Bee-crossed Lovers and a Forbidden *Castilleja* Romance:

Cross-breeding between *C. hispida* and endangered *C. levisecta* in prairie restoration sites

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Abstract

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Castilleja levisecta (golden paintbrush) is one of Washington's characteristic endangered species, restricted to the Pacific Northwest's disappearing prairies. To establish new populations, *C. levisecta* was seeded into restoration plots with other prairie natives including a closely related but more common *Castilleja*, *C. hispida*. Together these two *Castilleja* species could provide host plants for the Taylor's checkerspot butterfly (*Euphydryas editha taylori*), another endangered prairie species. However, suspicion of the two *Castilleja* species hybridizing in the wild if their populations overlap has led to confirmation of viable hand-crossed hybrids. Maintaining genetic separation of the two species is a high priority. This field investigation was conducted to determine the potential to hybridize via natural pollinators, and how the growing-context of the plants affects their reproduction. Analyzing the reproductive

characteristics and the seeds of plants from plots with both *Castilleja* species and from plots with each grown alone, as well as pure-bred off-site controls, indicate the species differ in their flower and seed production, with *C. levisecta* having higher fitness than *C. hispida* in this study, as well differing in seed germination and their sensitivity to context differences. *C. levisecta* reproduction was more inhibited by growth with *C. hispida* and pollinators showed no preference between the species. The restoration site quality also influenced *Castilleja* reproduction, partly due to decreased pollinator activity.

Analysis of the produced F1 generation from control (single-species) contexts and mixed-species contexts, using pollen and flower characteristics indicate a similar pattern of context-effect on *Castilleja* reproduction. The growing context of the species and site characteristics were found to influence *Castilleja* reproduction and increase the occurrence of hybrids when growing within a 40 square meter plot. Analyzing the reproductive characteristics identified distinguishing flower measurements for each species, particularly their proportions of bract lobing and galea beak proportions, as well as the angle of the flowers from the stem. Their pollen viabilities also indicate *C. levisecta* may have lower male fecundity than *C. hispida*, with hybrids having further decreased viability. A relatively small proportion of hybrid offspring were identified from mixed-species growing contexts, and with a higher incidence in one species' offspring than the other.

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Chapter 1

Pollinator Driven Cross-breeding between *Castilleja hispida* and Endangered *Castilleja levisecta* in Prairie Restoration Sites

Literature Review

Introduction

Tailoring prairie habitat restoration to optimize *Castilleja levisecta* survival requires careful selection of associated species, and understanding their interaction with, and effect on, the *C. levisecta* population. *Castilleja levisecta* conservation in the Pacific Northwest uses reintroduction of individuals or populations to restored prairie habitat, with the possibility of creating new sympatry with relatives. When introduced with a close relative or congener (*C. hispida*), because the *Castilleja* genus is well known for interspecies hybridization, the risk of genetic contamination and fitness-loss becomes a factor of concern. Hand-crossing between these *Castilleja* has produced hybrid F1 plants (Kaye & Blakeley-Smith 2008). Understanding the interactions of these two species, through their yet unidentified respective pollinators, and the outcome of possible hybridization events, is important for achieving stable reproductive populations *C. levisecta* in Pacific Northwest prairies. This literature review will focus on aspects of prairie and *Castilleja levisecta* restoration, pollinator behavior, hybridization dynamics, and seed characteristics as early hybridization indicators. Identifying early potential responses to hybridization in the F1 population would be valuable for preserving the genetic isolation of *C. levisecta* as a species. This could be done by analyzing seed production and other early reproductive measures of the pure-breeding species and their putative hybrids, potentially

linking these results to pollinator activity such as their degree of interspecies movements and constancy.

Prairie restoration

Optimizing prairie restoration techniques and revising theories are required for effective reintroduction and preservation of this very rare ecosystem. Prairies are unique as a habitat that usually has no opportunity for post-disturbance recovery on its own, since after a significant disturbance they do not regenerate unaided (Jordan 1997). A significant contributor to this is that prairies are continually occupied landscapes, by urbanization and agriculture (Jordan 1997; Moncada 2003). Conversion to different habitat via forest encroachment after fire suppression also changes the natural prairie dynamics, similarly yielding minimal chance of natural regeneration (Jordan 1997; Moncada 2003). This is partly why the prairie ecosystem is considered an endangered one across the globe, with less than 10% of North American prairies remaining now (Jordan 1997; Moncada 2003; Samson et al. 2004; Hamman et al. 2011), and why the science of ecological restoration began with them (Jordan 1997; Sperry 1983, as cited in Jordan et al. 1987, pg. 75). Some estimate there is as little as one percent of historic prairie left in the United States, most significantly due to their conversion to agriculture, as well as grazing on the lesser agriculturally suited prairie areas (Moncada 2003; Samson et al. 2004). Prairies are species-rich ecosystems, characterized as grasslands of tallgrass, mixed-grass, or shortgrass types with less than one mature tree per acre (Jordan et al. 1987), historically spanning a large area of the central United States (Moncada 2003; Samson et al. 2004). Prairies vary highly in their diversity and taxa composition depending on their geographic region, and once occupied nearly 24% of the Earth's land cover, making them formerly the largest terrestrial biome and distributed over most continents (Jordan et al. 1987; Savage 2004). Due to varying climate and stochastic

events, including fire patterns, a given prairie can change composition and diversity from year to year (Jordan et al. 1987), reflecting their highly dynamic character and close tie to disturbance.

Prairies of the Pacific Northwest (PNW) occupy even less historical area than those once occupying the other areas of North America, with fewer than 3% of pre-European settlement PNW prairie acreage currently left (Lawrence & Kaye 2011). Many of these remnant PNW prairies, like those across the world, are low quality due to fragmentation, fire suppression, and invasive plant introduction (López-Pujol et al. 2007; Dunwiddie & Bakker 2011; Lawrence & Kaye 2011). The small prairie system of the PNW, a small fraction of the system spanning central North America, was primarily maintained anthropogenically, as opposed to natural lightning-originated fires, for its resource value (Hamman et al. 2011). Anthropogenic fires halted in the mid-1800s after European settlement in the PNW, leaving the open prairies to be overgrown by encroaching shrub and tree species from the area, replacing prairie with forest habitat (Hamman et al. 2011). Of the few prairies/grasslands persisting in the PNW, typically those with less than twenty percent of their species richness consisting of non-native species are considered to be "high quality habitat", yet are still twenty percent invaded (Severns 2008).

The threats to prairies primarily involve habitat loss and degredation in various forms, via invasive species, conversion and fire suppression. Prairies are notably adapted to and reliant on fire, with deep root systems permitting fire-resistance (Moncada 2003). Suppression of fires by humans in remaining prairies allows fire-impeded forest and shrub systems to establish, making regular fire, or other disturbances, a requirement for prairie maintenance (Moncada 2003; Caplow 2004; CPC 2014). Fire suppression, with its influence on the majority of species, is also

a major threat considered to contribute significantly to the reduced population of *Castilleja levisecta,* in addition to the ubiquitous presence of anthropogenic habitat conversion (USFWS 2000; Caplow 2004; CPC 2014). *Castilleja levisecta* is the central species of a number of prairie restoration efforts across the Pacific Northwest (Caplow 2004; Caplow & Chappell 2005; Dunwiddie 2009). What prairie landscape is left is often fragmented, with restored sites also isolated due to habitat destruction and existing constructions blocking habitat connections and inhibiting natural migration or colonization (Moncada 2003). However, these restored sites can have similar diversity to larger remnant prairies, at least for surveyed insects, and size of restoration site was determined to not be a factor in insect population diversity (Moncada 2003).

Two basic restoration methods are used for prairies: increasing the quality/functionality of an extant degraded prairie, and creating a new prairie on land lacking any current prairie species (Jordan et al. 1987). Among the most common restoration tools of both methods is fire, establishing a regime suited for prairie-genesis or maintenance (Jordan et al. 1987). Typical problems all prairie restoration projects aim to solve are: 1) extirpating or at least decreasing the population of non-native species and native invading or encroaching species (typically woody species); and 2) boosting the diversity of less competitive prairie species. Specific methods depend on the region, current and desired populations, and current characteristics of the site. One effective method of restoring prairies, from a political and funding standpoint, is to target the restoration to be around a rare species endemic to that prairie system, thus gaining more persuasion power to conduct the restoration, brought with the recovery of the rare species. The majority of current restoration and conservation work in the PNW prairie and oak woodland

ecoregion is oriented towards the habitat of its 46 imperiled or vulnerable species (Hamman et al. 2011).

Endangered Species Restoration

Endangered species are particularly targeted for restoration due to their delicate status between survival and extinction, increasing their need for and benefit from restoration efforts. Rare taxa functional groups particularly at risk of extinction include insect-pollinated taxa, non-wetland taxa, those with small dispersal range for their seeds, and those at the northern edge of their range, according to a study conducted in New England (Farnsworth & Ogurcak 2008). Recovery of a rare species to a stable status requires wild populations be protected and able to sustain their population for the long term (Bowles & Whelan 1994). The survival and recovery of some species requires active restoration of degraded or extirpated habitats for the creation of new populations due to their extant populations being extremely low and particularly fragmented or isolated (Bowles & Whelan 1994; Caplow 2004; Maschinski & Haskins 2012). Some of these species are listed, or are in the process of listing, under the Endangered Species Act (ESA) and thus protected by the federal government with the end goal of de-listing them when their populations have become stable (NWF 2014). Getting a species listed is often the first step toward restoring its population, because listing under the ESA protects the species against further losses and funds can be allocated to its recovery, though only for plant populations on federal or state lands (NWF 2014). The U.S. Fish and Wildlife oversees listing and protection of these rare species (NWF 2014). Efforts by third parties must still be made to protect populations on private land, though with more persuasion power for listed species. Depending on the species' threats, characteristics, and the habitat it lives in, restoration methods for increasing the population could be as simple as changing management practices on the land it occurs on, or as extensive as total

ecosystem recreation and reintroduction to the area (Jordan et al. 1987). For many endangered or rare species, and particularly endemics, habitat loss and degradation is the primary reason for population declines and losses (Drake et al. 1998; Kofron & Chapman 2006; Fairbarns & Egger 2007; López-Pujol et al. 2007; USFWS 2010), especially for prairie endemics, because their habitat type is itself endangered (Walck et al. 2001; Dunwiddie & Bakker 2011; CPC 2014).

Common tools for applied restoration ecologists that are applicable to rare species include the various forms of species translocations, defined by the International Union for Conservation of Nature as the "movement of living organisms from one area with free release in another" (Weeks et al. 2011). These forms of translocations create new populations, which decreases the likelihood that the species will go extinct (Bowles & Whelan 1994). Reintroduction translocation involves placement of the species into a location within its natural or historic range where it has been extirpated, and is used for both rare species recovery and abundant species maintenance, (Bowles & Whelan 1994; Weeks et al. 2011). Reintroduction of rare species, in contrast to common ones, "occurs in a political glasshouse", meaning it is contained in an arena of increased regulation, often with higher concern for the project's success, though still depending on the species (Bowles & Whelan 1994). Introductions differ from reintroductions only in that the location is not where the species had previously been recorded, thus moving it beyond its historic range (Weeks et al. 2011). Assisted migration (i.e. 'assisted colonization' and 'managed relocation') is a more extreme form of introduction translocation, often used in response to climate change (Weeks et al. 2011). It involves moving or introducing the species to a location outside its current and historic ranges, but ostensibly to where climate changes will produce appropriate habitat (Weeks et al. 2011). Assisted migration is usually aimed at assisting

the species with longer-term population migration that is obstructed now by anthropic structures or anthropogenically produced habitat fragmentation (Weeks et al. 2011).

The genetic implications of any form of translocation should be accounted for in the restoration plan (Weeks et al. 2011), especially for rare species, which tend to have limited genetic variability (Bowels & Whelan 1994). Self-incompatible species would particularly benefit from genetic assessments for translocations because they require a higher amount of gene flow between unrelated individuals among their populations, likely requiring multiple populations be used for their sources (Weeks et al. 2011). Community size and density can also have strong effects on endangered species due to often being present at low densities. Flower patches of higher density attract more pollinators (discussed more in the pollinator section), and thus these plants tend to have a higher fitness than those of lower density and smaller community size (Robson 2010). Self-incompatible plants are even more inhibited when they have low population densities, and thus less compatible mates (Real 1983). This increases the chance of incompatible pollinations between relatives, or even self-pollination, which are unsuccessful fertilizations, thus causes reduced seed set (Real 1983). This creates a self-sustaining cycle of rareness, keeping the pollinator-popular species in the reproduction spot-light by increased outcrossed fertilizations. Reintroductions of endangered species should thus address population density, in addition to genetics, as factors in recovery. Augmentation of low density populations can help the entire species increase individual plant fitness.

In contrast to the outward spreading nature of translocations for restoration, *ex situ* conservation is a more inward pattern of species conservation, keeping smaller populations safe by clustering

them into a small protected facility. *Ex situ* ("off-site") conservation is a valuable tool in endangered species conservation and restoration because it allows a population to be very closely monitored, preserved, and even bred for reintroductions. *Ex situ* conservation usually takes place in botanic gardens or nurseries, and involves the collection of species of interest, often rare, into a protected site for close protection and study (Frankel et al. 1995; Ye et al. 2006). It provides the unique ease of access to research plants, and convenience for study and experimentation, as well as public accessibility (Frankel et al. 1995). An indirect benefit of *ex situ* collections, particularly those in botanic gardens, is the demonstration to the public of local biodiversity and education of species in need of human help to prevent extinction (Ye et al. 2006). Populations in *ex situ* conservation are also often used as a source of seed, plants, or clonal material for translocation restoration work (Jusaitis 2005). *Ex situ* conservation is treated more generally as an emergency action for species conservation, rather than the primary conservation effort, and used as a backup to *in situ* conservation and restoration efforts, in addition to being a source for *in situ* materials (Frankel et al. 1995). There is also potential for *ex situ* collections to undergo inbreeding due to small population sizes, and have artificial selective pressures different from those experienced by *in situ* populations (Margan et al 1998; Briggs 2009), due to their artificially controlled living environment, including novel pests & diseases, soils, water regimes, and even differential seed survival of their storage methods (Briggs 2009). This is compounded by a lack of diversity preservation among the collected plant species, since most *ex situ* efforts prioritize that simply "some material of each species" be conserved, without consideration to maintaining their natural variability (Briggs 2009). Since *ex situ* conservation can involve collecting multiple species into an artificially sympatric condition, it can also bring with it a novel risk of interspecies hybridization, if the collection species are compatible (Ye et al. 2006).

Hybridizing would contaminate the gene pool of each species receiving heterospecific pollen, and thus undermine the translocation population's integrity (Ye et al. 2006). Potentials for hybridization should thus be assessed if similar species are protected in the same *ex situ* site without isolation barriers to block pollinator exchange (Ye et al. 2006). The pollination system of endangered plants is most often unknown (Pitts-Singer et al. 2002), but the recovery of their populations is dictated by their successful pollination, and thus study of their pollinators is included in recovery plans (Neal 1998). Typically, though, pollinator management is not included in the plans and there is increasing encouragement for it to be (Neal 1998).

The allee effect is the lack of positive feedbacks from conspecifics when populations are small, thus inhibiting growth of small populations (Deredec & Courchamp 2007). It particularly applies to endangered and rare species because their extant populations tend to be at low densities and often in isolated patches (Deredec & Courchamp 2007). Allee effects increase the extinction risk for a population at lower densities due to lower fitness of the individuals (Deredec & Courchamp 2007). The level of conspecific benefit exceeding that of intraspecific competition depends on the species and conditions of the habitat (Deredec & Courchamp 2007). Pollination success is notably density dependent, especially with self-incompatible plant species (Real 1983; Robson 2010), and only one of a number of factors encompassed by the allee effect, including cooperative social interactions more applicable to animal species (Deredec & Courchamp 2007). Any restoration method applied to rare species should take this phenomenon into account and aim for high enough densities in restored or conserved populations, in order to achieve intraspecific benefits and avoid the allee effect. Reintroductions, in particular those of rare

species, are most often on a small scale, thus their susceptibility to allee effects should be assessed to optimize project success and, of course, budget (Deredec & Courchamp 2007).

Castilleja **Restoration**

Castilleja **Species**

Castilleja spp. are in the Scrophulariaceae (Figwort) family, previously classified in the Orobanchaceae (Broomrape) family, containing 200 or more species in the genus (Wentworth 2005; USDA 2014). They are short-lived perennials, often with showy flowers in a raceme (Wentworth 2005; CPC 2014). The racemes bear multiple bracted flowers and can continue to bloom until the end of the growing season (University & Jepson Herbaria 2014). The *Castilleja* genus spans the entire continent, including Alaska and parts of Canada (Hersch & Roy 2007; USDA 2014). Most species of the genus are characterized as hemiparasites, or green root-parasites, that will form haustorial root connections with neighboring host plants when nutrients and water are limited, making them photosynthetic and facultative, rather than obligate, parasites (Wentworth 2005; Hersch & Roy 2007; University & Jepson Herbaria 2014). *Castilleja* spp. will still complete their life cycles without a host plant in greenhouse experiments (Wentworth 2005; CPC 2014). *Castilleja* species have a broad range of host plants rather than specialize on one, and individuals could be able to form associations with several different species at the same time (Shen et al. 2005; Fairbarns & Egger 2007). Thus specific host availability is not likely a limiting factor in their growth, though some hosts may actually hinder growth and/or reproduction (Schmidt unpublished data 2014).

Two *Castilleja* species are the focus of this study- *C. levisecta* and *C. hispida*. *Castilleja hispida* (harsh Indian paintbrush) ranges widely from eastern Canada to southern California and is

considered fairly common across its range (Caplow & Chappell 2005; Crowe, eta l. 2014; USDA 2014). *Castilleja hispida* (CAHI) is documented across Washington and throughout the US in remnant grasslands (Palmer 2007; Burke Museum 2014). *C. hispida* blooms in racemes ranging from dark orange to less common yellow in color that continue to produce flowers from about April or May into August as the growing season concludes and the soil dries (Crowe et al. 2014). It inhabits both open grassy areas and forest gaps spanning sea level to mountain elevations (Crowe et al. 2014).

Castilleja levisecta (golden paintbrush) also blooms in continuing racemes beginning between the end of April and early May and concluding in June or early July, but consisting exclusively of yellow coloration (Evans et al. 1984; WDNR 1997; Delvin & Bakker 2013). Some *Castilleja levisecta* populations average two to three flowering stems per plant, but other field sites are reported to have as many as eight to fifteen stems per plant, and less common reports of as many as 50 stems or more (Caplow 2004; Wentworth 2005). The site and its community likely are what causes such variation in *C. levisecta*'s reproductive capacity. It is listed under the federal Endangered Species Act, as of 1997, with only eleven known populations remaining across its range (USFWS 2000). *Castilleja levisecta*'s federal status is categorized as threatened, and locally in Washington & Oregon as endangered, with a high recovery priority of 2 (on a scale of 1 to 18) (USFWS 2010). Since its listing on the Washington Natural Heritage Program's advisory endangered species list in 1981, *C. levisecta's* populations have been recorded as in decline, and 20 or more populations are now gone from previously inhabited sites, due to prairie habitat destruction or disturbance for human use in agriculture or development (USFWS 2000; Gamon et al. 2001; Kaye & Lawrence 2003; CPC 2014). The extant populations of *Castilleja levisecta* (CALE) are in Washington & British Columbia in isolated patches of remnant prairie

(Wentworth 2005; IAE 2014), ranging from flat to mounded topographic grasslands to grass-dominated rocky bluffs of the Pacific coast (USFWS 2000). They once spanned from southern Canada's Vancouver Island, south to Oregon, on more than 30 recorded sites, but are considered extinct in most of these locations and completely absent in OR (USFWS 2000; Kaye & Lawrence 2003; Wentworth 2005; Severns 2008). The majority of *C. levisecta* populations survive on islands. There are only nine populations in Washington, restricted to the Puget Sound trough and the San Juan Islands that maintain the most populations, and two populations on islands in British Columbia (USFWS 2000). Five of the eleven total remaining populations are on public land, and the remaining six are on private (USFWS 2000). Significant efforts are currently taking place in OR to reestablish populations there (USFWS 2000; Kaye & Lawrence 2003; IAE 2014), and in WA to strengthen extant populations and reestablish more in restored prairies (Wentworth 2005; Dunwiddie 2009; CPC 2014).

The stable recovery of *Castilleja levisecta* was determined to require twenty populations of 1,000 individuals or more and to be located on managed lands protected from further damage (USFWS 2000; Caplow 2004; USFWS 2010; Maschinski & Haskins 2012). Populations are considered stable when they average a minimum of 1,000 flowering individuals for a 5-year period (USFWS 2000; Caplow 2004). Recovery of the species also requires 15 of the 20 populations be on protected land of a government agency or conservation agency (Caplow 2004). Recovery to a stable population size would include augmenting existing smaller populations with more plants, in addition to establishment of new populations, in order to reach the twenty populations needed for species stability and delisting (Caplow 2004). *C. levisecta* seems to only reproduce sexually via seed resulting from outcrossing, and are self-incompatible (Kaye & Lawrence 2003),

therefore wild seed collection and mass nursery propagation for outplanting is important for reintroduction (USFWS 2000; Caplow 2004; Wayne 2004). Kaye & Lawrence (2003) state *C. levisecta* is protogynous, with stigmas maturing first, but a report of another *Castilleja* species, *C. linariaefolia*, state those *Castilleja* flowers are protandrous, with anthers maturing first (Caruso & Alfaro 2000). Protandry is the more commonly occurring type of differential floral organ timing, or dichogamy, and characteristic of plants with fused corollas and zygomorphic flowers like *Castilleja*, while protogyny is more common to wind pollinated flowers (Proctor et al.1996). This may be an issue of stem reference point, as the flowers at the top of the raceme are the youngest, thus organs would mature from bottom to top of the stem in either order of anther or stigma first. Confirmation of the dichogamy type may be useful in fitness assessments using pollinator activity.

Threats to *Castilleja*

Castilleja levisecta survival is limited by several major factors: low seed germination in wild populations and low seedling survival, low competitive ability with woody encroaching and non-native species, habitat loss, and herbivory (USFWS 2007). Similarly, the threats to *C. levisecta*, and to a lesser degree to *C. hispida*, include the same broad threats of prairies nationwide-agriculture, residential, and urban conversion and spread, as well as fire suppression, non-native plant invasion, native plant encroachment, and cattle grazing (USFWS 2000; Caplow 2004; Wentworth 2005; CPC 2014). Some of these particularly threaten *Castilleja* species like, *C. levisecta*, because the plants are poor competitors. More specific threats, such as human collection/flower picking, affect *Castilleja* species because the plants are aesthetically pleasing (Caplow 2004; Wentworth 2005). Removing entire inflorescences would greatly reduce seed-set

per plant since their continual blooming occurs on single stems, with typically only a few per plant. Another reproductive threat, self-pollination via geitonogamy, applies to both *Castilleja* species. Because *Castilleja* species bloom with several simultaneously flowering stems, and thus several flowers dehiscing pollen at the same time, a pollinator could transfer pollen between flowers of the same plant. This form of self-pollination, involving the movement of pollen between flowers on the same plant, can be considered a threat to the small populations of *C. levisecta* because they are more sensitive to decreased reproductive success (Proctor et al. 1996; Kaye & Lawrence 2003). Generally, seedlings produced from self-fertilizations tend to be poorer performers as well (Real 1983). Even if no or minimal self-fertilization can occur for self-incompatible species, geitonogamy can still decrease fitness by wasting pollen, clogging the recipient stigma, and/or wasting ovules that will be later aborted by late-acting reproductive barrier mechanisms (Proctor et al. 1996; Mitchell et al. 2009). The extent of geitonogamy depends on the pollinator's behavior, pollen carryover amount (the amount of pollen remaining on the animal after the first new flower pollinated, i.e. the percent not deposited per visit), and the number of flowering stems available (Proctor et al. 1996). The genetically identical threat of autogamy self-pollination, where pollen is moved from the anthers to the stigma of the same flower, is minimal due to the flower's structure. *Castilleja* flowers have stigmas extending beyond the stamen that are often enclosed in the galea, and either protogyny or protandry acts as a temporal autogamous self-pollination barrier for individual flowers (Kaye & Lawrence 2003).

Castilleja levisecta is also at risk from stochastic natural events like unusual fire timing and erosion, due to the isolated and mostly small populations being more likely to be extirpated from their site, and a near zero chance of natural recolonization (Caplow 2004; Wentworth 2005).

About half of the extant populations of *C. levisecta* are either in decline, possibly in decline, or temporarily stable for the four largest populations (Gamon et al. 2001; Caplow 2004; CPC 2014). Early data indicate that even the largest populations are not safe from the possibility of significant loss, and without active management even these stable populations could be extirpated within 50 years (CPC 2014), making management and restoration important to the species' survival.

Unlike the major threats of habitat loss and degradation, a more subtle threat to *C. levisecta* comes from it being such a rare species, with a very close relative that can co-occur in native prairies of the Pacific Northwest. The native *Castilleja hispida* is itself a potential threat to its endangered cousin, though it does not encroach habitats like native tree and shrub species threatening prairie plants (Hamman et al. 2011). Because these two plants are closely related, and *Castilleja* species are generally a genetically promiscuous species (Egger 1994; Hersch-Green & Cronn 2006; Hersch & Roy 2007), *C. levisecta* is threatened by genetic contamination from *C. hispida* hybridizing with it in natural settings, if pollinators transfer pollen between the plants. Though it is thought that novel species are commonly created through interspecies crosses like this throughout natural evolution history, and the creation the *Castilleja* genus' diversity has been partly credited to its high degree of hybridization, hybridization is still a significant risk for the rare *C. levisecta* (Egger 1994; Clay et al. 2012). Hybrids could potentially alter the ecology and evolution of their communities, and increase the risk of extinction when one of the species is rare (Wolf et al. 2001; Ayres et al. 2004; Hersch-Green & Cronn 2006).

Concern of *C. levisecta* hybridization has increased since proof of successful hybridization was demonstrated via hand-pollinated greenhouse crossings (Kaye & Blakeley-Smith 2008). Despite extensive overlap of the historic ranges for at least potential habitats of both *C. levisecta* and *C. hispida*, they have only been reported as naturally co-occurring at the Rocky Prairie site in the 1980s, though *C. hispida* has been extirpated there for ten or so years now (Kaye & Blakeley-Smith 2008; P. Dunwiddie email correspondence 2011). Hybridization concerns have also been fueled by observations of high *C. hispida* floral color variation at the Fort Lewis Military Reservation site including an unusually high number of yellow color variants (P. Dunwiddie email correspondence 2011; Egger 2014) possibly indicating a former site of both co-occurrence and hybridization. Though hybridization is a natural process occurring across the globe for millions of years, it poses a threat because *C. levisecta*'s small numbers could be significantly altered by the presence of hybrids, more so than a more populous species that could withstand genetic changes and sterile or less-fit hybrids.

Castilleja Restoration Techniques

Potential restoration work for this study's *Castilleja* species spans the historic range of *Castilleja levisecta* (Caplow 2004), which overlaps the western range of *C. hispida.* Restoration efforts are not focused on *C. hispida,* which is restored through prairie community restoration in general, rather than specifically targeted like *C. levisecta* as an endangered species with higher attention and need. Like the conservation plans of many endangered plant species, *Castilleja levisecta* restoration entails two basic methods: expanding current populations and introducing new populations to suitable sites (Caplow 2005; Caplow & Chappell 2005; Lawrence & Kaye 2011). Higher priority is given to the stabilizing and protecting of extant populations, due to uncertainty and the still experimental nature of reintroduction methods (Caplow 2004). It is suggested that

any reintroductions use a minimum of three to four times as many propagules as the target population size requirement (Caplow 2004). Sites proposed for *C. levisecta* reintroduction should be prepared to a habitable state prior to the introduction of *Castilleja* plants (Caplow & Chappell 2005) to increase survival. However, the habitable state may be determined as clear of non-native plants and success with co-seeding *C. levisecta* with its associated prairie community has proven successful (Delvin & Bakker 2013). Despite being a hemiparasite, C. levisecta does not need established hosts before seeding into a site, as seedling survival does not appear to require hosts (Delvin & Bakker 2013). Site preparation may only require edaphic characteristics favorable for *C. levisecta* introduction (Delvin & Bakker 2013). Restoration practices for *C. levisecta* plants include reintroducing them to an historic location- one of the 30 previously documented sites- and introduction to a site within its historic range but not previously known to grow there (Caplow 2004). Methods used for restoring habitat for *Castilleja levisecta* include the reestablishment of a fire regime into a prairie system (Delvin & Bakker 2013), which has resulted in increases in blooming, seed production, and seed germination of *C. levisecta* for years following the burn, as well as increased survival of post-burn established plants (Caplow 2004; CPC 2014). Prairie maintenance requires regular fire, either natural or anthropogenic, to prevent woody plant encroachment, thus many prairie endemics are adapted to low-intensity fire regimes, generally requiring regular fires to survive (Hamman et al. 2011; Grant et al. 2010; Tompkins et al. 2010). Prescribed fires should be set in the fall to allow for maximum *Castilleja* seed maturation and dispersal (USFWS 2007). *Castilleja* species have also benefited from mowing practices, partly due to increasing seed dispersal when mowed in the fall (Caplow 2004). Assisted seed dispersal within the population area could help the fitness of following generations by reducing the neighborhood effect, which creates patches of closely related

individuals that would potentially cross less successfully than with those further away (Bowles & Whelan 1994). Inbreeding is likely to increase within these neighborhoods, due to offspring staying close to the parents via their limited dispersal ability, and thus increasing the risk of inbreeding depression and lowered fitness from less successful fertilizations and lower germination (Talve et al. 2012). These related patches, or neighborhoods, also create areas that are equally susceptible to extinction or the creation of bottlenecked populations if only single patches survive (Bowles & Whelan 1994).

Standard reintroduction practice for *C. levisecta* was planting plugs, grown *ex situ*, into sites (Caplow 2004; Dunwiddie 2009), before success with direct seed sowing was achieved, sowing in fall for natural winter stratification (Kaye et al. 2011; Delvin & Bakker 2013). Sowing is a less labor intensive method and increasingly preferred for *C. levisecta* restoration (Kaye et al. 2011). *Ex situ* conservation is a major component of *C. levisecta* conservation due to its low dispersal capacity and isolation of the extant populations (Lawrence & Kaye 2011). The currently known primary host plants used for *C. levisecta* association are *Eriophyllum lanatum* and *Festuca roemeri*, though it is known that *C. levisecta* can parasitize a variety of host species (even attempting to form haustoria with inanimate soil materials), which is common among *Castilleja* species (Adler 2003; Caplow 2004; Lawrence & Kaye 2008; Schmidt unpublished data 2014). Though *C. levisecta* was found forming haustoria with its own roots when grown alone (Caplow 2004), it was not found forming haustorial connections with a separate individual of its own species when grown together (Schmidt unpublished data 2014). The host species can both directly and indirectly affect the performance of the parasitizing *Castilleja*, changing the number of stems and flowers, plant height, herbivory, survival, and even the number of

pollinator visits (Adler 2003; Lawrence & Kaye 2008). Due to increased growth and blooming when growing with an optimal host, these should be considered for use in restoration plant populations (Caplow 2004), though variable results with field applications should also be investigated further (Lawrence & Kaye 2008). Direct sowing of seed, as well as plug transplanting of *C. levisecta,* should include consideration of seed source genetics and applicability to the target habitat (Kaye & Lawrence 2003; Caplow 2004).

Castilleja Genetics

The current genetic condition of extant *C. levisecta* populations should also be considered in restoration projects to optimize success. Both rare and endemic species generally have lower allelic diversity and less heterozygosity than more widespread species, lending additional complication to their conservation and restoration (López-Pujol et al. 2007). Lower genetic diversity creates increased opportunities for inbreeding and extinctions (Margan et al. 1998; López-Pujol et al. 2007; Weeks et al 2011). Inbreeding depression can be detrimental to populations, particularly affecting smaller and less diverse ones (Caplow 2004; Godefroid et al. 2011; Talve et al. 2012). Augmentation by introducing lesser-related individuals from different populations for beneficial outcrossing through new plant genotype introduction can rebound the whole population's fitness (Kaye & Lawrence 2003; Caplow 2004). On the other hand, outbreeding depression can also be a risk if introduced plants are too distantly related, possibly disturbing co-adapted gene associations that are required together to create ecological resistance or adaptations (Proctor et a. 1996; Kaye & Lawrence 2003; Caplow 2004; Hersch-Green 2012). Outcrossing depression is less likely, as controlled crosses between self, siblings, and non-siblings of either the same or a different population demonstrate fitness increases at least in the

F1 generation via pollination by individuals of a different population (Kaye & Lawrence 2003). Outcrossing depression theoretically is a higher risk between populations at further distances from each other; however *C. levisecta* populations nearest each other may not be the most genetically similar, according to allozyme comparisons between all eleven populations at 16 loci (Godt et al. 2005). Further confounding the prediction of outbreeding depression is the fact that it may not be revealed in the first generation, thus continued breeding of F2 to F3 generations may be necessary to determine likelihood of outbreeding depression (Kaye & Lawrence 2003). Inbreeding may also be of minimal threat to *C. levisecta,* as a genetic analysis of several populations' allozyme diversity showed no evidence of inbreeding, and demonstrated high genetic diversity both within and across populations (Godt et al 2005). Inbreeding could still be possible, since smaller populations did have lower genetic diversity, as is the general trend among plant populations (Godt et al. 2005) and *Castilleja* species are moderately compatible with close relatives that would be nearby due to low dispersal ability (Lawrence & Kaye 2011). Smaller populations would also potentially have lower reproduction due to fewer individuals carrying the compatible alleles that are used to recognize self-incompatibility, yielding less compatible mating pairs (Bowles & Whelan 1994), though this risk is similarly abated by high genetic diversity. Higher than expected genetic diversity seems to indicate that augmenting populations through new genetic introduction would not be necessary or particularly helpful for this endangered plant species. The genetic information should still be useful in establishing new populations and appropriating compatibility plant stock for population expansion. While inbreeding can still pose a threat to the species, for example if a population experiences a bottleneck from a drastic decrease, the discovery of currently high genetic diversity shines a hopeful light on the stabilization of the rare golden paintbrush.

Association with Taylor's checkerspot

Castilleja species are often predated by a wide variety of caterpillars, despite a profusion of sticky trichomes. Evans et al. (1984) found as many as eight types of caterpillars feeding on *C. levisecta*. Among these, Evans et al. (1984) found larvae of the rare Edith's checkerspot butterfly (*Euphaedryas editha,* formerly *Occidryas editha*), which seemed to show no differential feeding preference between *C. levisecta* and *C. hispida* (Evans et al. 1984), indicating similar palatability of the *Castilleja* species to certain insect predators. *Castilleja hispida* is a well-documented host plant for the caterpillar of a subspecies of Edith's checkerspot butterfly, the endangered Taylor's checkerspot (*Euphydryas editha taylori*) (Vaughan & Black 2002; Aubrey 2013). Taylor's checkerspot has only fourteen known populations, with two containing the majority of their numbers (Black & Vaughan 2005). It is endemic to the PNW region between Oregon's Willamette Valley and the islands of British Columbia (Vaughan & Black 2002; Schultz et al. 2011). Though *C. levisecta* is still being explored as a possible host for Taylor's checkerspot with very recent positive results (Aubrey 2013), combined restoration and conservation efforts for the two are in place in the South Puget Sound (Schultz et al. 2011; Delvin & Bakker 2013; South Sound Prairies 2014). One concern for use of *C. levisecta* as a host plant is a possible incongruous phenology between the plant's vegetative availability and the butterfly's need to feed active caterpillars (Severns 2008). This concern may not be a significant one however with projects already underway and phenologies varying by year and locations for both species, plus the two may overlap more than predicted (Aubrey 2013). Schultz et al. (2011) state that when multiple rare prairie species occur together, such as with *C. levisecta* and *E. editha*, "a balance must be struck in managing and restoring habitat to meet the needs of each". Taylor's checkerspot is most threatened by habitat loss & degradation, similar to *C.*

levisecta and very typical of endangered species (Black & Vaughan 2005). With some extant populations in Oregon, and increasing efforts to reintroduce extirpated *C. levisecta*, which could serve double biodiversity duty as a larval host to Taylor's checkerspot, coordinated restoration and management efforts are imminently needed for these two rare species (Dunwiddie 2014).

Castilleja **hybridization**

When sympatric, two similar species can hybridize if genetically and physiologically compatible (Hersch & Roy 2007; Flegr 2008). Hybridization can alter the plant species in a variety of ways addressed more in chapter two. Compatibility between the species involves both matching sets of chromosomes (Heckard & Chuang 1977; Rieseberg et al. 1998; Hersch-Green 2012) and physiologic compatibilities for fertilization, based on genetic content (Proctor et al.1996). The *Castilleja* genus is well known for its tendency to hybridize across species, even between more than two species, especially where their ranges distinctly overlap (Egger 1994; Hersch-Green & Cronn 2006; Hersch & Roy 2007; Clay et al. 2012). Promiscuous species like these can hybridize in certain instances but not others, depending on any variety of genetic, physiological or even abiotic factors, as well as differences in pollinators or their preferences (Hersch & Roy 2007). Different pollinators will contribute more or less to hybridization due to differing constancy, or loyalty to a plant species, defined as the tendency of an individual pollinator to restrict their foraging to specific flower morphs or species while rewarding alternatives are available (Waser 1986; Hersch & Roy 2007). Their constancy entails bypassing close by alternative flowers in order to reach the preferred ones (Waser 1986). Wind pollinated plants would experience higher reception of interspecies pollen because of the randomness of wind as a vector for pollination, thus some may have stronger genetic cross-breeding barriers, exempting

several conifer groups that readily form fertile F1 hybrids (Rong et al. 2004; Williams 2009). Wind-pollinated plants have less control over their pollination than species using animal-mediated pollination, due to an ability to influence pollinator behavior to control pollination (Proctor et al.1996).

When compatible and sympatric species have successful pollen transfer and produce hybrid generations, they create a hybrid zone or hybrid swarm, typically along the meeting edges of their ranges that only partially overlap (Egger 1994; Ayres et al. 2004; Hersch-Green & Cronn 2006; Clay et al. 2012). Established hybrid zones increase the risk of further hybridization because the presence of hybrids may decrease pollinator constancy, thus increasing the interspecific pollinations (Hersch & Roy 2007). However, even areas lacking mature hybrids but containing a mixture of closely related plant species experience high risk of interspecific crossing as pollinators have decreased constancy in these sympatric contexts as well (Hersch & Roy 2007). Inconstant pollinators can create hybrids, but moving between species does not guarantee successful pollen deposition on another species' recipient stigma (Ye et al. 2006). Flower structure and pollinator activity both dictate cross-pollination rates.

Pollinator behavior

> *"That insects should visit the flowers of the same species for as long as they can, is of great importance to the plant, as it favours the cross-fertilisation of distinct individuals of the same species; but no one will suppose that insects act in this manner for the good of the plant. The cause probably lies in insects being thus enabled to work quicker; they have just learned how to stand in the best position on the flower, and how far and in what direction to insert their proboscides."* (Darwin 1876, p. 419.)

Pollinators are vectors of gene flow between immobile plants that don't wind or auto-pollinate. They are crucial to the survival of these plants' populations by providing a method of seed

production and thus successful reproduction (Pitts-Singeret al. 2002). The acknowledged importance of animal pollination has seen a recent increase partially due to the increased die-off of bee colonies and particularly the effect on the agricultural industry, even making its way into popular TV shows like *Doctor Who* (Davies & Nation 2008; OPS 2014). Yet pollinator fauna are responsible for roughly 90% of global angiosperm pollination and have experienced decline well before they became a popular public concern (Menz et al. 2011; ARS 2014). Despite bee declines originating from natural & anthropic causes, rather than returning to their home world like Dr. Who proposes, the public should still be interested with their fate (Davies & Nation 2008). A major part of our agriculture depends on bee pollinators, mostly introduced honey bees (Lindsey 2008). Animal pollinators are said to be responsible for 35% of our global food sources (Winfree et al. 2007), with honey bees contributing an estimated $11.68 billion value to US agriculture as of 2009, through at least 50 crops, and a minimum of 38 crops in the United Kingdom (Dramstad et al. 2003; Calderone 2013). But it's not just agriculture's pollinators in decline, nor is that the only system highly dependent on animal pollinators. Invaluably, bees pollinate as much as 57% of all wild flowering plants (Dramstad et al. 2003). Most plant species, as assessed recently, exhibit pollination limitation, though crop pollination is receiving preferential attention as a directly anthropic issue (Menz et al. 2011).

Prairies are synonymous with flowers and prominent pollinators. Truly successful prairie restoration requires the establishment of prairie pollinator communities with the plant communities, especially for recovery of plant species that are pollinator-dependent for reproduction such as *Castilleja* species (Wentworth 1994; Neal 1998; Menz et al. 2011). Luckily, animal pollinators are often able to colonize new habitat sites easier than less mobile

plant species. Intact remnant prairies were documented as having similar bumblebee diversity to historical records of the area, having ten of the fifteen previously recorded species (Hatten et al. 2013). Pollinator numbers were also found to be very similar between restored and remnant prairies, with size of the restoration site not affecting the number of pollinators present, particularly when near remnants for easier native pollinator colonizing (Reed 1995; Moncada 2003). However, barriers to insect migration, like roads, fields, and developments, that cause habitat fragmentation, restrict pollinator colonization of isolated prairies patches (Moncada 2003; Menz et al. 2011). Loyalty to specific forage sites can also limit colonization for bumblebees, despite an ability to fly long distances through non-hostile habitat for foraging (Moncada 2003). Colonization of new restoration sites may require habitat corridors or connections for new bumblebee queens in early spring searching for nest sites and other fauna sensitive to habitat types as they travel. Habitat fragmentation particularly affects bees as a general pollinator group, because they tend to be poor dispersers, especially the more social (eusocial) species, due to their aggregation habit (Real 1983). Sites closer to tropical forest fragments, sources for wild pollinators, receive higher visitation rates from surveyed bees in Costa Rican coffee crops adjacent the fragments (Ricketts 2004). This difference was caused by an increase in native bees that forage close to their forest fragment, bringing more pollinator species and higher pollen-deposition rates for the adjacent coffee plants (Ricketts 2004). Honey bees were found to travel further distances then other eusocial native species from fragments to pollinate coffee crops in Costa Rica (Ricketts 2003). Other factors effecting bee and other pollinator colonization of a site include availability of nesting locations and materials, food sources, and sheltering areas for predator protection, and all must be in easy flight range for a colony (Moncada 2003).

Pollinator movements and community make-up are strongly influenced by various ecological factors (Real 1983). The types of pollinators present on a site can vary significantly with changes in plant community, such as population density, distribution and number of species (Hersch & Roy 2007), with lower plant densities attracting less pollinators and less pollinator visits to each flower (Real 1983). Pollinator behavior also significantly influences plant populations and patterns in the communities (Koch et al. 2012). Pollinators change their foraging patterns depending on floral density and availability, which in turn change with season and year (Real 1983). This means floral distributions affect pollinator behavior thus coming full circle to affect the flowers' pollen flows (Real 1983).

Pollinator Influence on *Castilleja*

Since *Castilleja* plants do not self-fertilize, cross-pollination is essential for their reproduction (Moncada 2003), and *Castilleja* species even benefit from fertilization with more distant genotypes rather than siblings or individuals from closer populations (Kaye & Lawrence 2003). Bees in general are found to forage upward on inflorescences with flowers opening from the bottom up, like *Castilleja* racemes (Real 1983; Proctor et al. 1996; Richards 1997). This is beneficial behavior especially to protandrous plants, so that pollen is not moved downward to those with mature female organs, but rather across to one on the bottom of the next inflorescence. In this way, older flowers with mature stigmas are visited first at the bottom and get pollen from a different inflorescence deposited, then the bee moves upward to reload from the newly maturing anthers above (Proctor et al. 1996). This upward foraging pattern, stopping before reaching the top-most flowers, is likely reinforced by reward availability, as nectar volume of flowers tends to decrease upward (Real 1983; Richards 1997). Pollinators stop

moving up the flower spike once the reward (nectar) per flower is below the mean for the flower population (Richards 1997). Protandrous dichogamy is, more often than not, associated with self-incompatibility (Proctor et al. 1996), creating a redundancy for protection against inbreeding. Protogyny, in contrast, is associated with self-compatible species, and the stigma of these plants may remain receptive when the anthers later dehisce, if no other pollen has been received (Proctor et al. 1996). Some plants demonstrate both types of dichogamy, varying them between simultaneously flowering individuals or with each type opening at different times of the day (Proctor et al. 1996).

With the confirmation of viable hybrids of *C. levisecta* and *C. hispida* in human-pollinated greenhouse experiments (Kaye & Blakeley-Smith 2008), and given the restricting floral structures and self-incompatibility of these plants, the behavior of wild pollinators in the field must be established to assess likelihood of wild hybrids. *Castilleja* species require insect pollinators to transfer their genetic material to new plants due to envelopment of their stamens in the beak of the galea and behind the floral bract. It's likely only large enough animals that can push their way between the galea and bract to reach the nectar at the base are able to open the galea beak and become dusted with pollen, however small insects that do not visit for nectar can still access the stamen for pollen collection and potentially transfer pollen to the stigma (personal observations). To date, little is known about the pollinators of either *Castilleja levisecta* or *C. hispida*, which is surprising given the importance of the pollinator's role, the recent surge of active research surrounding the endangered *C. levisecta*, as well as the commonness of *C. hispida* increasing the likelihood of pollinator reports. Generally it is assumed they are similar to other *Castilleja* species in being pollinated by bumblebees and/or hummingbirds without

verification (Duffield 1972; Anderson & Taylor 1983; Caruso & Alfaro 2000; Lindsey 2008; Tank et al. 2009). Identification of pollinators and their biological and habitat requirements are included in the *Castilleja levisecta* recovery plan (USFWS 2000). Visual observations of presumably *Bombus* species are the only reports of *C. levisecta* pollinators, with the species estimation of *B. californicus* based on color patterns, but with no entomologist verification (Evans et al. 1984; Caplow & Chappell 2005). These observations were somewhat discredited when it was suggested the bee may actually belong to a leaf cutter group and mistakenly identified (Caplow & Chappell 2005). Thus definite confirmation of wild pollinators of both species is a high priority.

Pollinator Types

The morphology of a plant's flower and placement of stigma relative to the anthers are good indicators of what pollinates it. Flowers with bilateral symmetry like those in the *Fabaceae* (pea) and *Scrophulariaceae* (figwort) family, which includes *Castilleja*, are often bee pollinated, while flowers with long narrow tubular structure are more likely Lepidoptera (butterflies and moths) or hummingbird pollinated (Real 1983). Prairie habitats are iconic for both these groups of pollinators but the focus here will be on bee pollinators for relevance to *Castilleja* species.

Important bee types in prairies include bumblebees, miner, carpenter, leafcutter, and mason bees, though most insect conservation efforts may focus on butterflies (Moncada 2003), despite thirty-five species of bumblebees thought to be threatened (Moncada 2003). European honey bees are also useful across North America as introduced generalist pollinators and can forage on many plant species, though not as efficiently as native bees (Turpin 1999; Harmon et al. 2011)

However honey bees are primarily valuable for agriculture rather than native plants (Moncada 2003; Harmon et al. 2011). Native bees have been found to effectively pollinate several crops with higher efficiency than other managed species (Ricketts 2004). Most bee pollinators, including honey bees, usually forage within small areas, exploring a forage location of about 16 km in diameter for a season but sticking to small portions at a time, and moving to new portions as they become valuable for forage and others wane (Real 1983). It was initially thought that distances between a colony's nest and its floral sources would be as short as possible to reduce time and energy spent in flight but several studies have found distances of several miles being common to bumblebee flights (Dramstad & Shaffer 2003).

Very rarely do prairie plants have specialized associations with pollinators and if a plant has evolved to utilize a specialist pollinator that is missing, generalists may fill in the niche as they colonize the site (Moncada 2003). However, since generalist pollinators may not visit the plants sufficiently and tend to be less efficient at pollen transfer than specialists (Menz et al. 2011), the recipient plant will likely suffer from decreased seed set, unviable seeds, and/or possible inbreeding when susceptible, which will all decrease future population size and overall fitness (Moncada 2003). Maximizing pollinator diversity is important for stable landscape restoration efforts in order to fill as many pollination niches as possible and allow for temporally shifting pollinator roles without decreased plant fitness (Ricketts 2004; Menz et al 2011).

Pollinator effectiveness

Both time allocated to foraging and number of flowers visited differ by pollinator types depending on their metabolic needs and physical capabilities (Hersch & Roy 2007). Pollinator

communities of various habitats tend to consist of a few generalists, rarer specialists, and a large portion of slightly specializing pollinators, with temporal variation of what roles the different pollinators play between seasons and years as the floral community shifts its phenology (Menz et al. 2011). The few generalist species provide the majority of pollination (Menz et al. 2011).

Bees, in general, are effective pollinators and are typically generalist in many habitats, in contrast to other fauna such as flies, beetles, and birds. Bumblebees are central place foragers (Dramstad et al. 2003), making short flights centered around one point, and visit more flowers than hummingbirds, and both flies & hoverflies visit even fewer flowers than hummingbirds (Hersch & Roy 2007). Different pollinator types also move different amounts of pollen as they forage between flowers depending partly on their load capacity as well as forage behavior, yielding more or less efficiency in pollination (Richards 1997; Real 1983). Some pollinators groom themselves between flower visits, particularly when they specialize on nectar collection rather than pollen (Taylor 2008). Even if a pollinator stays dusted with pollen as it forages between different flowers, so that pollen is in a position to be transferred to the next flower's stigma, not all of it will be deposited. Generally as much as 50% of the transferred pollen will be deposited on the next flower visited, which tends to be a close neighbor, with original pollen deposition decreasing rapidly with each flower visit (Real 1983; Richards 1997). Because not all pollen is deposited on the next flower, pollen carryover can move pollen more than would be presumed based on pollinator movement alone (Real 1983). Carryover amount depends on both the flower and the pollinator, possibly even the amount of nectar reward in the flower as well (Real 1983). Grooming behavior, the time spent on the flower and the way the pollinator accesses or manipulates the flower all affect the amount of pollen transferred to the next flower's

stigma and vary by pollinators (Taylor 2008). Pollen foraging bees are more effective pollinators than nectar foraging bees (Kevan 1999; Evans & Spivak 2006).

Honey bees stay faithful to small forage areas over several days, minimizing the risk of poor foraging in unknown areas (Real 1983). Though not the most effective pollinators of cranberry crops, when honey bees were not introduced during the blooming period as is common practice, the cranberry yield was significantly lower than fields with honey bees introduced (Evans & Spivak 2006). They produce a 19% lower seeds yield than leaf-cutter bees in alfalfa test gardens, but slightly higher cross-pollination rates (Real 1983). The honey bees also carried the alfalfa pollen further distances from the plants (Real 1983). The presence of non-native honey bees can create negative effects for the extant native bee populations via competition (Moncada 2003).

Bumblebees serve as generalist pollinators, collecting both pollen & nectar from flowers as they learn the most rewarding species or morphs to forage on (Koch et al. 2012). Bumblebees are also important pollinators to open areas like prairies because of their high efficiency. Bumblebee colonies need a more constant supply of resources than honey bees because they store their supplies for only a few days (Dramstad & Shaffer 2003). Bumblebees are long-lived pollinators, so they require usually multiple plant species to have consecutively open flowers to forage on longer or species that match their varying peak forage times (Moncada 2003). This makes *Castilleja*'s inflorescences a valuable floral source to prairie bumblebees because their racemes continue to produce flowers for several months and into the end of summer for *C. hispida*. Bumblebees are considered "keystone species" and also suffering declines in populations like the

honey bees (Kevan 1991; Hatten et al. 2013), but still representing the dominant pollinators of the northern hemisphere, serving as valuable native and crop plant pollinators (Hatten et al. 2013). Decreasing bumblebee populations can change plant populations by reducing their seed production due to lesser quantity and quality of pollinations (Barron 1998; Hoehn et al. 2008). Bumblebees are known as the most efficient pollinators of cranberry crops, though seldom managed by cranberry farmers because the farming practices disturb their ground nest sites (Evans & Spivak 2006). Bumblebees fly up to 1.2 km, regularly flying 450 or more meters from the nest to floral resources (Hatten et al. 2013) but usually traversing less than 50 meters at a time (Talve et al. 2012). Compared to butterflies, bumblebees visit nearer plants (shorter inter-plant flights) while foraging away from the nest, but visit more flowers in an inflorescence per plant than butterflies (Real 1983). They often fly past the nearest forage area in preference for another which they are faithful to (Real 1983; Osborne et al. 1999). They demonstrate patch faithfulness depending on the habitat, because homogenous fields induce no faithfulness and fields with clusters of flowers have high faithfulness (Real 1983). Bumblebees also forage longer through the season than many solitary bees and are thus able to pollinate a larger variety of flower species (Hatten et al. 2013).

Less is known about the foraging habits of solitary bees like halictids (Real 1983; Proctor et al. 1996). Compared to bumble and honey bee flights, those of halictids like sweatbees (including *Lasioglossum* spp.) are short at between only 7.6-15.8 cm on average in different density flower patches, depending on floral density (Real 1983). However they follow the same leptokurtic pattern of bumble and honey bees, making short flights close to a central point (Real 1983). Halictids are effective pollinators for apple trees, and more efficient than honey bees because

they can carry more pollen and stay abundant through changing temperature (Boyle-Makowski 1987; Gardener & Ascher 2006). The *Lasioglossum* genus was not included in this assessment and may be too small and sparsely haired for effective apple pollination (Boyle-Makowski 1987; Gardener & Ascher 2006). The sweat bee *Halictus farinosus* produces more seed on onion plants than honey bees do (Real 1983). Halictid bees visiting rare flowers of a longleaf pine ecosystem spent between 30 seconds & 3 minutes foraging on the observed flowers (Pitts-Singer et al. 2002). Halictid bees are a major pollinator of the rare flowers of these Florida longleaf pine forests (Pitts-Singer et al. 2002). Despite very different physical traits, halictids and bumblebees often pollinate the same flowers, such as the rare *Scutellaria floridana* and *Harperocallis flava* of Florida's longleaf pine forests (Pitts-Singer et al. 2002), and *Lobelia spicata* of Midwest prairies (Griffin & Byers 2012). Studies from 1940s & '50s showed that a number of different halictids demonstrate high constancy for various fruit tree pollen (Proctor et al. 1996). Various *Lasioglossum* spp. have been recorded as strongly constant pollinators to various yellow flower species, bypassing other purple-hued flowers and even different yellow species for their preference (Proctor et al. 1996).

The presence of any or all of these pollinator groups, and others, would have varying effects on the pollination regimes of the local plant community. Correlative evidence from a study across multiple different habitats showed that functional group diversity in pollinators explains the most variation in cross-pollination dependent pumpkin plants' seed set (Hoehn et al. 2008). Seed set and pollinator richness were strongly positively correlated, suggesting higher diversity of pollinator types is beneficial to plant fitness (Hoehn et al. 2008). This supports other assessments that a diversity of pollinators can maintain pollination services to a region in the

long term, increasing resistance to problems from decline or loss of individual species (Ricketts 2004).

Pollinator foraging behaviors

Pollinators tend to follow the optimal foraging strategy of visiting flowers economically to maximize resource gathering per amount of time and energy expended (Hersch & Roy 2007; Dramstad et al. 2003; Gegear & Thomson 2004; Real 1983; Richards 1997). The energetic costs of foraging include the energy spent per distance travelled, the average distance travelled per flower visit, and the energy spent maintaining homeostasis during foraging (Richards 1997). Optimizing these costs in foraging entails visiting more rewarding flowers as well as minimizing foraging time, either via less flight distance or less floral handling (Dramstad et al. 2003; Hersch & Roy 2007; Real 1983). Flights of bumblebees have been recorded from 18.3 m up to 8 km (Dramstad et al. 2003). Minimizing flight time between nest and foraging patch is likely not the only variable for optimal foraging, as some evidence indicates bumblebees use floral resources more when placed further from their nests (Dramstad et al. 2003). They tend to be loyal to a specific forage patch (Real 1983), and when their nest is moved further from that patch, their visits increased, possibly to compensate for the longer flight (Dramstad et al. 2003).

To reduce floral handling, a pollinator will tend to make a flower preference, in order to learn how to best access that flower's nectar and/or pollen for efficient repeated gathering (Waser 1986; Richards 1997). Continually relearning new flowers reduces foraging efficiency by prolonging time spent gathering resources. Bees also show preference for flowers with less

variable but equal rewards, again showing an optimization to their learning behavior for optimal foraging (Real 1983).

When pollinators choose specific flower species to visit more than others, the selected species can exhibit increased fitness (via more seed production) due to the favored pollination (Hersch & Roy 2007). Pollinator preference for certain morphs of a plant species can even lead to a division of the species by creating a reproductive barrier to the morphs (Hersch & Roy 2007). Bee preference can also be affected by their own anatomy, such as tongue length, which dictates the types of flowers they can physically feed from without nectar robbing from drilled holes lower on the flower (Newman & Thomson 2005; Koch et al. 2012). While pollinators have some innate preferences for flower color, shape, or scent, specific flower preferences can be learned and changed over a season, even overcoming innate selections in changing floral environments with reward reinforcement changes (Real 1983). Both honey and bumblebees can see colors from red to near UV wavelengths and despites some inherent color preference, they can be trained to visit any color in this range but do learn certain wavelengths faster than others (Real 1983).

Learning and Social Behavior

Bee pollinator forage behaviors are also influenced by social interactions and observations of other individuals, shaping constancies via intraspecies learning and communication (Real 1983). The first foragers of the season are considered "naïve", and must sample and then make decisions about what flowers to visit (Real 1983; Richards 1997; Baude et al. 2008). Foragers must maximize the resource collection and thus need to discriminate between flowers of

different nectar or pollen quality. Foragers may use trial and error to gather information for themselves, retaining the information through associative learning (Baude et al. 2008). Via this method, each bee would vary its choice of flower between equally rewarding species, but would stay relatively loyal to one. When new foragers have the chance to observe experienced individuals however, they can gain information about available floral resources via this social interaction (Richards 1997; Baude et al. 2008), similar to the communication of an individual to fellow hive members through their waggle dance about recently visited flowers (Barron et al. 2009). Bumblebee species have been recorded showing preference to one floral species but when the majority of their hive-mates were removed from the area, the remaining bumblebees started foraging on the originally less-preferred flowers, lacking the social reinforcement of preference (Real 1983). The ability to gain information from social behavior with other bees is beneficial to those inexperienced in the area particularly when floral resources are in certain spatial distributions (Baude et al. 2008). At a small scale flowers are often in non-random or clustered spatial distributions such as inflorescences (Baude et al. 2008). Having demonstrators helps foraging success and consistency in these aggregated flower distributions, helping bees overcome the challenge of spatial heterogeneity in their floral resources especially when given a choice of species to visit (Baude, et al. 2008). Patchiness or non-random heterogeneous distribution slightly decreases the likelihood (from 20% to 16%) of a bee choosing a non-rewarding flower directly after visiting a non-rewarding flower, possibly because of the closer proximity and decreased time spent between flower visits. This non-rewarding choice also decreases with the presence of demonstrator bees (Baude et al. 2008).

Characteristics that influence pollinator foraging patterns include floral structure, color, scent, nectar and pollen properties, floral number, density and arrangement, and phenology (Real 1983). Many of these traits, plus the style length, stigma positioning pollen anthesis timing, and stigma receptivity period, also influence the pollen source plant- the paternal line of the offspring that will be produced (Real 1983). Floral traits are interpreted by pollinators to set a floral preference or constancy. When comparing three *Castilleja* species of western Colorado with red, pink, or yellow bract color, the pink species received significantly more total pollinator visits than the others, and both pink and yellow received more than red (Hersch & Roy 2007). Pollinators visit plants more that match the context, or when the plant is near similar plants morphs or species, possibly based on flower color (Hersch & Roy 2007). Generally bumblebees visit yellow flowers more and make more visits per raceme than red flowers, when comparing similar species' flowers, and vice versa for hummingbirds, when given equal choice between plants (Hersch & Roy 2007). When a pollinator demonstrates floral preference and/or constancy the recipient flower species benefits from outcrossing with others conspecifics and priority pollen transfer, increasing that species' fitness as a whole (Real 1983; Gegear & Thomson 2004).

Constancy

Bees (the Apoidea subfamily) are known as the most distinctly loyal pollinators, showing higher constancy as a group than others (Real 1983). Pollinator constancy originates from intrinsic qualities of the pollinator and is distinguished from other specialization such as fixed preference or labile preference, referring respectively to specialization on certain flowers to optimize learning the flowers' access, and focus on the most abundant rewarding flowers though less abundant rewarding ones may still be in their flight path (Waser 1986). Constancy demonstrates

both a choice of flowers and a temporary preference. There is still debate about whether optimal foraging tactic is the cause for constancy, as the actual energetic costs of passing up rewarding flowers had not been well assessed (Gegear & Thomson 2004). Pollinator constancy would imply that there are varying costs to switching between flower types, and to specializing on only one flower type (Gegear & Thomson 2004). Limitations to pollinator memory and/or learning have been implicated in the occurrence of constancy and may be the most likely hypothesis (Real 1983; Waser 1986). Perhaps pollinators can only learn to access a certain number of flowers that yield successful foraging, or only retain memory of a few at any one time. By this theory, pollinators with higher capacity memory should not demonstrate floral constancy, as they can efficiently forage on multiple flowers at a time (Waser 1986). Whatever cognitive limitations, pollinators can easily learn flower colors as rewarding and can change their preferred flower when the floral environment changes (Gumbert 2000). Constancy can be influenced by a number of factors. Initial encounter of a rewarding flower, or encounter history, can dictate the flower type that is learned for foraging (Waser 1986). Pollinators that are inconstant augment interspecies pollen movement, increasing the likelihood of cross-species reproduction and production of a hybrid community in the absence of postzygotic reproductive barriers (Hersch & Roy 2007).

While there is always a varying proportion of bee foragers acting as scouts, exploring the attractive objects in the area to assess new forage possibilities and relaying the information to the colony, these low constancy bees only make up a fraction of the foragers (Proctor et al. 1996). These are probably the ones that seem determined to visit humans in colorful clothing. Scouting behavior is typically excluded or separately addressed when assessing general constancy. The

different pollinator groups exhibit different constancies, as well as different constancies for particular plant species they must choose between (Hersch & Roy 2007). More prominent flower color or structural differences could be a cause for higher constancy when choosing between certain flowers. While honey bees are more generalist foragers, they exhibit higher constancy than bumblebees, and both are more constant than solitary bees like halictids (Real 1983). Honey bees are also more constant while collecting pollen than when collecting nectar (Proctor et al. 1996). Bumblebees exhibit more constancy to plant species that match their neighbors, or the context, and constancy decreases when hybrids or intermediates are present in the community (Hersch & Roy 2007). This would then cause more hybrids among compatible species, creating positive feedback for hybridizing (Hersch & Roy 2007). Hybridization is more likely between morphologically similar flower species in either color, structure or both, but especially in similarly rewarding flowers (Real 1983; Hersch & Roy 2007). Between very similar legumes (*Pultenaea densifolia, Dillwynia hispida,* and *Dillwynia uncinata*), all pollinated by a solitary *Tricholletes* bee species and growing sympatrically, *Tricholletes* constancy was only 43% for 183 interplant forages (Gross 1992). All the legumes are accessible to the bees in the same way, offer either nectar or pollen rewards, and have a similar UV-absorbing area on their petals (Gross 1992). Further compounding pollinator constancy, it has been found that individual bees of the same *Bombus impatiens* colony can have varied constancies for controlled artificial flowers (Gegear & Thomson 2004). Honey bees had no preference between equally rewarding artificial blue and yellow flowers, usually choosing the next closest flower to visit (Real 1983). Color is simply an advertisement with many similar products to choose from, and bees learn which ones work for them, and what to keep visiting, based on their experience.

Effects on Constancy

Constancy to a particular species or flower morph can be altered by various environmental and biological factors. When floral nectar volume is increased in artificial flowers, bumblebees show lower constancy (Gegear & Thomson 2004). Bumblebees are less constant to various artificial flowers when they are clustered and equally rewarding (Real 1983). This seems to show reward is the priority over flower traits for pollinator preference. However, decreasing the sucrose concentration of nectar rewards in artificial flowers did not change pollinator constancy, only the amount of time the bees spent on each flower, spending more time on the higher rewarding (higher sucrose concentration) flowers (Gegear & Thomson 2004). Gegear and Thomson's (2004) findings support the optimal or "economic" foraging theory of pollinator constancy, as it decreases as flowers are more rewarding and/or easier to visit so that the bee does not need to be as economic or strategic in gaining resources. However, Gegear & Thomson (2004) also found increasing the distance between flowers, from 7 to 15 cm, lowered bumblebee constancy, possibly due to the increased time the bees have to process their choices of rewarding flowers or due to the increased cost of selecting even further flowers of the same species. It is generally found that when resources are more scarce (at lower volumes or further distance) pollinators will forage more economically, demonstrated through increased constancy. But the foraging constancy of even a specific pollinator is a complex behavior. The hypothesis that pollinators use reward economics as their choice basis may be the most applicable to optimal/economic foraging, since color and structure seem to be reinforcements for a pollinator's rewarding flower preference rather than the primary choice basis, given equal accessibility to the reward (Real 1983). Color may be more important for contrasting to the background environment and other

flowers to be the most attractive to pollinators (Real 1983). Pairing economic foraging with learning patterns may explain constancy to one species when another is equally rewarding.

Floral Reward Effects

Nectar and pollen are both collected as food by bees and other pollinators, and can be the attractant of a pollinator to specific flowers, changing their foraging pattern and visitation rate, thus altering the plant community (Hersch & Roy 2007). According to Hersch & Roy (2007), nectar volume and sucrose concentrations vary across *Castilleja* species. These floral reward characteristics influence bee visitation choices and can even be relayed to the entire colony from individual scouts. Bees can assess the sucrose concentration and volume of the nectar, and honey bees relay the information back to hive mates through their symbolic waggle dance (Barron et al. 2009) or pheromone dispersing dance for bumblebees (Dornhause et al. 2003; Granero et al. 2005). Bees are more likely to visit artificial flowers that are closer to the last visit, rather than move to the next of the same flower species, when all flowers had higher nectar volume (Real 1983; Gegear & Thomson 2004). This shorter travel distance between more rewarding flowers did not, however, make significantly shorter travel time (Gegear & Thomson 2004). Larger nectar volumes (of the bottom-most flower) generally yield larger pollen collection per flower, higher number of flowers visited, and shorter distance traveled between inflorescences (Richards 1997). Flowers with higher rewards thus tend to attract more flower visits per inflorescence, as well as cause pollinators to spend more time nearby, via shorter flight distances and more frequent turns (Real 1983). This, in theory, keeps the pollinator within the highly rewarding floral patch. When flowers become less rewarding, usually later in the season as newly opened flowers are less common, bees will increase their flight distances and decrease

the rate of turns during flights in order to explore new and possibly untapped floral patches (Proctor et al. 1996). Significant differences in rewards may also increase pollinator constancy, as they learn one species is more or less valuable to visit than others (Real 1983).

Constancy in a pollinator is very beneficial to the plant in regards to fitness, helping ensure it receives pollen from another conspecific plant, and without the costs of foreign pollen from a more promiscuous pollinator (Real 1983). Constancy helps female fecundity by reducing the introduction of interspecific pollen that blocks access to the stigma for conspecific pollen, and can even actively inhibit stigma receptiveness, even if they don't germinate or fertilize ovules (Real 1983). It also helps male productivity by reducing the waste of pollen transferred to an unreceptive species (Real 1983). Pollinator constancy has also been linked to lower fitness in the rare species *Symphyotrichum sericeum* when compared to the co-occuring & common *Solidago nemoralis* that receives higher pollinator constancy and more seed set with no significant difference between their pollinator visitation rate (Robson 2010). Constancy can thus increase pollination quality and boost a plant's fitness.

Pollinators interpret outer floral traits like color, UV absorbance, and corolla dimensions that may communicate the flower's reward quality/ quantity and accessibility, then sample and learn what to visit again (Real 1983; Gross 1992; Hersch & Roy 2007). Hybrid zones, areas where hybrids are produced, tend to show more variation in these floral traits (Hersch & Roy 2007). Hybrid plants in the pollinator's foraging context are considered "bridges" between the parental plant species and augment pollination between the parent species to facilitate further hybrids (Floate & Whitham 1993; Leebens-Mack & Milligan 1998). Tracking various pollinator types

on three *Castilleja* species, Hersch and Roy (2007) conclude that closely related, pollinator-reproductive, and sympatric plant species have hybridization patterns that depend on their pollinators' visitation patterns, pollen transfer ability, and the reproductive barriers present to cross breeding, with implications on the fitness of the recipient plants due to differential pollination. Hersch & Roy (2007) also determined that pollinator preference works in conjunction with constancy. Bumblebees were more likely to move between the two species that they have a higher preference for (closed yellow & semi-closed pink), thus moving less, and showing more constancy, between two more different flowers (closed yellow & open red). Pollinators will show more constancy to a species that they prefer more strongly than another choice (yellow), by making less interspecies flights between a high and low preference. The presence of an intermediate flower morph, like pink (a moderate preference), actually encourages more interspecies flights (Leebens-Mack & Milligan 1998; Hersch & Roy 2007). These intermediates, acting like stepping stones between the more different morphs, thus lower constancy (Leebens-Mack & Milligan 1998; Hersch & Roy 2007). In these instances, the prezygotic interspecies reproductive barrier of pollinator behavior is considered "leaky" due to variable and only moderate maximum constancy (Hersch & Roy 2007).

Seed production & germination

Most *Castilleja* species are likely mostly or entirely self-incompatible, producing minimal seed without cross-pollination via animal pollinators (Kaye & Lawrence 2003). Experiments with four *Castilleja* species comparing caged plants to pollinator accessible plants, yielded from 50% to 80% more fruit set in uncaged plants, with the exception of *C. cryptantha*, which produces 89% fruit with pollinator exclusion and is reported to be self-compatible (Duffield 1972). *Castilleja levisecta* produced five times more seed via pollinator activity than from pollinator-

excluded flowers (Wentworth 1994) and only 0.7% of available ovules produce normal seeds via self-pollinations (Kaye & Lawrence 2003). Its seed production has been found to increase as the relatedness of the parent plants of controlled crosses decreases (Kaye & Lawrence 2003). Unrelated crosses within the same population produced 71% of normal seed and crosses from different populations produce 80%, while sibling crosses produced less than 40% seed set (Kaye & Lawrence 2003). Conspecific *Castilleja* crosses yielded still higher seed production than heterospecific crosses (Hersch-Green 2012), indicating a threshold of decreased relatedness in increasing seed production. The different paternal plant species also effected seed production, with one species (*C. sulphurea*) producing less seed than the others (*C. rhexifolia* and *C. miniata*) when involved in the heterospecific crosses, due to variation in fitness and reproductive isolation barriers between species (Hersch-Green 2012).

Castilleja levisecta seed germination may not be affected by parent relatedness according to Kaye & Lawrence's 2003 controlled *C. levisecta* crosses, averaging 85% germination, despite differential seed set amounts. Other *Castilleja* hybridization analyses did find both paternal effects on F1 seed germination and significantly lower germination of hybrid seeds than conspecific seeds (Hersch-Green 2012). Seedling growth after three months was affected by parent plants' relatedness, with the few seedlings of self-pollinated plants only reaching 55 cm, less than half the size of unrelated crosses of 130 cm of growth (Kaye & Lawrence 2003). Seed germination rates also differed between interspecific crosses of one region than another, with higher germination success among crosses from where an active hybrid zone exists.

Castilleja seeds develop in upright bisected capsules, which only partly opens at maturity and seeds can retain attachment to the funiculus long-term or stay wedged between the carpel walls, limiting dispersal (Figure 1.1). Average seed number per matured capsule for *C. levisecta* was around 160 seeds in wild prairie populations, as determined by Jane Wentworth (USFWS 2000; Wentworth 2005) and about 180 by Tom Kaye (Kaye & Lawrence 2003). Seed abortion, possibly selective, is not uncommon for many plants, leading to more fertilized ovules than viable seeds produced (Real 1983). Many species have been found to have regular rates of seed abortion for certain ovule positions, but some species abort set proportions of ovules regardless of position (Real 1983). This potentially allows for female selectivity at the ovule level, with the possibility of genotype of the zygote being used to determine which ovule is aborted (Real 1983), possibly via similar mechanisms as pollen tube rejection though perhaps still unknown (Seavey & Bawa 1986).

Figure 1.1. Capsules collected in spring of year following production, photographed at three or more years after maturation, demonstrating capsule remaining intact (left photo) and seed persistence within the capsules, despite occasional disturbance (right photo)

Dispersal of *Castilleja* seeds seems to be minimal with no dispersal mechanism on the actual seed, and is either via wind or animals knocking seeds out of the capsule to a short distance from the parent (USFWS 2000; Fairbarns & Egger 2007), or possibly via flower-feeding rodents

dragging flower stalks with early matured fruits back to their burrows. They have a reticulated membrane or seed coat surrounding the seed, and an average length of under 1 mm (Wentworth 2005; Crowe et al. 2014). Seeds of *Castilleja* generally require cold stratification for successful germination, resulting in up to 80% germination following 6-8 weeks of moisture and cold temperature (Caplow 2004; Fairbarns & Egger 2007; Gibble pers. comm. 2011; CPC 2014) but with less than 50% germination in some experiments (Wentworth 2005). Experiments with *C. hispida* germination determined cold stratification for six to twelve weeks improves germination but still only to 2.3 % at maximum for those experiments (Drake et al. 1998) and 49% germination in others (Kaye & Blakeley-Smith 2008). Seed germination and viability can vary depending on source population and storage conditions, and viability can decline sharply with time in storage outside cold dry conditions such as a controlled seed vault (Wentworth 2005; Caplow 2004; Gibble pers. comm. 2011). Germination rates also vary by year and time of year when collected (Caplow 2004). Wild collected seed averages 75% germination (Kaye & Lawrence 2003). Preliminary experiments with *Castilleja* seeds soaked in 0.75 mg/ml gibberellic acid (GA3) for 24 hours yielded no difference in germination speed (as T50 week) or percent, though seedling growth was more developed, with longer roots, shoots, and more leaves than untreated seeds. No discernable survival affect was observed in the treated *C. levisecta* plants after 48 days of growth and potting in soil, though the GA3-treated seedlings did have more leaves and longer stems than those untreated, but there was a possible negative effect on *C. hispida* survival. As preliminary data, analyses had not been done at the time of this write up.

Despite high germination rates for an endangered plant, (Vovides & Iglesias 1996; Cerabolini et al. 2004; Gillespie & Andersen 2005; Gimâenez-Benavides et al. 2005; Wenk & Dawson 2007;

Talve et al. 2011), *C. levisecta* has low recruitment rates due to low juvenile survival and regression of individuals back to smaller vegetative states before achieving larger flowering status (Wentworth 2005; CPC 2014; personal observation 2013). Fire application post-flowering increases seed germination and plant survival rates (Caplow 2004), possibly due to increasing the open ground for seedlings to grow on (Lawrence & Kaye 2011; Schafer et al. 2013). However, despite optimal growing locations, *Castilleja* species will produce minimal seed without the availability of appropriate pollinators in enough abundance to thoroughly pollinate the flowers.

In this study of *Castilleja levisecta* and *C. hispida* hybridization potential in the field, restoration plot characteristics, pollinator foraging behavior, and seed production and germination were assessed to examine three questions regarding the likelihood and patterns of hybridization. First, do pollinator visit duration and pattern depend on the species or species context they visit? Second, do seed set and germination differ between species or between their contexts? Third, is the pattern of hybridization influenced by the pattern of pollinator behavior or plot characteristics? These are addressed in addition to the confirmation of the pollinators of *Castilleja levisecta* and *C. hispida* in restoration sites.

Methods

Study Sites

Two prairie restoration sites located in Thurston County, Washington were used for this project: Glacial Heritage and West Rocky. Glacial Heritage and West Rocky are located about 8 miles from each other near Littlerock, WA in the south Puget Sound region of Washington. Glacial Heritage restoration plots were located at N +46.870899, W -123.052334, southwest of Littlerock, WA in the South Puget Sound off of Mima Road SW. West Rocky restoration plots were located at N +46.895261,W -122.872943, east north-east of the Glacial Heritage site and east of Interstate 5 near Old Highway 99 SE. The Glacial Heritage Preserve is owned by Thurston County and Washington Department of Fish and Wildlife and managed first by The Nature Conservancy (TNC), then recently by the Center for Natural Lands Management (CNLM) (WFW 2014). Glacial Heritage is among the largest remnant prairies still extant in Western Washington (South Native Plants 2014; WFW 2014). West Rocky Wildlife Area is owned by the Washington Department of Fish and Wildlife (Kessler 2006). West Rocky is one of two remaining sites in the Puget Sound Trough containing all local prairie specialist butterflies, in addition to several rare plants and vertebrates on its prairie-oak-wetland site (WWRC 2014). The restoration plots at these sites were established by Eric Delvin, a PhD student at the University of Washington Seattle, in 2008 as a USFWS project with the purpose of prairie restoration and habitat creation for *Castilleja levisecta*. Each plot measures approximately 40 meters squared, arranged in an array of various preparation & community treatments. Only some of the plots containing significant numbers of both or one of each *Castilleja* species were used for this hybridization study, and each received

different original treatments for establishment, such as burning or herbicide with either forb-rich or a mix of grass & forb seed mixes sown in each plot. At both Glacial Heritage and West Rocky, five mixed-species plots were used, as well solo-species plots. These original treatments were not the interest of this study, but rather the interaction between the *Castilleja* species when grown in significant numbers together. Mixed species plots containing both *Castilleja* species were located within the same array, established and broadcast seeded in 2008 at Glacial Heritage, and 2009 at West Rocky (Delvin & Bakker 2013) (Figure Appendix B.5) and the solo plots containing only *C. hispida* (CAHI) or *C. levisecta* (CALE) were located at least 70 meters from plots containing the other *Castilleja* to create on-site controls with potentially minimal cross-pollination between the species. CALE's seed source for this restoration was produced at a nursery from a mix of seed from the large population of Rocky Prairie, as well as some Whidbey Island sites (Dunwiddie email comm. 2011). The closely related species of CAHI was sown into these sites as well for the purpose of increasing diversity, floral sources for pollinators, and host plant for the endangered butterfly Taylor's checkerspot (*Euphydryas editha taylori*), which is known to use CAHI as a host. Seed for CAHI originated from Fort Lewis prairie (Dunwiddie email comm. 2011). CALE is suspected, but yet to be documented, as a host for Taylor's checkerspot larvae in the field but its relatedness to CAHI, with many physical and phenological congruities and similar palatability to the related Edith's checkerspot, all bode well for being another host source. Providing a site for Taylor's checkerspot colonization is an additional aim for Delvin's restoration sites in the Puget Sound Trough. Therefore CALE could be useful in the

recovery of the checkerspot's population, in addition to CAHI, (Vaughan & Black 2002) as well as valuable floral resources in the prairie.

Mapping

To test whether the plot context affects pollinator activity or seed characteristics, different plot contexts were used, based on the *Castilleja* species present. At the Glacial Heritage site, five mixed species plots (those containing both *Castilleja* species, see Figure 1.2) were used for hybridization and mixed-species growth analysis. One plot containing only CAHI was created inside a larger scaled-up plot using the same dimensions as the mixed plots, and used for an onsite-control CAHI solo-species plot. One plot of solo-CALE at Glacial Heritage, established in 2009, was used for an onsite-control of CALE, as well as two solo-CALE plots at West Rocky, established in 2008. A plot at Glacial Heritage was initially seeded with only CALE, but had two CAHI present and was used as a semi-solo plot to explore the effect of a few CAHI plants within a plot dominated by CALE. Similarly at the West Rocky site, five mixed species plots were used, as well as a semi-solo CAHI plot for the effect of a single CALE on CAHI. No solo-CAHI plots were present at West Rocky. West Rocky's semisolo *C. hispida* plot contained a single *C. levisecta* (#WR06), and Glacial Heritage's semisolo *C. levisecta* plot contained two *C. hispida* plants (GH#26). These semi-solo plots were not used in plot characteristic analysis due to the unnecessary complication they would cause and unlikely effect on plant characteristics within them. Plot numbers and treatments are listed in the Appendix A, Table Appendix A.7. Original seeding density of each species was different, due to differential germination rates, with CALE seeded at a lower density

then CAHI. The sowing mixes for the mixed plots at Glacial Heritage all forb-rich and 113 seeds per square meter for CAHI and 66 CALE. Seed mixes for West Rocky mixed-plots were both forb-rich and a mix of grass & forb, at 110 CAHI and 64 CALE seeds per square meter in forb-rich, 84 CAHI and 49 CALE in mixed grass & forb seeded plots (Delvin 2013). The mixed-species plots at Glacial Heritage were also pretreated with 2 years of herbicide to remove all previous vegetation, while the mixed-species plots at West Rocky were all burned as their pretreatment. The reverse was the case for solo-species plots, with Glacial Heritage's pretreated with burning and West Rocky's with herbicide, but both being forb-rich seed mixes. West Rock's solo CALE plots

The spatial location of each flowering *Castilleja* plant was mapped within each plot for reference of their distance to each other and their density. Mapping consisted of two measuring tapes laid along the south and west plot edges from the permanent corner markers and running strings out from each plant's center to the tape edges for an x-axis measure and a y-axis measure so that the center point of each plant could be mapped within the plot's coordinates. Each plant was also measured to determine square area of the plant's cover. The number of flowering stems was counted for each plant, as well as the color group of every CAHI flower recorded being either red, dark orange, orange, salmon/light orange, orange-yellow (having orange edges but yellow centers of the bracts & galeas), golden, and yellow. Then four selected individuals of each species per plot, serving as the parent generation, were flagged with a plant number (L1, L2, H1, H3, etc.) to track seeds of the next generation, or F1 plants. Flagged plants were selected by two categories of being either closer than 30 cm to an individual of the other *Castilleja*

species, when in mixed-species plots, or further than 30 cm from the other species, with one each of a larger and smaller plant per distance category, as well as selecting a range across each flower color of CAHI to represent all color varieties for hybridization study. Color was not a factor in selecting parent CALE for this study as CALE flowers are consistently yellow. Royal Horticulture Society color charts, including a UCL color identification, was used to identify color variation among the plants, particularly CAHI.

The statistical analysis program SPSS, by IBM, was used for all analysis of variance tests throughout this study. Assessment of plot characteristics included response variables of the number of plants of each *Castilleja* species, their floral density (measured as the number of flowering stems per square meter), and the mean number of stems per plant for each *Castilleja* species. Statistical analysis used univariate ANOVAs to assess significance of *Castilleja* species, plot context (mixed or solo species plots), and the site as explanatory factors, and the plot number nested within sites.

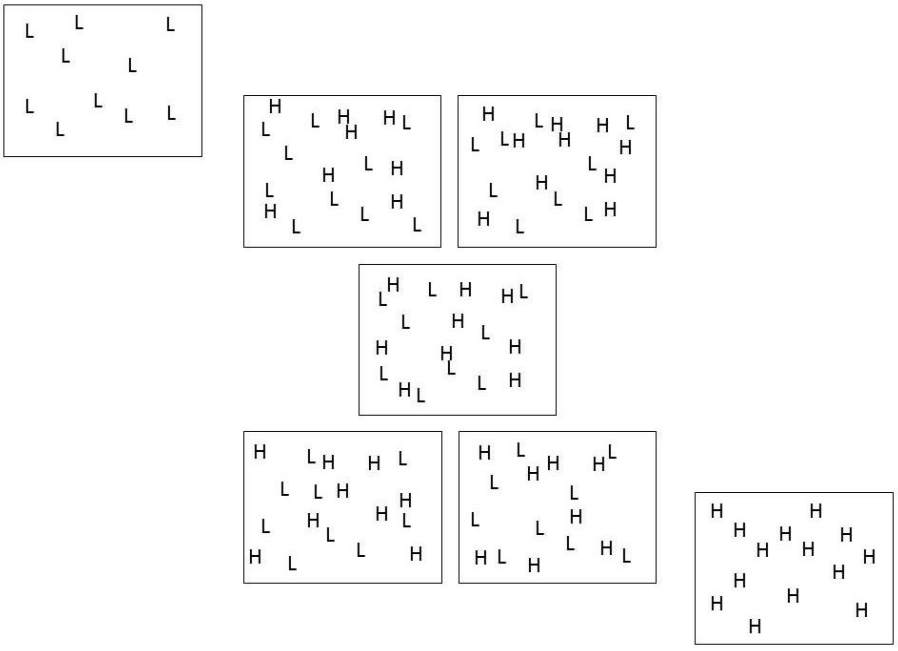

Figure 1.2. General plot arrangement at field sites, featuring 5 mixed-species plots and 2 solo plots of only either CALE or CAHI. "H" represents CAHI and "L" represents CALE.

Pollinators

Pollinators were recorded foraging at each study plot at the Glacial Heritage and West Rocky sites for at least 30 minutes per observation. Pollinator foraging on a flower raceme, not individual flowers, was recorded when it probed a flower, causing likely pollen contact, or was crawling on and collecting pollen from the flower's anthers. Visitation time was counted to the nearest five seconds or the nearest second when less than five. Sampling times were during the warmer parts of the day when pollinator activity would be highest, between 0900 hours and 1700 hours, with some sessions going until 2000 hours when the pollinators were still active later, specifically in June. The sequence in which the bee sampled pollen from the inflorescences was recorded, with the flower's species & color noted for CAHI to develop a visitation pattern. To test the effect of plant community context on pollinator visitation pattern two types of plots were observed at each site- plots with a mix of both species and plots that have only one species. A variety of eleven bee specimens were collected throughout observations to ensure identification of all species pollinating either CALE or CAHI. The collected bees were anesthetized on site and later identified by Dr. Evan Sugdan of the University of Washington, then pinned for future reference. The collection is currently located in Sarah Reichard's lab at the Center for Urban Horticulture, in Seattle, Washington.

Several sessions of observation at the West Rocky site yielded no visible pollinators, possibly due to the site's lower quality from persistent invasive *Leucanthemum vulgare* (ox-eye daisy) presence, and/or the lower density of *Castilleja* plants present. Time restrictions prevented repeated days of observation at that site, yielding a lack of

pollinators observed across repeated hour-long session at the only plot containing predominantly *C. hispida* at the West Rocky site, the semi-solo CAHI plot. Lack of observations at this on-site control plot restricts the strength of pollinator behavior analysis but is indicative of the lower ecological quality of the West Rocky site.

Visitation measurements were taken for each bee as individual samples, rather than for each raceme visit, because bees express individual choices during forages. One method of assessing pollinator visitation is via the average time each bee spent on each *Castilleja* species' raceme. This allows analysis of whether bees spend different amounts of time on the flowers. The total time, in seconds, each bee spent on recipient *Castilleja* species was also used as an assessment of pollinator visitation differences between flower species. Assessment of percent of total time on *Castilleja* species, the recipient plant species as a percent of the total plants visited, and the number of each *Castilleja* bees visited were used as an assessment of pollinator preferences between flower species. To assess these responses, multivariate ANOVAs were used with the recipient plant species, plot context, and site as factors, and the plot number nested within site. Plot contexts were reduced to solo- and mixed-species due to low sample size and unlikely effect on pollinator foraging behavior, to not confound other factor effects.

To assess whether pollinator behavior promotes interspecies pollen movement that could cause hybridization, individual pollinators were tracked visually, recording each plant species they visit and time spent per raceme. The total numbers of transitions between inflorescences of the same and of different *Castilleja* species were counted for assessing

constancy. Pollinator constancy was determined using Bateman's constancy indices (Bateman 1951).

		2nd visit (to) : 1	2
1st visit (from):	1	A	B
	2	C	D

Table 1.1. Notation for Bateman's 2x2 for pollinator transition frequencies between flowers 1 and 2. A & D are constant transitions between the same types. B & C are inconstant transitions, between different flower types.

Using Bateman's matrix, when pollinator transition frequencies are known, the Bateman Index (BI) is:

$$\text{Constancy} = [(AD)^{1/2} - (BC)^{1/2}] \; / \; [(AD)^{1/2} + (BC)^{1/2}] \; .$$

Bateman's index (BI) is a standard assessment of constancy when a pollinator has equal access to each flower type (Waser 1986). A BI of +1 denotes complete constancy with all pollinator transitions happening between the same flowers and a -1 denoting complete inconstancy with all transitions happing between different flowers. A BI of 0 denotes random transitions between flowers. Bateman's index is not useful when pollinators visit only one flower morph exclusively (Gegear & Thomson 2004). However, the pollinators in this study were not particularly loyal, thus Bateman's index is deemed appropriate.

Seed production and germination

Flagged plants were snooded in late June to prevent any seed loss or predation during maturity. Snooded seed stalks were collected in mid-August by clipping the stems below the snoods and attaching corresponding labels to track the plant ID later. Stems of each plant were counted and the most prominent stem selected for germination. Two of its seed capsules were collected in envelopes from the middle and bottom sections of each stem. The ranges of middle & bottom were used due to slight difference in flowering times between the species, causing possible lack of overlap for the first or bottom flowers. Therefore, the bottom flowers of the earlier blooming CALE may have been exposed only to other CALEs. Seeds from the fruits were counted, then cold stratified on moist filter paper in petri dishes at 5°C until germination ceased. Germination percentage and T50 week (the number of weeks when 50% germination is reached) were used to assess seed viability, in addition to the number of capsules per stem, length between the first and last capsule (the bloom zone), and number of seeds per fruit for assessing the effect of pollinator activity on parent plant reproduction. Samples of seed stock produced off-site, grown separate from the other *Castilleja* species, was used for off-site controls. These represented a maximum pure-bred sample of each species. The CALE seed was pulled from the Miller seed vault wild collection, and the CAHI seed was from the original seed mix used to seed the restoration plots and grown at a local production nursery.

To assess effect on seed production and germination characteristics, the number of seeds per capsule, percent germination, and T50 week (the week of 50% germination) for each

middle or bottom capsule (M- or B-capsules) were used as responses in a multivariate ANOVA. The factors of plot context, *Castilleja* species, site, and capsule (M & B) were used as explanatory variables to test their effects on these seed germination characteristics. One-way ANOVA was used to compare the off-site control plants separately for their seed germination and T50 week, with only species as the factor, due to the lack of capsules. Independent sample t-tests were conducted on all control plants (solo-context and off-site samples) compared to mixed-context plants for percent germination and T50 week, as well as t-tests between the parent *Castilleja* species, and between the sites. To test earlier characteristics of plant reproduction, the number of flowering stems per plant, length of the bloom zone (distance between top & bottom seed capsules), and the average number of capsules per stem were used in univariate ANOVAs with factors of plot context, *Castilleja* species, and site, to test their effects, and plot number nested within site.

Experiment Results and Observations

Study Sites

***Castilleja* counts**:

Glacial Heritage (GH) plots contained 394 CAHI total (*M*= 56/plot), with 245 in mixed plots, and 147 in the plots seeded only with CAHI. The remaining two CAHI were located in a plot originally seeded only with CALE, classified in this study as a semi-solo plot (#GH26). GH CALE counts consisted of 600 plants total (*M*= 86/plot), with 111 in the plot containing only CALE and 123 in the semi-solo plot of predominatnly CALE. West Rocky (WR) plots contained 243 CAHI total (*M*= 40/plot), with 224 in mixed plots, and 19 in the one plot seeded with only CAHI, but containing a single CALE (WR06, semi-solo plot, Figure Appendix A.4). This plot was heavily overgrown with Lupinus plants. WR CALE counts consisted of 335 plants total (*M*= 42/plot), with 197 in mixed plots, and 137 in solo-CALE plots plus the one in the semi-solo WR06 plot.

Plant sizes varied throughout the plots, some having as many as 82 stems on one CALE plant (Figure Appendix B.4), and 26 on the largest CAHI, both at GH. The largest diameter of a CALE plant was 1900 cm^2 and the largest CAHI was 1845 cm^2. The largest CALE at WR was 30 stems and the largest CAHI was 18 stems, and those with the largest diameters were 780 cm^2 and 272 cm^2 for CALE & CAHI respectively. CALE plants at GH had the most stems/plant on average at 13.3 stems/plant. GH CAHI were lower at 5.6 stems/plant, and WR plants following the same trend of 3.8 for CALE and 2.3 for CAHI. Sites were notably different due to the invasive ox-eye daisy (*Leucanthemum vulgare* Lam.) being prevalant in all plots at WR, and absent from GH.

***Castilleja* Color Variations**:

CALE was seen in two slightly different hues of yellow, consisting of the characteristic bright yellow and a slightly darker golden yellow, identified as 3B and 6B on the Royal Horticulture Society chart, both called "brilliant greenish yellow" and #98 on the UCL color chart (ASA 2014). CAHI varied widely in colorations at both sites, considered uncommon for most native sites. The most often represented color was an orange hue, with 51.7% of GH's CAHI color variation, and 61% of WR's (Table 1.2). The least common colors were yellow-hues, excepting a pink variation previously unknown among CAHI (Egger pers. communication June 2011). A single example of pink (RHS #32D, and UCL #29 "strong yellowish pink") CAHI was documented in GH's large plot of solo-CAHI (Figure Appendix B.3). It was not included in the CAHI control plot used for this study as it was near a rogue CALE at the edge of the plot. This plant was removed during the mass removal of CAHI at the end of the 2011 season to prevent possible future hybridization between the species. Yellow CAHI were more common at the large solo CAHI plots making up 8.5 and 4.9% of those populations. WR had less color diversity, lacking any pink variation, and having only 1% yellow coloration with several plots completely lacking it. Orange more extensively dominates the West Rocky CAHI at 61%, with salmon varieties taking the place of GH's red in the second tier of major colorations. Yellow, golden, and orange-yellow colorations are uncommon to both GH and WR.

CAHI Colors	Glacial Heritage	West Rocky
Red	10%	5.0%
Dark orange	20%	12%
Orange	52%	61%
Salmon	6.6%	12%
Orange / yellow	7.4%	5.0%
Golden	1.0%	4.0%
Yellow	2.8%	1.0%
Pink	0.50%	0.00%

Table 1.2. *C. hispida* color variations CAHI colors visually identified in the field during plot mapping at each site.

Mapping: Site, Plot, & Species Differences

The number of plants on each plot was noticeably different between the GH and WR study sites during field observations, and yielding a mean of 70.86 of all *Castilleja* plants per plot in GH, and 41.28 plants per plot in WR. The mean floral density of plots at GH was higher than at WR for both species of *Castilleja*, as was the mean number of *Castilleja* plants per plot and the mean number of flower stems per plant (Table 1.3). GH plots had an average of 27.3 CALE flowers/m^2 and 8.4 CAHI flowers/m^2, when omitting the two outlying CAHI present in plot #26 that was initially seeded with only CALE. CALE was also higher than CAHI for all measures. GH had a mean of 85.7 CALE and 56.0 CAHI plants per plot, and WR had 47.7 CALE and 40.5 CAHI plants per plot. GH's mean flowering stems per plant per plot was 13.3 CALE and 6.1 CAHI.

Table 1.3. Results of plot characteristics assessed via plot mapping, showing flower stem densities per square meter, mean number of plants per plot, and mean number of flowering stems per plot, for each site and *Castilleja* species and the mean of both *Castilleja* values as the total.

Site	*Castilleja* species	Mean flower density (# flowers/m^2)	Mean No. plants/plot	Mean No. flower stems
Glacial Heritage	CALE	27.3	85.7	13.3
	CAHI	8.4	56.0	6.1
	Total	17.8	70.9	9.7
West Rocky	CALE	4.4	47.7	4.4
	CAHI	2.1	40.5	2.3
	Total	3.4	44.4	3.4

Across both sites, mixed species plots had lower floral density, total number of *Castilleja* plants, and average number of stems per plant, for both species, than solo-species or semi-solo plots (Table 1.4). Semi-solo species plots had a low sample size (only two), and their data varies across species and measurements, thus were treated as solo plots and excluded the minority *Castilleja*. CALE also had a higher floral density, total number per plot, and number of stems per plant than CAHI. CALE in solo plots had about 21.4 flower stems per square meter compared to 13.6 in mixed plots, while CAHI had 8.1 in solo plots compared to 4.7 in mixed plots. There were also more CALE plants per solo plot than CAHI, with less of each in mixed plots. CALE averaged more stems per plant in solo plots than in mixed, as did CAHI. When both species of *Castilleja* were added for a total *Castilleja* floral density, plant number, and number of stems, they have higher values then the plants grown in solo plots (Table 1.4).

Table 1.4. Results of plot observations showing differences between floral densities as the number of flowering stems per square meters per plot, the total number of each *Castilleja* species per plot, and the mean number of stems per plant for each species. Differences are compared between the restoration sites, *Castilleja* species, and plot context (across both sites). Mixed total refers to totaling CALE & CAHI mixed-plot averages.

Factor	Floral Density	Total # plants	Mean # stems/plant
Site			
Glacial Heritage	17.81 ±10.8	70.85 ±24.8	9.69 ±3.9
West Rocky	3.38 ±2.5	44.38 ±25.5	3.41 ±2.5
Species			
CALE	14.8 ±12.9	62.33 ±36.7	8.37 ±5.3
CAHI	5.11 ±3.8	45.5 ±20.6	4.17 ±2.2
Context			
Solo- CALE	21.39 ±15.8	92.75 ±42.4	10.03 ±3.1
CAHI	8.12 ±5.7	55.33 ±31.6	5.43 ±1.6
Solo Avg.	15.70 ±13.6	76.71 ±40.4	8.06 ±3.4
Mix- CALE	13.63 ±11.4	56.30 ±25.0	8.35 ±5.9
CAHI	4.71 ±2.9	46.90 ±12.5	4.01 ±2.3
Mixed Total	18.34 ±14.0	103.2 ±33.5	12.36 ±8.0

Floral density, the total number of plants present on plots, and their mean stem number were all significantly affected by the site and species of *Castilleja*. Univariate analyses of floral density, total number of plants per plot, and average number of stems per plant for the mixed-species plots, with factors of restoration sites, *Castilleja* species, and plots nested within, yielded significant p-values for site & species factors, and plot (Table 1.5). The plot factor was only significantly for the total number of *Castilleja* plants in each plot, which was not affected by site or species. The GH site had significantly higher floral density, and average number of stems per plant than the plants of WR. The *Castilleja* species also differed significantly from each other, with CALE having significantly higher floral density and mean number of stems per plant than CAHI. The differences between sites and *Castilleja*

Table 1.5. Results from the univariate tests, for mixed-species plots, on the effect of restoration site, *Castilleja* species, and plot on the number of flower stems per square meter (floral density), the total number of each *Castilleja* per plot (total # of *Castilleja*), and the mean number of flowering stems per plant. Significant effects, at $\alpha = 0.05$, denoted with *

Response	Effect	DF	MS	F	p-value
Floral Density (#/sq.m.)	Site	1	852.56	207.68	0.000*
	Species	1	398.55	94.65	0.000*
	Plot(site)	8	4.11	0.97	0.514
	Site*Species	1	333.42	79.18	0.000*
Total # of Each *Castilleja*	Site	1	1805.00	4.47	0.067
	Species	1	441.80	4.00	0.080
	Plot(site)	8	403.73	3.66	0.042*
	Site*Species	1	1095.20	9.92	0.014*
Number of Stems/plant	Site	1	274.32	136.96	0.000*
	Species	1	93.87	96.71	0.000*
	Plot(site)	8	2.00	2.06	0.163
	Site*Species	1	58.65	60.42	0.000*

species can be seen in the representative plot maps, created by mapping each plant's x and y coordinates (as south and west) and size in each plot (Figure 1.3a and b).

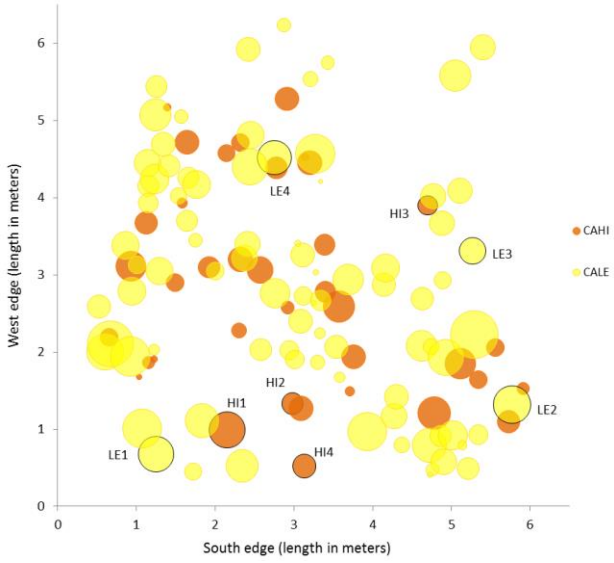

Figure 1.3a. A representative plot map from Glacial Heritage- plot GH18, showing flagged parent plants for F1 study.

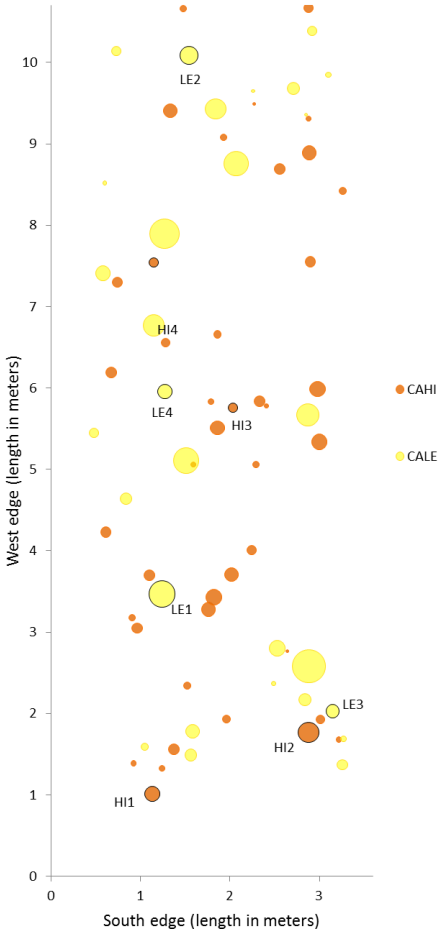

Figure 1.3b. A representative plot map for West Rocky- plot WR16, showing flagged parent plants for F1 tracking

The interaction of site and *Castilleja* species also affected each response significantly, indicating differences between sites for CALE more than CAHI (Figure 1.3c). GH CALE thus was significantly higher in density, number of plants, and number of stems than GH CAHI, with no difference between WR CALE and CAHI.

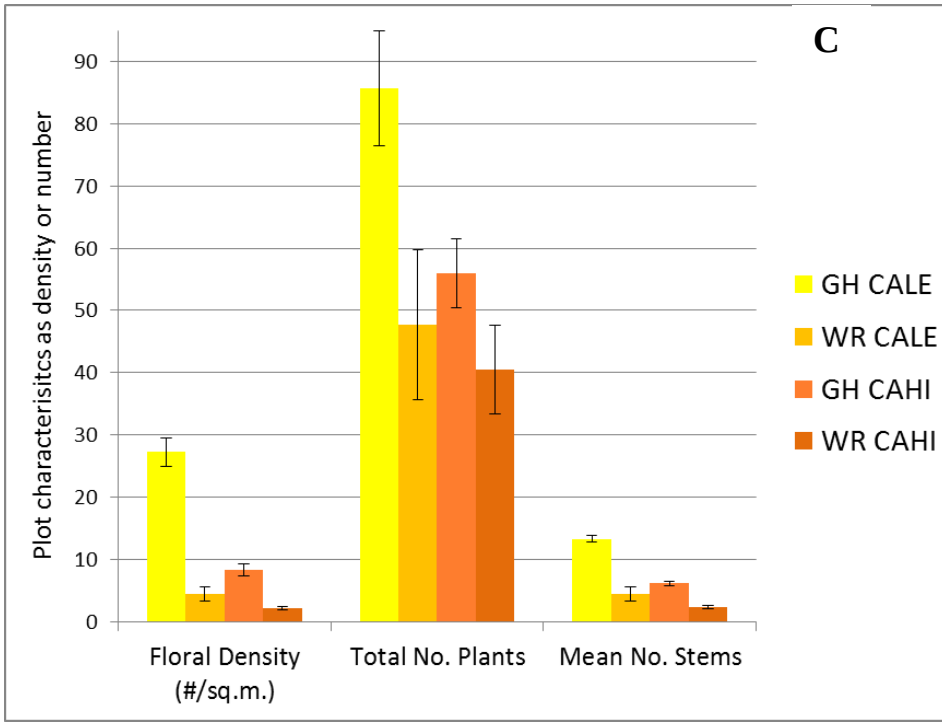

Figure 1.3c. Compilation of plot characteristic results, showing differences between sites and species for floral density, total number of plants per plot, and mean number of flowering stems per plant.

Pollinators

Two animal visitors were seen transferring pollen between *Castilleja* flowers during field observations, and were recorded on both *Castilleja* species as well as present at both sites. These floral visitors are presumed to be the pollinators for these plants at these sites, as pollen can be clearly seen on their bodies during visits to the *Castilleja* flowers (Figure1.4). One pollinator species predominated, the bumblebee *Bombus californicus*, including queens and workers, with the additional unexpected pollinators of sweat bees, of *Lasioglassum* spp. and *Sphecodes* sp. (Figure1.5). While not as commonly seen as the bumblebees, which are easy to both see and hear approaching each observed plot, the sweat bees were likely more common than observed, due to being partly hidden in a flower as they forage for pollen. This makes them harder to find from a distance. The bumblebees tended to forage on the upper most mature whorl of flowers in each raceme, visiting lower flowers only just under the first mature whorl. Honey bees were present at both restoration sites in abundance, but never seen on or attempting to visit either of the *Castilleja* species. Because of the few observed sweat bee pollinators representing a very small fraction of the pollinator sample size, and their fewer number of flower visits per forage, they were not included in the pollinator analysis, except for an estimate of their constancy. They were documented moving between *Castilleja* plants, and the two species. Observations were averaged per *Bombus* forager to represent their individual forage decisions. Observations almost only occurred with a single *Bombus* in a plot at a given time, with queens still frequently seen in the early June observations.

Figure 1.4. Pollinator on CAHI with visible pollen on its head.

Figure 1.5. *Bombus californicus* extending tongue to feed on *Castilleja hispida* (A) and *C. levisecta* (B). *Lasioglossum* sp on *C. hispida* (C), and *C. levisecta* (D).

Bees spent approximately the same amount of time on both species of *Castilleja* at GH, with 14.1 seconds on CALE and 13 seconds on CAHI (Figure 1.6). Their total forage time (totaling the time spent on each flower per forage) on each species per plot was higher for GH CALE than CAHI. WR bees had similar mean times spent on CALE and CAHI, but spent less time on both than at GH, with 5.8 seconds on WR's CALE and 9.4 seconds on its CAHI (Figure 1.6). Bees visited more CALE at GH than CAHI (*M*= 6.7 & 4.6 respectively), and WR bees visited less plants of both species than at GH (Table 1.6).

Table 1.6. Results of pollinator forage observations, showing differences between sites and *Castilleja* species for the mean time bees spent on the flower racemes and the mean number of each *Castilleja* species visited.

Site	*Castilleja* species	Mean time bee spent on racemes	Total time bee spent foraging	Mean number of plants visited
Glacial Heritage	CALE	14.13 sec.	106.4 sec.	6.70
	CAHI	13.03 sec.	75.0 sec.	4.64
West Rocky	CALE	5.78 sec.	24.4 sec.	2.20
	CAHI	9.44 sec.	33.9 sec.	3.17

Plot averages for mean time per raceme vary widely, with the GH plots having higher variability for CAHI & CALE (*SD*= 10.13 and 9.63 seconds respectively) than WR plots (*SD*= 9.45 and 7.38 seconds respectively) (Figure 1.7). Sites differed more than *Castilleja* species (Table 1.7). The number of bees that visited the *Castilleja* flowers was also distinctly fewer at West Rocky during the field observations, with just 20 West Rocky observations on CALE, compared to 47 at Glacial Heritage, and 17 versus 70 bees that visited CAHI at West Rocky versus Glacial Heritage.

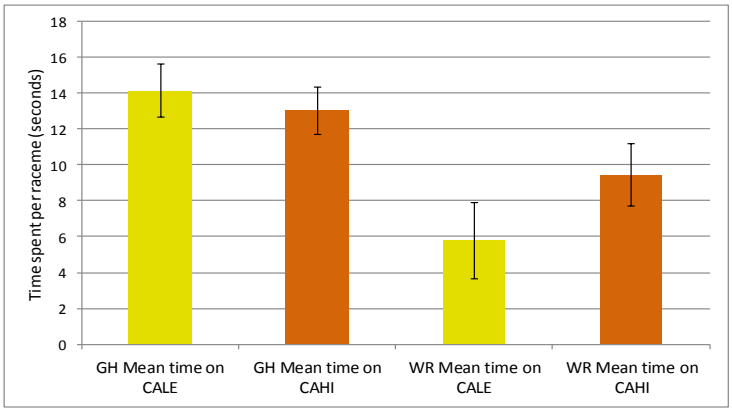

Figure 1.6. Graph of Glacial Heritage (GH) and West Rocky (WR) *Castilleja* mean times bees spent per *Castilleja* raceme.

Table 1.7. Results from the pollinator observations showing differences between restoration site, recipient *Castilleja* species, and plot context (averaging both species) for the mean time each bee spent per racemes, and their total time on racemes per forage.

Factor	Mean time on racemes	Total time on racemes
Site		
Glacial Heritage	13.55 ±9.8	89.75 ±101.9
West Rocky	7.52 ±8.6	28.89 ±37.9
Species		
CALE	11.64 ±10.6	81.91 ±97.9
CAHI	12.12 ±9.2	64.58 ±87.8
Context		
Solo	16.09 ±9.7	118.32 ±120.4
Mixed	10.51 ±9.6	58.17 ±77.1

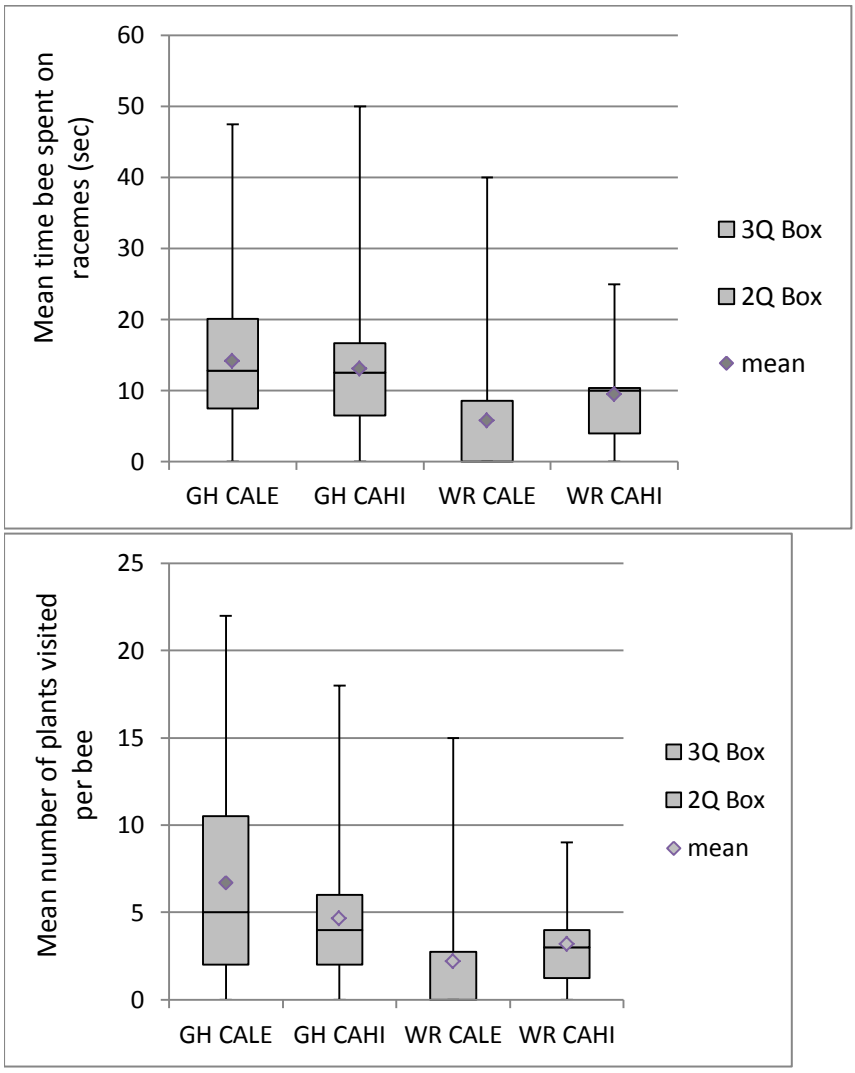

Figure 1.7. Boxplots of pollinator observations showing the range of variance for mean time bees spent on racemes (top), and mean number of plants visited per bee (bottom), both species at each site.

For pollinator observations, semi-solo plots, containing only one or two of the other *Castilleja* species in an otherwise solo plot, were counted as solo plots for their general effect on bee foraging behavior. This only affected the GH site data, as WR's plot #06 never received any pollinator visits during observations. Univariate ANOVAs conducted on the average time bees spent on each flower and total time spent on each species' flowers per forage, show that the site had significant effects on both measures (Table 1.8). GH bees spent significantly more time on racemes, and foraged significantly longer

than WR bees. The recipient *Castilleja* species did not affect either pollinator foraging measures. The species received the same length of visits, per forage and total forage. The plot (nested in site) did affect the bee average forage time per raceme. Site and species factor interactions also yielded no significant p-values.

Table 1.8. Results from univariate tests on the effects of restoration site and recipient *Castilleja* species on the mean time each bee spent per racemes, their total time on racemes per forage, and the mean number of flowers visited per forage. Significant effects, at α= 0.05, denoted with *

Response	Effect	DF	MS	F	p-value
Mean time on racemes	Site	1	571.09	6.21	0.015*
	Recipient Species	1	7.90	0.09	0.768
	Plot(site)	15	94.01	1.04	0.420
	Site*Species	1	357.86	3.96	0.049*
Total time on racemes	Site	1	47041.60	4.59	0.039*
	Recipient Species	1	4721.02	0.67	0.414
	Plot(site)	15	14563.46	2.07	0.016*
	Site*Species	1	23362.52	3.32	0.071

To further assess pollinator preference, the mean number of each *Castilleja* plant bees visited, the percentage of the total time on plants of each species visited, and the percentage of the total number of plants of each species visited by bees were used. These also showed similar preference for each *Castilleja* species, as similar mean numbers of plants each bee visited, with 5.4 CALE

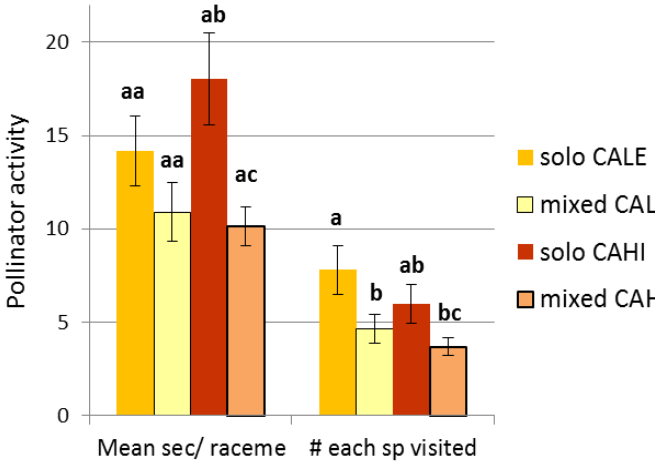

Figure 1.8. Compiled results of pollinator activity for solo & mixed-species contexts, showing mean seconds bees spent on each raceme, and the mean number of each *Castilleja* species visited by bees.

plants and 4.3 CAHI plants visited, as well as similarity between sites with 5.6 plants at GH site and 4.8 plants visited at WR site (Table 1.9 and Figure 1.8).

Table 1.9. Results from pollinator observations showing the differences between restoration site, recipient *Castilleja* species, and plot context (averaging both species) for the mean number of flowers visited per forage, the percent time a bee spent on each *Castilleja* species compared to total, and the percent total plants visited for each species compared to total visited.

Factor	Mean # plants visited	% total time visiting plants	% total number of plants visited
Site			
Glacial Heritage	5.61±5.1	52.2% ±32%	51.9% ±28%
West Rocky	4.83±4.8	48% ±44%	48.9% ±41%
Species			
CALE	5.36±5.6	48.3% ±35%	47.7% ±32%
CAHI	4.33±3.8	53.8% ±36%	54.3% ±32%
Context			
Solo	6.91±4.8	54.5% ±28%	54.5% ±26%
Mixed	4.17±4.6	50% ±38%	50% ±34%

Univariate ANOVA analysis yielded significant results for the percentages, and not the number of plants visited. The site had no effect on these preference measures (Table1.10). GH had slightly higher values for all measures but not statistically significant. The recipient *Castilleja* species did not affect the number of plants visited, but did affect both the percent of total time bees spent on each plant, the percent of the total number of plants visited by the bees. The interaction of site and species was significant for all measures of pollinator preference, indicating a difference between the plant species more at WR than at GH. WR CALE and CAHI were not significantly different from each other in number of bee visits, but GH CALE and CAHI plants were, with less CAHI plants visited by bees (Figure 1.9). GH and WR CALE visits made up a

significantly lower percent of the total time bees spent on racemes than CAHI visits, though the sites did not differ in any measures.

Table 1.10. Results from univariate tests on the effects of restoration site and recipient *Castilleja* species on the mean time each bee spent per racemes, their total time on racemes per forage, and the mean number of flowers visited per forage. Significant effects, at $\alpha = 0.05$, denoted with *

Response	Effect	DF	MS	F	p-value
Mean # plants visited/ species	Site	1	53.95	1.71	0.201
	Species	1	0.78	0.05	0.831
	Plot(site)	15	51.19	3.01	0.000*
	Site*Species	1	115.21	6.77	0.010*
% time on recipient species	Site	1	0.09	0.93	0.338
	Species	1	0.56	4.83	0.030*
	Plot(site)	15	0.08	0.71	0.767
	Site*Species	1	3.13	27.13	0.000*
% total recipient species visited	Site	1	0.03	0.36	0.552
	Species	1	1.14	14.12	0.000*
	Plot (site)	15	0.07	0.89	0.573
	Site*Species	1	3.84	47.58	0.000*

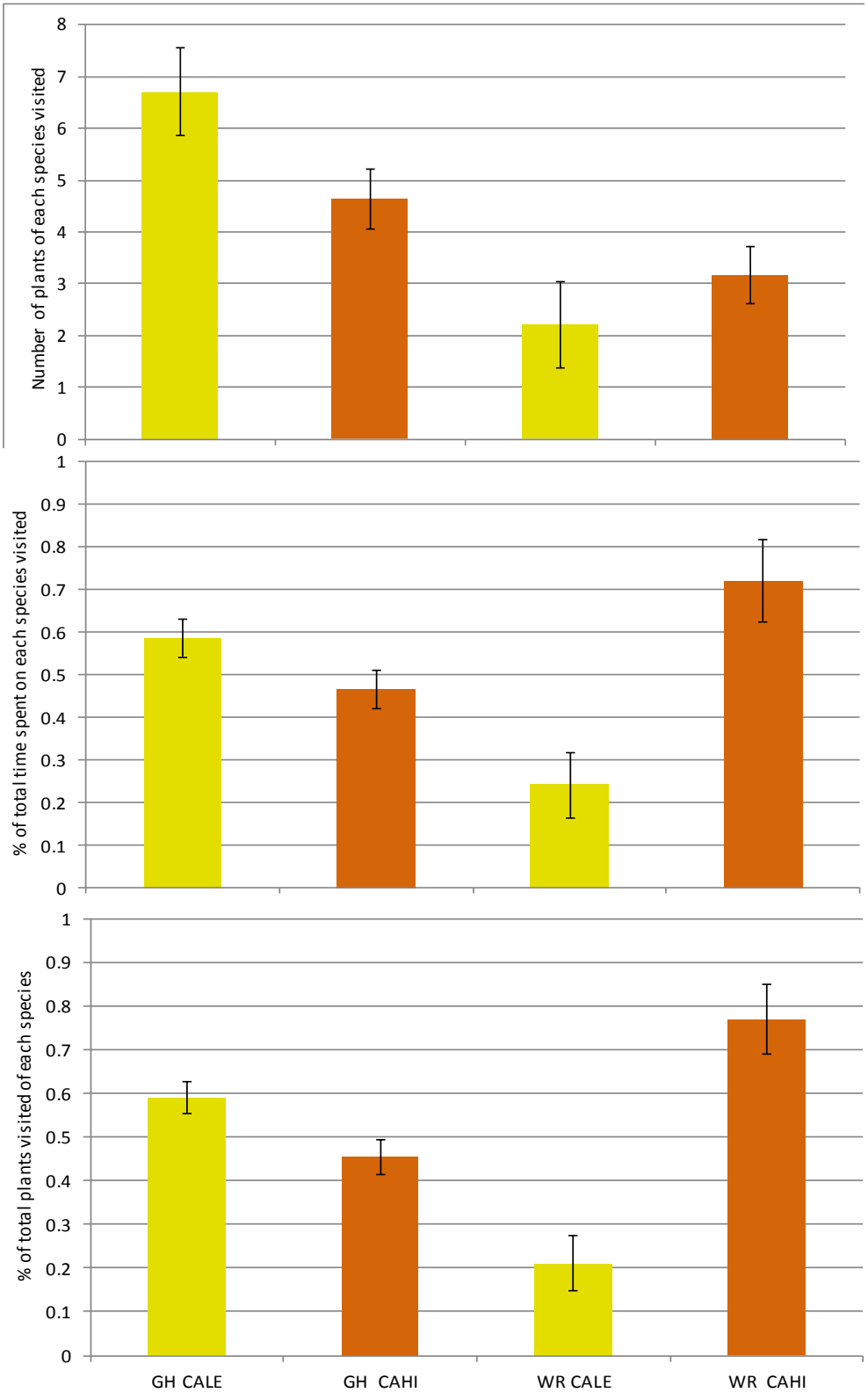

Figure 1.9. Graphs of pollinator preference measures as total number of each *Castilleja* species visited by bees (top), the percent of the total time spent on each species (middle), and the percent of the total plants of each species visited (bottom), showing differences across sites and *Castilleja* species.

Assesment of the pollinator constancy, for both *Bombus californicus* and the sweat bee visits, were calculated from the number of transitions between conspecific flowers (i.e. CALE to CALE) and between interspecific flowers (i.e. CALE to CAHI). These transitions are shown in Table 1.11.

Table 1.11. Counts of the total inter- and intraspecies transitions during each bee group's foraging activity across all sites. *Bombus californicus* (BC) bees are compared to *Lasioglosum* bees (LB).

		BC bees		LB bee	
From		CALE	CAHI	CALE	CAHI
To	CALE	124	81	0	5
	CAHI	82	80	5	1

BC choices	
CALE-CALE	33.79%
CAHI-CAHI	21.80%
CALE-CAHI	22.34%
CAHI-CALE	22.07%

An index of constancy close to zero indicates a low degree of loyalty to a flower species among the bees. *Bombus californicus* index of constancy was 0.10. *Lasioglosum* bees index of constancy was -0.667. However there was a very low sample size of *Lasioglosum*, with more transitions between species than among them. Comparing the mean constancy indexes for each mixed plot between the sites, using a t-test, shows a significant difference between sites for *Bombus californicus* constancy, with a two-tailed p-value of 0.020. WR bee transitions yielded a high mean constancy of 0.80, indicating an 80% loyalty to a preference species, with GH bees having a constancy index of 0.16. WR bees visited much fewer CALE, tending to remain on CAHI during forages (Figure Appendix A.1). GH bees tended to visit CALE for more consecutive transitions, but still transitioning frequently to CAHI for one or two flower visits. Of all bee transitions, 44% were made between different species, with 56% being between plants of the same species (Table 1.11). Only mixed plots could be used to assess constancy, due to the requirement of having different options for the pollinators to choice between.

Seed Production & Germination:

The mean seed number per matured capsule for *C. levisecta* was around 160 seeds in wild prairie populations, as determined by Jane Wentworth (USFWS 2000; Wentworth 2005). In this study GH CALE plants produced more seed per capsule than CAHI plants and Heritage had higher seed counts than WR plants, for both *Castilleja* species (Table 1.12). CALE at GH had 176.3 seeds per capsule, and CAHI had 161.9, while CALE at WR had 150.8 and CAHI had the least at 123.3 seeds per capsule. Germination percentage was also higher for CALE at GH than CAHI (86.3% versus 75.4% respectively), but CAHI at WR was higher than CALE germination percentage, with 84.6% for CAHI and 81.8% for CALE (Figure 1.10). Germination speed, as the mean week to reach 50% germination (T50), was lower for CALE than CAHI for both sites, with sites similar at 6.3 weeks for GH CALE and 7.5 weeks for WR, and 11.2 weeks for GH CAHI and 8.4 weeks for WR.

Table 1.12. Results of seed production and germination, showing differences between sites & *Castilleja* species for the number of seeds per capsule, percent germination, and T50 week.

Site	*Castilleja* species	Mean number of seeds/capsule	Mean % germination	Mean T50 week
Glacial Heritage	CALE	176.3	86.3	6.3
	CAHI	161.9	75.4	11.2
West Rocky	CALE	150.8	81.8	7.5
	CAHI	123.3	84.6	8.4

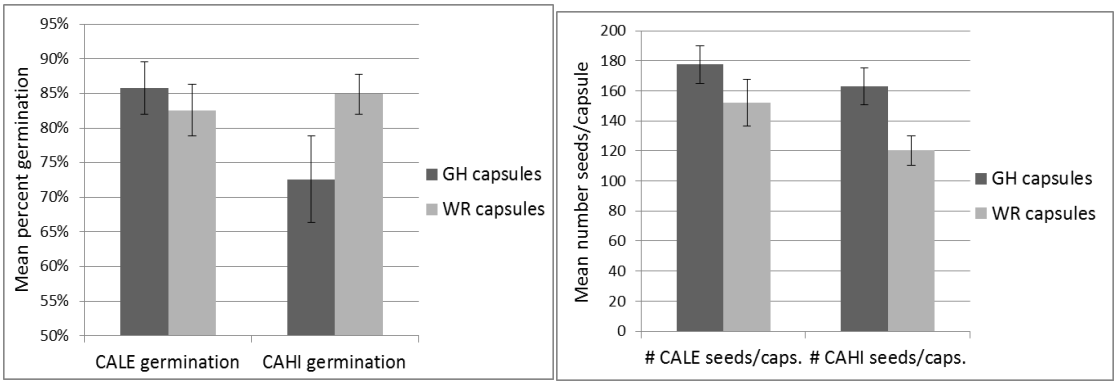

Figure 1.10. Mean percent germination (left) and mean seed count per capsule (right) for the restoration sites, showing differences between the *Castilleja* species.

Independent sample t-tests on each species' germination, comparing GH and WR sites, yielded significant effects of site on T50 week, but not percent germination, for both *Castilleja* species (Table 1.13). Seed counts also yielded significant p-values when comparing CALE and CAHI between sites.

Table1.13. Results from t-tests exploring the overall effects of the restoration site, on the mean seed germination percent, T50 week, and mean seed count/capsule. Significant effects at α= 0.05 denoted with *

Comparison	Response	DF	t-stat	p-value (1-tail)
CALE	% germination	61	1.03	0.153
GH vs WR	T50 week	47	-1.79	0.040*
	Seed count	62	1.96	0.027*
CAHI	% germination	54	-1.64	0.053
GH vs WR	T50 week	55	3.01	0.002*
	Seed count	65	2.49	0.007*

Capsule comparisons were also taken between the bottom (first produced) seed capsules and the middle (later produced) capsules. Off-site control seed samples of CALE and CAHI could not give capsule comparisons, since they were pulled from pooled seed stock of the species. Capsules towards the bottom of stems (B-caps.) had lower seed counts than mid-stem capsules, at 164.5 seeds for CALE & 161.9 for CAHI, compared to 188.1 for CALE and 161.9 seeds for CAHI at GH (Table 1.14). WR seed

Table 1.14. Capsule comparisons for sites and *Castilleja* species, showing mean seed count per capsule, the mean percent germination, and mean T50 week.

Parent species	**Site**	**Capsule**	**Mean # seeds/ capsule**	**% Germin-ation**	**T50 Week**
CALE	Glacial Heritage	Bottom	164.47	84.85	6.34
		Middle	188.12	87.64	6.26
	West Rocky	Bottom	133.63	77.26	8.33
		Middle	168.06	86.41	6.67
CAHI	Glacial Heritage	Bottom	161.94	74.58	11.28
		Middle	161.89	76.19	11.14
	West Rocky	Bottom	113.69	84.84	8.78
		Middle	132.88	84.34	8.05

counts had the same pattern, with more in middle capsules. Germination mean and speed were consistently lower in the bottom capsules of CALE, but varied for CAHI. Mean middle CALE capsule percent seed germination was 87.1% and 81.1% in B-caps., and the mean CAHI germination was 80% and 79.4% for B-caps. across sites. T50 was similar across capsules but varied between the sites, decreasing from GH CAHI to WR CAHI.

Mean off-site control CALE germination was 97.3% and 88.7% for CAHI (Table 1.15). On-site controls (solo-species context plots) had slightly less germination than the off-site controls, and the mixed-species context seed had the least for both *Castilleja* species (Figure 1.11). CAHI was also consistently lower than CALE germination across these treatments. T50 week did not show a pattern across the treatments or species, though the mixed-species plot CALE germinated faster than the mixed-species plot CAHI.

Table 1.15. Results of seed germination tests, showing mean percent germination and mean T50 for seeds of each *Castilleja* species from the off-site controls, on-site solo-species plot controls, and mixed species contexts.

Treatment	*Castilleja* species	Mean % germination	Mean T50 week
Off-site control	CALE	97.33	9.83
	CAHI	88.67	8.75
On-site control	CALE	90.42	7.33
	CAHI	87.50	8.88
Mixed-species	CALE	84.09	6.77
	CAHI	78.50	10.13

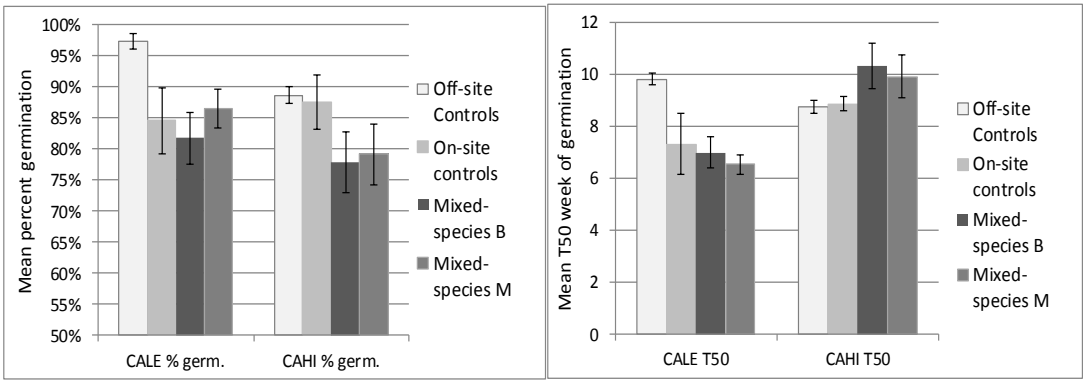

Figure 1.11. Mean percent germination for each treatment type (right), and T50 showing the speed of germination by the week at which 50% was reached (left). Each show the off-site control, assumed to be pure-bred for the species, the on-site control grown in the solo-species context, and the mixed-species contexts with distinction between the mid-stem and bottom-stem capsules.

According to a univariate ANOVA for the seed data set of only on-site mixed-plot plants (excluding off-site controls that had no capsules for seed counts, and solo-plots), the site, parent *Castilleja* species, and interspecies distance had mostly no significant effect on the number of seeds per capsule, percent of seed germination, or T50 week for the *Castilleja* seeds of the F1 generation

Table 1.16. Results from univariate tests, of only mixed-species plots, showing effects of restoration site, parent species, plot context, & capsule position on the number of seeds per capsule, percent seed germination, T50 week for F1 germination, and total germination. Significant effects, at $\alpha = 0.05$, denoted with *

Response	Effect	DF	MS	F	p-value
Number of Seeds/ Capsule	Site	1	49750.85	6.49	0.034*
	Species	1	9757.46	2.28	0.134
	Distance	1	2048.06	0.48	0.490
	Plot(site)	8	7694.47	1.80	0.086
	Sp.*Dist.	1	4468.33	1.05	0.309
Percent Germination	Site	1	0.05	0.47	0.511
	Species	1	0.07	1.56	0.214
	Distance	1	0.01	0.27	0.605
	Plot(site)	8	0.11	2.40	0.021*
	Sp.*Dist.	1	0.01	0.32	0.574
T50 Week	Site	1	398.88	3.31	0.106
	Species	1	44.07	0.50	0.480
	Distance	1	133.93	1.53	0.219
	Plot(site)	8	120.62	1.38	0.217
	Sp.*Dist.	1	235.12	2.68	0.105

from the sites (Table1.16). The species didn't diff in any measure, and no factor affected the T50 week. Restoration sites differed in their number of seeds per capsule, and plots differed in percent seed germination. Using the more specific distance between plants, dividing mixed-context plants into those closer than 30 cm and those further than 30 cm to the other *Castilleja* species, did not yield significant p-values for any factors. Table 1.17 shows these distances and the values of several response variables for the plants of these distances.

Univariate ANOVA analysis of CALE and CAHI seed data for per capsule seed counts were run separately for each species without off-site controls that lacked capsules. The ANOVAs compared site and distance factors, with plot number nested within site, with only significant p-values for plot for CAHI and for the species and distance interaction for

Table 1.17. Results from univariate tests, of only mixed-species plots, showing effects of restoration site, parent species and interspecies distance on the number of seeds per capsule, percent seed germination, T50 week for F1 germination, and total germination. Significant effects, at $\alpha = 0.05$, denoted with *

Response	Effect	Cast. sp.	DF	MS	F	p-value
Number of Seeds/ Capsule	Site	CALE	1	373.96	0.04	0.851
		CAHI		16981.1	4.04	0.080
	Distance	CALE	1	5156.49	0.95	0.333
		CAHI		498.74	0.12	0.731
	Plot(site)	CALE	9	10121.5	1.87	0.078
		CAHI		4207.01	1.01	0.440
	Sp.*Dist.	CALE	1	18342.0	3.39	0.071
		CAHI		2208.71	0.53	0.470
Percent Germin.	Site	CALE	1	0.04	1.37	0.272
		CAHI		0.15	0.59	0.464
	Distance	CALE	1	0.003	0.10	0.757
		CAHI		0.03	0.67	0.416
	Plot(site)	CALE	9	0.03	0.92	0.520
		CAHI		0.15	3.23	0.004*
	Sp.*Dist.	CALE	1	0.161	4.73	0.034*
		CAHI		0.07	1.45	0.234
T50 Week	Site	CALE	1	0.18	0.01	0.908
		CAHI		5387.4	0.26	0.626
	Distance	CALE	1	42.92	3.92	0.053
		CAHI		38932.4	2.82	0.099
	Plot(site)	CALE	9	12.74	1.16	0.338
		CAHI		20971.7	1.52	0.172
	Sp.*Dist.	CALE	1	17.89	1.63	0.207
		CAHI		40338.2	2.93	0.093

CALE (p= 0.034) (Table 1.17). This indicates that the distances between the *Castilleja* species only affected the on-site CALE, but only in T50 week. The similarities between interspecies distance responses can be seen in Table 1.18 and between plot contexts in Figure 1.12.

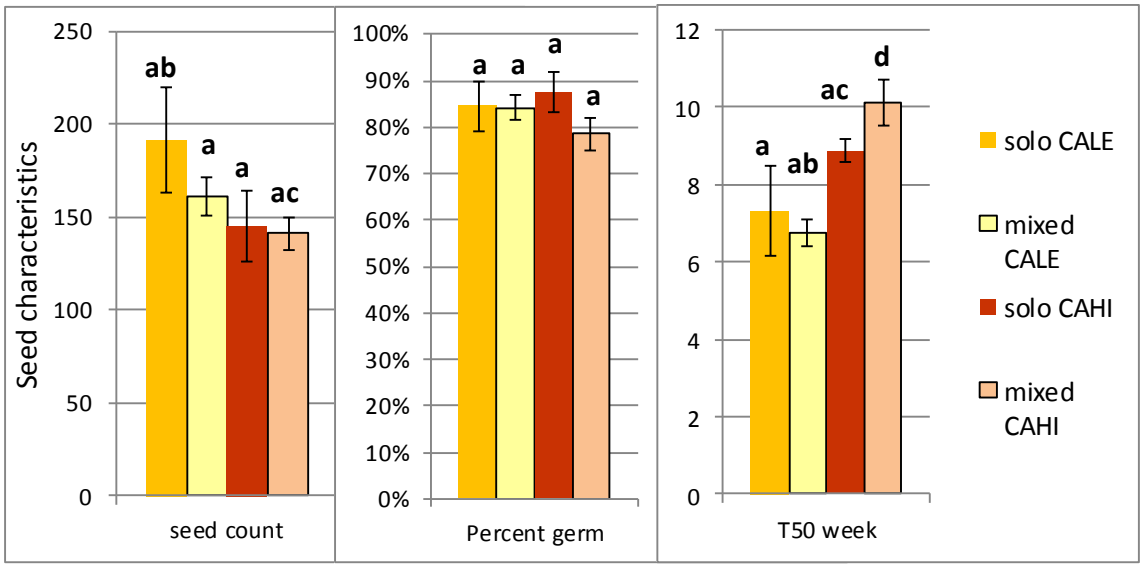

Figure 1.12. Compiled results of on-site plants' seed counts (left), germination percent (middle), and germination speed as T50 week (right), comparing solo and mixed-species contexts.

Table 1.18. Results for different contexts, as solo- and mixed-species plots comparing plants further than 30 cm and plants closer than 30 cm to the other *Castilleja* species. Results of mean seed count per capsule, mean bloom zone length, and mean number of capsules per stem.

Parent species	Distance to other species	Mean # seeds/B capsules	Mean # seeds/M capsules	Length of Bloom Zone (cm)	Mean. No. capsules/ stem
CALE	solo plots	162.67	220.83	13.92	11.47
	>30 cm from CAHI	166.23	185.00	20.00	13.99
	<30 cm from CAHI	128.36	154.07	16.55	12.37
	Total	149.52	178.39	17.43	12.84
CAHI	solo plots	163.75	167.33	22.50	15.14
	>30 cm from CALE	134.08	134.69	21.62	16.29
	<30 cm from CALE	142.60	152.33	18.48	15.26
	Total	139.24	148.24	20.39	15.63

One way ANOVA analysis of the off-site control seeds' percent germination and T50 values indicate significant difference between the pure-breeding controls' germination for the percent germination (p= 0.010), and for T50 week (p= 0.031). Mean off-site control CALE percent germination was significantly higher (M= 97%, SD= 0.023) than CAHI's (M= 89%, SD= 0.023), and mean T50 for CALE was significantly longer (M= 9.83, SD= 0.38) than CAHI's (M= 8.75, SD= 0.43) (Table 1.19).

Table 1.19. One way ANOVA on off-site control seeds of CALE and CAHI, comparing their percent germination and T50 week for differences between the pure-breeding individuals. Significant effects, at α= 0.05 ($p < 0.05$), denoted with *

		Sum of Squares	df	Mean Square	F	Sig.
% germ.	Btwn Groups	0.011	1	0.011	21.125	0.010*
	Within Groups	0.002	4	0.001		
	Total	0.013	5			
T50 week	Btwn Groups	1.760	1	1.760	10.563	0.031*
	Within Groups	0.667	4	0.167		
	Total	2.427	5			

Comparing the sites to each other in independent sample t-tests did not yield significant p-values (Table 1.20). T-tests between both on & off-site controls together and the mixed-species contexts did not yield significant p-values for percent germination or for T50, indicating the mixed-species context seeds had similar germination to seed produced away from the other *Castilleja* species. Using independent sample t-tests for the full data set to compare means between parent species of all the seeds, CALE to CAHI, yielded a significant p-value for T50 week (p= 0.001), but not for the percent germination (p= 0.201). Comparing mixed-plot seed capsules via t-tests yielded no significant difference between the capsules for seed count, germination or T50 week.

Table1.20. Results from t-tests exploring the overall effects of control contexts (on and off-site controls compared to mixed-species context), recipient *Castilleja* species, the restoration site, and the capsule level, on the seed counts/capsule, mean seed germination percent, and T50 week. Significant effects at α= 0.05 denoted with *

Comparison	Response	DF	t-stat	p-value (2-tail)
On & Off-site controls vs mixed-plot context	% germination	53	1.69	0.097
	T50 week	53	-0.43	0.670
Parent *Castilleja* species	Seed count	138	0.64	0.520
	% germination	131	1.29	0.201
	T50 week	136	-3.42	0.001*
GH vs WR sites	Seed count	131	2.51	0.013*
	% germination	122	-0.68	0.489
	T50 week	118	2.22	0.028*
Bottom vs Middle capsules	Seed count	106	-1.05	0.296
	% germination	105	-0.67	0.505
	T50 week	67	-0.41	0.681

Independent sample t-tests comparing both restoration sites' solo-species plots to mixed species plots, for each *Castilleja* species, yielded a significant difference between CAHI's T50 week but not CALE's, nor any differences between seed count per capsule, or the percent germination (Table 1.21).

Table1.21. Results from t-tests exploring the effects of contexts (solo-species compared to mixed-species context), for each *Castilleja* species, on the mean percent germination, and T50 week. Significant effects at α= 0.05 denoted with *

Comparison	Response	DF	t-stat	p-value (2-tail)
CALE solo vs mix	Seed count	14	0.98	0.171
	% germination	17	0.07	0.472
	T50 week	15	-0.16	0.436
CAHI solo vs mix	Seed count	7	0.17	0.434
	% germination	13	1.60	0.067
	T50 week	54	-2.18	0.017*

Assessing earlier reproductive characteristics at the parent plant level shows differences between the sites, and *Castilleja* species, for the number of flowering stems per plant, length of the bloom zone (distance between the bottom and top-most flowers), and the mean number of capsules per flower stem. These tracked plants at GH had more flower stems then those at WR, and CALE had more stems than CAHI, with 23.5 CALE at GH and 5.44 at WR, versus 9.83 CAHI stems at GH and 3.1 at WR (Table 1.22). The length of their bloom zones were reverse with CALE having shorter bloom zones than CAHI, with 19.7 cm for CALE and 22.6 for CAHI at GH, and shorter zones of the same pattern at WR. Matching this pattern, the average number of capsules per stem on CALE was fewer than those on CAHI. GH also had longer bloom zones than WR. Differences between the interspecies distance measures were not as clear as those between sites (Figure 1.13).

Table 1.22. Results of parental reproductive measures, showing differences between sites and *Castilleja* species for the mean number of stems per plant, the length of the bloom zone, and the mean number of capsules per stem. These data were taken from the F1 tracking plants for each plot, as representative reproducing plants.

Site	*Castilleja* species	Mean number stems/plant	Bloom zone length (in cm)	Mean number capsules/stem
Glacial Heritage	CALE	23.53	19.65	12.84
	CAHI	9.83	22.64	16.65
West Rocky	CALE	5.44	15.08	15.63
	CAHI	3.13	17.86	14.49

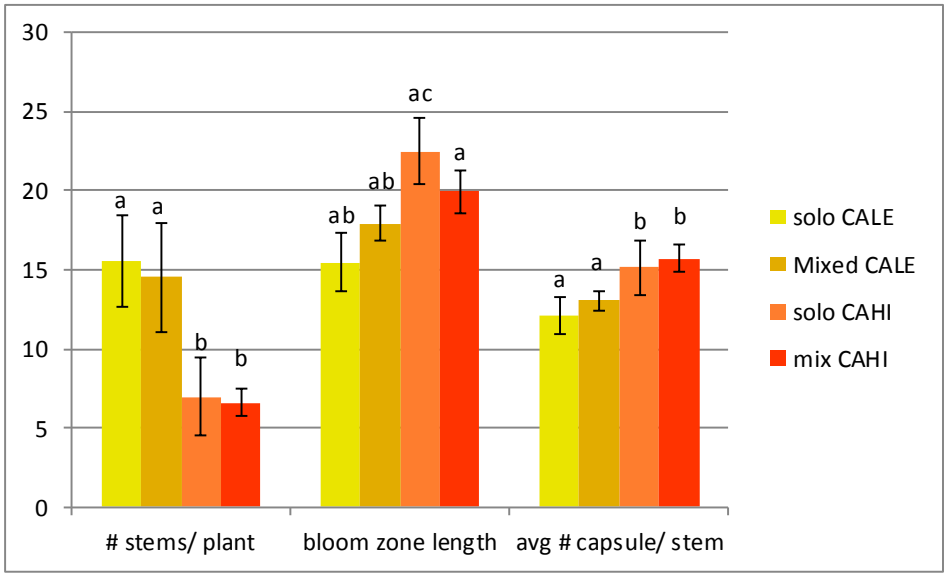

Figure 1.13. Early reproductive characteristics for each interspecies distance of both CALE and CAHI. The number of stems per plant, length (in cm) of the bloom zone, and average number of seed capsules per flowering stem are given.

The univariate ANOVA analyses of earlier reproduction characteristics also showed significant effects from the sites and parent *Castilleja* species on the number of stems per plant, the bloom zone length, and the average number of capsules per stem, but not plot context as interspecies distance (Table 1.23). GH plants had significantly more (p=0.000) flowering stems per plant (M= 16.68) than those at WR (M= 4.29), as did CALE have more stems than CAHI plants (M=14.49 and M= 6.48 respectively). GH plants also had significantly smaller bloom zones than WR plants (M= 21315 and M=

16.47 respectively), but with no significant difference between the species. GH plants' stems likewise had significantly less seed capsules than WR plants (*M*= 14.75and *M*= 15.06), as did CALE have less capsules than CAHI (*M*= 14.24and *M*= 15.57). The distance, as the distance between the species of either closer or further than 30 cm, in separate plots, or off-site, only affected the length of the bloom zone.

Table 1.23. Results from univariate tests on the effect of restoration site, parent species, and plot context on the number of stems per plant, bloom zone length, and average number of capsules per stem. Significant effects, at $\alpha = 0.05$ denoted with *

Response	Effect	DF	MS	F	p-value
Number stems/ plant	Site	1	2014.25	26.74	0.000*
	Species	1	879.43	8.51	0.005*
	Distance	1	35.00	0.34	0.563
	Plot(site)	1	71.03	0.69	0.744
	Species* site	1	682.77	6.61	0.013*
Bloom zone length	Site	1	396.08	9.67	0.008*
	Species	1	28.23	0.94	0.338
	Distance	1	189.31	6.28	0.016*
	Plot(site)	1	42.63	1.41	0.198
	Species* site	1	0.67	0.02	0.882
Average number capsules/ stem	Site	1	121.68	9.55	0.007*
	Species	1	74.85	4.99	0.030*
	Distance	1	29.81	1.99	0.165
	Plot(site)	1	12.39	0.83	0.615
	Species* site	1	1.06	0.07	0.791

Discussion

The first objective of this study, and important to endangered CALE conservation, was to obtain a positive identification of the pollinators of CALE plants in a field setting, as the pollination needs of a plant can strongly affect their fitness and the population's long term survival. Identifying the actual pollinators will help assess whether a site and its insect population will sustain a population of CALE. *Bombus californicus* was identified as the primary pollinator in these South Puget Sound prairie restoration sites, and these bees foraged almost exclusively on the present *Castilleja* species. Since they are also a fairly common species in the region, the likelihood that CALE will suffer from pollination limitation in these, and other regional restoration sites, is low. With several species of sweat bees also foraging on CALE flowers and likely pollinating them, though to a lesser degree than *B. californicus*, the overall pollination success of CALE seems high, and potentially resistant to pollinator losses by having multiple species. Introduction of additional non-native honeybees would not be needed or even helpful, as they don't even attempt to visit *Castilleja* flowers.

With the confirmation of active pollinators in the field, the assessment of pollinator visit duration and pattern being affected by the species of recipient *Castilleja* and the species context the pollinators visit can be addressed. The plot and site characteristics of these pollinator forage-contexts were linked to their effects on pollinator activity. GH had more *Castilleja* plants and flowers overall, but particularly more CALE while WR CALE and CAHI were more similar and less than at GH. These site differences are similar to the pollinator foraging times, with longer foraging on flowers at GH.

Mixed species plots had lower floral density, total number of *Castilleja* plants, and average number of stems per plant, for both species. However, with both species added for mixed plots, the *Castilleja* plants had slightly higher values of each plot characteristic than solo-grown *Castilleja* plants, possibly indicating no interspecies competition t. CALE was sown at a lower density than CAHI in each mixed plot and yet more CALE plants were present in these plots than CAHI, which may be due to CALE's higher germination and/or better seedling survival after each plot was initially seeded. Though, with different seeding mixes and ages across the plots and sites, no conclusions about species interaction can be made from this study, rather the plot characteristics can be used to interpret what the pollinator and seed results could be based on. The West Rocky site (WR) was significantly poorer in quality (as the size of *Castilleja* populations), with lower floral density, less plants per plot, and less flowering stems per plant, than the Glacial Heritage site (GH) (Table 1.4). This was evident during field observations by the predominance of invasive ox-eye daisy at WR rather than *Castilleja* flowers that stood out among the GH plots, like clumps of brilliant colors living up to the common 'paintbrush' name (Figure Appendix B.1). The differences in floral densities and plant sizes (by number of flowering stems) is likely due to the age difference of the mixed plots, with GH mixed plots being older by one year then WR's. However, the solo-CALE plots were also different ages, but the younger GH plot still had higher floral density, total plant number, and number of stems than the longer-established WR plot, with means of 31.9, 111, and 11.8 at GH, compared to 5.5, 30 and 5.5 at WR for each measure respectively. Therefore age of the plots is not the only contributor to GH having more *Castilleja* plants and flowers. Pollinator activity was also of poorer quality at WR

than at GH, with less *B. californicus* visits, and shorter visit times per plant and total forages (Table 1.6 and 1.7). *Castilleja levisecta* (CALE) was also more prevalent at the restoration sites than *C. hispida* (CAHI), with more flowers per square meter of plot, more plants per plot, and more stems per plant (Table 1.3). The *Castilleja* species did not, however, affect the pollinator activity, as bees spent the same amount of time on racemes of both species, as well as visiting the same number of plants, according to the ANOVA analysis (Table 1.8). While bees seemed to spend much longer time in total foraging on CALE, the difference was not significant, likely because they spent much less time on CALE at WR *M*= 24.4 sec, versus GH *M*= 106.4 sec. Bees showed no strong preference overall for one *Castilleja* over the other, though they visited more consecutive CALE at GH and more CAHI at WR (Figure Appendix A.1). Constancy, to CAHI, was high at WR, but still low for GH CALE due to frequent, but short, interludes to CAHI, following CALE visits.

Because CALE plants received the same length of visits, per flower and per forage, as CAHI did, as well as the same number of by bees, this indicates no bee preference difference between the *Castilleja* species. The floral structure and color differences may not be different enough to produce a preference in the bees, and/or nectar similarities may encourage bees to treat each *Castilleja* equally. Only a few instances were seen of a bee moving from CALE to a yellow CAHI, possibly in part due to the low occurrence of yellow CAHI in the sites, but also possible due to the bees not using color as a foraging search characteristic.

When CALE was grown with CAHI in the mixed-species context, CALE's number of plants per plot and number of stems per plant decreased, but CAHI was less affected when grown with CALE, potentially indicating sensitivity in CALE to interspecies competition, but not CAHI. The plot contexts were no different for pollinator activity except for the number of plants bees visited of each species, with bees visiting more plants in solo plots (M= 6.9) than in mixed-species plots (M= 4.2). Solo context is most likely affecting pollinator foraging optimization because the plants match their neighbors, which increases the number of visits and pollinator constancy, as studied in bumblebees (Hersch & Roy 2007). In the semi-solo plot of CALE at GH, having only two CAHI, one bee was observed passing over a CAHI in the middle of its CALE foraging, though stopping to attempt two *Vicia hirsuta* flowers throughout. The lower density of *Castilleja* flowers at WR may have been part of the reason for higher constancy the bees expressed there, due to resources being scarcer, which increases constancy by requiring more economic foraging (Real 1983). Since CALE was also lower than CAHI at WR, the bees were more likely to form a preference for CAHI as the dominant flower.

Site characteristics and pollinator activity predicted the patterns seen in seed production, germination and parent plant's flowering amount. The number of seeds per capsule followed the same pattern as the flower densities of the plots, with GH have higher values than WR, and with CALE having higher values than CAHI. Given that pollinator activity at WR was lower than GH, as the bees spent less time per raceme on WR flowers and less total forage time at WR, lower seed production would be a direct result of decreased pollination. This could lead to decreased fitness of plants at the lower quality WR site.

Comparisons of the *Castilleja* species controls, though lacking seed counts due to being outsourced, did indicate CALE seeds germinated slower but at a higher percentage than CAHI. Solo-species controls from the sites had a similar pattern of CALE out producing CAHI in seed counts and having higher germination percentages, though with no species effects across all restoration site seeds except for CALE's T50 week generally being shorter than CAHI's. Germination did decrease for CAHI when it grew in mixed-species contexts though not significantly, but CALE germination did not (Figure 1.12). Seed production in mixed contexts also decreased for CALE but not CAHI, indicating fitness costs for CALE more than CAHI when the species are grown together.

Parent plant flowering characteristics were less affected by their growing context, but were different between the *Castilleja* species, partly reflecting their slightly different phenologies. Bloom zone lengths for the *Castilleja* species matched their relative bloom periods, with CALE having smaller zone lengths, likely due to its shorter blooming period (Figure 1.14). CALE produced less seed capsules per stem, also reflecting the shorter bloom period and bloom zone length. The fewer flowers produced per stem is unlikely to create a lower competitive capacity with CAHI, because CALE produced more seed per capsule, though not found to be significantly different from CAHI, and a much larger number of flowering stems than CAHI. While no difference was found between the capsules, there was a pattern of lower germination in seed from bottom capsules, and lower seed production in these capsules in all plot contexts, but only in CALE (Table 1.14 and Figure 1.11).

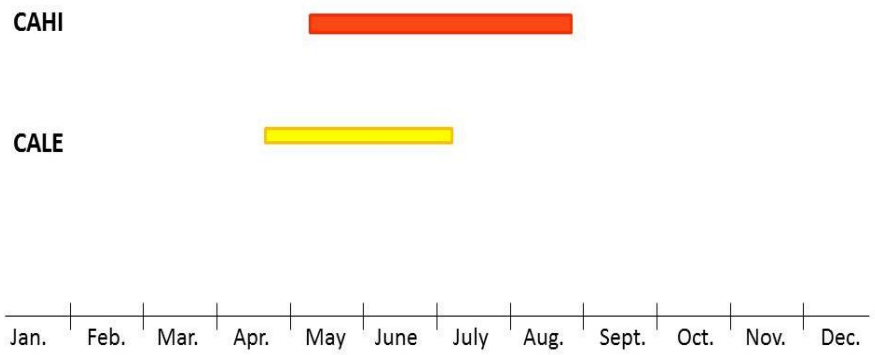

Figure 1.14. Phenology of CAHI and CALE, showing the degree of overlap, and length of blooming periods.

Because the bottom capsules would have been flowers primarily exposed to other CALE flowers, before CAHI initiated blooming, we would have expected higher fitness in these seeds. The decrease could be due to the lower density of flowers and fewer active pollinators at that early stage of CALE blooming. Lower flower density early in reproduction would attract fewer pollinators, of what small bee population is present early in the pollination season (Robson 2010). The cool spring in which the pollinator observations were started likely delayed the emergence of bumblebees from their underground nests. When both CALE and CAHI were in bloom, there were more bumblebees foraging at the sites.

The mixed-species plots in this study only slightly affected seed production and germination, but not the number of flowers that the parent plants produced, indicating growth with another *Castilleja* species could affect the offspring level of reproduction more than the parental level, but more investigation with more plots would be needed for accurate assessment. This offspring effect could be from the potential presence of hybrid

seeds. Experiments with controlled hybridization between an invasive and locally endemic species (*Silene vulgaris* & *S. uniflora* ssp. *petraea*) indicate that the F1 seeds are significantly affected by paternal line, with germination rates resembling those of the pollen donor (Andersson et al. 2008). Thus hybrids sired by a lower germinating species produced seeds with lowered germination, and vice versa (Andersson et al. 2008). Seed germination speed had a similar paternal effect (Andersson et al. 2008). Since CAHI is known to have low seed germination, which was supported in this study, hybrid seeds from maternal CALE could have decreased germination as a direct result of the CAHI paternal characteristics. Seed set trends were also towards lower production, from plants cross-pollinated with another species, while intraspecific seed sets were the same for both species (Andersson et al. 2008). Lower seed production from hybridization between CALE and CAHI could be an early sign of decreased fitness due to outcrossing depression.

These effects in mixed-species contexts could be from the production of hybrids due to low pollinator constancy and no indication of preference by the bees for one *Castilleja* over the other. It could also be from general interspecific competition for pollinators when they have options to choose from directly in their forage path. The F1 offspring from these mixed-plots were in need of analysis before the cause of reduced fitness when *Castilleja* were grown together can be more accurately determined.

Conclusion

The *Castilleja levisecta* and *C. hispida* flowers are likely protogynous, because the bumblebee and sweat bees visiting them appear to move primarily from the top most flowers to those directly below rather than from a bottom flower upward (Figure Appendix B.2), following the pattern of pollinator movement with the direction of floral organ maturation. This could be confirmed by pollen viability tests paired with *in vivo* stigma receptivity tests. The bumblebees would often continue to an adjacent raceme of the same plant due to the clustering of the flower stems. This movement pattern contradicts the usual pollinator movements of upward on protandrous flowers (Real 1983), but would be more reproductively advantageous for protogynous flowers by moving pollen first to a new flower then down to reload on fresh pollen from a flower with no less receptive stigmas. Bumblebees in this study tended to move more laterally to new racemes than further down the current raceme, making geitonogamy the most likely inbreeding risk to plants with multiple flowering stems of similar heights. The *Castilleja* plant's self-incompatibility will help prevent most geitonogamy inbreeding.

Not all animal visitors to a flower are active pollinators, moving pollen from anthers to the stigma for fertilization. However, since animal exclusion yields minimal seed production in *Castilleja* plants (Wentworth 1994) and only two animals were documented actively foraging on the *Castilleja* flowers on these sites, at least one of these should be assumed to play the role of pollinator for these plants. Honey bees were prolific but never seen visiting *Castilleja* flowers, likely due to an inability to open the bracts to access the actual flowers. Since the bees observed during this study were often still the queens, foraging before establishing their hive in spring to early summer, they were often foraging alone, with one seen at a time in each plot. By foraging solo, they were unable to observe others with experience of rewarding flowers, thus must make

their own choices of what flower to visit. This may increase the randomness of their initial choice and decrease their constancy for a species. Sweat bees are supposed to have lower constancy than bumblebees but they made fewer flower transitions due to their small size, probably reaching a full pollen load with fewer flowers. Low constancy for them is probably not an issue as much as their role in geitonogamy for the *Castilleja* plants, as they were more likely to move a short distance from one flower to another on the same plant. Longer observations later into the season, and multiple years of observations would be valuable to confirm longer-term pollinator constancy and what affects it in the site. The sample size for pollinator constancy in this study was too low for thorough analysis, because too often the pollinators made only a few transitions between any flowers. More inter-floral movements would be required for assessment of constancy using the Batemans Index.

Castilleja hybridizing pattern depends on their pollinators, being exclusively insect pollinated and self-incompatible. Bee constancy would be the driver of any *Castilleja* hybridizations, as the cross-species movements is the only means of interspecies pollination. Differential pollinator preference between the two *Castilleja* species would be expected, due to color and morphological differences in flower structure, with CAHI having longer galeas and more open bracts and CALE exclusively yellow colored. However, this is probably not what the bumblebees were using as their selection criteria while foraging since they showed no preference for either flower form. Despite the frequency of movement from a CALE to a CAHI at GH, rarely was it to a yellow CAHI, indicating color was probably not a factor in their flower choice. Nectar differences, or similarities, between CALE and CAHI are likely driving the pollinator choices between them. It is possible CALE was slightly more rewarding in nectar supply for the bumblebees, as they made repeat visits more often between CALE when both plants were fairly populous, at GH. Otherwise, floral density could have driven the selection choice in those

repeated visits. *Bombus californicus* has a long tongue, at 2.3 cm (Macior 1974; Koch et al. 2012), which would explain why it can feed from the shorter galeas of CALE as well as the longer galeas of CAHI, averaging 2.6 cm and 2.7 cm respectively. *Lasioglassum* & *Sphecodes* spp. are too small to forage nectar but were recorded crawling across the *Castilleja* galeas collecting pollen (Figure 1.5C & D).

We currently know more about CALE than CAHI, due to the higher need for conservation and restoration for CALE. However, assessment of the genetic and reproductive mode of CAHI would be valuable for comparison and further risk-assessment to the rare congeneric *C. levisecta* (Talve et al. 2012). If the pollinator movements are successfully transferring pollen, which can then fertilize the other species' ovules, as confirmed from hand crosses (Kaye & Blakeley-Smith 2008), the later effects on the F1 offspring, and differences from parentage direction, would be useful for further CALE conservation.

Implications for Practice

- Pollinator populations can be assessed before introducing CALE into new sites, to confirm adequate populations to support CALE reproduction.
- The growing context or distance to a related *Castilleja* species can reduce the fitness of CALE, and mixed species plantings may not be recommended in CALE restoration sites.
- High seed production and germination of CALE, as well as higher flower production, may help buffer fitness reductions in mixed plantings and make interspecies competition less likely, or occur in favor of CALE. Low seedling survival and recruitment should still be accounted for, and the growing site established to optimize CALE seedling survival.

- Restoration with the goal of high floral density to attract pollinators and increase their constancy, especially in cases of CAHI sympatry, will optimize CALE reproduction and population maintenance.
- Successful site establishment before the introduction of CALE populations will increase their fitness and likely long term survival, as site had wide effects on CALE reproduction.

In the instance that *Castilleja levisecta* does hybridizes with *C. hispida*, or possibly any other *Castilleja* species of the region, the hybrids formed should be identified quickly & removed to prevent positive hybridization feedback among the pollinators. Maintenance of hybrid zones to remove hybrids from populations as they arise would be key to maintaining pure species. The other *Castilleja* may also need to be removed or kept at a further distance from CALE for reducing hybridizing events, since plants present on the site had little effect on CALE, but plants within the same 40 square meter plot changed various reproductive responses.

Chapter 2

Field Potential for Hybridization between *Castilleja hispida* and *C. levisecta*, Estimated Using Flower and Pollen Characteristics

Literature Review

Introduction

Restoration of *Castilleja levisecta* in the Pacific Northwest requires careful selection of associated species for the stable maintenance its populations, and complex ecological interactions between species can strongly influence an endangered species' survival. When introduced with a close relative or congener (*C. hispida*), because the *Castilleja* genus is well known for interspecies hybridization, the risk of genetic contaminating and fitness-loss becomes a factor of concern. Suspicion of the possibility of *C. levisecta* hybridization with *C. hispida*, which is native to some of the same Pacific Northwest prairies and used in restoration projects, inspired experiments that confirmed their ability to create potentially reproductive hybrids (Kaye & Blakeley-Smith 2008). Understanding these two species, their potential to interbreed and its possible consequences, as well as the ability to identify putative hybrids, will help further conserve the endangered *C. levisecta*. This literature review will focus on aspects of *Castilleja* ecology, breeding, hybridization dynamics, and floral characteristics as hybrid identifiers. Identifying potential morphological and physiological responses to hybridization in the F1 population would be valuable for preserving the genetic isolation of *C. levisecta* from *C. hispida* in prairie restoration. This could be done by analyzing floral morphology and pollen viability measures of the pure-breeding species and their putative hybrids, potentially linking these results to factors of the plant's ecological interactions such as floral density and distance between species.

Castilleja breeding

The various types of plant breeding systems differ in their genetic diversity, likely as a result of these breeding methods and how they combine the parental genetic material. Surveys of several hundred species, conducted by Hamrick, Godt, and associates (1990), found self-pollinating plants demonstrate a lower number of polymorphic loci, at 41.8% compared to outcrossing animal pollinated plant's 50.1% and the significantly different outcrossing wind-pollinated plant's 66.1% (Bowels & Whelan 1994). Population-scale genetic diversity also varies by breeding system, with less genetic diversity within and more diversity between populations in self-pollinators, which are significantly more diverse between populations then outcrossing animal-pollinated plants, and both significantly higher than between population diversity of outcrossed wind-pollinated plants, having 0.510, 0.197, and 0.099 variation between populations respectively (Bowels & Whelan 1994). Outcrossing reproduction is very valuable to flowering plants, via its creation of genetic diversity, allowing increased likelihood of survival through changing environments (Rea & Nasrallah 2008). The ability to select optimal mates for fertilization to both prevent inbreeding and promote appropriate outcrossing requires various reproductive barriers, include genetic selectivity.

Inbreeding

Inbreeding occurs when closely related individuals breed, including siblings. Self-fertilization is the extreme form (Weeks et al. 2011). Though some plants reproduce successfully via inbreeding and even regular self-fertilization, often parents sharing many of the same alleles produce less fit offspring (Kaye & Lawrence 2003; Rea & Nasrallah 2008). Closely related parents producing offspring with reduced fitness or vigor indicates inbreeding depression in the population (Kaye & Lawrence 2003; Weeks et al. 2011). This may be due to the presence of

recessive deleterious alleles that become homozygous in offspring when shared by parents, thus allowing the expression of the harmful or less-beneficial trait in that generation (Kaye & Lawrence 2003; Talve et al. 2012). Small and isolated populations are more prone to inbreeding due to less access to a variety of compatible (unrelated) mates, thus inbreeding depression is more common to these populations (Caplow 2004; Kaye 2008; Godefroid et al. 2011; Talve et al. 2012). Inbreeding reduces genetic variation in a population and can inhibit a population's resistance to environmental changes and ability to continue its evolutionary trend (Bowles & Whelan 1994; Godefroid et al. 2011; Talve et al. 2012). When inbreeding depression occurs, population survivorship and fitness will also decrease (Bowles & Whelan 1994), amplifying the effects of inbreeding's reduced genetic variation.

Outbreeding

Outbreeding is the crossing of parents that are distantly or unrelated, different ecotypes, or even from different populations (Kaye & Lawrence 2003; Johansen-Morris et al. 2006; Weeks et al. 2011). A moderate degree of relatedness is typically optimal, without sharing too many alleles, but also without being too distantly related for successful interbreeding, such as between different species or populations from very different habitats (Johansen-Morris et al. 2006; Weeks et al. 2011). When parents are too distantly related, their offspring can have similarly reduced fitness and/or vigor as with inbreeding, indicating outbreeding depression (Kaye & Lawrence 2003; Johansen-Morris et al. 2006). Outcrossing between different species can create hybrids, not always with outbreeding depression displayed however. Often the first generation of hybrids, the F1 generation, demonstrates higher vigor than their pure bred parental lines (called hybrid vigor), while later generations of the hybrids' offspring demonstrate reduced fitness and outbreeding depression (Kaye & Lawrence 2003; Johansen-Morris et al. 2006). This and lesser forms of outbreeding depression may be due to lacking the ecological adaptations developed in

the initial parental populations, and possibly from disconnection of coadapted, thus codependent, gene complexes of the parents via their recombination (Kaye & Lawrence 2003; Caplow 2004; Johansen-Morris et al. 2006). Hybrid vigor can be due to breaking up dominant homozygotes when hybrids create heterozygosity, or possibly via overdominance or epistasis (interaction between genes to control a single phenotype) (Johansen-Morris et al. 2006). These can also contribute to the outbreeding depression (or "hybrid breakdown") in later generations (Johansen-Morris et al. 2006). Outbreeding depression is usually assessed in the F2 or later generations because of the hybrid vigor that typically affects the first hybrid generation or F1plants (Godefroid et al. 2011). Outbreeding depression risk depends on population size and structure, gene flow, the habitats each groups is adapted to plus their adaptive differentiation from each other, as well as their reproductive methods (Weeks et al. 2011). Outbreeding depression is a less thoroughly studied condition than inbreeding depression, but has been reported in a handful of animals and plants (Kaye & Lawrence 2003). It possibly occurs less often than inbreeding depression or is less of a threat to species, but ultimately depends on the parents' breeding systems and extent of their relation (Weeks et al. 2011). Over time, outbreeding depression can be selected out of a mixing population and produce a combined population with an outbred advantage over the parent populations (Weeks et al. 2011).

A plant's ability to resist inbreeding and outbreeding depends on its breeding system (self-incompatible, self-fertilizing, asexual, etc.) (Weeks et al. 2011). Depending on the floral structure, either foreign or self-pollen can very often be deposited on a flower's stigma, often even germinating on the surface (Nasrallah et al. 1994; Flegr 2008). The negative effects of both inbreeding and outbreeding produced a selective pressure in the evolution of plant species for the ability to recognize and select their mates (Nasrallah et al. 1994). Plants thus have the ability to select against gametes sharing too many of same alleles, to reduce likelihood of inbreeding

depression, but also to select for some in common, to distinguish its own species (Nasrallah et al. 1994; Franklin-Tong & Franklin 2003). Reproductive selectivity against inbreeding most commonly involves a genetic barrier called self-incompatibility (Nasrallah et al. 1994; Franklin-Tong & Franklin 2003). Gamete selectivity is possible because plant reproduction involves multiple stages where cell-cell interaction and recognition can occur prior to fertilization (Nasrallah et al. 1994). Gamete incompatibility greatly improves plant reproduction by controlling what pollen is able to reach the oocytes and fertilize its eggs (Flegr 2008). Both inbreeding and outbreeding selection are conducted with similar genetic mechanisms.

Reproductive barriers

Reproductive isolation barriers between species consist of two types, prezygotic and post zygotic. Prezygotic barriers are any that inhibit the initial formation of a cross-species zygote, thus are active prior to fertilization, and range from phenology differences to inhibition of pollen tube growth through the style (Flegr 2008). Postzygotic barriers are active after fertilization, preventing the development of a reproductive offspring, which can occur as seed abortion or sterility in the grown plant (Flegr 2008). These reproductive barriers are considered, a "wastage of ovules" (Seavey & Bawa 1986), because they allow fertilization but not the continuation of that generation. Studies indicate that prezygotic barriers occur more often in sympatric species' populations, thus maintaining species separation (Flegr 2008). Allopatric species are more likely to have postzygotic barriers to their introduced reproduction (Flegr 2008). There is evidence indicating recent speciation events were the results of a progenitor species splitting via differences in breeding systems, creating a reproductive isolation (Talve et al 2012). The quick speciation happened via a new group increasingly self-pollinating, compared to the obligate outcrossing in their close relative species and likely the progenitor (Talve et al 2012). This would indicate a group of self-compatible individuals splitting off from the outcrossers. Self-

incompatibility is common throughout angiosperms and credited with their high degree of success throughout plant evolution, controlled by highly polymorphic alleles to identify and inhibit related pollen (Nasrallah et al. 1994; Franklin-Tong & Franklin 2003). Self-incompatibility within a species can be early or late-acting, and is a form of prezygotic barrier like those between species (Nasrallah et al. 1994). The methods of self-incompatibility vary widely across plant families and in the mechanism of genetic control (Nasrallah et al. 1994).

Castilleja self-incompatibility

Self-incompatibility occurs in both widespread and rare species (Bowels & Whelan 1994), and is the most widespread method of preventing inbreeding in angiosperms (Franklin-Tong & Franklin 2003). It has been identified in the Scrophulariaceae family as well (Rieseberg et al. 1998; Franklin-Tong & Franklin 2003). A species could have complete self-incompatibility and yet still produce selfed offspring via the mentor effect, where self-pollen is able to reach the ovules if it is mixed with out-crossed pollen, like hitching a ride (Rieseberg et al. 1998). This effect would depend on the behavior of the pollinators. Many *Castilleja* species are known to be almost entirely self-incompatible, an adaptation common across plant taxa to prevent inbreeding depression (Bowles & Whelan 1994; Franklin-Tong & Franklin 2003). Experiments with four *Castilleja* species comparing caged plants to pollinator accessible plants, yielded from 50% to 80% more fruit set in uncaged plants, with the exception of *C. cryptantha*, which produces 89% fruit with pollinator exclusion and is reported to be self-compatible (Duffield 1972). *Castilleja levisecta* produced five times more seed via pollinator activity than from pollinator-excluded flowers (Wentworth 1994), and only 0.7% of available ovules produce normal seeds via self-pollinations (Kaye & Lawrence 2003). Fertilizing a *C. levisecta* flower with its own pollen yields less than ten percent normal seeds from the proportion of available ovules (Kaye & Lawrence 2003). However, normal germination of this small proportion of seeds produced from

self-crossing (Rieseberg et al. 1998; Kaye & Lawrence 2003) confirms a pre-fertilization or prezygotic intraspecific reproductive barrier to prevent self-inbreeding in *C. levisecta* (Nasrallah et al. 1994). *Castilleja levisecta*'s self-incompatibility manifests in nearly complete lack of selfed seed set (Kaye & Lawrence 2003). Only 50% of self-pollinations produced fruits with any normal seeds, and those being only one to two seeds in each, while cross-pollinations all produced seed-filled fruits (Kaye & Lawrence 2003).

With the few viable offspring produced from *C. levisecta* self-fertilization, it is possible that the self-incompatibility of *Castilleja* species lies in the development of the pollen tube when on the stigma of its own flower, or otherwise within the style, before the pollen tube is able to reach an ovule (Nasrallah et al. 1994). This form of reproductive barrier would be a gametophytic form of self-incompatibility, where the pollen tube (the male gametophyte) is blocked from reaching the ovule either at the stigma (as in many grasses) or lower in the style (as in legumes, poppies, lilies, and nightshades) (Seavey & Bawa 1986; Proctor et al. 1996). Often gametophytic incompatibility originates from binucleate pollen grains, but with the rejection of the self-pollen taking place in the style or ovary before reaching the ovule (Real 1983). Some plant groups have sporophytic self-incompatibility, where the pollen grain's outer proteins, originated from its sporophyte plant, are what is recognized and rejected by the recipient stigma (Seavey & Bawa 1986; Proctor et al. 1996). It includes the large plant groups of crucifers and asters (Proctor et al. 1996). Incompatibility mechanisms acting in the ovary, either before or after fertilization, are thought to be somewhat rare, though several examples have been identified, with notoriety given to the example in *Theobroma cacao*, established in the 1960s (Seavey & Bawa 1986). These are called late-acting self-incompatibility (Seavey & Bawa 1986).

Self-incompatibility is usually controlled via a single genetic locus (the S-locus) that has multiple possible alleles (Franklin-Tong & Franklin 2003). This allows female selection against pollen grains with S-alleles that are the same as those in the style or stigma of the recipient, because they are identified as self-alleles (Franklin-Tong & Franklin 2003). A species in the *Antirrhinum* genus, a member of Scrophulariaceae and relative to *Castilleja* species, is known to use an S-RNase system of gametophytic self-incompatibility, via this common single multiallelic S-locus (Franklin-Tong & Franklin 2003). This could be the mechanism used in the *Castilleja* genus, due to its commonality among self-incompatible species (Bowles & Whelan 1994; Nasrallah et al. 1994; Franklin-Tong & Franklin 2003). The method of this Scrophulariaceae specie's S-RNase self-incompatibility involves halting pollen tube growth within the first one-third of the style length (Franklin-Tong & Franklin 2003). It is theorized that S-proteins are the receptors or translocators that trigger the S-RNase to degrade the pollen's cytoplasmic RNA, in response to the pollen's alleles, which stops the tube growth, often via bursting the tube tip (Nasrallah et al. 1994; Franklin-Tong & Franklin 2003; Rea & Nasrallah 2008).

The degree of self-incompatibility can vary between individuals within and among populations, as well as changing with the age of the pollen and stigma and decreases with the increase of heterozygosity of individuals (Real 1983). Nearly complete self-incompatibility is important to *Castilleja* reproduction, as their flowers form in a raceme inflorescence, typically with multiple stems per plant, so that several flowers of the same plant bear receptive stigmas & dehisced pollen at the same time, increasing the risk of self-fertilization via geitonogamy or movement of pollen between flowers on the same plant (Kaye & Lawrence 2003; Hersch & Roy 2007).

Castilleja outcrossing

Maintaining a self-incompatible species' population requires a larger amount of gene flow (outcrossing) than for other diploid species, in order to maintain the sufficient levels of alleles used in compatibility recognition (Young et al. 2000a, as cited in Weeks et al. 2011), because all closely related individuals are unable to contribute to fertilization. *Castilleja levisecta*'s seed production has been found to increase as the relatedness of the parent plants of controlled crosses decreases (Kaye & Lawrence 2003). Experimental crosses between *C. levisecta* plants with different relatedness between individuals demonstrated both inbreeding depression and outbreeding advantage (Kaye & Lawrence 2003). Inbreeding depression manifested in both lower F1 inbred plant growth and lower seed production (Kaye & Lawrence 2003). Expression of inbreeding depression is commonly in the F1 seed characteristics, like in the small populations of the rare *Rhinanthus osiliensis*, which demonstrated significantly lower seed germination and increased homozygosity, due to inbreeding, than the related and common *Rhinanthus rumelicus* (Talve et al. 2012). Unrelated crosses within the same population of *C. levisecta* produced 71% of normal seed, and crosses from different populations produce 80%, indicating on outcrossing benefit, while sibling crosses produced less than 40% seed set (Kaye & Lawrence 2003). Self-pollinations produced even less normal seed (0.7%) (Kaye & Lawrence 2003), indicating a difference between *C. levisecta* inbreeding depression and self-incompatibility. *Castilleja levisecta* plants are also 1.1 to 4.6 times more likely to produce flowers when their parent plants were outcrossed from different populations than when the parents are in the same population (Kaye & Lawrence 2003). Plants produced from self-pollination have less than 5% probability of flowering (Kaye & Lawrence 2003). Late-acting self-incompatibility (like the somewhat rare ovular form) is thought to be an evolutionary response to inbreeding depression, and can be difficult to distinguish from inbreeding depression itself (Seavey & Bawa 1986). Inbreeding depression, particularly in selfing, and outbreeding advantage may be identified in experimental

conditions for *C. levisecta* (and are likely applicable to *C. hispida* as well), but so have negative outcrossing effects of hybrid vigor, which could create problems for the conservation of these plants as separate species.

Castilleja **hybridization**

In areas of sympatric growth, two genetically similar or compatible species can interbreed, depending on the extent or lack of prezygotic reproductive barriers, which may prevent such occurrences (Hersch & Roy 2007; Flegr 2008). Not only genetic barriers, but ecological and phenological barriers between the species, dictate the extent of genetic assimilation that could occur between them (Andersson et al. 2008). Hybridization between plant species impacts their populations and ecosystems in ways varying by the taxa, their relatedness, and location (Andersson et al. 2008; Hersch-Green & Cronn 2009). Some hybrids survive less but bloom more than one of their parent species, others spread faster than both parents (Ayres et al. 2004; Andersson et al. 2008). Often the hybrids characteristics fall intermediate to the parent characteristics, but can also exhibit novel traits of their own (Ayres et al. 2004; Hersch & Roy 2007; Kaye & Blakeley-Smith 2008). Some suggest that 70% or more species have origins in hybridization (Andersson et al. 2008). Currently, anthropogenic activities are bringing allopatric species together via introductions or reducing the natural barriers between their habitats, allowing new opportunities for unnatural hybridizations (Andersson et al. 2008). Creating F1 hybrids happens via the transfer of pollen from the male organs (stamen) of one species onto the receptive female organ (stigma) of another, and in most plants not using wind to move their pollen, this requires an animal vector for pollen movement (Hersch & Roy 2007). Other physiological steps following pollination must also take place before a mature F1 offspring can be created (Nasrallah et al. 1994). Organisms can have pre- and postzygotic reproductive

barriers at any of these steps to prevent or reduce hybridization. Prezygotic barriers include differences in the organisms' ecology, physiology or biochemistry, and even an associated organism's behavior that facilitates reproduction (i.e. pollinators) (Flegr 2008). Postzygotic barriers prevent a hybrid offspring from maturing into reproductive adults (Flegr 2008), allowing for possible survival of a hybrid plant but not the reproduction. Even if hybrid F1 plants do survive and reach reproductive status, they tend to have lower male and female fertility, with an average of 4.8% pollen viability and 0.8% seed set, and creating a late stage of postzygotic barrier, though not complete (Wolf et al. 2001). Without these reproductive barriers between species, hybridization can take place and alter the genetic and phenotypic characteristics of a population, even creating previously absent traits in the parental populations (Hersch-Green & Cronn 2009).

Hybridization in the wild poses a threat to many plant, and less often animal, species via increased extinction risk (Wolf et al. 2001; Ayres et al. 2004). Based on an individual-based model of two hybridizing annual plants' life cycles by Wolf et al. (2001), all parameters tested had an effect on extinction risk for these species, including population size, number of patches, number of ovules per plant, and pollen-tube growth. Parameters with the strongest effect were selfing-rate, competitive ability, and initial frequency, and prezygotic reproductive barriers to hybridization had a stronger effect on extinction likelihood than postzygotic barriers (Wolf et al. 2001). Wolf et al. (2001) also found that, without habitat differentiation, a stable hybrid zone will not persist, and instead one species will either overtake the other or produce a purely hybrid population. When one of these species is rare and a poorer competitor (Wentworth 2005; Lawrence & Kaye 2008), this risk is particularly worrying. A similar case is taking place with a native cordgrass being dominated by hybrids of an invasive species (Ayres et al. 2004). The non-native smooth cordgrass (*Spartina alterniflora*) hybridized with the native species (*Spartina*

foliosa) in the 1970s, resulting in a hybrid genotype that has outcompeted the native, and even sired most of the native plants' seeds, though both species contribute to cross pollination, causing hybrids (Ayres et al. 2004). The vigorously spreading hybrid outgrows both the parent species in height and lateral expansion, even flowering up to four times more than the non-native parent species (Ayres et al. 2004). This trend could lead to the native cordgrass's full extinction in time (Ayres et al. 2004).

Concern of *C. levisecta* hybridization has increased since proof of successful hybrid offspring was demonstrated via hand-pollinated greenhouse crossings (Kaye & Blakeley-Smith 2008). Despite extensive overlap of the historic ranges for at least potential habitats of both *C. levisecta* and *C. hispida*, they have only been reported as naturally co-occurring at the Rocky Prairie site in the 1980s, though *C. hispida* has been extirpated there for ten or so years now (Kaye & Blakeley-Smith 2008; P. Dunwiddie email correspondence 2011). Hybridization concerns have also been fueled by observations of high *C. hispida* floral color variation at the Fort Lewis Military Reservation site including an unusually high number of yellow color variants (P. Dunwiddie email correspondence 2011; Egger 2014), possibly indicating a former site of both co-occurrence and hybridization.

Though hybridization is a natural process occurring across the globe for millions of years, it is a concern because *C. levisecta*'s small numbers could be significantly altered by the presence of hybrids, more so than a more populous species that could withstand genetic changes and sterile or less-fit hybrids. The consequence of interspecific mating will depend on the amount of contact between the species and the interactions of their genetics, hybrid fitness, and the environmental pressures they experience (Hersch-Green & Cronn 2009). The direction of crossing can also affect the outcome, since some *Castilleja* species have stronger reproductive

isolation than others when acting as the maternal plant rather than the paternal pollen donor (Hersch-Green 2012). This is likely due to the recognition of relatedness occurring in the pistil of the parent plant.

Genetics of *Castilleja levisecta* and *C. hispida*

The potential for different species to hybridize with each other requires at the very least a genetic compatibility, as physical co-occurrence can be anthropically created. The species generally need the same number of chromosomes & ploidy for viable zygotes to develop (Heckard & Chuang 1977; Rieseberg et al.1998; Hersch-Green 2012). *Castilleja* has well documented intraspecific polyploidy (Egger 1994; Hersch-Green & Cronn 2009). Genetic analysis of *C. hispida* revealed that they are a polyploid species, with many populations having chromosome counts of 12 (the diploid), and some populations with polyploids having 24, and 36 (tetraploid & hexaploid) (Heckard 1968; Heckard & Chuang 1977). These are evenly distributed across six Washington sites even including one site (Yellow Island) with the possibility of more polyploids occurring due to low sample sizes (Kaye 2008). The perennial clade of *Castilleja* (as opposed to the annuals they evolved from) may owe its high degree of polyploidy to its perenniality (Tank & Olmstead 2008). *C. levisecta* populations have only been found to be diploid, with a single set of chromosomes (12 n) across all six to eleven sample sites (Godt et al. 2005; Kaye 2008). This provides a moderate likelihood of interbreeding where *C. hispida* populations are diploid like *C. levisecta*'s. However, differences in ploidy do not guarantee an inability to interbreed, but does reduce the likelihood (Hersch-Green & Cronn 2009; Clay et al. 2012; Hersch-Green 2012). The high level of hybridization in *Castilleja* has been linked to their high degree of polyploidy (Heckard & Chuang 1977; Clay et al. 2012), as well as being a recently diverged genus (Clay et al. 2012).

Despite numerous genetic analyses of these *Castilleja* species, and the diversification of the Castillejinae subtribe, no indication of the precise relatedness of *C. hispida* to *C. levisecta* could be found (Heckard 1968; Heckard & Chuang 1977; Egger 1994; Godt et al. 2004; Tank & Olmstead 2008; Tank & Olmstead 2008). However, genetic analyses have determined diversity among the *C. levisecta* populations. When eleven populations of *C. levisecta* were analyzed for variations in enzymatic alleles, or allozymes, all 16 loci examined were polymorphic and allele heterozygosity was found to be equal to or more than expected for all eleven populations, indicating high genetic diversity for a rare species (Godt et al. 2005). *Castilleja levisecta* populations have also proven to have higher heterozygosity than expected for a rare species, possibly due to their high degree of self-incompatibility and lack of inbred individual's flowering (Kaye & Lawrence 2003). The rare *Silene sennenii* of Spain, with around 5,000 individuals remaining across five highly fragmented populations, had expectedly low genetic variability of 0.071, assessed as the expected panmictic heterozygosity for allozymes (López-Pujol et al. 2007). Other multiple-species analyses of plant genetic diversity yield similar results. Endemic species had significantly lower genetic variability than widespread species, with variability measures of 0.096 for endemics and 0.202 for widespread species, as well as 0.063 for endemic species across 100 samples by Hamrick and Godt (1990) (Bowels & Whelan 1994; López-Pujol et al. 2007). Endemics also had significantly lower polymorphic loci (40.0% for endemics and 58.9% for widespread species (Bowels & Whelan 1994). The genetic diversity among versus within populations was similar for both types of plant species (Bowels & Whelan 1994). Interestingly, *C. levisecta* has high genetic diversity (assessed via allozymes), even relative to 27 other Scrophulariaceae species, as well as compared to a similarly endangered Scrophulariaceae (*Schwalbea americana* L.), which had genetic variation of 0.006 compared to CALE's 0.285 variation (Godt et al. 2005). Population size may not be an indicator of CALE's genetic diversity since the population with the second lowest variation had the largest population size at

the time of the study, at over 7,000 plants (Godt et al. 2005). This large population also had the lowest number of alleles and fewest per polymorphic loci (Godt et al. 2005). This large population's low diversity may be the result of a small founding population with similar genetics, creating a founder effect, or a large extinction in the past causing a bottleneck with a later population recovery (Godt et al. 2005). This population may be an outlier in the usual pattern of larger populations having higher genetic diversity though, because the other large populations of *C. levisecta* did have the highest allelic diversities (Godt et al. 2005). *Castilleja levisecta* populations also demonstrated moderate genetic divergence with six alleles unique to only one population each and the Rocky Prairie population having three of these unique alleles (Godt et al. 2005).

Flower characteristics

Use of floral characteristics for genetic or hybrid assessment is used as an alternative to genetic analysis, and a primary method of *Castilleja* hybrid documentation (Hersch-Green & Cronn 2009). Understanding of the floral characteristics of each species involved is required to understand their hybrid floral characteristics, as well as the likely method of inheritance of these traits. Traits used should be objectively measurable and taxonomically specific (Hersch-Green & Cronn 2009). Often, hybrids display traits intermediate to those of their parents (Hechard & Chuang 1977; Wolf et al.2001; Clay et al. 2012), though not always (Hersch-Green & Cronn 2009). Hybridization can create entirely new traits unseen in the parent species, complicating the analysis of hybrids via morphological characteristics (Hersch-Green & Cronn 2009). This analysis is notably limiting when the hybrids are closely related due to fewer trait differences (Hersch-Green & Cronn 2009).

Each flower of *Castilleja levisecta* and *C. hispida* contains a singular pistil with bilobed stigma, an ovary with multiple ovules, and four stamens lower in the galea (Kaye & Lawrence 2003; Clark personal observation). *Castilleja* flowers are characterized as protogynous or having hermaphroditic flowers with the stigma receptive before the anthers dehisce and release pollen (Kaye & Lawrence 2003), but a report of another *Castilleja* species, *C. linariaefolia,* states those *Castilleja* flowers are protandrous, with anthers maturing first (Caruso & Alfaro 2000). Protandry is the more commonly occurring type of differential floral organ timing, or dichogamy, and characteristic of plants with fused corollas and zygomorphic flowers, while protogyny is more common to wind pollinated flowers (Proctor et al. 1996). This may be an issue of reference point, as the flowers at the top of the raceme are the youngest, thus organs would mature from bottom to top of the stem in either order of anther or stigma first. Confirmation of the maturation type may be useful in fitness assessments via pollinator activity.

Castilleja plants have continued flowering from a terminal apical bud that results a long blooming period for each plant. If the flowers are protandrous, the top flowers will have newly mature pollen, and receptive stigmas are on the flowers below (Figure 2.1). Upward moving insects would transfer pollen in a beneficial pattern to the receptive stigmas below, then up to reload on pollen above. If the flowers are protogynous, the topmost flowers will have receptive stigmas but no, or less, available pollen, and flowers a few inches below will have the most recently dehisced anthers and presumably non- or less-receptive stigmas. Insects would need to move downward on these racemes to beneficially transfer pollen for the plant, starting from receptive stigmas to reload from mature anthers below. Most surveyed bees were found to forage from the bottom upward on inflorescences with flowers opening from the bottom up (Real 1983; Proctor et al. 1996). If this is the way pollinators move on the *Castilleja levisecta* and *C. hispida,* they may be inferred to be protandrous, as presumed (Kaye & Lawrence 2003), though

the foraging behavior is reinforced or ultimately driven by nectar availability in the flowers (Real 1983; Gegear & Thomson 2004; Hersch & Roy 2007). Either form of dichogamy (differential floral organ maturation) helps reduce self-pollination, with protandry most often being associated with self-incompatibility (Proctor et al. 1996). With a longer pistil protruding from the *Castilleja* flower above the enclosed anthers, autogamy self-pollination should be minimal (Kerner von Marilaun 1902).

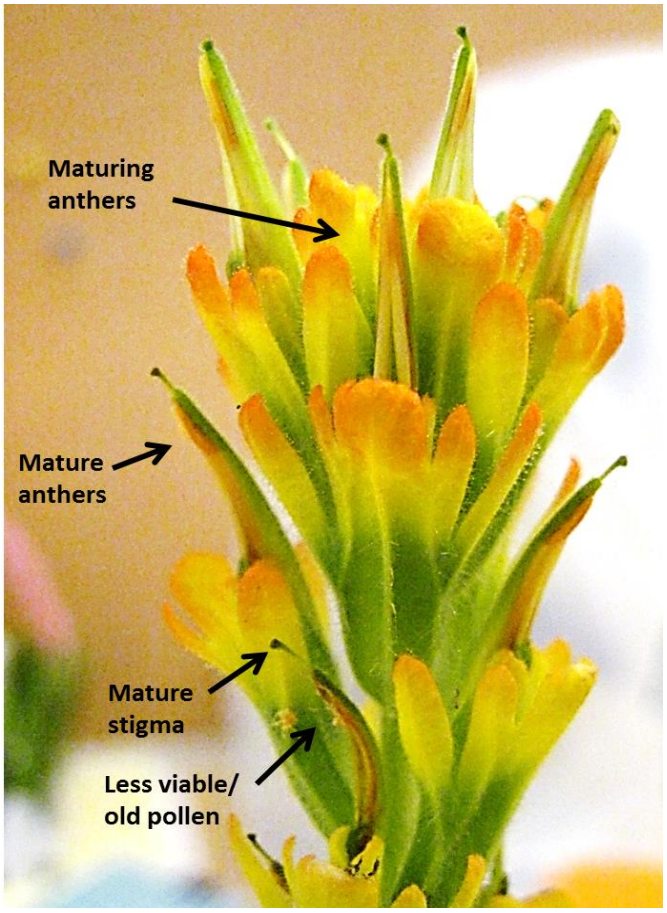

Figure 2.1. Example of a *Castilleja* flowering stem, showing multiple whorls and prominent galea with visible pollen covered stamen inside. Labels indicate the proposed pattern of protandrous floral maturation with mature anthers in flowers above those with mature stigmas as anthers would mature first.

A variety of morphological traits can be used for hybrid assessment, including flower color, size and anything else that distinguishes the parent species from each other. *Castilleja hispida* displays a higher degree of variation in flower size than *C. levisecta* (Kaye & Blakeley-Smith 2008). Flower galeas were measured at 2 cm long for all *C. levisecta*, with *C. hispida* ranging smaller and larger than 2 cm, up to 4 cm for some (Kaye & Blakeley-Smith 2008). Experimentally crossed hybrids of the species produced flowers with mostly intermediate morphology and coloration to the parent plants, but tending to exhibit traits more like one parent (Kaye & Blakeley-Smith 2008).

Typically, the most obvious morphological characteristic used for *Castilleja* hybrid assessment has been floral bract coloration, usually with uniformity among a species, like *C. miniata*'s red bracts and *C. sulphurea*'s yellow bracts (Hersch-Green & Cronn 2009). Hybrids tend to have variable and intermediate bract coloration with several hues present through a single bract, changing from the margin to the center (Hersch-Green & Cronn 2009). This color variation across the bract is rarely seen in pure-breeding parent species, if not absent entirely (Hersch-Green & Cronn 2009). Bract color in *Castilleja* appears to be inherited as continuous traits, where two different colors of parent plants interbreeding will produce offspring in the F1 generation of intermediate colors from a blending of the parent colors (McClean 2013). If those are then crossed, the F2 generation can have all variants from the original parent colors to the intermediate colors. Continuous traits, which allow genetic combinations to create a continuous range of the trait, are also called quantitative traits, because they are often measured and given quantitative values across a continuous spectrum, such as heights, lengths and colors, which can be measured by set scales (McClean 2013). The bracts of *Castilleja* flowers resemble the bracts of *Gaillardia* flowers by being typically bi-color (Figures 2.2 & 2.1), with the depth of each color varying across lineages (McClean 2013). Such traits have this inheritance pattern because there are multiple genes controlling them. *Castilleja* plants have been characterized to have quantitative inheritance for their bract color, identified in *C. hispida* (Figure 2.3), yielding intermediate flower colors when different parent plants cross breed (Griffiths et al. 2004; McClean 2013).

Figure 2.2. From Griffith et al. 2004, showing quantitative variation in *Gaillardia* flower bi-coloration

Figure 2.3. Images from Griffiths et al. 2004 depicting *Castilleja hispida* color variation ranges

Figure 20-1 **Quantitative inheritance of bract color in Indian paintbrush *(Castilleja hispida).*** The photograph on the left shows the extremes of the color range, and the one on the right shows examples from throughout the phenotypic range.

Other *Castilleja* morphological traits used for hybrid analysis, in addition to bract color include: presence/absence of color change across bract, percentage of bract lobing, mean bract width and length, galea length, stamen lengths, leaf lobing percentage, stem color (anthocyanic extent), and pubescence (Anderson & Taylor 1983; Hersch-Green & Cronn 2009). These quantitative traits placed hybrid plants in an intermediate position between their three parent species of *C. rhexiifolia*, *C. sulphurea*, and *C. miniata* and were not transgressive for any measured traits (Hersch-Green & Cronn 2009). Field-identified hybrids showed traits intermediate or similar to one of the parent species for nine of the eleven measured quantitative traits (Hersch-Green & Cronn 2009). Assessment of floral organ development at different flower development stages can also be used to compare species and transgenic plants (Bengtsson 2006). Morphology alone may not provide an accurate image of the relationship between these plants, with cytogenetic and AFLP (amplified fragment length polymorphism) data indicating a more complex dynamic (Hersch-Green & Cronn 2009). Thus, while morphological traits can identify hybrids and infer some parentage, unraveling the specific genetic interactions between species (especially when involving more than two) may require detailed genetic analysis.

If reliably distinct morphological traits cannot be found, genetic molecular markers can be used dependably (Hersch-Green & Cronn 2009), though likely at higher cost in both time and funds. Intermediate flower color is, however, a likely useful morphological trait for identifying hybrids between *Castilleja* species, due to the likelihood of qualitative genetic basis for their flower color, making bract coloration an "obvious feature" to use since it tends to be consistent across a species (Griffiths et al. 2004; Hersch-Green & Cronn 2009). However, *Castilleja hispida* is variable for its qualitative bract color across and even among populations (Dunwiddie email correspondence 2011; Egger 2014), yielding suspicion of previous hybridization events in its population. A study found evidence of *C. hispida* hybridization with other *Castilleja* species in the Mazama, WA Slate Mountains area (Anderson & Taylor 1983). Lobe lengths were among the most discriminating variable defining the three *Castilleja* species measured for taxonomic accuracy and possible hybrid identification (Anderson & Taylor 1983). One hundred representative *Castilleja* plants were measured, 31 of which had characteristics of two or more species to indicate a hybrid zone (Anderson & Taylor 1983). Early pollen viability tests supported the evidence of hybrids among that mixed population of three *Castilleja* species (Anderson & Taylor 1983). Earlier studies of *Castilleja* hybrid populations, and including the Anderson & Taylor study (1983), have used ploidy differences, along with morphology variation measurements (Egger 1994; Hersch-Green & Cronn 2009), while genetic marker tracking optimization now has increasing utility for ecological hybrid studies (Hersch-Green & Cronn 2009; Clay et al. 2012), though it is still a time consuming technique if not outsourced to specialists. Genetic sequencing identified a stable hybrid between reddish-orange *C. miniata* and purplish-red *C. linariifolia,* to create the separate *C. christii* species as a yellow hybrid, all located in Idaho (Egger 1994). This is an example of the hybrid having an entirely unique coloration from the parent species. Such transgressive traits in hybrids are often linked to

ecological divergence from the parent species, allowing for speciation through hybridization (Hersch-Green & Cronn 2009).

Pollen testing

Pollen characteristics can be a measureable response for assessing changes in the species' genetic content and even new reproductive methods like selfing and hybridization (Cresti & Tiezzi 1992; Wolf et al. 2001). Pollen viability may be essential to the successful production of cross-species hybrids (Bots & Mariani 2005), with endangered species also suffering from pollen limitation and less reliable reproduction, due to a low pollen-contributing population size (Moncada 2003). Pollen is the male gametyphyte stage of a plant's life cycle, and is itself a separate living organism (Cresti & Tiezzi 1992). As such, pollen is affected by both its genotype and the conditions it develops and disperses in (Cresti & Tiezzi 1992). It is the vector of genetic transfers between seeded plant species that are generally otherwise immobile (Taylor 2008). Pollen's effectiveness in fertilization can vary by both the rate of germination at the stigma and the speed with which it reaches the egg, or the speed of pollen tube growth (Real 1983). These can both influence a pollen strain's overall viability, but both are not necessarily accounted for in most viability tests. Competitive behavior between pollen can also influence fertilization success, (Neuffer & Paetsch 2013) but is not as often tested.

Speed of growth was linked to an increase in self-compatibility in normally incompatible *Capsella* species, with self-incompatible plants taking between 150 - 255 min. from pollen deposition to ovule fertilization, and recently evolved self-compatible species taking less time, as 15 - 180 min. for one and 15 - 300 min. at the maximum for another species (Neuffer & Paetsch 2013). Flower characteristics of the maternal recipient can also alter a pollen strain's viability

and competiveness with other pollen strains if, for example, a longer-styled flower may be pollinated by a faster-growing pollen tube strain after a slower-growing one, but still be fertilized first by the later arriving pollen, if it had enough time to bypass the slower growing pollen tube (Real 1983). Thus faster-growing pollen has an increased chance of fertilizations if the physical distance between the stigma and ovary is increased (Real 1983). Pollen viability is influenced by both environmental factors, such as drought stress, and by genetic incompatibility of the parent plants, such as crossing between two closely related species.

Germination rate, like pollen tube growth rate, can be measured for plant reproduction analyses. The paternal plants among the same species, or pollen donors, can differ in their pollen germination rates on the maternal plant's stigma, as in *Capsella* species crosses (Neuffer & Paetsch 2013). This would lead to individual plants siring more offspring than others, even with equal pollen deposition. Increased pollen production on the anthers would also yield higher paternal fitness, but via increased pollen deposition, which also differs across species and decreases with inbreeding (Neuffer & Paetsch 2013). Germination rates vary across species and genotypes, ranging from about 33% to 58% for *Passiflora* plants of different genotypes, when in optimal germination medium (with higher sucrose and neutral pH), and with 90% for *Colchicum steveni*, 29% for *Crocus hyemalis*, 60% for *Narcissus tazetta*, and 89% for *Cyclamen persicum* pollen (Rodrigues-Riano & Dafni 2000). Pollen germination ability decreases rapidly with time since removed from the flower, often reaching zero germination after only two hours (Rodriguez-Riano & Dafni 2000).

Male floral sterility can be caused either from decreased pollen production, pollen inviability or defective anther dehiscence, as found in *Arabidopsis* mutants (Preston et al. 2004). If the anthers can't disperse the pollen inside, the flower can't contribute it's genetics to the next generation via

pollination whether or not its pollen is viable. Viability is generally defined as "having the capacity to live, grow, germinate, or develop", but generally with more focus on the ability to germinate and/or fertilize ovules (Firmage & Dafni 2001). Pollen viability differs across the stages of anther development, with the highest viability during anthesis and lowest pre-anthesis (differing by one day) for both germination and staining tests (Soares et al. 2013). The most sensitive time of pollen to influencing factors is between dehiscence from the anthers and reaching the pistil of a recipient flower (Bots & Mariani 2005). Environmental factors like humidity and temperature, as well as both the male and female genotypes, can reduce pollen viability in the anther, during dispersal, or post-pollination (Bots & Mariani 2005). Inviability can be due to aborted pollen grains that lack cytoplasm and/or nuclei (Kapoor & Takatsuji 2006; Coimbra et al. 2009). Aborted pollen grains may be caused by abnormal meiosis, which were found closely correlated in tests of the percent of each of aborted or abnormal pollen and abnormal meiosis (Whelan 1965).

Pollen viability tests fall under three categories, staining, *in vitro*, and *in vivo* testing, with several methods for each (Whelan 1963). *In vivo* tests typically require fluorescent microscopy to see the stained pollen tubes growing through the live stigma and/or style (Cresti & Tiezzi 1992). *In vitro* tests usually consist of germinating the pollen in supportive media (Cresti & Tiezzi 1992; Rodriguez-Riano & Dafni 2000). Staining is commonly used to clearly and quickly visually distinguish viable from nonviable pollen grains, however recent studies have shown this method often results in false positives, with dead or inviable pollen also being stained as well (Firmage & Dafni 2001; Pline et al. 2002; Soares et al. 2013). Some staining methods also use highly toxic components (Peterson et al. 2010). When compared to same-species pollen germination, stains tend to overestimate pollen viability as 80-99% (Pline et al. 2002; Soares et al. 2013). Complimentary seed set tests for cotton varieties yielded between 25 and 37 seeds per

boll, and most closely matching *in vivo* germination of pollen than any of the four staining methods used (Pline et al. 2002). Seed set measures are not often used due to the time required and increased complexity involved in fertilization, but can be used to assess a pollen sample's minimum ability to fertilize ovules (Cresti & Tiezzi 1992; Firmage & Dafni 2001). Stains yielding consistently inaccurate estimates are said to simply demonstrate the stainability rather than viability of pollen (Firmage & Dafni 2001). Extensive stain comparisons indicate that there probably is not one single stain that is best, and accuracy of tests for the species should be investigated before use (Firmage & Dafni 2001), and all tests only give a likelihood estimate and cannot guarantee a pollen sample will not be able to fertilize any con- or heterospecific plant (Cresti & Tiezzi 1992). Staining assays conducted in the field can be highly variable, possibly due to the influence of variable microenvironments on the collected pollen (Bots & Mariani 2005).

The accuracy of staining depends on the method, such as stains that bind to proteins on the pollen grain, which may be present in non-viable grains as well. Vital stains like cotton blue can clearly distinguish between sterile and partially sterile plants, but not demonstrate changes in viability (Firmage & Dafni 2001). The stain thiazolyl blue (MTT) has yielded many positive results in staining comparisons, and detects dehydrogenase in the pollen, indicative of active cell respiration (Rodriguez-Riano & Dafni 2000). All viability tests still have an inherent margin for error depending on the type of inviability that pollen exhibits. An inviable pollen cell can still produce alcohol dehydrogenase, as detected by Baker's solution (another supposed more accurate test), and giving false positives with every such test (Rodriguez-Riano & Dafni 2000). This is why the fast and easy test of acetocarmine stain, which stains chromosomes (Cresti & Tiezzi 1992), was used for the staining test in this study, since aborted grains are unlikely to

contain chromosomes due to greatly reduced nuclei (Kapoor & Takatsuji 2006). Acetocarmine staining is a long-standing standard method for pollen testing (Whelan 1965).

Pollen tube germination is also used as a standard for the pollen's viability because it is most likely the pollen is truly viable, thus able to fertilize an egg, if it can grow a pollen tube for sperm delivery (Cresti & Tiezzi 1992). The *in vitro* media is meant to mimic the stigma and style of the recipient plant (Soares et al. 2013). However, *in vitro* germination tests often produce highly variable results, with numerous factors affecting them, including time period between the collection and testing of pollen, time of collection, flower age, humidity, temperature, and solution pH level (Whelan 1965; Firmage & Dafni 2001). Some early researchers have considered versions of acetocarmine staining a more accurate measure of functional pollen over a pollen germination method (Whelan 1963). Typically boron and calcium are required, in addition to a carbohydrate source like sucrose, for the germination of a pollen tube (Firmage & Dafni 2001; Bots & Mariani 2005; Bengtsson 2006). Different species of plants will have their own requirements for successful pollen tube germination, and may not demonstrate viability without specific preconditioning such as humidifying (Cresti & Tiezzi 1992).

Differences in pollen viability ratios, as well as viability period (measure either via staining or *in vitro* germination), can contribute to rates of outcrossing and hybridization (Bots & Mariani 2005). This is because species with higher viability or longer viable pollen will contribute more to reproduction on recipient flowers (Bots & Mariani 2005). Pollen typically loses viability the longer it is away from the humid environment of the flower, thus assays done in the field or quickly after collection may be optimal, if conditions reducing viability can be controlled (Firmage & Dafni 2001; Bots & Mariani 2005). Germination and staining results can be inconsistent across tests of the same pollen sample, due to a lack of optimized and consistent

solution recipes (Peterson et al. 2010). Multiple tests are recommended for more accurate estimates of actual viability, particularly germination testing used as a baseline because physical germination means the grain had to have been alive and able to grow (Firmage & Dafni 2001).

In this study of *Castilleja levisecta* and *C. hispida* hybridization potential in the field, restoration plot characteristics, herbarium sample measurements, live flowers measurements, and pollen viability measures were assessed to examine three questions regarding the identification of putative hybrid individuals and the patterns of hybridization in relation to inter-species distances. First, are the floral characteristics of each species distinct enough for measureable differences to distinguish them? Second, does the growing context of these plants effect their floral measurements? Third, do their pollen viabilities differ between pure-breeding individuals and putative hybrids? These are addressed across both restoration sites using off-site sourced seed grown as pure-bred control plants.

Methods & Materials

Plant production

Parent plants were selected for use in F1 analysis to achieve a maximum range of variables, including distance to other species, size of plant, and color for CAHI. This resulted in 2.5 plants of each species from each plot (alternating either 2 or 3 between species throughout plots) were used for the parent generation, with multiple F1 plants grown from the each. Seed originating from an off-site source, such as a production nursery for CAHI and Miller seed vault collections for CALE, were used as assumed guaranteed pure-breeding plants for each species for primary controls. The five mixed-species plots from GH and WR were used, along with one solo-CALE plot from each site and the solo-CAHI plot from GH, as well as the semi-solo CALE plot at GH and CAHI plot at WR.

Seedlings from plants selected for testing were potted in 2" round pots filled with a 4-to-6 ratio of Sunshine 4 to seedling-mix soil. Seedlings were grown in the lab on a light rack for up to one month, until large enough to then be moved to the greenhouse & grown under high pressure sulfur laps. Plants were up-potted to 4" pots with 4-to-6 ratio of Sunshine 4 to Miracle Gro potting mix soil once they were more than 1" tall and grown to bloom for flower morphology measurements and pollen testing. Blooming plants were tested when more than four flowers were present on the inflorescence. Variable survival rates and regression to vegetative state rather than flowering of the F1 plants inhibited equal sample sizes for each tested category tested.

Plant production was hindered by several issues while growing in the University of Washington research greenhouse. *Castilleja* seedlings experience high fatality rates largely due to prevalent

fungus gnat infestation of their soil, inhibiting growth by the larvae predating the seedling's roots. Up-potted plants also experienced a virulent powdery mildew infection, identified as likely *Podosphaera phtheirospermi* by Dr. Dean Glawe of Washington State University, assisted by Shyam affecting most of the plants and reducing flowering when unchecked (Figure Appendix C.3). No powdery mildew was found on the restoration site plants. Winter light changes also halted flowering for about four months every year of F1 plant growth, regressing to vegetative growth until spring increased light availability. Growth regression occurred frequently with the *Castilleja* plants, happening seemingly randomly throughout their growing season. These issues all reduced sample size and lengthened plant production time to two years for both species.

Flower morphology and color

Herbarium samples

Herbarium samples are a valuable resource for species assessment, and have been used for floral analysis to compare the floral traits of changing breeding systems in Brassicaceae (Neuffer & Paetsch 2013). Initial *Castilleja* floral assessment for this study used preserved herbarium samples from the University of Washington Herbarium collection of *Castilleja* vouchers. Local *Castilleja* expert Mark Egger was consulted for optimal traits that could be used to distinguish pure-bred *Castilleja levisecta* from *C. hispida*, resulting in floral traits of the bract lobing and galeas. Nine *C. levisecta* and twelve *C. hispida* vouchers were measured for the number of flowers per stem, flower bract and lobe lengths, and galea angle from the stem. Only three CALE specimens had measurable galea lengths without damaging the voucher, and ten CAHI were measurable, including galea beak lengths. These herbarium tests were used to confirm

measurable traits to distinguish between the *Castilleja* species. Independent-sample t-tests were used to compare means for each species' floral measurements.

Field crossed F1 generation

Floral traits assessed on live flowers included bract lobe depth as a percent of the total bract length (referred to as "lobe ratio"), galea beak length as a percent of total galea length (referred to as "beak ratio"), floral angle off the stem, and flower color. Floral angle was added to capture the distinct look of the two species as looking either 'closed' for CALE, or 'open' for CAHI, referring to flowers that are pressed against the stem or protruding out from it respectively. No difference was obvious in the calyx around the galeas, all having four slight lobes in early observations, thus calyx traits were no used. Blooming plants were pulled from the greenhouse weekly for assessment in the lab, with floral morphologies and pollen tests conducted concurrently as time can decrease pollen viability. Digital photographs of the F1 plant's flowers were taken for reference and color ID later, using the same camera setting for each to reduce digital color alteration. Flower stems were measured for height and floral angle measured from the horizontal, aligning the stem with 90° (Figure 2.4A). Thus flowers that stay closer to the stem, forming a tighter 'closed' look (like typical CALE) have higher degree angles closer to 90°, than those pointing more outward or 'open' type flowers (like CAHI), having lower degree angles. For CAHI, the bract is often at a lower angle than the galea, in which case a center-line between the two was used for the floral angle (Figure 2.4B). CALE galea & bracts are typically pressed together at the same angle. Three or more flowers between the top-most whorl & those about two inches down the stem (or the three flowers that precede the top whorl when internodes were long) were carefully removed from the stem. They were arranged for photographing on a printed 1 cm x 1 cm grid between acrylic plexiglass sheets in order of oldest to newest flower.

Image J was used for increased accuracy in measuring the traits from the photographs, scaling each to the grid. Bract, lobe, galea, and beak lengths were measured for each flower. After photographs were taken, pollen viability was assessed from the same flowers. Royal Horticulture Society color charts were used to identify the range of flower color variations present on the restoration sites via photographs taken in the field, in addition to the photos of each F1 plant tested to compare these to the parent plant's coloration and to their siblings, which can have different paternal plants. Bract color was used as the overall flower color but occasionally varied from the center to the upper edge, usually in a yellow to orange pattern. In the instances where the colors were similar and the distinction between them is unclear, the middle section between both colors effectively mixing them in an intermediate golden-orange color. But in instances when the flower color is labeled as yellow-orange (Figure 2.5) because it distinctly contains both a yellow center and orange edge to the bract, then both colors were identified.

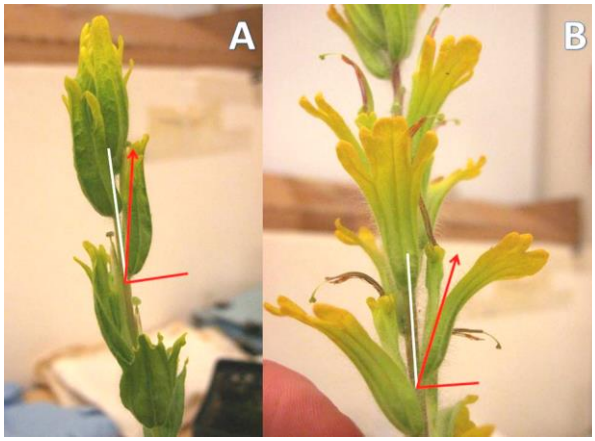

Figure 2.4. Technique for measuring floral angle on live F1 flowers. A) *C. levisecta* with bracts flush with the galea, B) *C. hispida* with bracts angled out more than the galea

Figure 2.5: Yellow-orange coloration in *C. hispida*, in both field site plants (left) and greenhouse F1 generation (right).

Flower color was categorized into common hues among the F1 generation, and used to support patterns of interbreeding but no statistical analysis was conducted at the time of this write up. Assessment of F1 flower characteristics used the floral angle, bract and lobe lengths, the ratio of

lobe-bract lengths, galea and its beak lengths, and the ration of beak-galea lengths. Independent sample t-tests were used to compare seed capsules to investigate differences between the bottom and middle capsules for both species. Univariate ANOVAs were used to determine the effects of the restoration site, plot context (or interspecies distance, as greater than 30 cm or less than 30 cm from the other *Castilleja* species), the capsule level (middle versus bottom), and maternal *Castilleja* species as factors, with the plot numbers nested within the sites. One-way ANOVA analysis was used to compare the off-site control plants for distinguishing floral characteristics between CALE and CAHI. Univariate ANOVA analysis on the floral data with control treatments consolidated as off-site and on-site (solo-context plots) controls versus mixed-species contexts were used to test differences between control contexts, the *Castilleja* species, the site, and capsules. Principal component analysis was used to test for relationships among the floral characteristics and between F1 individuals of each species and any putative hybrids, via SPSS factor analysis.

Pollen viability

All pollen tests took place in the same lab conditions and at roughly the same time of day to control for the environment's effect on pollen viability, and pollen assays were started as soon as flowers were photographed after they were removed from the stem to reduce time-dependent viability loss. Two separate methods of viability testing were used due to discrepancies between many tests and possible over or under estimation of any one test. Germination of the pollen to create a pollen tube was used as the most reliable representative to actual viability due to the high likelihood of a germinated pollen grain being alive and able to fertilize an ovule, having successfully grown its own structure (Cresti & Tiezzi 1992). Acetocarmine staining was also used as an easy test of viability that stains the nuclei and faintly the cytoplasm of pollen (Cresti & Tiezzi 1992), easily distinguishing aborted pollen grains that lack cytoplasm (Coimbra et al.

2009). A minimum of one hundred grains were counted for each sample tested, excepting those with less than hundred grains distinguishable in the sample, denoting very low pollen production.

Acetocarmine Stain

Acetocarmine stain from Carolina Biological Supply was diluted to 1/20 ratio with acetic acid as the undiluted acetocarmine did not stain clearly. This acetocarmine solution stained the pollen grain's cytoplasm a deep red by staining DNA, thus red grains are counted as viable. Grains that did not have stained cytoplasm appear clear or pink to light-blotchy-red and are considered non-viable, as well as distinctly smaller but red-stained grains, in accordance to Whelan's aborted versus normal grain distinctions for *Prunus avium* pollen via acetocarmine (Whelan 1965). The staining technique consisted of scraping a sample of pollen from the anthers and suspending it in a droplet of acetocarmine solution on a glass microscope slide. Each drop is allowed to dry in a closed but not airtight container for roughly 24 hours before counting. Pollen for staining must be taken separately & removed prior to immersion in germination solution because the germination solution neutralizes acetocarmine staining and pollen will not take on the color, although very occasionally a pollen grain was observed germinated in the stained slides (without exposure to germination solution).

***In Vitro* Germination**

The hanging drop method was used for germinating pollen tubes *in vitro* (Cresti & Tiezzi 1992; Rodriguez-Riano & Dafni 2000; Kumari et al 2009). All the anthers of the test flower were placed in an eppendorf tube with 100 ul of germination solution after the staining sample was taken. The solution of the hanging drops contained 10% sucrose with 6 e-3 M calcium chloride & 2 e-3 M boric acid (both sourced from Carolina Biological Supply). The base solution for the hanging drop method, used to moisten filter paper in the dish below the drops, was 10% sucrose

in DI water, as complementary sucrose concentrations for the base and the hanging solution work best (Firmage & Dafni 2001). The anthers in solution were disturbed to release as much pollen as possible, then a 65 ul sample was removed and suspended under the petri dish lid as the 'drop' over the 10% sucrose filter paper. Initial tests showed a 1:1 relationship of drop:base solution, as well as the presence of boron & chlorine in the solution, were the best combinations for pollen tube germination. *Castilleja* pollen would not germinate without the presence of boron and chlorine, except for very rare instances seen in acetocarmine staining (Figure 2.6).

After 18-24 hours of tube germination at room temperature, the entire hanging drop was transferred to a microscope slide, mounted with a coverslip and the germinated grains-to-total pollen grains counted in two separate view fields of a compound light microscope at 200x magnification. The views consisted of the primary cluster of germinated grains, when present, with tubes grown intertwining and the grains dispersed outside the primary cluster with stray and mostly ungerminated grains. This is meant to cover the full range of pollen grain distribution on the slide as the germinated grains tended to mass in the center together via gravity and would be unrepresentative of the entire sample. Germinated grains tended to also be lighter or empty-looking due to cytoplasm moving out through the tube (visible in Figure 2.7), though shorter tubes still have a darker yellow cytoplasm in the grain, so color was used as an occasional guide but not distinguishing factor of pollen germination. Germinated pollen is counted at the grain, since tubes can be long and tangled, and a grain is considered germinated when having a tube that exceeds the grain's diameter.

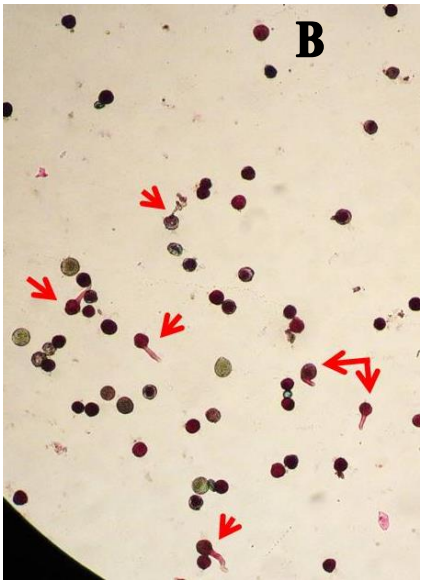

Figure 2.6. A- Pollen stained with Alexander stain exhibiting an unstained but germinated grain at center (sampled from #GH16 H3M-2. B- Multiple germinated pollen grains in the acetocarmine stained sample, from the same GH16 H3M-2 plant.

Figure 2.7: Germinated pollen grain with long extending tube and cytoplasm visible down the tube.

Analysis of the F1 generation plants' pollen data included a univariate ANOVAs to assess the effects of the restoration site, plot context as interspecies distance (greater than 30 cm or less than 30 cm from the other *Castilleja* species), and maternal *Castilleja* species as factors, with the plot numbers nested within the sites. One-way ANOVA analysis was used to compare the off-site control plants, to test for differences in pollen viability between CALE and CAHI pure-breeding individuals. Each species' pollen data was analyzed separately with univariate ANOVAs to test differences between the interspecies distances, site and capsules. Independent sample t-tests were done on each species to compare control contexts to mixed-species contexts.

Experiment Results and Observations

As assessed in the pollinator & seed chapter of this study, the study sites differed from each other, and between the *Castilleja* species. The site, species and plot context all affected plot characteristics of floral density, number of each *Castilleja* present, and number of stems per plant. These site and species differences can then be related to the differences in floral characteristics and pollen viabilities found between sites and species, via higher flower density's influence on measures of fitness for plants in that area.

Herbarium data

Differences between the *Castilleja* species can be seen in the herbarium data. CALE specimens had longer bract lengths, shorter lobes, lower ratios of lobe-bract lengths, and higher floral angles than CAHI (Table 2.1). CALE lobe-bract ratios averaged 0.27, meaning roughly 30% of the bract length was lobed, while CAHI ratios averaged 0.41, 40% of bract length lobed (Figure 2.8). CALE flowers were also closer to their stems, with higher floral angle (M= 70^0), compared to the more open aspect of CAHI from smaller angles (M= 62^0). One sampled CAHI had the unusual yellow-orange coloration present in its bracts.

Table 2.1.Herbarium specimen measurements of mean bract length, mean lobe length, mean lobe-bract ratio, and mean floral angle for both *Castilleja* species.

Castilleja Species	Mean bract length (cm)	Mean lobe length (cm)	Mean Lobe: Bract ratio	Mean Floral angle
CALE	2.53	0.07	0.27	69.6^0
CAHI	2.28	0.90	0.41	62.3^0

Independent sample t-test comparisons between CALE and CAHI show they differ significantly in their lobe:bract length ratios (Table 2.2). As predicted, CAHI had significantly higher percent lobing in their bracts (p= $7.92x10^{-6}$) than CALE (Figure 2.8 C). The floral angle was

significantly higher in CALE specimens than CAHI (p= 9.668x10^{-4}). The lobe and bract lengths were also significantly different between the two species (p= 0.036 and 0.019 respectively), indicating longer lobes in CAHI and longer bracts in CALE.

Table 2.2. Herbarium specimen t-test analyses for lobe-bract ratio, lobe and bract lengths, and floral angle, comparing CALE and CAHI for difference between species.

Floral measurements	Lobe:Bract Ratio	Lobe Length	Bract Length	Floral Angle
mean (CALE) (CAHI)	0.273 0.412	0.70 0.90	2.56 2.28	69.56° 62.32°
p-value (one-tail)	7.92e-06	0.036	0.019	9.67c-4
t-statistic	-6.426	-2.296	2.558	3.661
variance (CALE) (CAHI)	7.82e-04 4.14e-03	0.012 0.076	0.051 0.074	19.55 19.08
degrees freedom	14	15	19	17

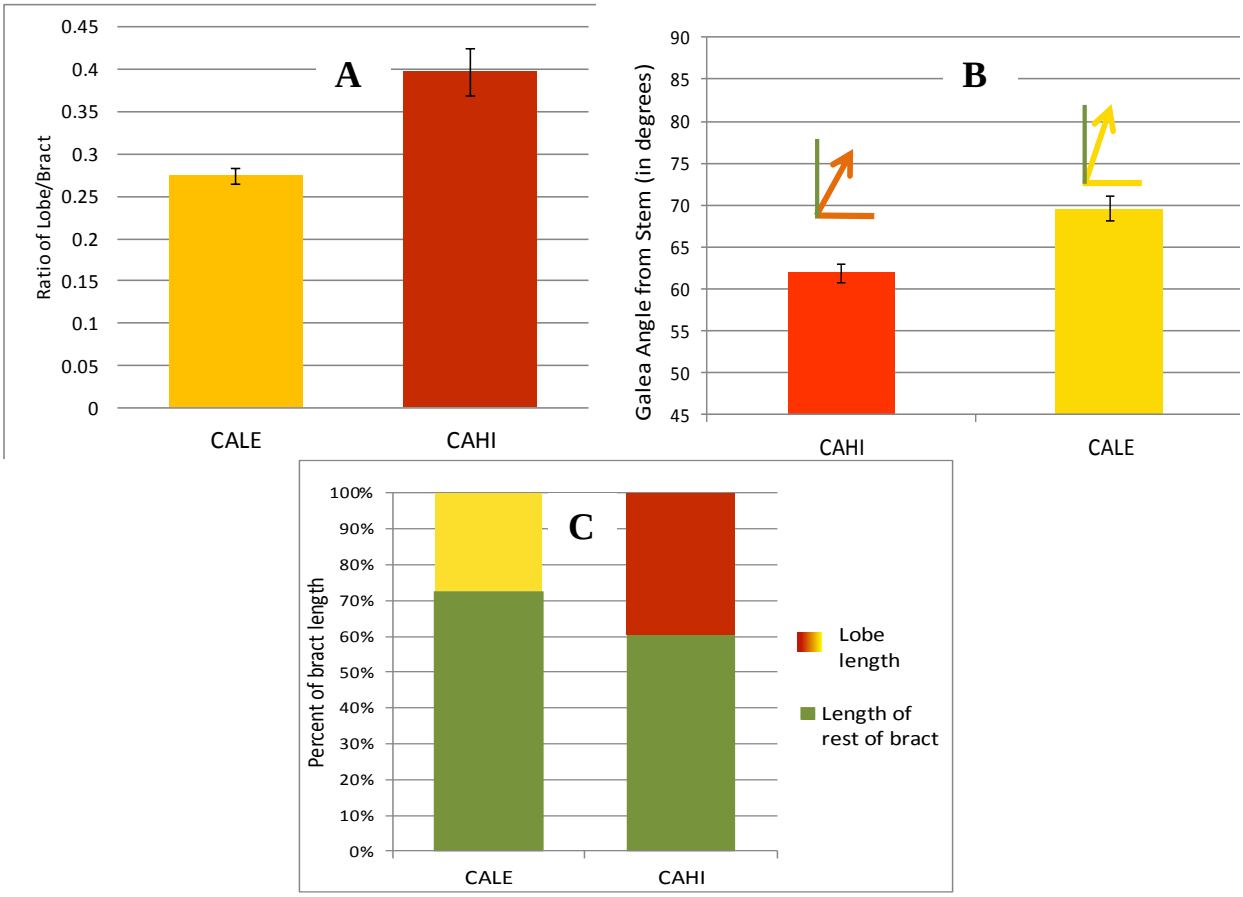

Figure 2.8. Flower measurements of herbarium specimens. A- Lobe-bract length ratios for CALE and CAHI. B- Floral angle, measured from horizontal plane, of each *Castilleja*. C- Depiction of bract lobing depth of both *Castilleja* species, proportionate to their bract lengths to visualize lobe ratios.

F1 Generation Flower Characteristics

The most surprising discovery was a new color morphology in a few of the F1 generation plants. These F1 plants were abnormally variegated, with leaves and flowers having white striation. This had not been previously seen in either CALE or CAHI (Egger pers. communication, June 2013). Some plants had both a normal green stem with normal flower coloration and a separate stem with variegated leaves and flowers, such as the WR10 H1B #6 (Figure 2.9, additional photos in Appendix C.1). The coloration was rare, only recorded in CAHI, and only two flowered.

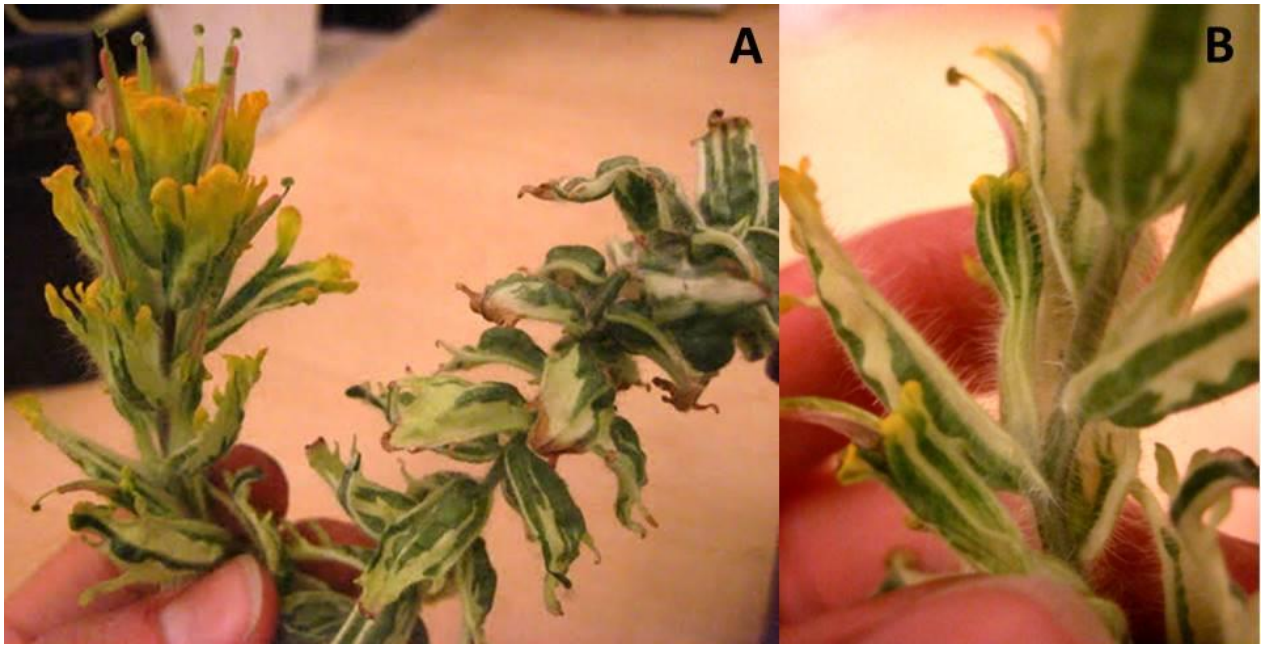

Figure 2.9. The variegated stem (A) of the WR CAHI from a mixed-species context, WR10 H1B 6, with detailed view of the bract and galea variegation (B).

Plot color variations for both restoration sites were heavily orange in their range, with 61% orange and 17% darker hues at GH, and 52% orange and 30% darker hues at WR (Figure 2.10). Yellow hues were the least common variation at both sites, with 13% making up yellow or a darker golden color (between 7A 83 and 13A 82 color IDs) at GH, and 4% at WR. All CALE identified on the sites and growing in the studied plots were yellow in coloration, with structural morphologies matching the species description and herbarium samples measured.

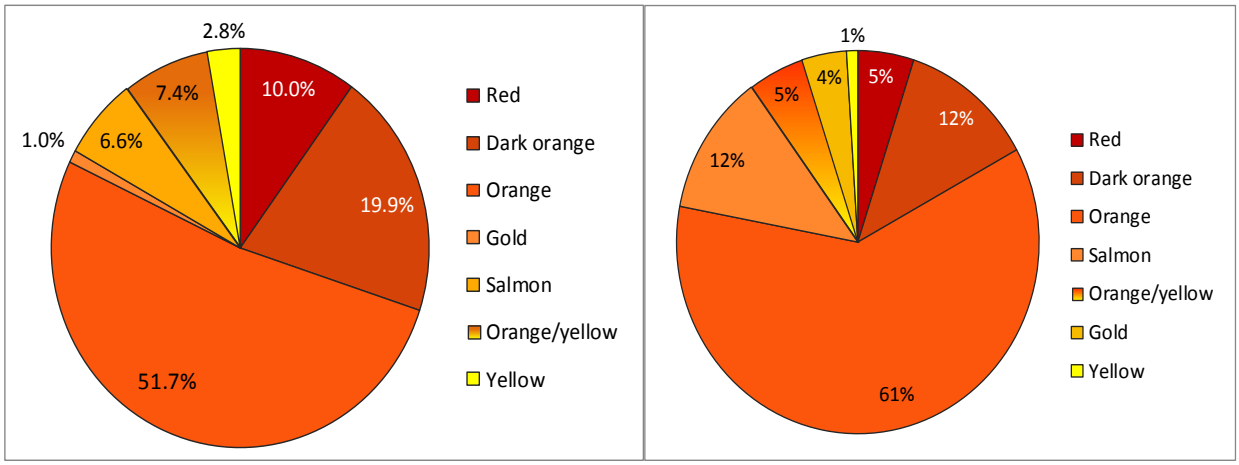

Figure 2.10. Total site CAHI color variations for Glacial Heritage (left) and West Rocky (right) restoration sites, showing the percent of the CAHI population making up each color morph. Color varieties are in order clockwise from the top.

The F1 plant color variations were heavily yellow across the 370 plants analyzed in this study. Solo CALE plots of both sites only yielded yellow F1 plants, as did the semi-solo CALE plot with two CAHI present. WR's semi-solo plot had a yellow CAHI plant present in the plot during the study's field work, and three yellow F1 plants, one being from a dark orange parent that also sired three yellow-orange and one salmon (soft pale orange- between 14B 82 & 16A 82 color ID) offspring. The other two yellow plants from WR's semi-solo plot were from the same salmon parent plant that was growing fairly close to a yellow-orange individual, but overlapped growth with a dark orange or red individual. Several of the other offspring were orange. The densely flowering solo-CAHI plot at GH only produced one yellow F1 plant and one yellow-orange plant, from different maternal CAHI. A single yellow-orange offspring was also produced by the off-site CAHI control seed stock, plus two dark yellow ones, with all others being orange or salmon colored. The maternal plants of these are unknown. Of the mixed-species plots, only a few CALE-mothered individuals were found with orange coloration, with only one salmon F1 plant from CALE parents grown further than 30 cm from a CAHI ('mix-F' interspecies distance), and two yellow-orange F1 plants, all from different maternal plants and plots. One

yellow CALE in this intermediate distance category had a distinctly open aspect, with very low floral angle averaging 59.3°, resembling the open aspect of CAHI plants. A single CALE-mothered F1 plant grown within 30 cm of a CAHI ('mix-C' distance) had dark orange color, with no yellow-orange morphs among that distance population. CAHI from mix-F interspecies distance had color variations of 22% orange or darker, with two red-hued individuals, 15% salmon, 12% yellow-orange, 50% yellow, and one variegated orange individual, with 68 total F1 CAHI at this interspecies distance (Figure 2.11). CAHI plants from mix-C interspecies distance had color variations of 9% orange, 14% salmon, 19% yellow-orange, 52% yellow, and one variegated salmon individual, with 75 total F1 CAHI at this close interspecies distance.

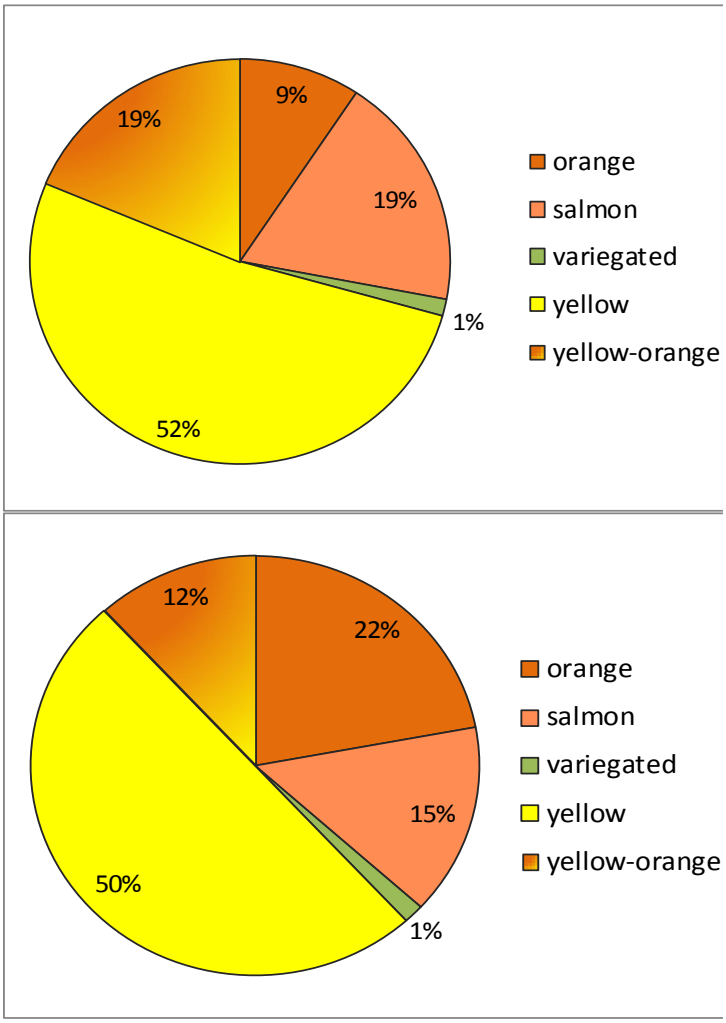

Figure 2.11. F1 mixed-context plots' CAHI color variations, showing the variation of offspring from a close-interspecies distance (left), and the >30 cm distance (right). Color varieties are in clockwise order.

Live F1 generation plants followed the same pattern of the herbarium samples, with measurable differences between the species flower characteristics. Means from all F1 CALE offspring from the sites were 71.60° for floral angles, 2.73 cm bracts, 0.33 lobe-bract ratios, and 0.28 beak-galea ratios. The F1 CAHI plants from the sites averaged 66.98° floral angles, 2.45 cm bracts, 0.37

lobe-bract ratios, and 0.35 beak-galea ratios. Comparisons of off-site controls, as representative pure-breeding individuals of each species, yielded similar results, with CALE floral angles higher than CAHI (*M*= 74.8° & 64.3° respectively), longer bract lengths, and smaller ratios than CAHI (table 2.3). These values varied between on-site control CALE and CAHI , and mixed-species context CALE and CAHI. Mixed-species context CAHI had higher angles, longer bracts and smaller ratios than did the control CAHI contexts (Figure 2.12).

Table 2.3. Treatment comparisons for each *Castilleja* species, showing the mean floral angles, bract lengths (in cm), mean lobe-bract ratios, and mean beak-galea ratios of the live off-site control plants, on-site or solo-plot controls, and the plants from mixed-species context plots.

Treatment	*Castilleja* species	Floral angle	Bract length	Lobe-bract ratio	Beak-galea ratio
Off-site controls	CALE	74.8°	2.94	0.30	0.29
	CAHI	64.3°	2.32	0.42	0.39
On-site controls	CALE	72.2°	2.72	0.32	0.27
	CAHI	61.6°	2.43	0.45	0.39
Mixed-species context	CALE	71.5°	2.74	0.33	0.28
	CAHI	66.0°	2.49	0.36	0.35

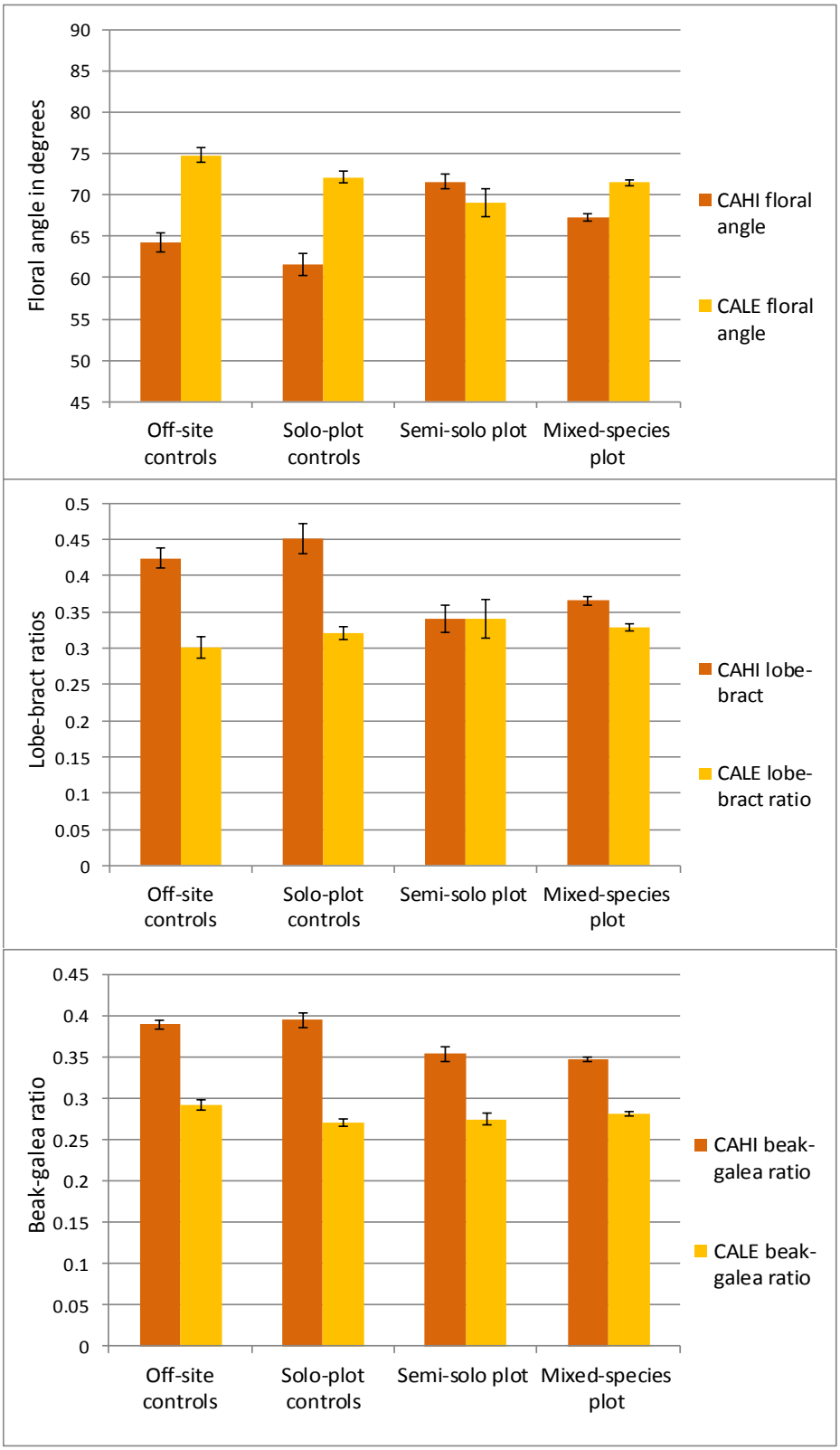

Figure 2.12. Graphs of each treatment type for floral measurements, comparing off-site controls, solo-plot context, semi-solo context, and mixed-species context plots separately, for each *Castilleja* species, including both restoration sites. Floral angle, lobe-bract ratio, and beak-galea ratio values shown, from top to bottom respectively.

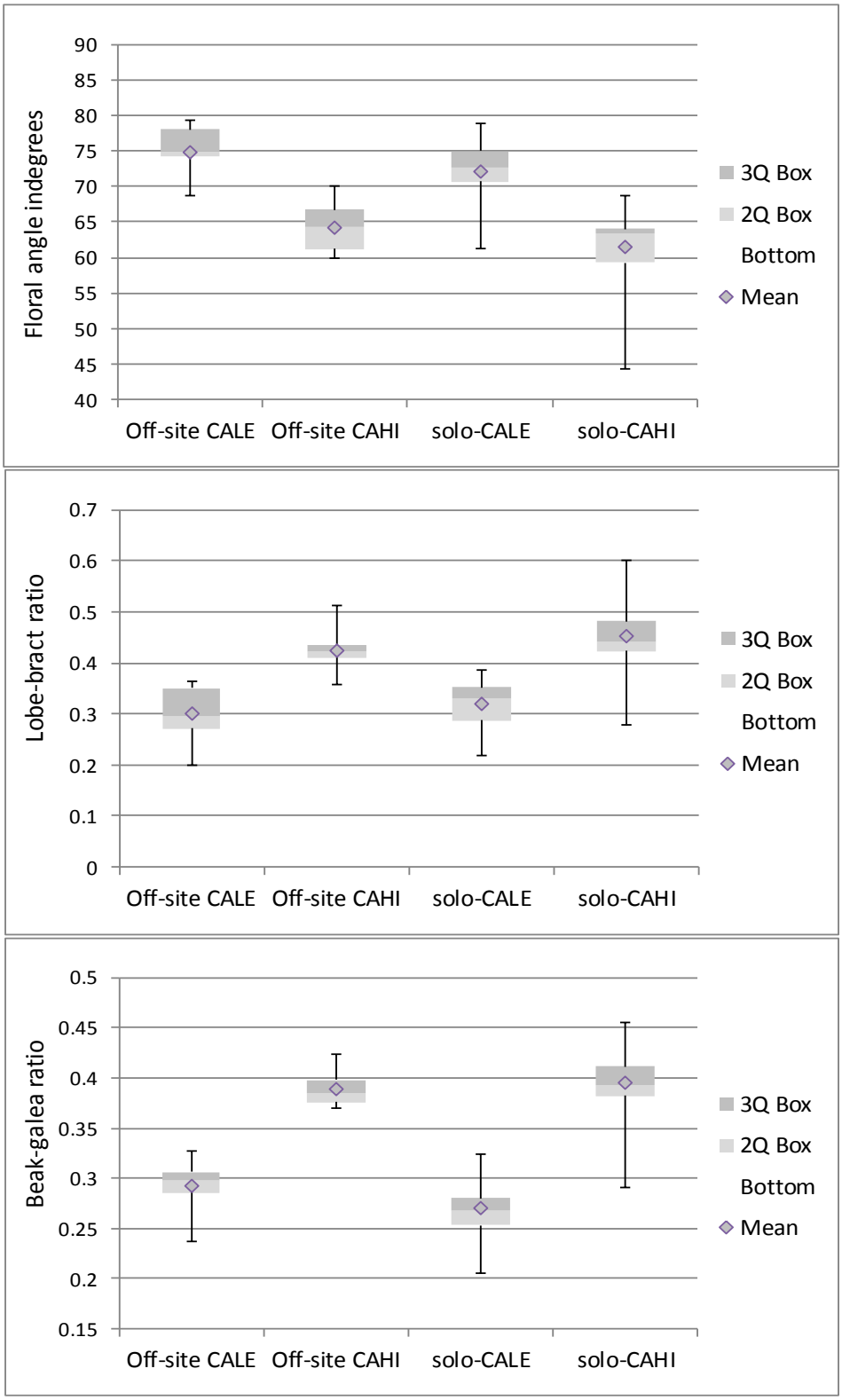

Figure 2.13. Boxplot graphs for floral measurements variances, depicting minimum and maximum values, upper and lower quartiles, and the means. All plots compare the off-site controls and the solo-site controls for each species. Floral angle, lobe-bract ratio, and beak-galea ratio values shown, from top to bottom respectively.

Off-site control CALE and CAHI had similar values to on-site control CALE and CAHI for all measures, but generally with higher variance (Figure 2.13). The GH and WR sites had very similar values for floral angle, lobe-bract ratio, and beak-galea ratio, for both *Castilleja* species (Table 2.4). These three characteristics were suspected to be the most distinguishing between CALE and CAHI, therefore they are given higher focus in the presentation of these results than the bract, lobe, beak, and galea lengths. However, bract and beak lengths differed for the species in control contexts (Figure 2.14). Galea length was typically longer for CAHI, but occasionally a very short galea was found on an F1 CAHI, along with occasional long CALE galeas, making the measure less distinguishing between the species.

Table 2.4. Results of CALE & CAHI floral measurements, including the floral angle, the ratio of lobe-bract lengths, and the ratio of beak-galea lengths. Differences are compared between sites.

Castilleja species	Floral angle	Lobe-bract ratio	Beak-galea ratio
CALE			
Glacial Heritage	71.5±4.7	0.33±0.05	0.28±0.03
West Rocky	71.5±4.1	0.33±0.05	0.28±0.04
CAHI			
Glacial Heritage	67.0±5.6	0.38±0.08	0.36±0.04
West Rocky	66.9±5.7	0.36±0.06	0.35±0.03

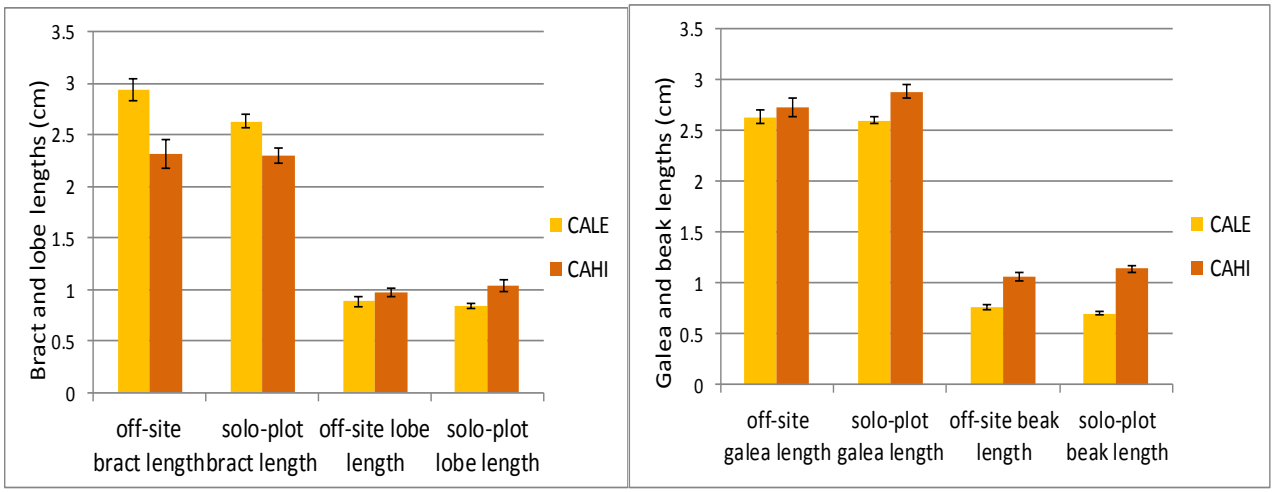

Figure 2.14. Floral measures for both *Castilleja* species. Left: bract and lobe lengths, comparing off-site and solo-plot control values. Right: galea and beak lengths, comparing off-site and solo-plot control values.

One-way ANOVA analysis of off-site control plants for the *Castilleja* species' effect on floral angle, bract and lobe lengths, lobe-bract ratio, galea and beak lengths, and the beak-galea ratio indicate differences between CALE and CAHI in some measures, but not others. Floral angle, bract length, lobe-bract ratio, beak length, and beak-galea ratios all had p-values below 0.003 (Table 2.5), indicating CALE had significantly higher floral angles, lower ratios, longer bracts, and shorter beaks than CAHI, visible in the Figure 2.14 graphs, and Figure Appendix A.5.

Table 2.5. One-way ANOVA results for off-site control flowers as representative pure-breeding plants of their species. Tests analyzed floral angle, bract and lobe lengths, lobe-bract ratio, galea and beak lengths, and beak-galea ratios. Significant effects at $\alpha = 0.05$ denoted with *

		Sum of Squares	df	Mean Square	F	Sig.
Floral angle	Between Groups	586.49	1	586.49	50.22	0.000*
	Within Groups	233.57	20	11.68		
	Total	820.05	21			
Bract length	Between Groups	2.03	1	2.03	12.59	0.002*
	Within Groups	3.23	20	0.16		
	Total	5.26	21			
Lobe length	Between Groups	0.042	1	0.042	1.42	0.248
	Within Groups	0.60	20	0.030		
	Total	0.64	21			
Lobe-bract ratio	Between Groups	0.08	1	0.081	34.10	0.000*
	Within Groups	0.05	20	0.002		
	Total	0.13	21			
Galea length	Between Groups	0.05	1	0.05	0.73	0.402
	Within Groups	1.37	20	0.07		
	Total	1.42	21			
Beak length	Between Groups	0.47	1	0.47	47.95	0.000*
	Within Groups	0.20	20	0.01		
	Total	0.66	21			
Beak-galea ratio	Between Groups	0.05	1	0.050	111.03	0.000*
	Within Groups	0.01	20	0.00		
	Total	0.06	21			

Independent sample t-test analysis of the capsule level, for each species in mixed plots only, yielded no significant difference between either CALE's or CAHI's seed capsules in any of the floral measures (Table 2.6). Capsule level thus had no effect on the floral angle, lobe length or ratio, beak length, or beak ratio, and was not included in the other analyses due to being a confounding factor.

Table 2.6. Results of independent sample t-tests comparing mixed-plot seed capsule levels, for each *Castilleja* species. Significant effects at α= 0.05 denoted with *

Effect	DF	t-stat	p-value (1-tail)
CALE: Floral angle	140	0.73	0.468
Lobe length	145	0.46	0.645
Lobe ratio	143	-0.50	0.615
Beak length	138	-1.47	0.144
Beak ratio	140	-1.22	0.226
CALE: Floral angle	127	-0.98	0.328
Lobe length	122	-1.17	0.245
Lobe ratio	121	-0.38	0.706
Beak length	130	0.28	0.780
Beak ratio	122	0.11	0.913

Univariate ANOVA test of each *Castilleja* species' floral data set separately, using both control contexts (off- and on-site solo-plots) treated as one control factor, yielded significant p-values only for the control treatment factor. Floral angle, lobe length, and lobe ratio were all affected by the difference between control (solo grown) plants, while beak length and ratio were not. Control CALE floral angles were higher than mixed-species plots, and the lobe ratios were lower than mixed-species plots (Figure 2.15). Between control treatments however, the control CALE lobe ratio was 0.33 and CAHI 0.44, but 0.32 and 0.36 in mixed-species treatments. Thus, growing within the same plot as CAHI, affected CALE's floral angle and lobing (Table 2.7).

Table 2.7. Results from CALE univariate tests of the effects of restoration site, control (as on & off-site, versus mixed-species plots) and interspecies distance, on floral angle, lobe and beak lengths, the lobe-bract ratio, and the beak-galea ratio. Significant effects at $\alpha = 0.05$ denoted with *

Effect	Response	Floral angle	Lobe length	Lobe-bract ratio	Beak length	Beak-galea ratio
Site	p-value	0.857	0.796	0.684	0.266	0.488
	MS	1.15	0.002	0.001	0.03	0.001
	F	0.03	0.07	0.18	1.40	0.52
	df	1	1	1	1	1
Distance	p-value	0.213	0.682	0.781	0.598	0.343
	MS	75.54	0.008	0.001	0.004	0.001
	F	1.79	0.18	0.082	0.30	1.00
	df	1	1	1	1	1
Control (distance (plot(site))	p-value	0.014*	0.042*	0.002*	0.516	0.518
	MS	42.69	0.04	0.007	0.01	0.001
	F	2.39	2.00	3.08	0.91	0.91
	df	9	9	9	9	9
Plot(site)	p-value	0.667	0.668	0.831	0.290	0.428
	MS	33.77	0.04	0.004	0.02	0.001
	F	0.75	0.74	0.51	1.53	1.17
	df	9	9	9	9	9

For CAHI, the control treatment also had the most effect on floral traits, affecting all measures except lobe length (Table 2.8). The control treatment affected the floral angle and lobe-bract ratio, beak length and beak ratio, while the site affected only the floral angle ($p = 0.050$). CAHI's mean floral angle at GH was 69.2°, compared to the angles at WR as 66.9° for CAHI. Control CAHI floral angles were lower than in mixed-species plots, and the lobe and beak ratios were higher in controls than in mixed-specie plots (Figure 2.15).

Table 2.8. Results from CAHI univariate tests of the effects of restoration site, control (as on & off-site, versus mixed-species plots) and interspecies distance, on floral angle, lobe and beak lengths, the lobe-bract ratio, and the beak-galea ratio. Significant effects at $\alpha = 0.05$ denoted with *

Effect	Response	Floral angle	Lobe length	Lobe-bract ratio	Beak length	Beak-galea ratio
Site	p-value	0.050*	0.618	0.167	0.069	0.146
	MS	93.64	0.01	0.008	0.07	0.001
	F	4.77	0.26	2.18	4.05	2.32
	df	1	1	1	1	1
Distance	p-value	0.858	0.268	0.787	0.521	0.860
	MS	1.55	0.07	0.001	0.02	0.000
	F	0.03	1.36	0.08	0.44	0.03
	df	1	1	1	1	1
Control (distance (plot(site))	p-value	0.025*	0.051	0.031*	0.018*	0.031*
	MS	50.75	0.06	0.008	0.04	0.003
	F	2.19	1.93	2.12	2.31	2.12
	df	9	9	9	9	9
Plot(site)	p-value	0.907	0.927	0.890	0.868	0.989
	MS	19.04	0.02	0.003	0.02	0.000
	F	0.38	0.34	0.41	0.44	0.17
	df	8	8	8	8	8

These show changes between control & mixed-species treatments in both CAHI and CALE floral measures, but in different measures (Figure 2.15), as well as significant differences across all floral measures between the *Castilleja* species. Site only affected CAHI floral angle.

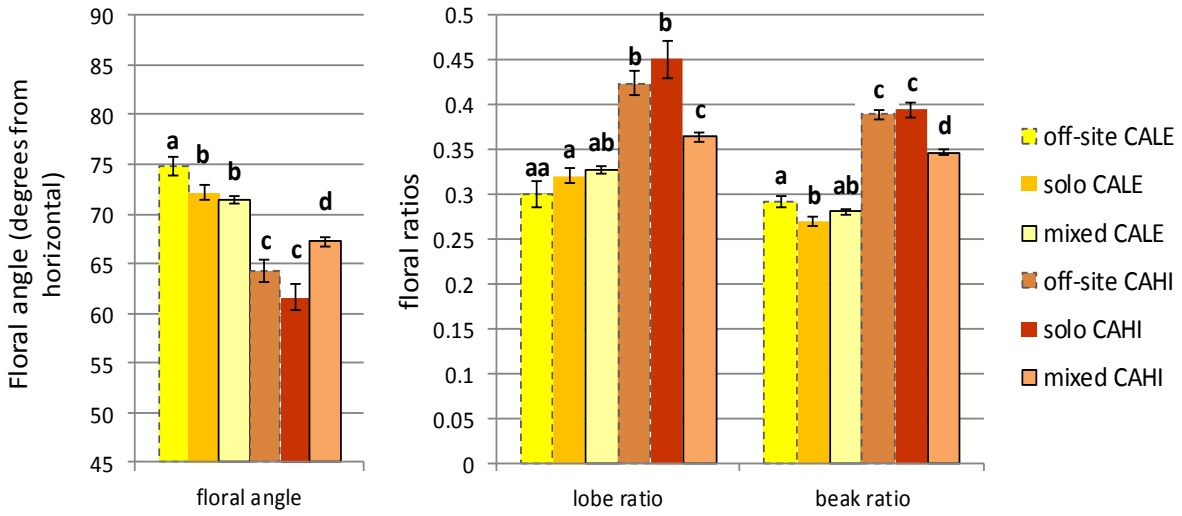

Figure 2.15. Compiled results of floral measures, comparing off-site and on-site solo plot, and mixed-species plot contexts, with floral angles (left), lobe-beak ratio (middle), and beak-galea ratio (right). Differences indicated bv different letters.

Principal component analysis (PCA) yielded two principal components (PC) axes that accounted for 79% of the variation in floral data, for all 370 individual plants measured. Lobe-bract ratio, beak-galea ratio, lobe length and floral angle were used for this PCA to reach a Kaiser-Meyer-Olkin measure of sampling adequacy of 0.522. The component score coefficients of each PC are given in Table 2.9. The first PC accounted for 53.02% of the data's variance and was produced primarily from lobe length and lobe ratio (Table 2.9). The second PC accounted for 25.96% of the variance, and was produced primarily from floral angle and beak ratio, with floral angle in the opposite direction (negative) from beak ratio. This is because the angle decreases in CAHI while beak ratio increases. The control treatment plants (off-site and solo-species contexts) of each *Castilleja* species separated along the first two PC axes distinctly (Figure 2.16). Separation of the species is primarily due to PC2, using floral angle and galea ratio. The cluster of semi-solo CAHI plants ranges far into the cluster of control CALE, due to the unusual WR06

Table 2.9. Component score coefficient matrix for the principle component analysis using lobe-bract and beak-galea ratios, lobe length and floral angle.

	Component	
	1	2
Lobe length	0.633	-0.227
Lobe-Brt ratio	0.488	0.073
Floral angle	0.153	-0.616
Beak-Galea ratio	-0.066	0.576

plot with both a low density CAHI and a single CALE present. The mixed-species context CAHI also infiltrate far into the CALE cluster, which stays closer to the CALE controls, which matches the floral measurement differences in mixed-context CAHI but not the mixed-context CALE.

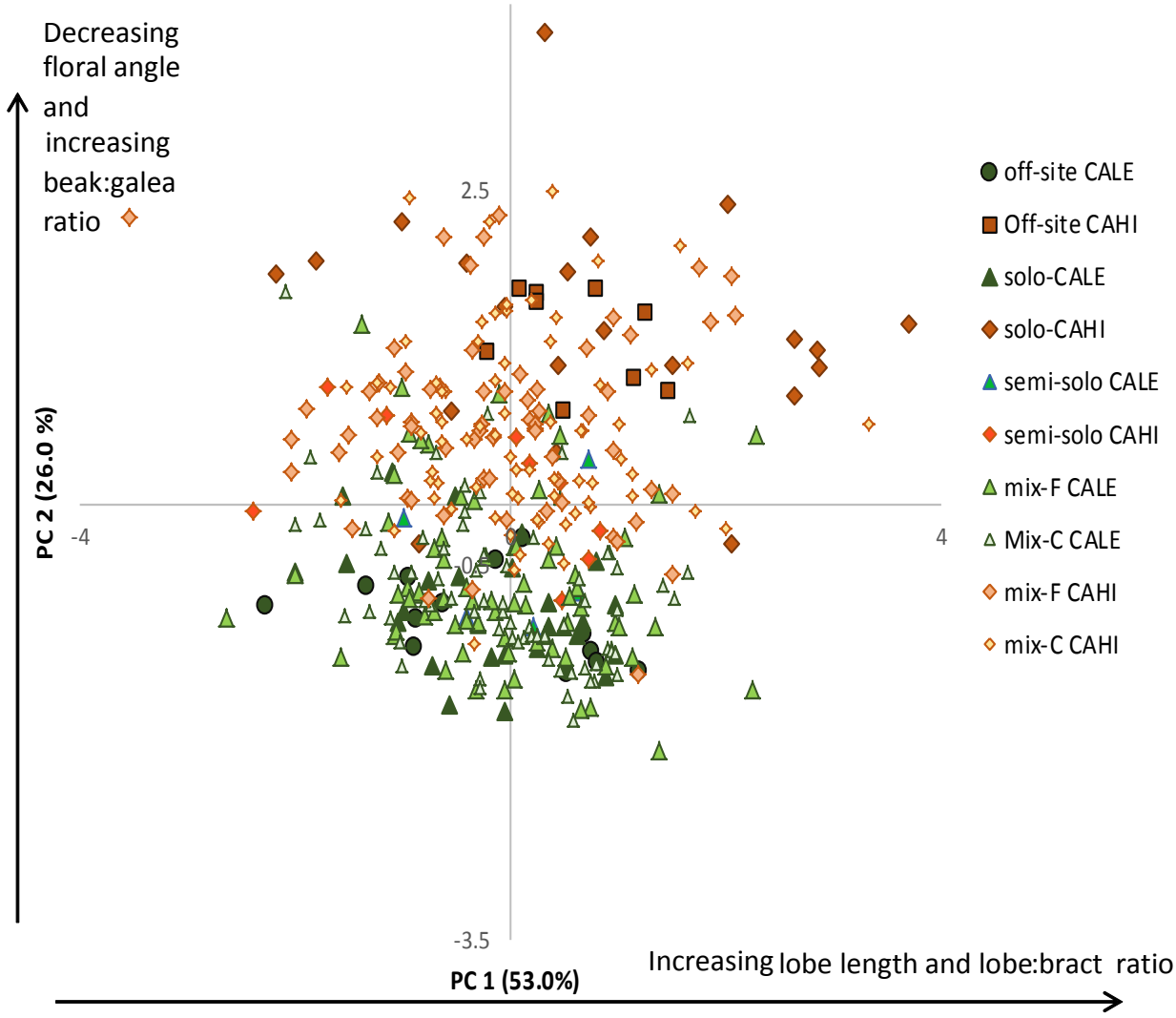

Figure 2.16. Principal component analysis of four traits (lobe-bract ratio, lobe length, beak-galea ratio, and floral angle) for the two *Castilleja* species across their treatment contexts. The principle component (PC) scores for PC1 and PC2 are shown, with the percent of the data's variation explained by each axis given in parentheses. All treatment contexts are shown, with mixed-species context plants divided by interspecies distance, as those further than 30 cm (mix-F) and those closer than 30 cm (mix-C).

Pollen tests:

Initial observations

Castilleja pollen required boron & chlorine in sucrose solution to germinate tubes, as initial tests lacking boron & chlorine failed to germinate. Pollen viability decreased quickly the lower flowers were on the stem, with flowers below the first four or five whorls (approximately one to two inches below the stem apex) having near zero pollen tube germination, but less of a viability decrease assessed via acetocarmine staining. Findings of interest but not directly applicable to this study included sterility in variegated CAHI plants, with one from GH yielding 63 and 61% viabilities from tube germination and acetocarmine staining respectively, and another plant from WR yielding a 2% pollen viability via tube germination and 25% via acetocarmine staining. The WR plant was more severely variegated but with only one flowering stem affected. Its non-variegated stem had 36% pollen tube germination and 91% acetocarmine staining, which were normal high viabilities for each test. Pictures of the plant are in the picture Appendix C.1. This sterile stem was not counted as an individual for this pollen analysis.

Pollen viability analysis

In vitro pollen tube germination (PTG) was consistently lower than acetcarmine staining (ACS) for viability percentages, with a high viability being about 30% for PTG and 90% for ACS tests in general. Mean PTG viability for off-site control CALE was 16% and mean ACS viability for CALE was 67.3%, while CAHI had higher PTG at 53.3% and higher ACS at 91.2% (Table 2.10). Low CALE viability was due to several of the off-site control CALE having very low germination and some of the same samples having low staining viability (Figure 2.17). Low CALE survival inhibited the sample size of this control treatment. The cause of these low numbers is unknown, as the plants looked normal and healthy. Excluding the PTG results lower than 10% yielded a CALE off-site control viability of 28.6%. On-site controls (solo-species plot

contexts) had higher CALE PTG and ACS viabilities than the controls (*M*= 26.8% and *M*= 82.2% respectively), but still lower than CAHI viabilities. Mixed- species contexts had more similar viability between the *Castilleja* species than the off-site control plants, for both PTG and ACS test (Figure 2.18). This was due to mixed-context CAHI having a decrease in pollen viability percentages for both test, compared to off-site controls, with little change in CALE between the on-site control (solo-context plots) and mixed-species plots.

Table 2.10. Treatment comparisons for each *Castilleja* species, showing the mean pollen tube germination (PTG) and acetocarmine stain (ACS) viability percentages of off-site control plants, on-site or solo-plot controls, and the plants from mixed-species context plots.

Treatment	*Castilleja* species	PTG %	ACS %
Off-site controls	CALE	16.3 - 28.6	67.3
	CAHI	53.3	91.2
On-site controls	CALE	26.8	82.2
	CAHI	46.7	82.8
Mixed-species context	CALE	29.1	84.3
	CAHI	29.5	75.3

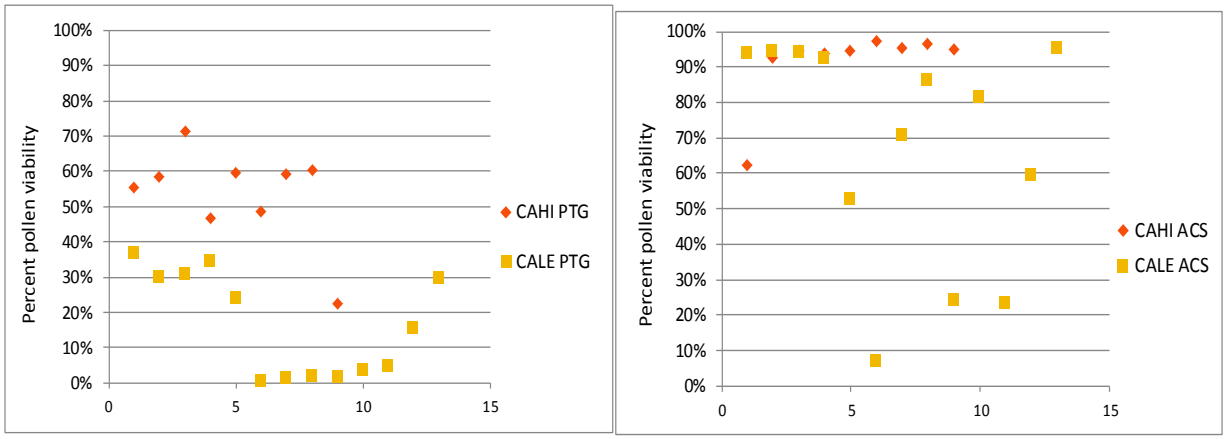

Figure 2.17. Pollen tube germination (PTG) for CALE and CAHI (left), and acetocarmine stain (ACS) for both species (right), from off-site control plants. Sample numbers are across the x axis.

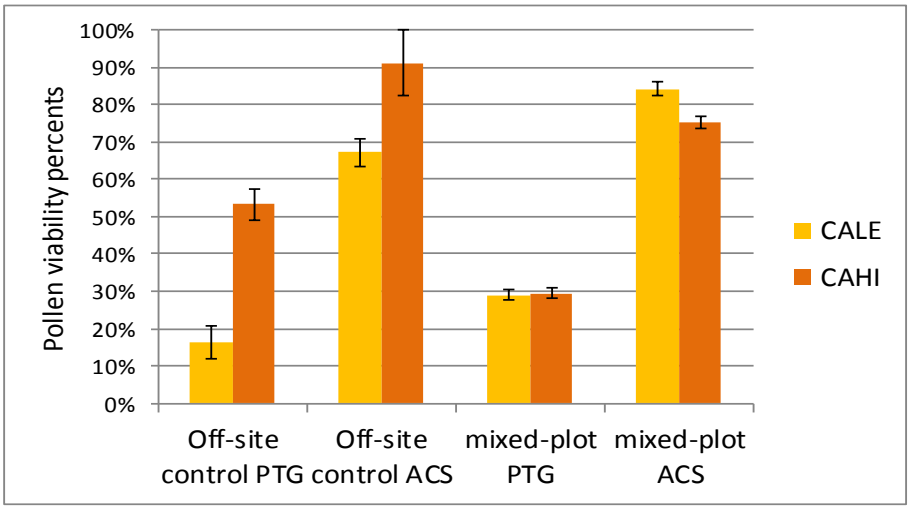

Figure 2.18. Pollen viability percentages for CALE and CAHI plants from off-site controls, compared to mixed-species plot context plants.

The restoration sites were slightly different for pollen viabilities, with GH having higher PTG (*M*= 32.1% versus WR's *M*= 27.2%) and ACS viability (*M*= 82.8% versus WR's *M*= 75.9) for F1 offspring grown from parent plants there (Table 2.11). In general, CALE had lower PTG but higher ACS viabilities than CAHI, at 28.5% and 84% respective, compared to CAHI's lower 31.4% and higher 74.8%. Plants had the highest viabilities when grown in solo contexts, and the lowest when grown in a semi-solo context, with only one or two individuals of the others species. GH's semi-solo plot of CALE had 37% PTG and 85% ACS, but WR's unusual semi-solo plot #WR06 of CAHI had 24% PTG and 71 % ACS, with more plants in its sample size. Both semi-solo plots had low seedling survival but the WR plot seemingly more so. Off-site controls and solo-species plot controls varied in their pollen viabilities, with CALE

Table 2.11. Results of pollen viability tests, including PTG and ACS, comparing sites, *Castilleja* species, and plot contexts.

Factors	PTG	ACS
Sites		
Glacial Heritage	32.1% ±17	82.8% ±18
West Rocky	27.2% ±18	75.9% ±24
Castilleja species		
CALE	28.5% ±17	84% ±21
CAHI	31.4% ±19	74.8% ±21
Contexts		
Solo	34.2% ±17	82.5% ±24
Semi-solo	27.9% ±19	74.9% ±27
Mixed	29.2% ±18	79.4% ±20

having produced more low values in their off-site controls than CAHI (Figure 2.19).

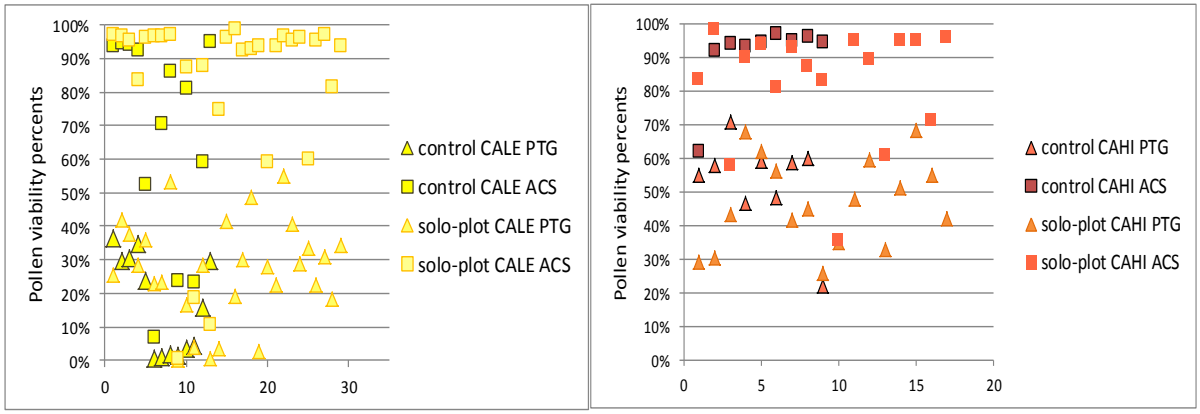

Figure 2.19. Pollen viability percentages for CALE (left) and CAHI (right), showing pollen tube germination (PTG) and acetocarmine staining (ACS) results for off-site controls and solo-species context plants. Sample numbers are across the x-axis.

Table 2.12. One way ANOVA results for off-site control plants' pollen viability tests (pollen tube germination and acetocarmine stain).

		Sum of Squares	df	Mean Square	F	Sig.
PTG	Between Groups	1.033	1	1.033	53.890	0.000
	Within Groups	0.556	29	0.019		
	Total	1.589	30			
ACS	Between Groups	.433	1	0.433	9.088	0.005
	Within Groups	1.380	29	0.048		
	Total	1.813	30			

One way ANOVA analysis of off-site controls, to compare the pure-breeding *Castilleja* individuals, yielded significant differences between the species for both pollen viability tests. CALE had a significantly lower PTG (p= 0.000) and ACS (p= 0.005) viabilities (*M*= 16.3% and 37.3%, *SD*= 0.15 and 0.31 respectively) than CAHI (*M*= 53.3% and 91.2%, *SD*= 0.14 and 0.11 respectively) (Table 2.12).

Univariate ANOVA analysis of the on-site pollen data set from the restoration sites, excluding off-site controls to test for site differences, indicates significant effect of species for only ACS tests. Interspecies distance also affected the PTG test, but not ACS, and restoration site affected the ACS (Table 2.13). This indicates restoration site CALE have significantly lower pollen viability for PTG tests (*M*= 28.7%, *SD*= 0.17) than CAHI (*M*= 30.9%, *SD*= 0.19).

Table 2.13. Results from univariate tests of effects of restoration site, plot, parent *Castilleja* species, capsule, & interspecies distance as context on pollen viability tests. Off-site controls were excluded to test restoration site and capsule effects. Significant effects at α= 0.05 denoted with *

Response	Effect	DF	MS	F	p-value
Pollen tube germination	Site	1	0.02	0.55	0.475
	Species	1	0.000	0.000	0.985
	Distance	1	0.17	5.71	0.017*
	Plot(site)	9	0.05	1.60	0.116
Acetocarmine stain	Site	1	0.31	6.42	0.027*
	Species	1	0.76	18.41	0.000*
	Distance	1	0.11	2.54	0.112
	Plot(site)	9	0.05	1.20	0.295

Independent sample t-tests on each species, comparing control (on & off-site plants) to mixed-species contexts, indicate similar effects of the mixed-species context on each species. When each control context is treated together, CALE PTG is significantly higher in mixed plots, compared to off- and on-site controls, but its ACS is affected (Table 1.14). CAHI PTG and ACS decrease in mixed-species plots, similar to the pattern between solo and mixed site plants (Figure 2.20).

Table 2.14. Results from t-tests exploring the overall effects of control contexts (on and off-site controls compared to mixed-species context), on pollen viability for each *Castilleja* species separately. Significant effects at α= 0.05 denoted with *

Comparison	Response	DF	t-stat	p-value (2-tail)
CALE control vs mixed context	PTG viability	21	2.22	0.038*
	ACS viability	17	1.42	0.175
CAHI control vs mixed context	PTG viability	22	-6.55	0.000*
	ACS viability	25	-4.32	0.000*

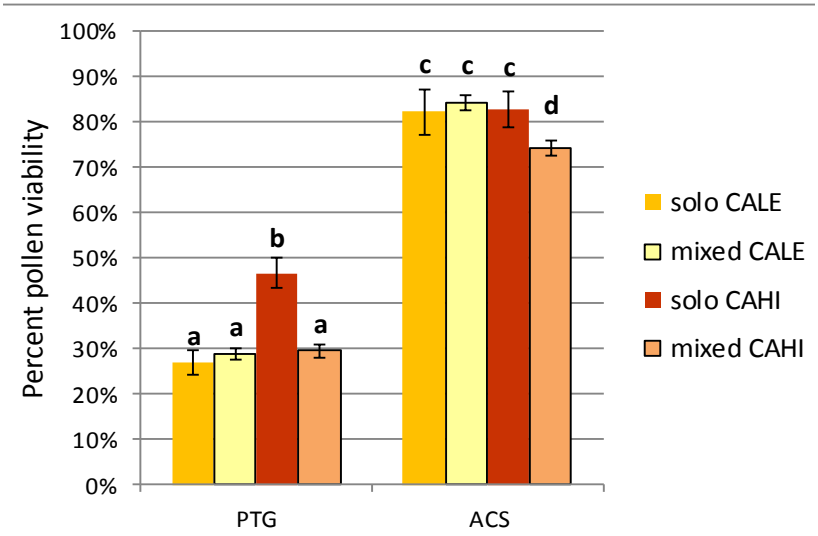

Figure 2.20. Compiled results of pollen viabilities, comparing off-site and on-site solo plot, and mixed-species plot context, with PTG and ACS tests. Differences indicated by different letters.

Changing the context to include all interspecies distances greater than 30 cm as a treatment compared to plants within 30 cm of another *Castilleja* species, had no effect on CALE pollen t-test significance, but significant p-values for CAHI PTG tests (Table 1.15). This may indicate CAHI PTG is affected particularly by growing within 30 cm of CALE.

Table 2.15. Results from t-tests exploring the overall effects of interspecies greater than 30 cm versus less than 30 cm, on pollen viability for each *Castilleja* species separately. Significant effects at $\alpha = 0.05$ denoted with *

Comparison	Response	DF	t-stat	p-value (2-tail)
CALE > 30 cm vs < 30 cm	PTG viability	79	1.34	0.185
	ACS viability	62	1.34	0.186
CAHI > 30 cm vs < 30 cm	PTG viability	61	-3.42	0.001*
	ACS viability	59	-1.83	0.072

Separate univariate ANOVA analyses on each *Castilleja* species indicated a difference in their pollen responses to factors. CALE had the only significant p-value for any factor, and CAHI had none (Table 2.16). CALE was significantly affected by the plot, nested within the sites (p=0.040). The site and interspecies distance had no effect on pollen viability tests for either of the *Castilleja* species.

Table 2.16. Results from univariate test of the effects of restoration site, parent *Castilleja* species, and interspecies distance as context on pollen viability tests. Significant effects at α= 0.05 denoted with *

Response	Effect	DF	*Castilleja* species	MS	F	p-value
PTG	Site	2	CALE	0.07	1.75	0.209
		1	CAHI	0.001	0.03	0.871
	Distance	3	CALE	0.04	1.39	0.248
		3	CAHI	0.07	2.31	0.078
	Plot(site)	10	CALE	0.05	1.79	0.066
		9	CAHI	0.05	1.56	0.133
ACS	Site	2	CALE	0.20	2.68	0.103
		1	CAHI	0.007	0.11	0.749
	Distance	3	CALE	0.05	1.04	0.376
		3	CAHI	0.09	2.12	0.100
	Plot(site)	10	CALE	0.09	1.97	0.040*
		9	CAHI	0.07	1.80	0.072

Putative hybrid plants via flower & pollen characteristics

Using both floral measures and pollen characteristics, plants with ranges outside the range of their pure-breeding control plants for each species were identified and tallied. Floral ranges of the control CALE and CAHI were separated from each other, but their ranges were very close, so that the bottom boundary of CAHI was close to the top boundary of CALE. For example, CALE floral angle, for example, had its lowest value as 68.7°, while CAHI's highest floral angle was 70.0°, overlapping CALE's lower range. Each off-site control *Castilleja* species' upper and lower range boundaries are given in Table 2.17 The pollen viability ranges were higher for CAHI for both tests, with both the top boundary of their ranges, and the bottom boundary higher than CALE's (Table 2.18).

Table 2.17. Ranges of each floral measure for the pure-bred species, from off-site controls. The floral angle, lobe-bract and beak-galea ratios were used as optimal measures to distinguish CALE from CAHI.

Castilleja species	Range boundary	Floral Angle	Lobe-bract ratio	Beak-galea ratio
CALE	Top	79.3°	0.35	0.33
	Bottom	68.7°	0.25	0.24
CAHI	Top	70.0°	0.51	0.42
	Bottom	60.0°	0.36	0.27

Table 2.18. Ranges of each pollen viability test for the off-site controls. Tests include pollen tube germination (PTG) and acetocarmine stain (ACS). The unusually low CALE control viabilities were excluded as outliers.

Castilleja species	Range boundaries	PTG	ACS
CALE	Top	36.6%	95.2%
	Bottom	15.5%	52.4%
CAHI	Top	71.1%	97.3%
	Bottom	22.2%	62.3%

Castilleja hybrid plants are assumed to be intermediate to their parent species' floral measures, and have lower than normal pollen viability. The floral angle for CALE is estimated to be greater than 70°, with off-spring from a CALE maternal plant that have a lower than 70° floral angle being putative hybrids. Offspring from maternal CAHI with floral angles higher than 68° are estimated to be CALE-sired hybrids. The lobe-bract ratio for CALE is estimated to be lower than 0.32 (Figure 2.21), with CALE off-spring that have higher than 0.32 lobe-bract ratios being putative hybrids. CAHI lobe ratios that are lower than 0.35 are estimated to be CALE-sire hybrids. The beak-galea ratio for CALE is estimated to be lower than 0.30, with CALE off-spring that have higher than 0.30 beak-galea ratios being putative hybrids. CAHI beak-galea ratios that are lower than 0.34 are estimated to be CALE-sired hybrids. Hybrids from CALE maternal plants, sired by CAHI are estimated to have lower floral angles, and longer lobe and beaks to give higher ratios.

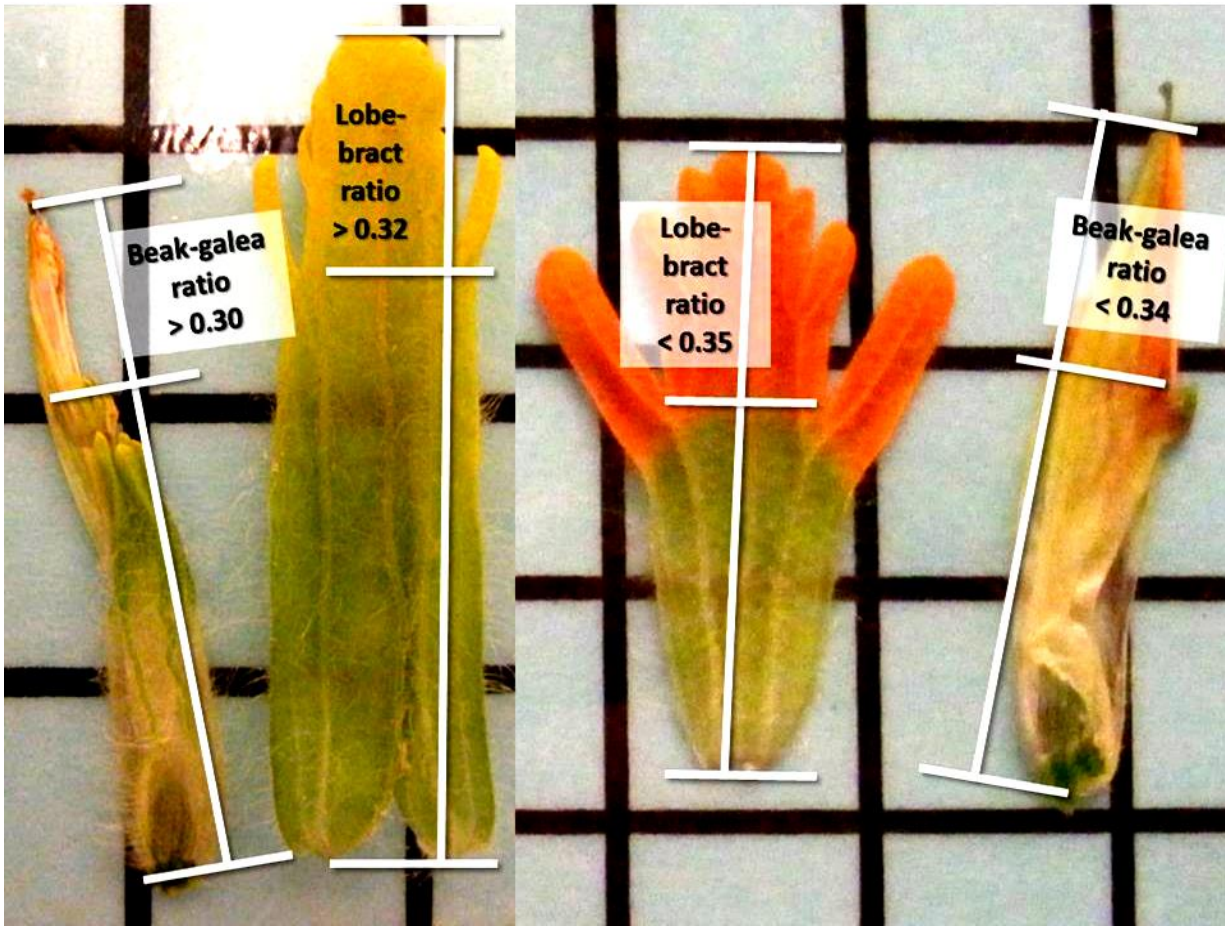

Figure 2.21. CALE (left) galea and bract example, from off-site control plants, and CAHI galea and bract example (right) from off-site control plants, showing the proportions of each floral structure that is beaked, for the galeas, and lobed, for the bracts. The upper range for CALE ratios and the lower range for CAHI are given for each structure's ratios.

Plants from the F1 generation of mixed-species context plots were grouped by those with floral angle, lobe-bract and beak-galea ratios that were beyond that of their maternal plants' species. Those with more than one measure beyond their species' range were tallied as putative hybrids. Rarely did any plants have all floral measures beyond the ranges. The species differed in their proportions of flowers with a single measure beyond the species range compared to the total F1 population for that group. CALE plants from the mixed-species contexts had a lower proportion of plants with floral angles beyond their species' range than CAHI, for both sites (Table 2.19). However, CALE had a higher proportion of plants with lobe ratios beyond the range of their

species than CAHI for both sites, and a lower proportion of beyond-range plants for the beak-galea ratio.

CALE and CAHI normal pollen viability is estimated as equal to or higher than 25% in PTG tests and equal or higher than 70% for ACS. The GH site had a lower percent of CALE plants with lower than normal pollen viabilities than the WR CALE plants, but similar percentages of the CAHI plants across both sites had lower than normal viabilities (Table 2.20). The plants from both maternal *Castilleja* species had higher proportions of lower than normal viability in the PTG test than in the ACS test. The unusual WR semi-solo plot (WR06) had 38.5% lower than maternal pollen viability in ACS tests, and 46.2% lower in PTG tests. Most of the plants from this plot had a combination of low pollen viability and outlying floral measures, including most of those with yellow colored flowers.

Table 2.19. Percentages of each floral measure that falls outside the pure-bred species' range, from plants grown in mixed-species context plots. Each site, GH and WR, is calculated separately.

Site	*Castilleja* species	Floral Angle	Lobe-bract ratio	Beak-galea ratio
Glacial Heritage	CALE	26.4%	52.8%	15.3%
	CAHI	53.2%	41.6%	37.7%
West Rocky	CALE	26.0%	46.6%	21.9%
	CAHI	35.5%	37.1%	43.5%

Table 2.20. Percentages of each pollen viability test that falls below the pure-bred species plants' range, from the mixed-species context plots. Percentages are given for pollen tube germination (PTG) and acetocarmine stain (ACS). The unusually semi-solo CAHI West Rocky plot was not included.

Site	*Castilleja* species	PTG	ACS
Glacial Heritage	CALE	35.6%	12.2%
	CAHI	26.3%	26.3%
West Rocky	CALE	45.2%	9.6%
	CAHI	24.2%	22.6%

Plants with both multiple floral traits beyond their species' range and low pollen viability for either of the viability tests were most likely hybrids of the two *Castilleja* species, produced in the field sites. For both sites, more CAHI plants had two floral measures beyond their parental range than CALE plants, with 30 CAHI at GH and 27 at WR, compared to 16 and 17 CALE at GH and

WR respectively. Roughly half of the CAHI floral outliers also had at least one low pollen viability measure, with 17 of the 30 outliers from GH and 15 of those from WR. CALE only had three plants with both outlying flower measures and low pollen viability in GH, and 12 at WR. Pollen test results for these likely hybrids varied, with some having only one test yielding low viability and others having low measures in both tests. There were multiple families of hybrids identified as well, with several off-spring from the same parent plant having similarly outlying floral measures. The number of these hybrids from each interspecies distance within the mixed-species plots (further than 30 cm or closer than 30 cm) was 21 for further plants and 27 for closer plants, with many of these being from the same parent plant (Table 2.21). CAHI hybrids identified via this floral measurement and pollen viability analysis were predominantly yellow, yellow-orange, or soft 'salmon' orange in coloration, with only one having the more common orange color of wild CAHI population flowers. From these measures, the single orange CALE identified earlier in this study was likely a mislabeled CAHI due to very high pollen viability, and all floral measures high in the CAHI range for floral angle and the ratios.

Table 2.21. Interspecies comparisons for number of likely hybrids identified via two outlier floral measures and at least one low pollen viability measure. Percent of is based on the total number of mixed-species plot CALE and CAHI.

Treatment	*Castilleja* species	# putative hybrids	% of hybrids
Mixed-species context- "Far"	CALE	7	4.9%
	CAHI	16	11.9%
Mixed-species context- "Close"	CALE	8	5.6%
	CAHI	14	10.4%
Total hybrids	CALE	15	10.6%
	CAHI	32	23.7%

Discussion

Distinguishing species

For the conservation of CALE, genetic isolation from related *Castilleja* may be important for the species' long-term survival due to the various risks interbreeding brings. It is possible, though, that minimal fitness loss can occur, and rather hybridization can lead to a new species from the parents. Until the stable recovery of CALE populations, maintaining genetic isolation from CAHI, as a known compatible hybridizer, would be the safest conservation method. The ability to easily identify hybrids in instances where the species do cross, via low constancy pollinators, would be a valuable tool for preserving CALE's genetic integrity without risking removing variable CALE. The high genetic diversity of the species will be important during its population's recovery and continued survival through changing environments and influences and should be maintained.

Morphological differences between CALE and CAHI were found in this study, in both in live plants, and preserved specimens from herbarium collections. The maintained floral distinctiveness in pressed samples could be useful to land managers lacking the time for field measurement and calculations. Suspected hybrid specimens can be pressed and measured later with maximum accuracy, and removal of the marked plant done another day. Due to the high genetic variation of CALE, it may be prudent to establish each population's normal range of floral measures. The control CALE of this study had bract lengths that ranged from 2.56 cm in herbarium specimens to 2.94 cm in live plants, and lobe lengths of 0.70 cm and 0.89 cm in herbarium and live plants respectively. Changes

to lengths from the drying process may occur and could be accounted for. The control CALE lobe-bract ratios were 0.273 and 0.301 and the floral angles 69.6° and 74.8° for herbarium and live plants respectively. CAHI measures ranged from 2.28 cm and 2.32 cm herbarium and live bracts, with 0.96 cm and 0.96 cm lobe lengths, and 0.412 and 0.389 lobe-bract ratios respectively. The CAHI floral angles were 62.3° and 64.3° for herbarium and live plants. Lobe lengths did not differ between live plants of the *Castilleja* species, though they did for herbarium plants. The lobing is probably kept at the same proportions by genetic control and its length would depend on the bract's length. Beak length and beak-galea ratios could also be used to distinguish CALE from CAHI, though herbarium specimens could not be measured due to the galeas hidden behind the bracts. In this study the beaks but not galeas differed between CALE and CAHI with CALE having 0.77 cm beaks and CAHI 1.06 cm beaks on average. Their beak ratios were distinguishable at 0.29 for CALE and 0.39 for CAHI. These floral measures create an easily calculable way to distinguish CALE from CAHI, even without requiring damaging the plants, in the field or from collected specimens for lab measurement. The cost of these measures would be negligible, if any, compared to the cost and time required for genetic analysis.

Pollen viability was also different between the species but would be less likely to be a reliable identifier than an indicator of general plant fitness. CALE did have lower viability in both tests than CAHI but several of the CALE controls had very low percent viabilities. Whether these could be considered outliers or showing a natural high variation in pollen viability would require more sampling. Though the on-site controls

also had some variation, they were higher in viability for both tests, yet still lower than CAHI. CALE plants kept their viability up more than CAHI when grown in different contexts though. If CALE populations do have lower overall pollen viability than CAHI, this would have reproductive implications where the plants could interbreed. Lower viability would mean a decreased competitive capacity between the species pollen. It could also contribute to the reason CALE is rare and the very similar CAHI is not.

Patterns of variation by growing context

Pollen viability decreased quickly the lower flowers were on the stem, which coordinates with pollinator observations of primarily visiting the upper-most flowers on each stem (discussed in Chapter 1). This indicates a short distance of stem that a pollinator will be able to visit and pick up reproductive pollen to then transfer to flowers of the next plant, likely within the same short stem distance where flowers are viable, but to a receptive stigma. This short stem interval is also indicated from the pollinator foraging pattern, as they only foraged from a few flowers at the apex of the stem, moving down to the next whorl from an upper-most whorl, as discussed in the first chapter of this study.

Pollen viabilities, and floral characteristics, had similar patterns of variation across the plot contexts as the seed characteristics (Chapter 1). The highest pollen viabilities were from solo-context plants and lower from mixed-context (though the lowest from semi-solo context plants), the floral ranges became more similar between the species when grown in mixed-plots, and seed counts and germination were highest from solo-context plants. These all support the hypothesis that the growth context of the plants influences

their reproductive responses and potential to hybridize. The plot characteristics also changed between solo and mixed contexts, with a decrease in floral density, and total plant number of each species, as did pollinator activity, with bees spending less time foraging on mixed-context flowers and visiting less plants (Chapter 1). The seed production loss is likely a direct effect of the decreased pollinator activity, but the seed germination and floral changes of the F1 offspring may be from the more complicated interactions of interbreeding, or general fitness loss from less generally conspecific pollination. Successful geitonogamous pollination can be achieved in a self-incompatible species when the pollen is mixed with an unrelated species (Rieseberg et al. 1998), making it possible that pollen viability and seed germination decreases could be from an inbreeding effect allowed by the mixing of pollen via low constancy pollinators. Plants with low seed germination in this study were rarely from the same maternal plant as F1 plants exhibiting outlying floral measures and low pollen viabilities. However, most of the WR seeds with low germination were from maternal plants that also produced F1 offspring with low pollen viabilities, and many of the WR CAHI with outlying floral measures as well. Since their floral measures were also altered, and several likely hybrids were identified from these WR CAHI F1 groups, the low seed germination and pollen viability of the WR CAHI are likely due to hybrid offspring present.

The sites did differ in their occurrence of low pollen viability plants and floral measure outliers, likely due to the site differences overall since WR had less *Castilleja* plants and flowers, and fewer pollinators. The pollinators were more constant at WR, tending to visit CAHI more, and the proportion of WR CAHI that are likely to be hybrids was lower

than those CAHI at GH. However, more CALE at WR were likely to be hybrids, with only three at GH. GH bees did favor CALE, though exhibiting low constancy overall. This possibly helped reduce the creation of hybrids in the CALE population but not the CAHI, which received very few consecutive visits from bees, so that most of their received pollen was from a CALE plant. The reverse was the case at WR, with few consecutive CALE visits, bringing primarily CAHI pollen to the occasional CALE plant visited. Paired with CAHI's higher pollen viability, this would have created a significant hybrid population in WR's CALE, despite fewer CALE transitions at the site.

The low constancy of GH's bees would be expected to create a roughly equal hybrid population between the species, if the bees are able to pick up and deposit each *Castilleja* species' pollen equally. This may not be the case due to the differences in galeas, and thus probable differing positions of the stigma and stamens. Stamen and stigma relationships could be studied, and pollen transfer tests conducted to assess this. It could also be that the reproductive barriers of each species are different, if pollen is roughly equally transferred. Other *Castilleja* species have been found to differ in their reproductive barriers, with the direction of the pollen movement affecting the production of hybrids (Hersch-Green 2012). The lower effect of mixed-species contexts on CALE than CAHI could be from a stronger reproductive barrier in CALE than in CAHI, allowing for stronger selection against CAHI pollen on a CALE flower, and weaker on CAHI flowers receiving CALE pollen. The high incidence of yellow coloration in the F1 CAHI population, despite a predominantly orange parent population, is a strong indicator of CALE-sired hybrids from maternal CAHI, at both sites. Similarly, non-yellow F1

offspring from maternal-CALE was rarely found, despite the 13 plants from WR with low pollen viability and outlying floral measures. This collective evidence indicates that of the 48 plants identified as likely hybrids, making up 16.9% of the mixed-context plant population, most were sired by CALE rather than CAHI, at 67% of the hybrids. Of the GH population, where constancy was low, 85% of the hybrids were sired by CALE. While any hybridization could be detrimental to either or both *Castilleja* species, particularly because the presence of intermediate plants like hybrids reduces constancy to create a positive feedback of hybridization, at least the endangered CALE population experienced less genetic intrusion from CAHI. In terms of CALE conservation, this is a positive sign that hybridization will affect CALE less than CAHI.

The discontinuity between F1plants with outlying floral measures and F1 plants with low pollen viability can be due to a variety of factors influencing both. Low pollen viability can be caused by other physiological issues affecting male fecundity, as well as environmental influences. Some F1 flowers' development could have been affected by the greenhouse conditions. The onset of winter completely halted blooming in the plants, despite the addition of extra lights, and flowers measured during this transition could have developed abnormally. It could also be possible that some hybrid plants could have normal pollen viability, or the tests used here could not detect the decrease after hybridization for all crossing genotypes. The viability tests of the earlier hybrid study investigating the effects of hand-crossed pollinations use a different staining method than acetocarmine and no pollen tube germination (Kaye & Blakeley-Smith 2008). While multiple measures of hybrid detection are more likely to identify the hybrids among a

population, they are not a guarantee for complete detection. The PTG tests were more sensitive to changes in the plant's population than ACS, which is expected due to the nature of stains tending to be misrepresentative of actual pollen viability (Soares et al. 2013; Pline et al. 2002). However, *in vitro* pollen viability tests like germination only show one side of the reproductive equation for the F1 generation. The female sporophyte is not accounted for, as it is more labor intensive to assess, but female choice for different pollen phenotypes and female receptivity can also influence reproductive viability of hybrids and pure-breeding plants (Real 1983). It could be that plants with other indicators of hybridization, particularly flower measurement changes, but lacking the characteristic decrease in male fecundity, could have an altered female fecundity in stigma receptiveness or weaker reproductive barriers.

Bract colorations of extant mature plants, and a significant number of various maternally unrelated F1individuals, expressed the multiple color hues across the bract noted present in hybrids and rarely in pure-breeding plants in Hersch-Green and Cronn's study (2009). Only in the F1 offspring of two maternal-CAHI, did this multi-colored bract morph line up with being likely hybrids. The maternal CAHI (GH16 H3 and GH16 H4) for these yellow-orange offspring were both orange in flower color. Both of them were, however, in close proximity to one or more CALE, though one was outside the 30 cm distance for mix-C interspecies distance category (Figure Appendix A.6). Because this yellow-orange coloration was seen in the parent generation of plants on the sites, it is possible this pattern is not identifying as hybrids, or that these plants were already establish hybrids,

possibly even from a contaminated seed stock. This is another reason why off-site controls for both species were used.

Conclusion

Castilleja hybridizing behavior depends on their pollinators, being exclusively insect pollinated and self-incompatible, and on their growing conditions, particularly the distance between plants of different species. Distance between species had the clearest effect on *Castilleja* reproduction and putative hybrid production, with no evidence found for flower color influencing crossings, as indicated by pollinator activity (Chapter 1). Since flower color does overlap slightly between these species, with some yellow CAHI morphs, and the bees favoring yellow CALE rarely visited a yellow CAHI when making the species transition, color is not likely the trait bees are using to make their forage choices. Nectar quantity and/or quality is more likely influencing their selection (Real 1983; Richards 1997), and *Castilleja* species do differ in nectar properties (Hersch & Roy 2007). Pollinator observations later in the season, or in warmer years when workers emerge sooner and have more experienced individuals to learn a preference from, may show different constancies and possibly a color choice used, but more study would be needed.

Floral measurements separated the species from each other clearly, but with only a small separation between them, with some measurement range overlap at extremes. This would make clear identification of intermediate flower measurements, as hybrids, more difficult. For more precise identification, other measures, like pollen viability or the possibility of

measurements that are less variable in conspecifics, is recommended. Measurement data could also be tested by genetic analysis for precise estimation of its accuracy. While the species did differ in pollen characteristics, with CALE having generally lower viability, and pollen tube germination probably giving a more accurate measure, the ranges varied for CALE and decreased viability can be a symptom of a factors other than hybridization. Hybrids should be estimated not by viabilities lying between those of the parent species, but rather a significant decrease in viability. However, accuracy should be assessed for several tests on multiple known hybrids to better understand the fecundity alterations by hybridization. There are more aspects of the complicated reproductive interactions between these species and the effects on their offspring that can be investigated.

Implications for Practice

- Field-measurable hybrid identifying traits can be used to identify and remove hybrids and prevent establishing hybrid zones.
- Probable lower pollen viability in CALE could be a factor in its low competitive ability and rare status, though a large sample size for further testing of their viability is recommended, due to high variation.
- The lower occurrence of maternal-CALE hybrids may indicate a competitive edge over CAHI in the form of an stronger reproductive barrier to hybridization than CAHI, or morphology differences that decrease the likelihood of pollinator pollen transfer from CAHI to CALE, but not CALE to CAHI.
- The plants can likely grow sympatrically in the same site with minimal crossing, as long as distances are more than 50 meters between the specie, as

the on-site control plants had no recorded hybrids and were potentially growing within 50 meters of the nearest other species.

- Where already in a mixed growing context, the CAHI should be removed, at least to a further distance, and any intermediate flowers tested for hybrid traits, and removed when confirmed.
- Site characteristics will influence the occurrence of hybrids, potentially through changes to pollinator constancy.

Hybridization is a naturally occurring process that may have aided the diversification of the *Castilleja* species we have today. If the hybridization populations are large enough to resist the stresses of hybridization, then it would not pose a problem and could even create a novel species in time, possibly filling a niche opened by climate shifts or anthropic disturbance. However, until the stable recovery of *Castilleja levisecta* is achieved, prevention of hybridization *with C. hispida* could safeguard the species from reduced fitness effects of hybridization and even potential population loss. Since bees are the vectors of interspecies pollination, and little analysis of their activity has been done for either species, more study of their behavior and selection criteria for *Castilleja* foraging could help increase the success of active and new restoration sites. Restoration for *C. levisecta* should be aimed towards attracting its pollinators, in addition to increasing *C. levisecta* numbers, keeping in mind the factors that influence interspecies crossing if *C. levisecta* and *C. hispida* are both present.

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Appendix A
Additional Figures

Glacial Heritage BC foraging example:

species	flr 1	flr 2	flr 3	flr 4	flr 5	flr 6	flr 7	flr 8	flr 9	flr 10	flr 11	flr 12	flr 13	flr 14	flr 15	flr 16	flr 17	flr 18	flr 19	flr 20	flr 21
BC	H-O	L	H-DO	H-O	L	L	H-O	H-R	L	H-O	L	L	L	L	L	L	H-DO		H-O	L	H-O\
time:	20	20	5	20	10	30	40	30	2	3	35	5	35	5	25	20	10		2	3	
BC	L	H-O	H-O	L	L																
time:	5	5	20	20	5																

West Rocky BC foraging example:

species	flr 1	flr 2	flr 3	flr 4	flr 5	flr 6	flr 7	flr 8	flr 9	flr 10
BC	HI	HI	HI	HI	L	HI	HI	HI	HI	HI
time:	10	8	12	3	10	15	6	3	7	32
BC	H-O	H-O	H-S	passed 3 lev without stopping						
time:	15	55	3							

Figure Appendix A.1. Representative examples of pollinator foraging patterns at GH and WR, showing different *Castilleja* recipients as CALE, "L", and CAHI, "H" with color classes for CAHI. Time spent per raceme is below each species designation.

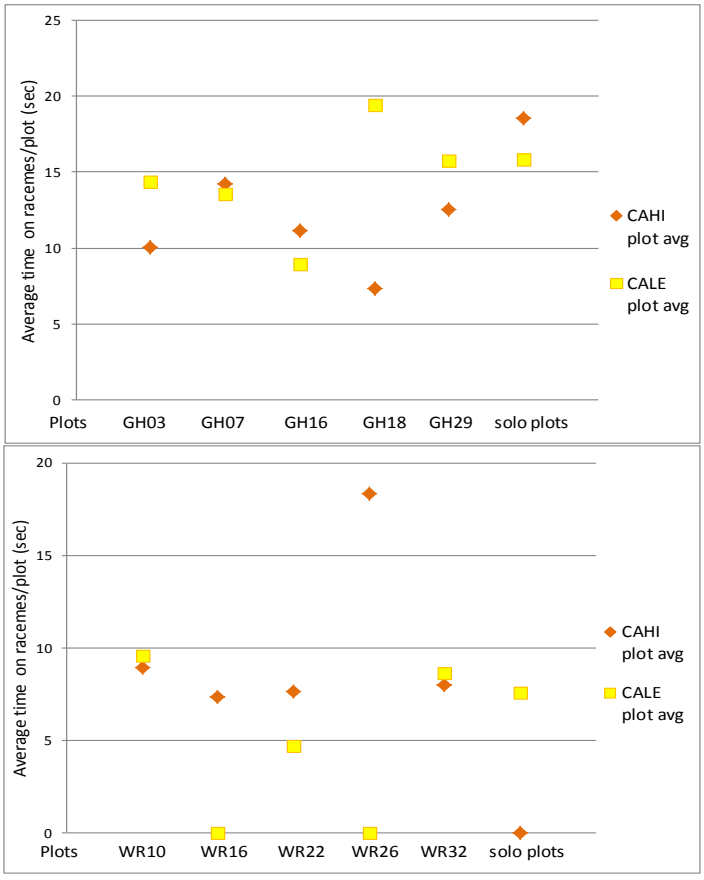


Figure Appendix A.2. Plot averages for bee visit durations, showing the variation for the mean time bees spent on racemes in each plot. GH plots (top) and WR plots (bottom) shown.

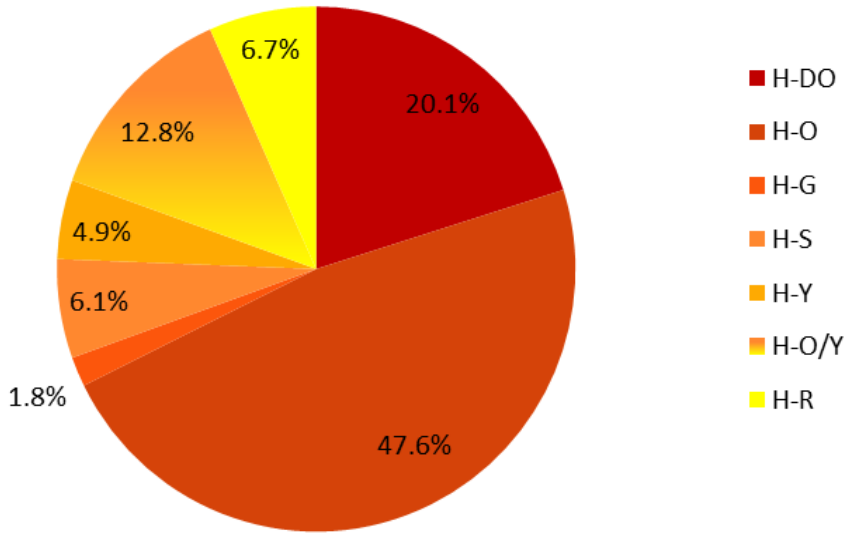


Figure Appendix A.3. Color distribution for all parent generation CAHI of both sites Color designations, from top to bottom, are dark orange, orange, golden, salmon, yellow, yello-orange and red.

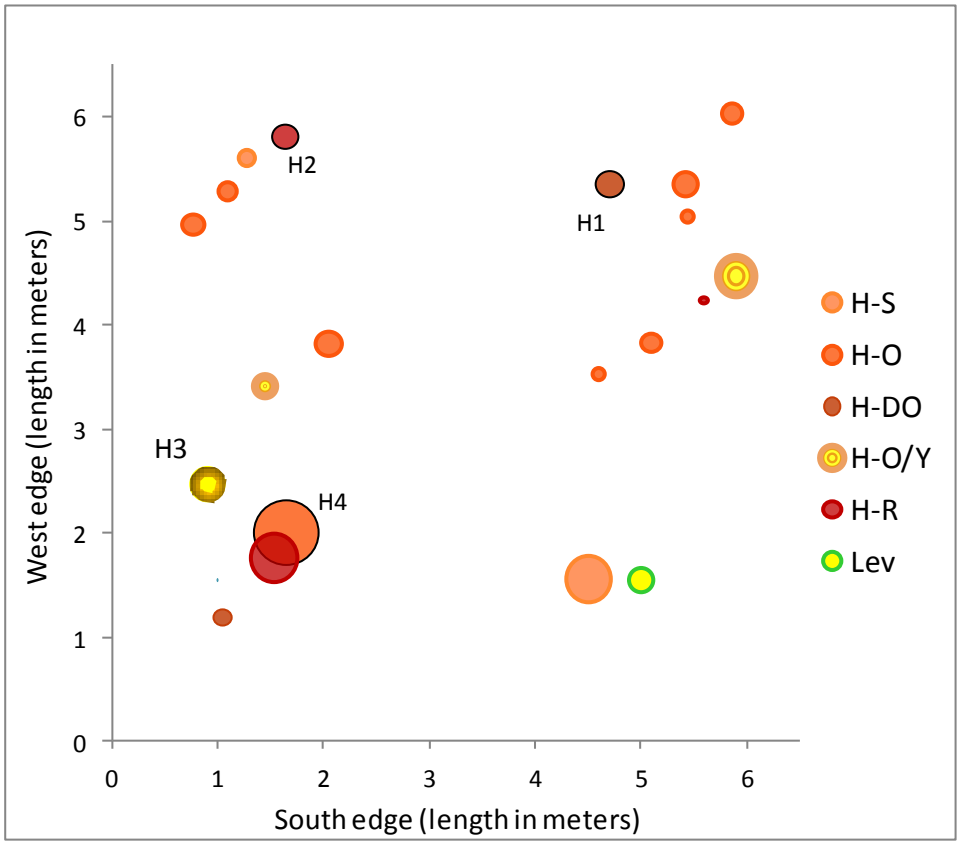
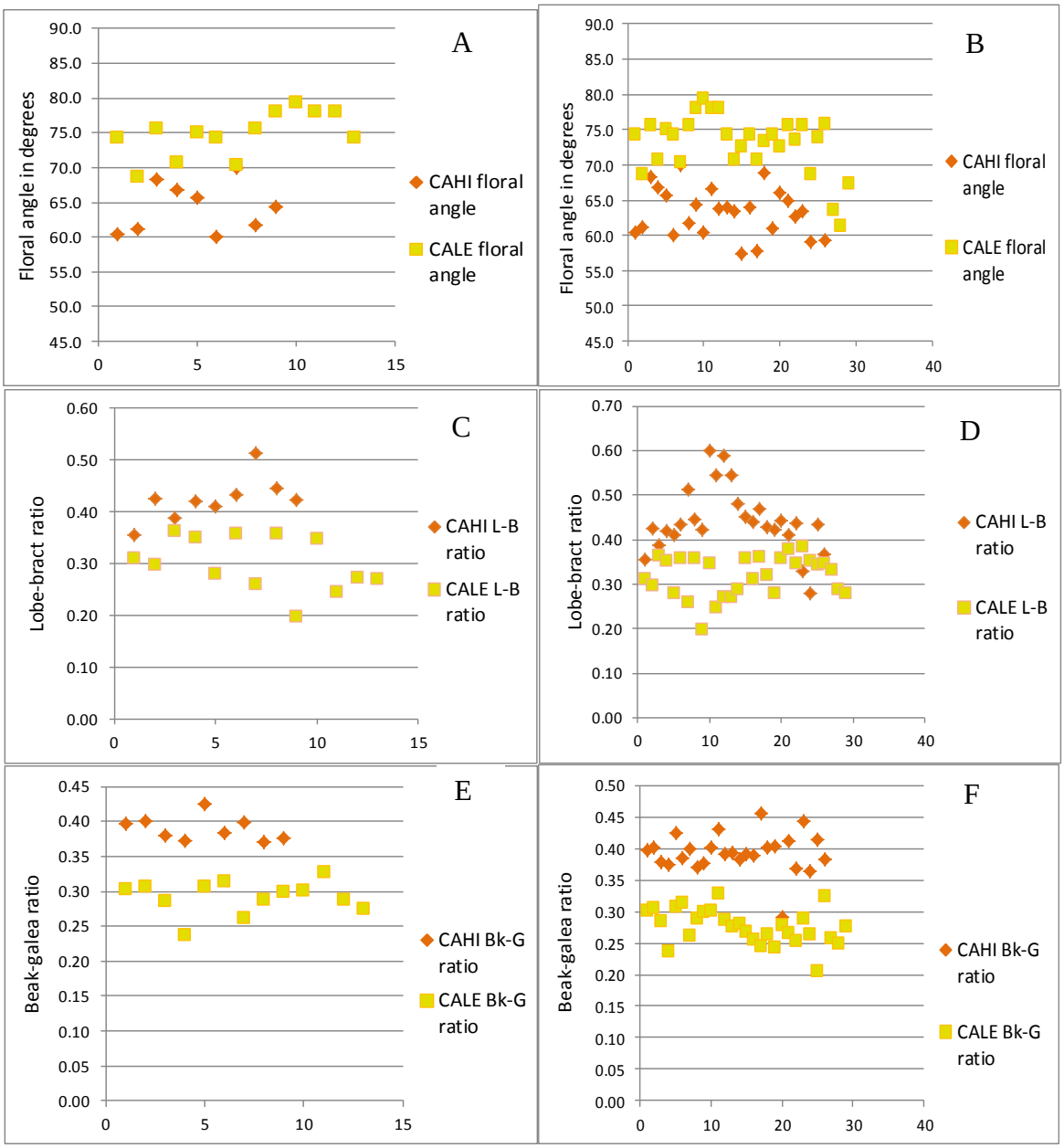


Figure Appendix A.4. Plot map of the unusual WR06 semi-solo CAHI plot, with a single CALE present, and a high proportion of yellow and yellow-orange F1 color variants.



Appendix A.5. Graphs of floral measures for off-site controls only (A, C, and E) and all control plots, including solo-species plots (B, D, F). Floral angles for these controls- top, lobe-bract ratios- middle, and beak-galea ratios- bottom.

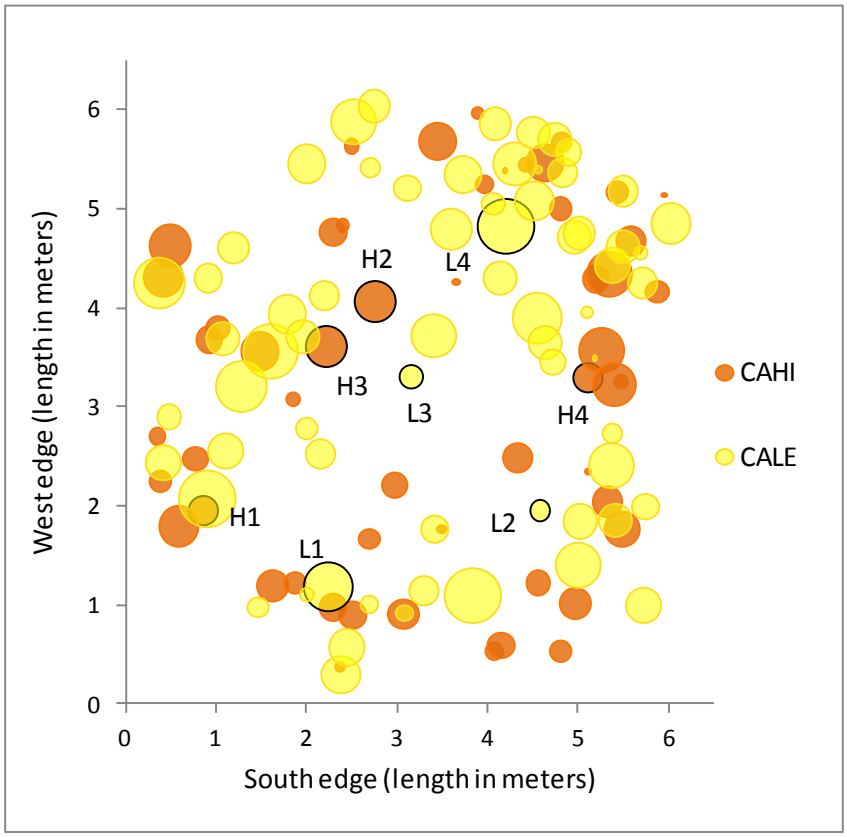
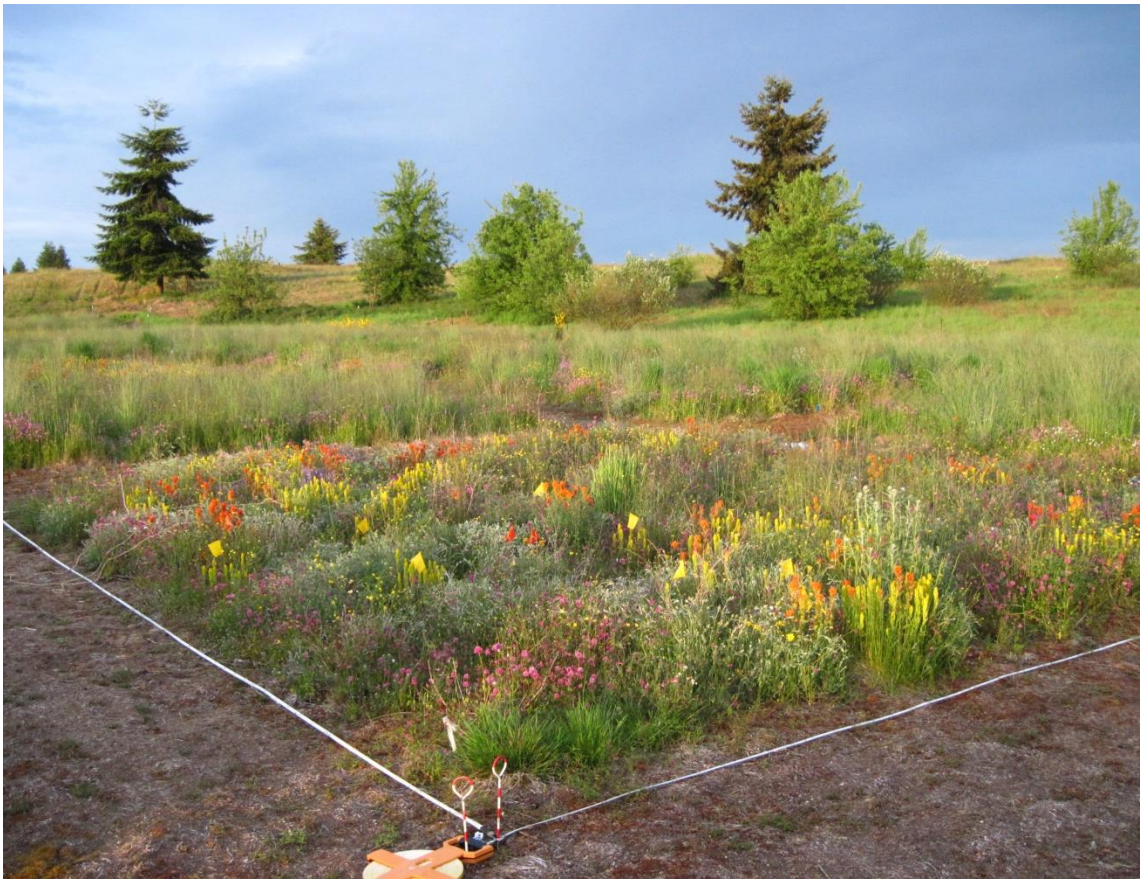


Figure Appendix A.6. Plot map for GH16, showing the plant numbers for all those that were analyzed in the F1 generation, and the relative size of each plant. GH16 had the most identified hybrids of all plots, with three of the CAHI producing the hybrids (H1, H3, and H4).

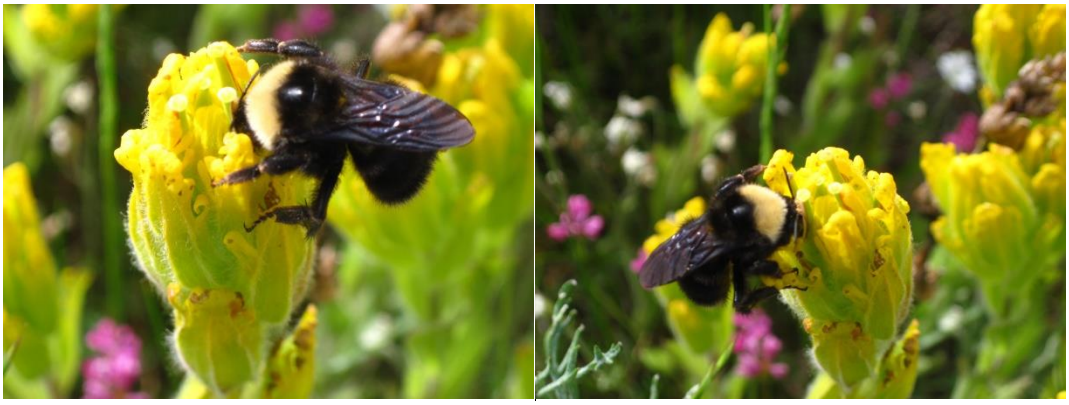
Table Appendix A.7. Plot numbers and treatments for plots used in this study. Burn treatment indicates plot was burned before seeding. Seed mix indicates if the mix was forb-rich, grass-rich, or an intermediate 'mix' level of forbs and grasses.

Glacial Heritage Plots	Pretreatment	Seed mix	West Rocky Plots	Pretreatment	Seed mix
GH03	Herbicide	Forb	WR10	Burn	Mix
GH07	Herbicide	Forb	WR16	Burn	Mix
GH16	Herbicide	Forb	WR22	Burn	Forb
GH18	Herbicide	Forb	WR26	Burn	Mix
GH29	Herbicide	Forb	WR32	Burn	Forb
GH21	Burn	Forb	WR06	Solarize	Mix
GH26	Burn	Mix	WR14	Herbicide	Forb
GH scaled up North #2 (GHSN2)	Burn	Forb	WR35	Herbicide	Forb

Appendix B
Photographs from field sites and experiments



Appendix B.1. Glacial Heritage mixed-species context plot with visible CALE and CAHI together and prominent among the plot population.



Appendix B.2. *Bombus californicus* queen foraging on apical flowers

Figure Appendix B.3. Pink variation of CALE at GH site.

Figure Appendix B.4. Largest recorded CALE on the sites, at GH, with 82 flowering stems.

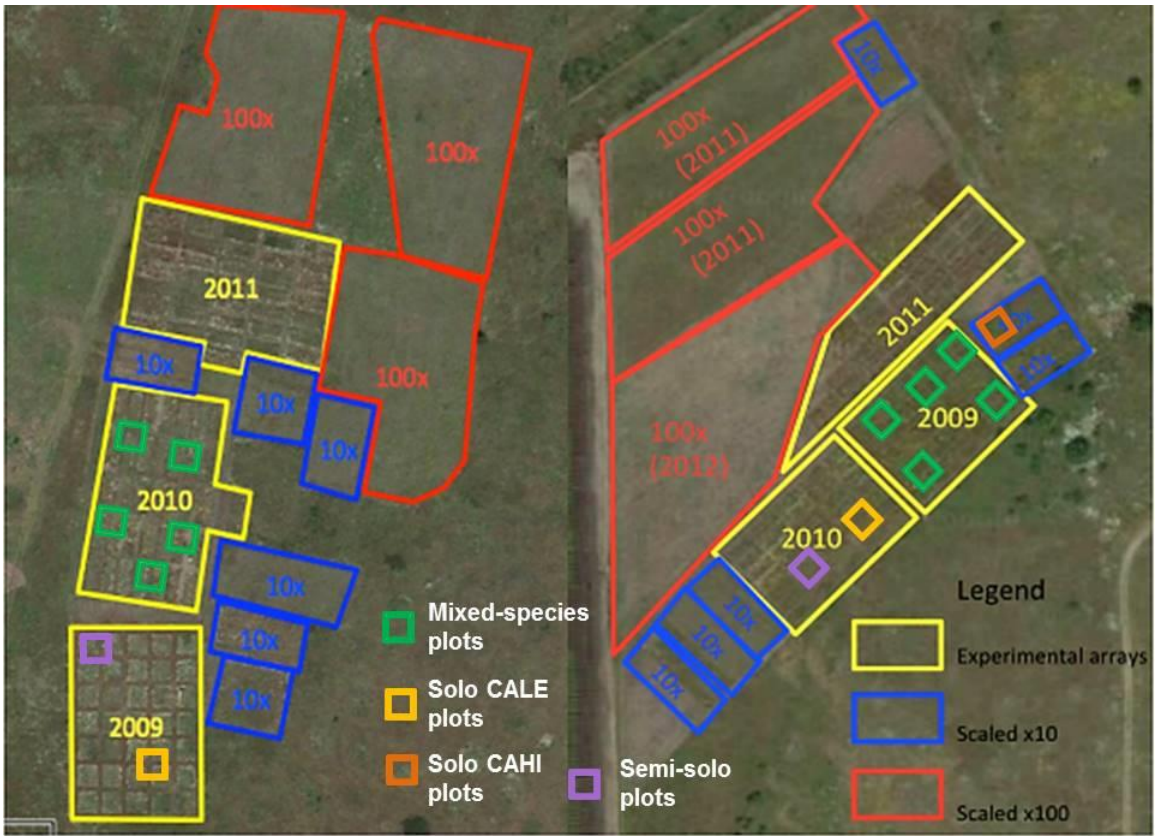


Figure Appendix B.5. Site maps by Eric Delvin, showing his array set up, and year each section was established, with stars denoting general locations of plots used in this study. WR on left and GH on right

Figure Appendix B.6. Seed capsules on the stem of a *Castilleja* during seed collection from tracked plants. Removed capsule is marked with red ink, and represents a bottom-capsule.

Appendix C
F1 Generation Photos

Figure Appendix C.1. The novel variegated individuals from the F1 generation. Picture A and B are from the same plant with variegation, and picture C-F are from the variegated stem of plant #WR10 H1B 6. The normal green stems can be seen in C and D.

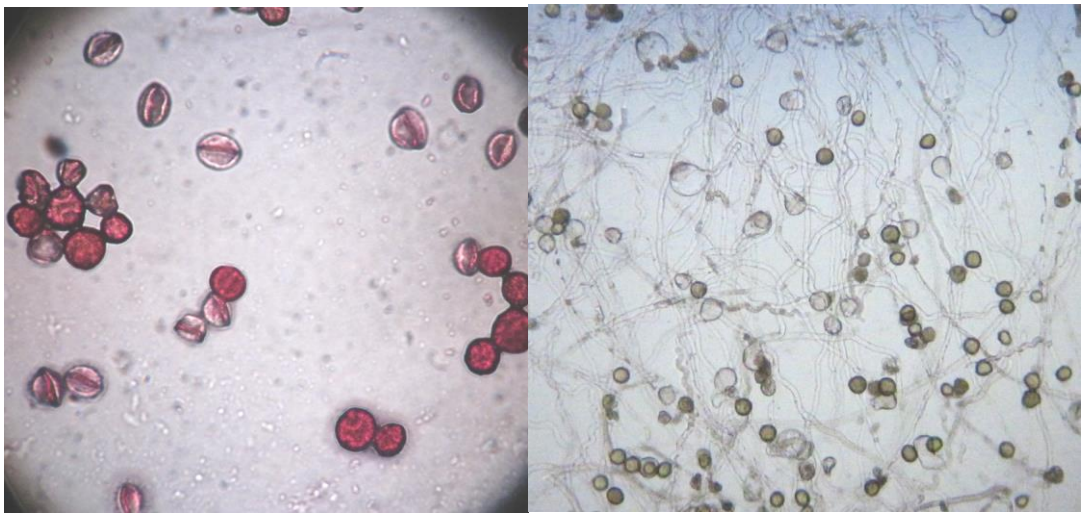
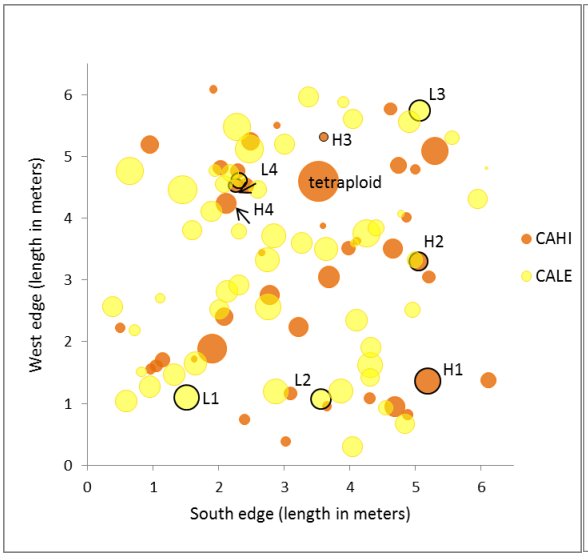


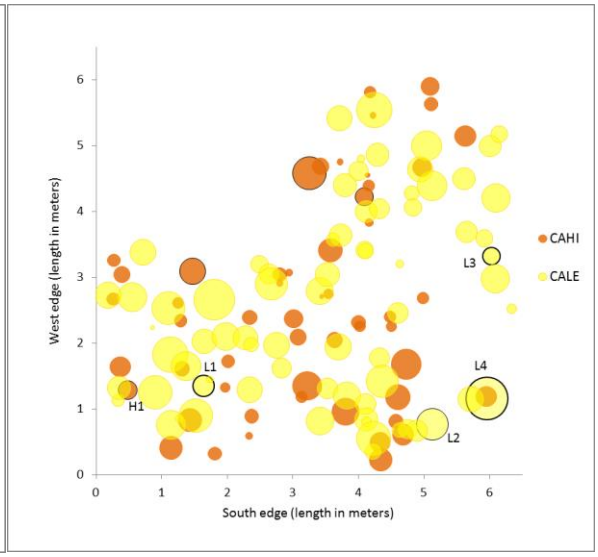
Figure Appendix C.2. Acetocarmine stained pollen (left), showing non-viable clear grains distinctly from the stained viable grains, and *in vitro* germinated pollen tubes (right), showing lighter germinated and darker non-germinated grains.

Figure Appendix C.3. Untreated powdery mildew infection of a F1 *Castilleja* growing in the research greenhouse, showing near complete leaf cover by the fungus.

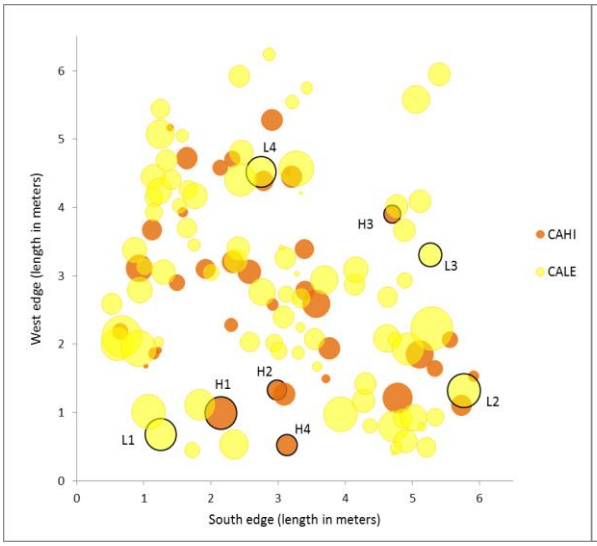
Appendix D
Additional Plot Maps



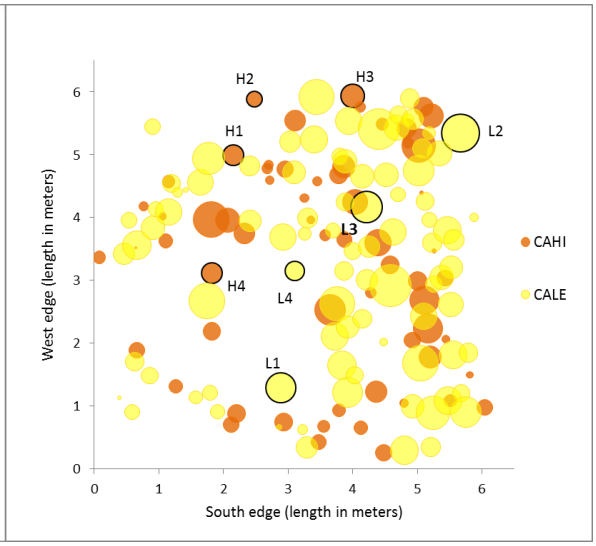
GH03 mixed-species plot



GH07 mixed-species plot

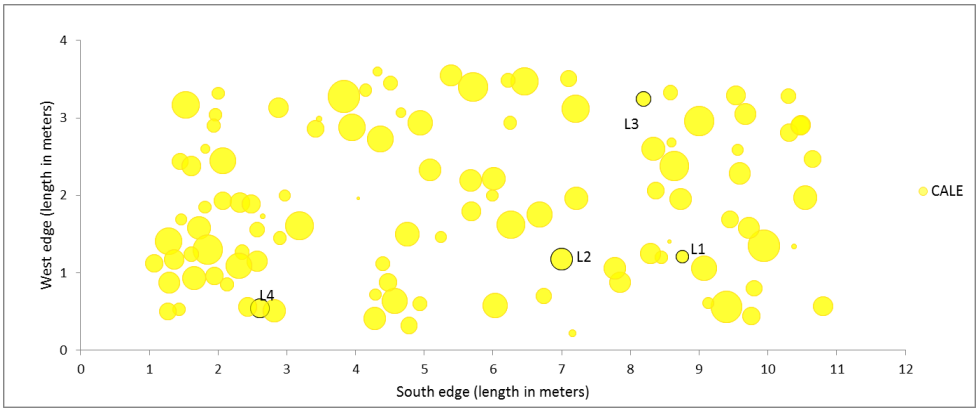


GH18 mixed-species plot

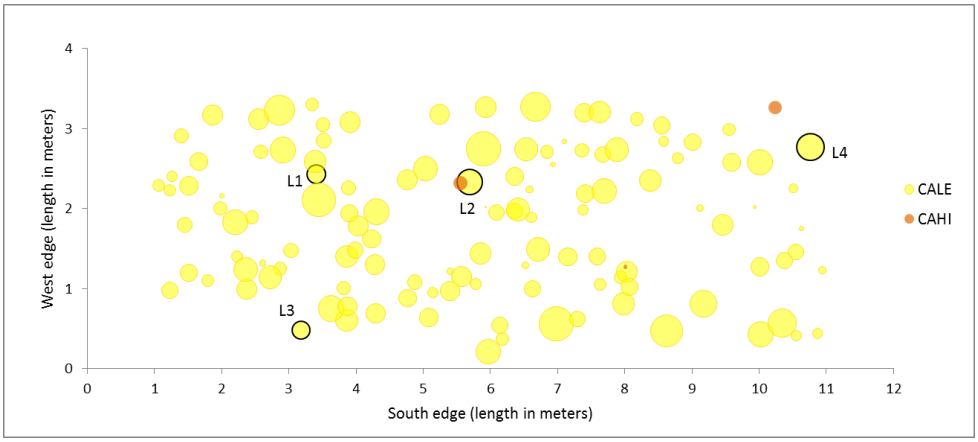


GH29 mixed-species plot

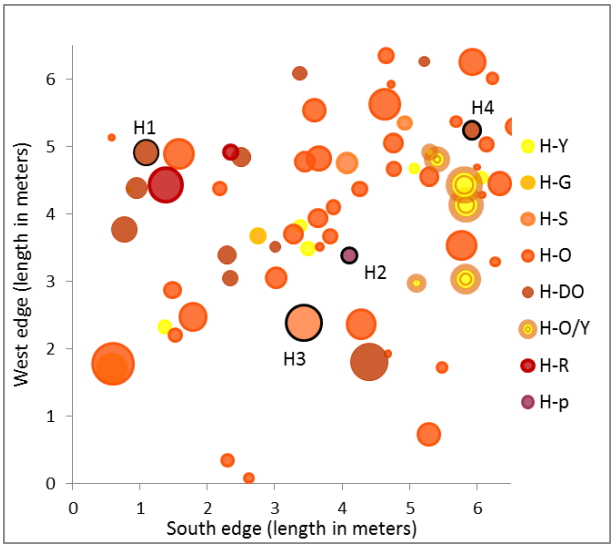
GH 16 plot is in Appendix A.



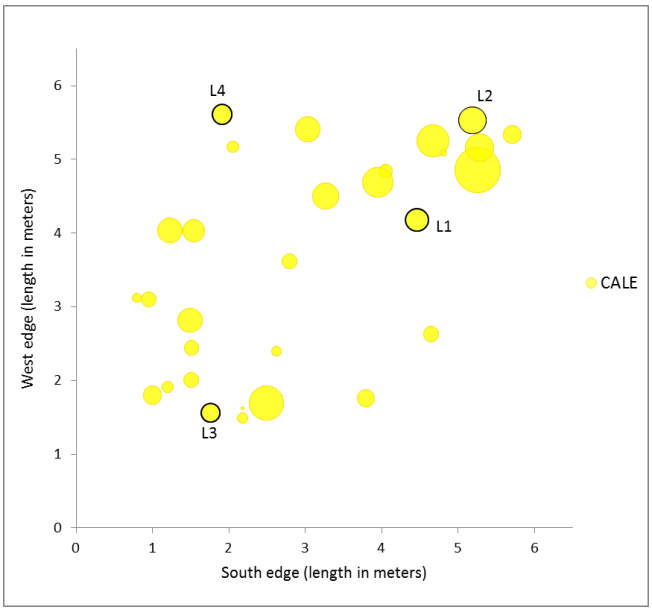
GH21 solo-CALE plot



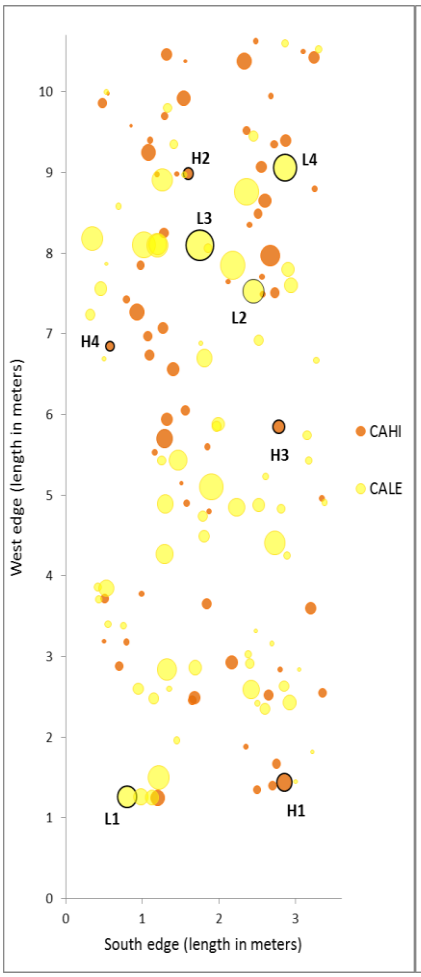
GH26 semi-solo plot



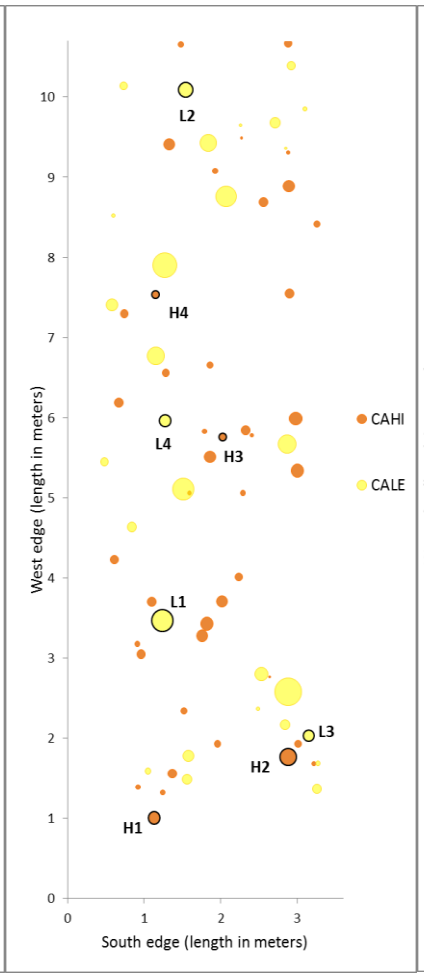
GHS-N2 (scaled-up north #2) solo-CAHI plot



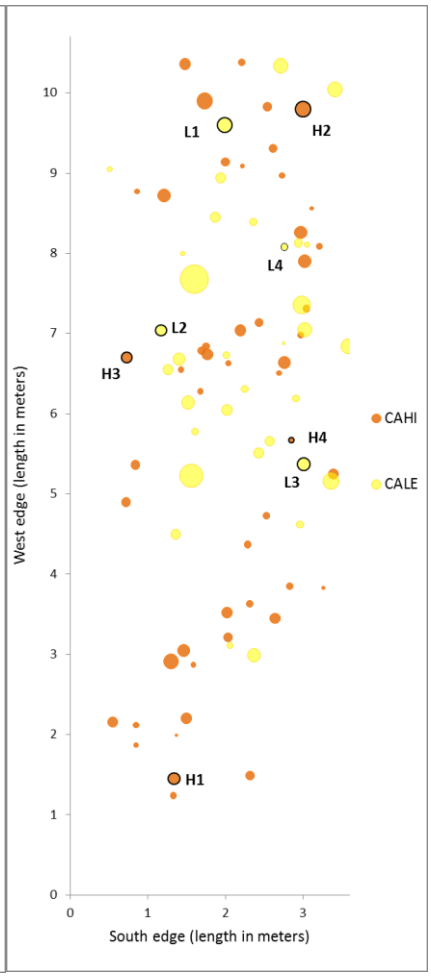
WR14 solo-CALE plot



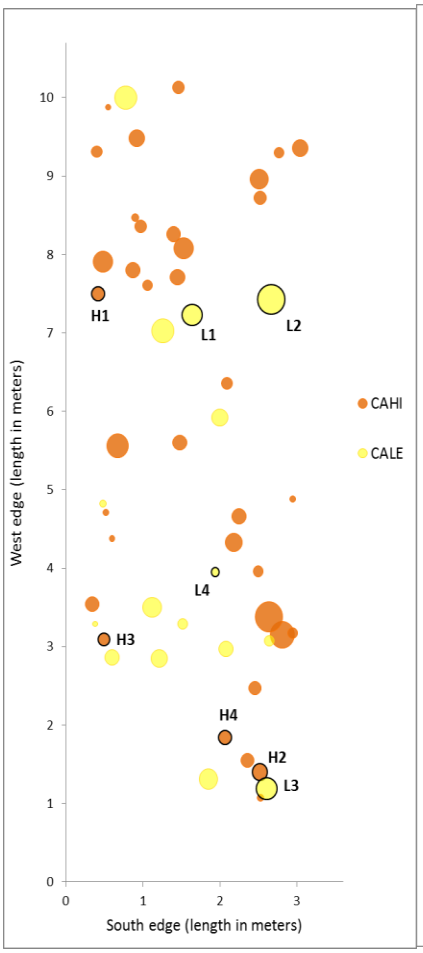
WR10 mixed-species plot



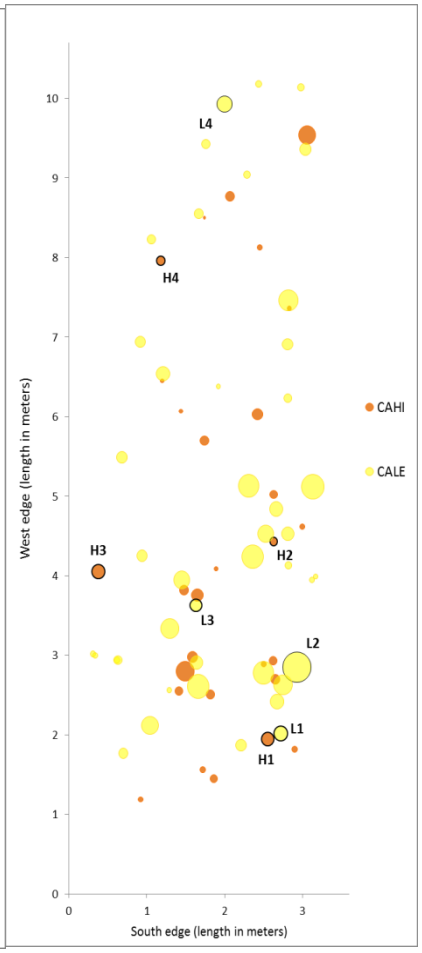
WR16 mixed-species plot



WR22 mixed-species plot



WR 26 mixed-species plot

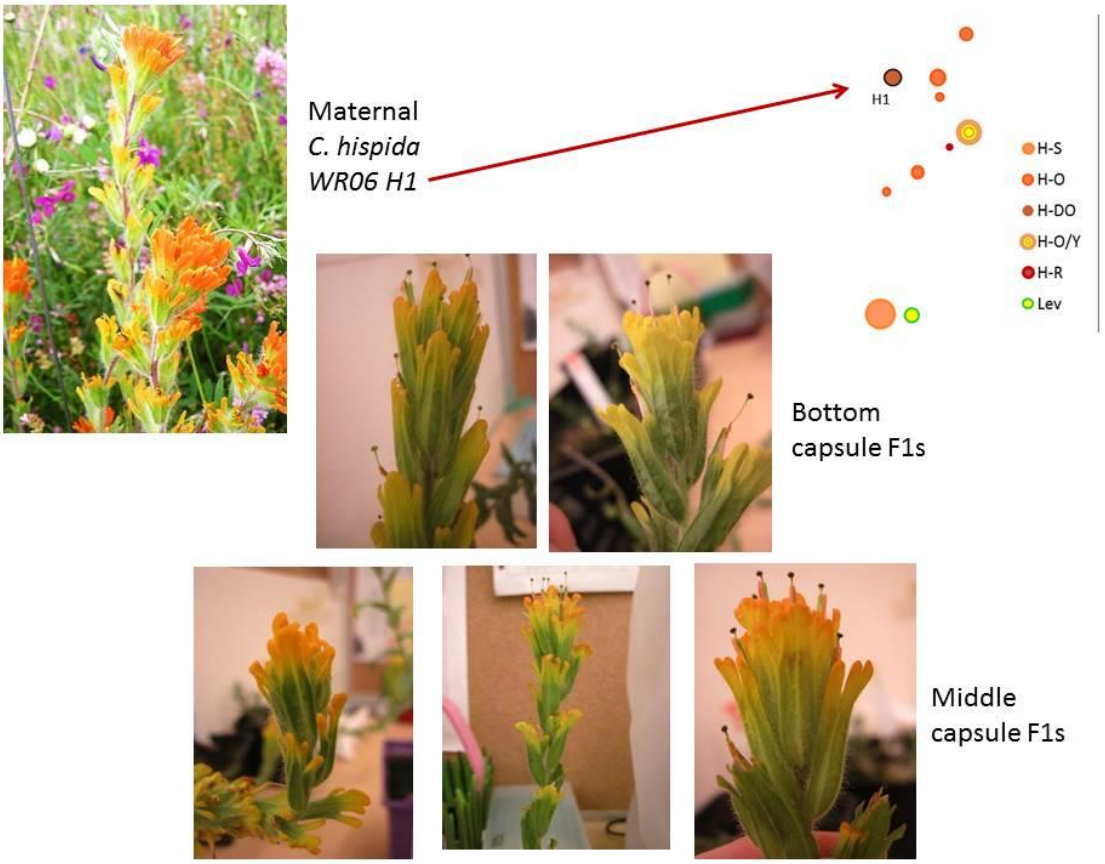


WR32 mixed-species plot

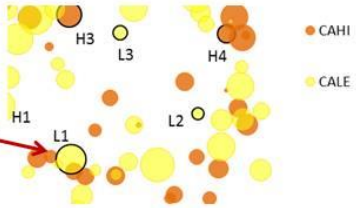
WR06 semi-solo CAHI plot is in Appendix A.

Appendix E
Family Trees of Hybrids

Family trees show maternal CALE or CAHI plant in top left corner with its offspring below and to the right. A plot map shows the maternal plant's location and the *Castilleja* plants nearby. Offspring are divided by capsule level, with those from the bottom capsule, produced first in the season, above those from the middle capsule, produced later when more CALE and CAHI blooming overlap was occurring.



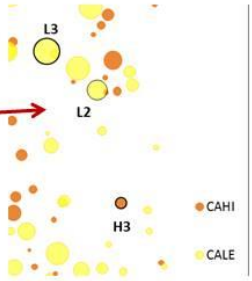
Maternal
C. levisecta
GH16 L1



Bottom
capsule F1s

Middle
capsule F1s

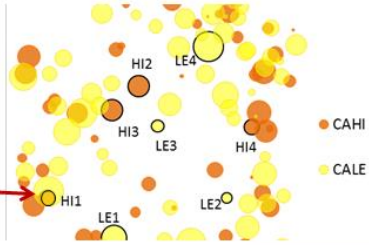
Maternal
C. levisecta
WR L3



Bottom
capsule F1

Middle
capsule F1s

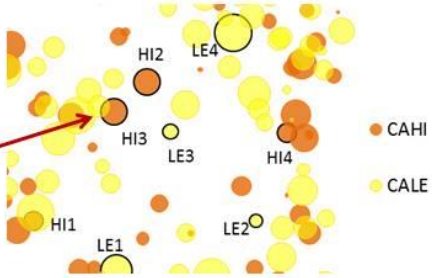
Maternal
C. hispida
GH16 H1



Bottom
capsule F1s

Middle
capsule F1s

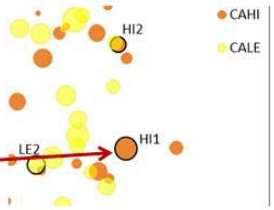
Maternal
C. hispida
GH16 H3



Bottom
capsule F1s

Middle
capsule F1s

Maternal
C. hispida
GH03 H1



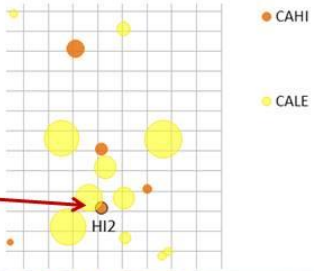


Bottom
capsule F1s



Middle
capsule F1s

Maternal
C. hispida
WR32 H2





Bottom
capsule F1s



Middle
capsule F1s

Appendix F

Data

Plot characteristics

Site	Plot	Species	context	Floral density/ plot (#/sq.m.)	Total # plants/ plot	Avg # stems/ plant
GH	GH21	CALE	solo	31.91	111	11.77
GH	GH26	CALE	solo	37.67	123	12.38
GH	GH29	CALE	mixed	26.15	89	12.03
GH	GH18	CALE	mixed	29.08	84	14.18
GH	GH16	CALE	mixed	22.20	66	13.82
GH	GH07	CALE	mixed	22.51	73	12.63
GH	GH03	CALE	mixed	21.29	54	16.15
GH	GHSN1	CAHI	solo	11.38	76	6.13
GH	GHSN2	CAHI	solo	11.38	71	6.56
GH	GH29	CAHI	mixed	8.50	61	5.61
GH	GH18	CAHI	mixed	6.05	37	6.70
GH	GH16	CAHI	mixed	9.64	54	7.31
GH	GH07	CAHI	mixed	5.49	53	4.17
GH	GH03	CAHI	mixed	6.08	40	6.23
WR	WR35	CALE	solo	10.47	107	10.47
WR	WR14	CALE	solo	5.49	30	5.49
WR	WR10	CALE	mixed	4.88	75	2.67
WR	WR16	CALE	mixed	3.1	29	4.38
WR	WR22	CALE	mixed	2.27	34	2.74
WR	WR26	CALE	mixed	0.93	16	0.93
WR	WR32	CALE	mixed	3.93	43	3.93
WR	WR10	CAHI	mixed	2.98	68	1.79
WR	WR16	CAHI	mixed	2.51	41	2.51
WR	WR22	CAHI	mixed	2.37	51	2.37
WR	WR26	CAHI	mixed	1.9	36	1.9
WR	WR32	CAHI	mixed	1.54	28	1.54
WR	WR06	CAHI	solo	1.59	19	3.59

Pollinator Observations

Site	plot	Bee #	Context	Recipient plant species	Avg sec/ raceme	Total sec on racemes	# each sp visited	% of Total time on plants	% of Total plants visited
GH	GH S-N	ghsn-1	solo	CAHI	15.00	60	4	20.3%	30.8%
GH	GH S-N	ghsn-2	solo	CAHI	15.83	95	6	39.6%	37.5%
GH	GH S-N	ghsn-3	solo	CAHI	50.00	100	2	60.6%	22.2%
GH	GH S-N	ghsn-4	solo	CAHI	32.50	260	8	67.4%	57.1%
GH	GH S-N	ghsn-5	solo	CAHI	21.67	130	6	53.1%	42.9%
GH	GH S-N	ghsn-6	solo	CAHI	25.00	250	10	52.6%	50.0%
GH	GH S-N	ghsn-7	solo	CAHI	30.29	515	17	92.0%	89.5%
GH	GH S-N	ghsn-8	solo	CAHI	15.00	30	2	6.4%	10.5%
GH	GH S-N	ghsn-9	solo	CAHI	7.50	15	2	50.0%	50.0%
GH	GH S-N	ghsn-10	solo	CAHI	17.50	35	2	70.0%	50.0%
GH	GH S-N	ghsn-11	solo	CAHI	16.67	50	4	45.5%	40.0%
GH	GH S-N	ghsn-12	solo	CAHI	5.75	23	4	14.1%	20.0%
GH	GH S-N	ghsn-13	solo	CAHI	12.14	85	7	70.8%	58.3%
GH	GH S-N	ghsn-14	solo	CAHI	18.75	270	15	94.7%	88.2%
GH	GH S-N	ghsn-15	solo	CAHI	14.82	163	11	56.6%	42.3%
GH	GH S-N	ghsn-16	solo	CAHI	15.00	30	2	100.0%	100.0%
GH	GH S-N	ghsn-17	solo	CAHI	7.50	15	2	100.0%	100.0%
GH	GH S-N	ghsn-21	solo	CAHI	13.57	95	7	100.0%	100.0%
GH	GH21	g21-1	solo	CALE	26.11	235	9	79.7%	69.2%
GH	GH21	g21-2	solo	CALE	14.50	145	10	60.4%	62.5%
GH	GH21	g21-3	solo	CALE	9.29	65	7	39.4%	77.8%
GH	GH21	g21-4	solo	CALE	21.00	126	6	32.6%	42.9%
GH	GH21	g21-6	solo	CALE	14.38	115	8	46.9%	57.1%
GH	GH26	g26-1	solo	CALE	22.50	225	10	47.4%	50.0%
GH	GH26	g26-2	solo	CALE	22.50	45	2	8.0%	10.5%
GH	GH26	g26-3	solo	CALE	25.76	441	17	93.6%	89.5%
GH	GH26	g26-4	solo	CALE	7.50	15	2	50.0%	50.0%
GH	GH26	g26-5	solo	CALE	7.50	15	2	30.0%	50.0%
GH	GH26	g26-6	solo	CALE	10.00	60	6	54.5%	60.0%
GH	GH26	g26-7	solo	CALE	8.75	140	16	85.9%	80.0%
GH	GH03	g03-1	mixed	CAHI	0.00	0	0	0.0%	0.0%
GH	GH03	g03-3	mixed	CAHI	15.43	108	7	46.8%	38.9%
GH	GH03	g03-4	mixed	CAHI	15.00	135	9	41.5%	45.0%
GH	GH03	g03-5	mixed	CAHI	9.67	58	6	32.2%	54.5%
GH	GH07	g07-1	mixed	CAHI	17.50	35	2	26.5%	28.6%
GH	GH07	g07-2	mixed	CAHI	13.33	40	3	57.1%	50.0%

GH	GH07	g07-3	mixed	CAHI	10.20	51	5	15.5%	25.0%
GH	GH07	g07-4	mixed	CAHI	17.50	35	2	83.3%	50.0%
GH	GH07	g07-5	mixed	CAHI	15.50	279	18	49.6%	45.0%
GH	GH07	g07-6	mixed	CAHI	31.50	252	8	50.5%	29.6%
GH	GH07	g07-7	mixed	CAHI	8.20	41	5	21.2%	29.4%
GH	GH07	g07-8	mixed	CAHI	0.00	0	0	0.0%	0.0%
GH	GH16	g16-1	mixed	CAHI	13.20	132	10	64.4%	52.6%
GH	GH16	g16-2	mixed	CAHI	10.00	20	2	100.0%	100.0%
GH	GH16	g16-3	mixed	CAHI	30.00	30	1	75.0%	50.0%
GH	GH16	g16-4	mixed	CAHI	12.50	25	2	45.5%	40.0%
GH	GH16	g16-5	mixed	CAHI	5.00	5	1	11.1%	25.0%
GH	GH16	g16-6	mixed	CAHI	5.25	21	4	100.0%	100.0%
GH	GH16	g16-7	mixed	CAHI	5.00	15	3	83.3%	75.0%
GH	GH16	g16-8	mixed	CAHI	8.33	25	3	83.3%	75.0%
GH	GH18	g18-1	mixed	CAHI	6.50	13	2	6.0%	11.8%
GH	GH18	g18-2	mixed	CAHI	0.00	0	0	0.0%	0.0%
GH	GH18	g18-3	mixed	CAHI	7.00	28	4	15.4%	25.0%
GH	GH18	g18-4	mixed	CAHI	10.00	50	5	11.7%	25.0%
GH	GH18	g18-5	mixed	CAHI	6.70	67	10	32.7%	52.6%
GH	GH18	g18-6	mixed	CAHI	12.50	75	6	24.8%	30.0%
GH	GH29	g29-1	mixed	CAHI	17.50	35	2	23.3%	40.0%
GH	GH29	g29-10	mixed	CAHI	5.00	10	2	50.0%	66.7%
GH	GH29	g29-2	mixed	CAHI	0.00	0	0	0.0%	0.0%
GH	GH29	g29-3	mixed	CAHI	0.00	0	0	0.0%	0.0%
GH	GH29	g29-4	mixed	CAHI	5.00	5	1	13.5%	20.0%
GH	GH29	g29-6	mixed	CAHI	3.00	3	1	100.0%	100.0%
GH	GH29	g29-7	mixed	CAHI	10.00	10	1	9.5%	33.3%
GH	GH29	g29-8	mixed	CAHI	6.00	30	5	23.1%	55.6%
GH	GH29	g29-9	mixed	CAHI	24.00	120	5	78.9%	50.0%
WR	WR10	w10-1	mixed	CAHI	3.50	7	2	31.8%	50.0%
WR	WR10	w10-2	mixed	CAHI	10.67	96	9	90.6%	90.0%
WR	WR10	w10-3	mixed	CAHI	0.00	0	1	0.0%	33.3%
WR	WR10	w10-4	mixed	CAHI	7.33	22	3	100.0%	100.0%
WR	WR10	w10-5	mixed	CAHI	25.00	25	1	100.0%	100.0%
WR	WR10	w10-6	mixed	CAHI	7.20	36	5	100.0%	100.0%
WR	WR16	w16-1	mixed	CAHI	2.00	9	3	100.0%	100.0%
WR	WR16	w16-2	mixed	CAHI	10.00	30	3	100.0%	100.0%
WR	WR16	w16-3	mixed	CAHI	10.00	40	4	100.0%	100.0%
WR	WR22	w22-1	mixed	CAHI	5.25	21	4	30.9%	44.4%
WR	WR22	w22-2	mixed	CAHI	10.00	10	1	100.0%	100.0%
WR	WR26	w26-1	mixed	CAHI	20.71	145	7	100.0%	100.0%

WR	WR26	w26-2	mixed	CAHI	10.00	30	3	100.0%	100.0%
WR	WR26	w26-3	mixed	CAHI	24.33	73	3	100.0%	100.0%
WR	WR32	w32-1	mixed	CAHI	3.50	10	1	100.0%	100.0%
WR	WR32	w32-2	mixed	CAHI	10.00	7	2	11.7%	28.6%
WR	WR32	w32-3	mixed	CAHI	10.50	50	5	31.8%	41.7%
GH	GH03	g03-1	mixed	CALE	3.50	7	2	100.0%	100.0%
GH	GH03	g03-3	mixed	CALE	11.18	123	11	53.2%	61.1%
GH	GH03	g03-4	mixed	CALE	18.50	190	11	58.5%	55.0%
GH	GH03	g03-5	mixed	CALE	24.40	122	5	67.8%	45.5%
GH	GH07	g07-1	mixed	CALE	19.40	97	5	73.5%	71.4%
GH	GH07	g07-2	mixed	CALE	7.50	30	3	42.9%	50.0%
GH	GH07	g07-3	mixed	CALE	18.60	279	15	84.5%	75.0%
GH	GH07	g07-4	mixed	CALE	3.50	7	2	16.7%	50.0%
GH	GH07	g07-5	mixed	CALE	12.86	283	22	50.4%	55.0%
GH	GH07	g07-6	mixed	CALE	13.00	247	19	49.5%	70.4%
GH	GH07	g07-7	mixed	CALE	12.67	152	12	78.8%	70.6%
GH	GH07	g07-8	mixed	CALE	20.71	145	7	100.0%	100.0%
GH	GH16	g16-1	mixed	CALE	8.11	73	9	35.6%	47.4%
GH	GH16	g16-2	mixed	CALE	0.00	0	0	0.0%	0.0%
GH	GH16	g16-3	mixed	CALE	10.00	10	1	25.0%	50.0%
GH	GH16	g16-4	mixed	CALE	10.00	30	3	54.5%	60.0%
GH	GH16	g16-5	mixed	CALE	13.33	40	3	88.9%	75.0%
GH	GH16	g16-6	mixed	CALE	0.00	0	0	0.0%	0.0%
GH	GH16	g16-7	mixed	CALE	3.00	3	1	16.7%	25.0%
GH	GH16	g16-8	mixed	CALE	5.00	5	1	16.7%	25.0%
GH	GH18	g18-1	mixed	CALE	13.60	204	15	94.0%	88.2%
GH	GH18	g18-2	mixed	CALE	35.00	35	1	100.0%	100.0%
GH	GH18	g18-3	mixed	CALE	12.83	154	12	84.6%	75.0%
GH	GH18	g18-4	mixed	CALE	25.13	377	15	88.3%	75.0%
GH	GH18	g18-5	mixed	CALE	13.80	138	9	67.3%	47.4%
GH	GH18	g18-6	mixed	CALE	16.29	228	14	75.2%	70.0%
GH	GH29	g29-1	mixed	CALE	38.33	115	3	76.7%	60.0%
GH	GH29	g29-10	mixed	CALE	10.00	10	1	50.0%	33.3%
GH	GH29	g29-2	mixed	CALE	3.00	3	1	100.0%	100.0%
GH	GH29	g29-3	mixed	CALE	4.00	8	2	100.0%	100.0%
GH	GH29	g29-4	mixed	CALE	8.00	32	4	86.5%	80.0%
GH	GH29	g29-6	mixed	CALE	0.00	0	0	0.0%	0.0%
GH	GH29	g29-7	mixed	CALE	47.50	95	2	90.5%	66.7%
GH	GH29	g29-8	mixed	CALE	25.00	100	4	76.9%	44.4%
GH	GH29	g29-9	mixed	CALE	6.40	32	5	21.1%	50.0%
WR	WR10	w10-1	mixed	CALE	7.50	15	2	68.2%	50.0%

WR	WR10	w10-2	mixed	CALE	10.00	10	1	9.4%	10.0%
WR	WR10	w10-3	mixed	CALE	40.00	80	2	100.0%	66.7%
WR	WR10	w10-4	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR10	w10-5	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR10	w10-6	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR16	w16-1	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR16	w16-2	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR16	w16-3	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR22	w22-1	mixed	CALE	9.40	47	5	69.1%	55.6%
WR	WR22	w22-2	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR26	w26-1	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR26	w26-2	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR26	w26-3	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR32	w32-1	mixed	CALE	0.00	0	0	0.0%	0.0%
WR	WR32	w32-2	mixed	CALE	10.60	53	5	88.3%	71.4%
WR	WR32	w32-3	mixed	CALE	15.29	107	7	68.2%	58.3%
WR	WR17	w17-1	solo	CALE	7.00	35	5	29.2%	41.7%
WR	WR09	w09-1	solo	CALE	7.50	15	2	5.3%	11.8%
WR	WR35	w35-1	solo	CALE	8.33	125	15	43.4%	57.7%
WR	WR06	none	solo	CAHI	0.00	0	0	0.0%	0.0%

Seed Characteristics

maternal plant #	site	plot	Matern. Species	capsule	Parent	context	distance	#seeds in pod	% germ	T50 week
SV L1	Off	1.0	CALE			Off	Off		0.96	9.75
SV L2	Off	1.0	CALE			Off	Off		1	9.5
SV L3	Off	1.0	CALE			Off	Off		0.96	10.25
GH03 L1	GH	3.0	CALE	M	311	Mix	C	157	0.9	8.25
GH03 L4	GH	3.0	CALE	M	314	Mix	F	155	0.875	7.25
GH07 L4	GH	7.0	CALE	M	714	Mix	F	79	0.975	6.75
GH07 L3	GH	7.0	CALE	M	713	Mix	C	157	0.95	6.75
GH16 L1	GH	16.0	CALE	M	1611	Mix	F	194	0.975	5.5
GH16 L2	GH	16.0	CALE	M	1612	Mix	C	181	0.8	8
GH16 L4	GH	16.0	CALE	M	1614	Mix	C	278	0.9	6.5
GH18 L1	GH	18.0	CALE	M	1811	Mix	C	197	0.95	5
GH18 L2	GH	18.0	CALE	M	1812	Mix	F	116	0.85	7
GH18 L4	GH	18.0	CALE	M	1814	Mix	F	231	0.8	8
GH29 L2	GH	29.0	CALE	M	2912	Mix	C	319	1	6.25
GH29 L3	GH	29.0	CALE	M	2913	Mix	F	200	0.35	99
GH29 L4	GH	29.0	CALE	M	2914	Mix	C	226	0.95	6.75
GH03 H1	GH	3.0	CAHI	M	321	Mix	C	133	0.975	8.75

GH03 H2	GH	3.0	CAHI	M	322	Mix	F	260	0	20
GH03 H3	GH	3.0	CAHI	M	323	Mix	C	170	0.35	20
GH03 H4	GH	3.0	CAHI	M	324	Mix	F	27	0.74	9.75
GH07 H1	GH	7.0	CAHI	M	721	Mix	F	120	0.975	9
GH07 H2	GH	7.0	CAHI	M	722	Mix	C	190	0.95	8
GH07 H3	GH	7.0	CAHI	M	723	Mix	F	129	0.925	9.5
GH16 H1	GH	16.0	CAHI	M	1621	Mix	F	72	0.95	8
GH16 H3	GH	16.0	CAHI	M	1623	Mix	F	265	0.825	9
GH16 H4	GH	16.0	CAHI	M	1624	Mix	C	134	0.975	8.5
GH18 H1	GH	18.0	CAHI	M	1821	Mix	F	302	0.975	9.25
GH18 H2	GH	18.0	CAHI	M	1822	Mix	C	54	0.225	20
GH29 H1	GH	29.0	CAHI	M	2921	Mix	F	260	0.875	9
GH29 H2	GH	29.0	CAHI	M	2922	Mix	C	228	0.975	6
GH29 H3	GH	29.0	CAHI	M	2923	Mix	F	140	0.375	20
GHS-N2 H2	GH	2.0	CAHI	M	222	Solo	Solo	175	0.775	8.25
GHS-N2 H3	GH	2.0	CAHI	M	223	Solo	Solo	115	0.875	8.25
GHS-N2 H4	GH	2.0	CAHI	M	224	Solo	Solo	140	0.975	9.25
GH21 L2	GH	21.0	CALE	M	2112	Solo	Solo	135	0.95	6.25
GH21 L3	GH	21.0	CALE	M	2113	Solo	Solo	327	0.925	5.75
GH21 L4	GH	21.0	CALE	M	2114	Solo	Solo	202	0.95	6.25
GH26 L2	GH	26.0	CALE	M	2612	SS	SS	44	0.8	6.25
WR10 L1	WR	10.0	CALE	M	1011	Mix	C	113	0.925	7
WR10 L2	WR	10.0	CALE	M	1012	Mix	F	236	0.975	4.75
WR10 L3	WR	10.0	CALE	M	1013	Mix	C	302	0.95	5.75
WR16 L3	WR	16.5	CALE	M	16513	Mix	F	105	0.925	4.75
WR16 L4	WR	16.5	CALE	M	16514	Mix	C	105	0.925	6.5
WR22 L1	WR	22.0	CALE	M	2211	Mix	F	84	0.95	6.5
WR22 L3	WR	22.0	CALE	M	2213	Mix	F	120	0.975	7
WR22 L4	WR	22.0	CALE	M	2214	Mix	C	245	0.625	7
WR26 L1	WR	26.5	CALE	M	26511	Mix	F	65	0.5	11
WR26 L3	WR	26.5	CALE	M	26513	Mix	F	242	0.975	7.25
WR32 L1	WR	32.0	CALE	M	3211	Mix	F	254	0.925	7
WR32 L3	WR	32.0	CALE	M	3213	Mix	F	97	0.925	6
WR32 L4	WR	32.0	CALE	M	3214	Mix	C	60	0.65	7.5
WR10 H1	WR	10.0	CAHI	M	1021	Mix	C	154	0.9	7.75
WR10 H4	WR	10.0	CAHI	M	1024	Mix	F	145	0.925	6.75
WR16 H1	WR	16.5	CAHI	M	16521	Mix	C	163	0.9	7.75
WR16 H2	WR	16.5	CAHI	M	16522	Mix	F	198	0.95	8
WR16 H3	WR	16.5	CAHI	M	16523	Mix	C	123	0.95	8
WR22 H1	WR	22.0	CAHI	M	2221	Mix	C	90	0.475	10
WR22 H2	WR	22.0	CAHI	M	2222	Mix	F	127	0.85	9

WR22 H4	WR	22.0	CAHI	M	2224	Mix	F	106	0.9	8
WR26 H1	WR	26.5	CAHI	M	26521	Mix	C	114	0.925	9
WR26 H2	WR	26.5	CAHI	M	26522	Mix	F	36	0.694	8
WR26 H4	WR	26.5	CAHI	M	26524	Mix	C	108	0.85	7.5
WR32 H2	WR	32.0	CAHI	M	3222	Mix	F	98	0.95	6.5
WR32 H3	WR	32.0	CAHI	M	3223	Mix	C	90	0.8	7
WR06 H1	WR	6.0	CAHI	M	621	SS	SS	224	0.825	8.25
WR06 H2	WR	6.0	CAHI	M	622	SS	SS	280	0.625	10.5
WR06 H4	WR	6.0	CAHI	M	624	SS	SS	70	0.975	6.75
WR14 L1	WR	14.0	CALE	M	1411	Solo	Solo	242	0.975	6
WR14 L2	WR	14.0	CALE	M	1412	Solo	Solo	342	0.975	6.25
WR14 L3	WR	14.0	CALE	M	1413	Solo	Solo	77	0.65	6.5
GHNC CAHI 1	Off	1.5	CALE			Off	Off		0.86	9
GHNC CAHI 2	Off	1.5	CALE			Off	Off		0.9	9
GHNC CAHI 3	Off	1.5	CALE			Off	Off		0.9	8.25
GH03 L1	GH	3.0	CALE	B	311	Mix	C	136	0.975	7.5
GH03 L4	GH	3.0	CALE	B	314	Mix	F	134	0.925	7
GH07 L4	GH	7.0	CALE	B	714	Mix	F	43	0.975	6.75
GH07 L3	GH	7.0	CALE	B	713	Mix	C	170	0.9	6
GH16 L1	GH	16.0	CALE	B	1611	Mix	F	122	0.975	7
GH16 L2	GH	16.0	CALE	B	1612	Mix	C	199	0.875	8.5
GH16 L4	GH	16.0	CALE	B	1614	Mix	C	248	1	6
GH18 L1	GH	18.0	CALE	B	1811	Mix	C	198	0.725	6.5
GH18 L2	GH	18.0	CALE	B	1812	Mix	F	194	0.925	6.75
GH18 L4	GH	18.0	CALE	B	1814	Mix	F	127	0.7	8
GH29 L2	GH	29.0	CALE	B	2912	Mix	C	314	0.875	6.75
GH29 L3	GH	29.0	CALE	B	2913	Mix	F	150	0.2	20
GH29 L4	GH	29.0	CALE	B	2914	Mix	C	220	0.975	5.25
GH03 H1	GH	3.0	CAHI	B	321	Mix	C	124	0.675	10.25
GH03 H2	GH	3.0	CAHI	B	322	Mix	F	240	0	20
GH03 H3	GH	3.0	CAHI	B	323	Mix	C	111	0.4	20
GH03 H4	GH	3.0	CAHI	B	324	Mix	F	56	0.825	22
GH07 H1	GH	7.0	CAHI	B	721	Mix	F	184	0.975	7.25
GH07 H2	GH	7.0	CAHI	B	722	Mix	C	173	0.925	8
GH07 H3	GH	7.0	CAHI	B	723	Mix	F	169	0.975	9.5
GH16 H1	GH	16.0	CAHI	B	1621	Mix	F	71	0.95	8.25
GH16 H3	GH	16.0	CAHI	B	1623	Mix	F	160	0.85	9.75
GH16 H4	GH	16.0	CAHI	B	1624	Mix	C	234	0.95	7.75
GH18 H1	GH	18.0	CAHI	B	1821	Mix	F	256	1	9.25
GH18 H2	GH	18.0	CAHI	B	1822	Mix	C	67	0.25	20
GH29 H1	GH	29.0	CAHI	B	2921	Mix	F	290	0.85	8.5

GH29 H2	GH	29.0	CAHI	B	2922	Mix	C	200	0.875	8.75
GH29 H3	GH	29.0	CAHI	B	2923	Mix	F	140	0.3	20
GHS-N2 H2	GH	2.0	CAHI	B	222	Solo	Solo	224	0.9	8.25
GHS-N2 H3	GH	2.0	CAHI	B	223	Solo	Solo	104	0.725	9.5
GHS-N2 H4	GH	2.0	CAHI	B	224	Solo	Solo	112	1	9.75
GH21 L2	GH	21.0	CALE	B	2112	Solo	Solo	81	0.95	4.75
GH21 L3	GH	21.0	CALE	B	2113	Solo	Solo	128	0.975	6.25
GH21 L4	GH	21.0	CALE	B	2114	Solo	Solo	267	0.675	6.75
GH26 L2	GH	26.0	CALE	B	2612	SS	SS	65	0.8	8
WR10 L1	WR	10.0	CALE	B	1011	Mix	C	148	0.9	7
WR10 L2	WR	10.0	CALE	B	1012	Mix	F	90	0.25	20
WR10 L3	WR	10.0	CALE	B	1013	Mix	C	208	0.975	6
WR16 L3	WR	16.5	CALE	B	16513	Mix	F	189	1	5.25
WR16 L4	WR	16.5	CALE	B	16514	Mix	C	23	0.609	8
WR22 L1	WR	22.0	CALE	B	2211	Mix	F	158	0.925	6
WR22 L3	WR	22.0	CALE	B	2213	Mix	F	120	0.925	7.25
WR22 L4	WR	22.0	CALE	B	2214	Mix	C	182	0.725	6.25
WR26 L1	WR	26.5	CALE	B	26511	Mix	C	100	0.55	7
WR26 L3	WR	26.5	CALE	B	26513	Mix	F	196	0.9	7
WR32 L1	WR	32.0	CALE	B	3211	Mix	F	196	0.975	6.5
WR32 L3	WR	32.0	CALE	B	3213	Mix	F	13	0.769	4.75
WR32 L4	WR	32.0	CALE	B	3214	Mix	C	15	0.733	9
WR10 H1	WR	10.0	CAHI	B	1021	Mix	C	68	0.95	7.75
WR10 H4	WR	10.0	CAHI	B	1024	Mix	F	135	0.875	7.75
WR16 H1	WR	16.5	CAHI	B	16521	Mix	C	215	0.975	7.75
WR16 H2	WR	16.5	CAHI	B	16522	Mix	F	83	0.925	6.75
WR16 H3	WR	16.5	CAHI	B	16523	Mix	C	157	0.95	8
WR22 H1	WR	22.0	CAHI	B	2221	Mix	C	80	0.425	20
WR22 H2	WR	22.0	CAHI	B	2222	Mix	F	42	0.95	7
WR22 H4	WR	22.0	CAHI	B	2224	Mix	F	103	0.95	8
WR26 H1	WR	26.5	CAHI	B	26521	Mix	C	90	0.675	10.25
WR26 H2	WR	26.5	CAHI	B	26522	Mix	F	117	0.875	8
WR26 H4	WR	26.5	CAHI	B	26524	Mix	C	76	0.925	8.25
WR32 H2	WR	32.0	CAHI	B	3222	Mix	F	93	0.9	7.25
WR32 H3	WR	32.0	CAHI	B	3223	Mix	C	148	0.625	7.5
WR06 H1	WR	6.0	CAHI	B	621	SS	SS	215	0.95	7.75
WR06 H2	WR	6.0	CAHI	B	622	SS	SS	123	0.8	10.5
WR06 H4	WR	6.0	CALE	B	624	SS	SS	74	0.825	8
WR14 L1	WR	14.0	CALE	B	1411	Solo	Solo	272	0.95	6.25
WR14 L2	WR	14.0	CALE	B	1412	Solo	Solo	179	0.775	7
WR14 L3	WR	14.0	CALE	B	1413	Solo	Solo	49	0.4	20

Early Reproductive Characteristics

maternal plant #	site	plot	Matern. Species	context	distance	# stems/ plant	bloom zone length	avg # pods/ stem
GH03 L1	GH	3.0	CALE	Mix	C	20	27.5	16.33333
GH03 L4	GH	3.0	CALE	Mix	F	14	20	13.66667
GH07 L4	GH	7.0	CALE	Mix	F	82	17	14
GH07 L3	GH	7.0	CALE	Mix	C	14	15.5	10.66667
GH16 L1	GH	16.0	CALE	Mix	F	16	11	13.33333
GH16 L2	GH	16.0	CALE	Mix	C	8	13.5	15.33333
GH16 L4	GH	16.0	CALE	Mix	C	41	24.5	15
GH18 L1	GH	18.0	CALE	Mix	C	26	25	14.33333
GH18 L2	GH	18.0	CALE	Mix	F	36	27.5	14.33333
GH18 L4	GH	18.0	CALE	Mix	F	17	21.5	15.66667
GH29 L2	GH	29.0	CALE	Mix	C	32	22.5	15
GH29 L3	GH	29.0	CALE	Mix	F	11	15	10
GH29 L4	GH	29.0	CALE	Mix	C	9	27	15.33333
GH03 H1	GH	3.0	CAHI	Mix	C	21	39	21.33333
GH03 H2	GH	3.0	CAHI	Mix	F	8	28.5	13
GH03 H3	GH	3.0	CAHI	Mix	C	6	27.5	15.66667
GH03 H4	GH	3.0	CAHI	Mix	F	6	15	14.66667
GH07 H1	GH	7.0	CAHI	Mix	F	6	29	16
GH07 H2	GH	7.0	CAHI	Mix	C	8	28	22.66667
GH07 H3	GH	7.0	CAHI	Mix	F	7	27.5	24.66667
GH16 H1	GH	16.0	CAHI	Mix	F	13	14	16.66667
GH16 H3	GH	16.0	CAHI	Mix	F	15	22	17
GH16 H4	GH	16.0	CAHI	Mix	C	8	12	13.66667
GH18 H1	GH	18.0	CAHI	Mix	F	13	20	15
GH18 H2	GH	18.0	CAHI	Mix	C	10	16	4.333333
GH29 H1	GH	29.0	CAHI	Mix	F	11	19	12.66667
GH29 H2	GH	29.0	CAHI	Mix	C	5	16.5	16
GH29 H3	GH	29.0	CAHI	Mix	F	10	17	21.66667
GHS-N2 H2	GH	2.0	CAHI	Solo	Solo	5	23	15
GHS-N2 H3	GH	2.0	CAHI	Solo	Solo	19	25	18.33333
GHS-N2 H4	GH	2.0	CAHI	Solo	Solo	6	28.5	21.33333
GH21 L2	GH	21.0	CALE	Solo	Solo	17	12.5	16
GH21 L3	GH	21.0	CALE	Solo	Solo	10	14.5	12.33333
GH21 L4	GH	21.0	CALE	Solo	Solo	23	14.5	11.33333
GH26 L2	GH	26.0	CALE	SS	SS	24	25	16
WR10 L1	WR	10.0	CALE	Mix	C	5	15.5	12.33333

WR10 L2	WR	10.0	CALE	Mix	F	5	18.5	13.66667
WR10 L3	WR	10.0	CALE	Mix	C	8	16.5	11.66667
WR16 L3	WR	16.5	CALE	Mix	F	5	13	12.33333
WR16 L4	WR	16.5	CALE	Mix	C	6	17	9.333333
WR22 L1	WR	22.0	CALE	Mix	F	4	13	10.66667
WR22 L3	WR	22.0	CALE	Mix	F	2	11	12.5
WR22 L4	WR	22.0	CALE	Mix	C	2	12	9.5
WR26 L1	WR	26.5	CALE	Mix	F	3	26.5	23
WR26 L3	WR	26.5	CALE	Mix	F	2	11.5	8
WR32 L1	WR	32.0	CALE	Mix	F	4	8.75	5
WR32 L3	WR	32.0	CALE	Mix	F	3	19	14
WR32 L4	WR	32.0	CALE	Mix	C	3	17	14
WR10 H1	WR	10.0	CAHI	Mix	C	4	16.5	10.66667
WR10 H4	WR	10.0	CAHI	Mix	F	2	10	12
WR16 H1	WR	16.5	CAHI	Mix	C	2	18	16.5
WR16 H2	WR	16.5	CAHI	Mix	F	5	20.5	11.66667
WR16 H3	WR	16.5	CAHI	Mix	C	3	22	14
WR22 H1	WR	22.0	CAHI	Mix	C	4	19.5	18.66667
WR22 H2	WR	22.0	CAHI	Mix	F	2	11	13
WR22 H4	WR	22.0	CAHI	Mix	F	2	23.75	20
WR26 H1	WR	26.5	CAHI	Mix	C	2	24	21.5
WR26 H2	WR	26.5	CAHI	Mix	F	4	10	10.33333
WR26 H4	WR	26.5	CAHI	Mix	C	4	14	18.33333
WR32 H2	WR	32.0	CAHI	Mix	F	2	10	10.5
WR32 H3	WR	32.0	CAHI	Mix	C	2	28	18.5
WR06 H1	WR	6.0	CAHI	SS	SS	5	22.5	14.33333
WR06 H2	WR	6.0	CAHI	SS	SS	3	13	9.5
WR06 H4	WR	6.0	CAHI	SS	SS	4	23	12.33333
WR14 L1	WR	14.0	CALE	Solo	Solo	18	11.5	9
WR14 L2	WR	14.0	CALE	Solo	Solo	15	11.5	7.666667
WR14 L3	WR	14.0	CALE	Solo	Solo	2	19	12.5

Herbarium Samples

UW Herbarium #	species	lobe:bract ratio	bract length (cm)	lobe length (cm)	galea length (cm)	floral angle
L143587	CALE	0.243	2.5	0.617		74.7
L16255	CALE	0.315	2.4	0.767	2.3	68.3
L292155	CALE	0.280	2.4	0.667		71.3
L139265	CALE	0.311	3.0	0.933		62.7
L291809	CALE	0.234	2.6	0.600	2.1	70.3
L355882	CALE	0.280	2.7	0.750		69.7
L45447	CALE	0.275	2.2	0.600		76.3
L323290	CALE	0.274	2.7	0.725	2.2	64.0
L139266	CALE	0.250	2.6	0.650		68.7
H374966*	CAHI				2.1	63.0
H15413	CAHI	0.538	2.3	1.250	3.0	65.0
H332074	CAHI	0.364	2.2	0.800		61.0
H16292	CAHI	0.331	2.3	0.750	3.0	68.3
H16291	CAHI	0.446	2.2	0.967	3.2	64.0
H354224	CAHI	0.385	2.2	0.833	2.6	65.3
H353696	CAHI	0.425	2.4	1.033	2.8	60.7
H373664	CAHI	0.442	2.9	1.267		58.0
H332507	CAHI	0.315	1.8	0.567	2.5	54.5
H15420	CAHI	0.474	2.3	1.083	2.9	59.7
H360516**	CAHI	0.391	2.6	1.017	2.5	68.7
H380350	CAHI	0.419	2.3	0.950	2.6	60.3

***** outlier CAHI with only one flower visible for bract & lobe measure

** yellow-orange CAHI coloration

F1 Flower Data

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
GH	GH03	321	H1B 3	M	F	b	360	yellow	67.7	2.487	0.960	*0.386*	2.583	0.823	*0.319*
GH	GH03	321	H1B 4	M	F	b	226	Y/O	65.7	2.350	1.020	*0.434*	2.750	1.007	*0.366*
GH	GH03	321	H1B 7	M	F	b	213	yellow	65.7	2.777	1.180	*0.425*	3.153	0.987	*0.313*
GH	GH03	321	H1M 3	M	F	m	249	bright yellow	74.3	2.253	0.713	*0.317*	2.580	0.720	*0.279*
GH	GH03	321	H1M 4	M	F	m	157	gold	63.3	2.300	0.910	*0.396*	2.780	0.853	*0.307*
GH	GH03	321	H1M 8	M	F	m	262	yellow	76.0	2.530	0.813	*0.321*	2.870	0.897	*0.312*
GH	GH03	323	H3B 8	M	C	b	205	light yellow	65.7	2.473	0.903	*0.365*	2.927	1.107	*0.378*
GH	GH03	324	H4B 4	M	F	b	393	gold w/org tips	69.8	2.253	0.670	*0.297*	3.057	1.157	*0.378*
GH	GH03	324	H4B 6	M	F	b	301	gold-salmon	72.7	2.803	0.980	*0.350*	3.443	1.213	*0.352*
GH	GH03	324	H4M 2	M	F	m	84	soft org	75.7	2.417	0.590	*0.244*	2.570	1.077	*0.419*
GH	GH03	324	H4M 6	M	F	m	205	Y/O	66.7	2.637	0.817	*0.310*	3.357	1.227	*0.365*
GH	GH03	324	H4M 7	M	F	m	271	salmon	73.0	2.427	0.773	*0.319*	2.750	0.910	*0.331*
GH	GH03	324	H4M 8	M	F	m	74	yel pale org edge	69.7	2.600	0.750	*0.288*	3.290	1.130	*0.343*
GH	GH03	311	L1B 1	M	F	b	147	gold	76.3	2.617	0.930	*0.355*	2.600	0.740	*0.285*
GH	GH03	311	L1B 2	M	F	b	438	yellow	73.0	2.770	0.730	*0.264*	2.700	0.667	*0.247*
GH	GH03	311	L1B 3	M	F	b	146	yellow	76.3	3.097	1.123	*0.363*	2.737	0.820	*0.300*
GH	GH03	311	L1B 5	M	F	b	167	yellow	76.7	2.783	*1.003*	0.360	2.557	*0.650*	0.254
GH	GH03	311	L1B 6	M	F	b	118	yellow	69.7	2.460	1.090	*0.443*	2.393	0.813	*0.340*
GH	GH03	311	L1B 8	M	F	b	96	yellow	74.3	2.923	1.080	*0.369*	2.997	0.900	*0.300*
GH	GH03	311	L1M 2	M	F	m	208	yellow	78.3	2.290	0.433	*0.189*	2.543	0.720	*0.283*
GH	GH03	314	L4B 1b	M	C	b	264	yellow	76.0	2.660	0.873	*0.328*	2.583	0.787	*0.305*
GH	GH03	314	L4B 3	M	C	b	232	yellow	74.3	2.585	0.858	*0.332*	2.420	0.660	*0.273*
GH	GH03	314	L4M 7	M	C	m	322	gold	77.5	3.227	1.063	*0.330*	2.847	0.857	*0.301*
GH	GH03	314	L4M 8	M	C	m	256	yellow	72.3	2.633	1.127	*0.428*	2.757	0.820	*0.297*
GH	GH03	314	L4M 2cot	M	C	m	107	yellow	69.3	3.083	0.863	*0.280*	2.680	0.770	*0.287*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
GH	GH07	721	H1B 1b	M	C	b	211	yellow	68.3	3.040	1.093	*0.360*	3.307	1.257	*0.380*
GH	GH07	721	H1B 4	M	C	b	212	soft yell-org tips	64.7	2.390	0.857	*0.358*	2.740	1.040	*0.380*
GH	GH07	721	H1B 6	M	C	b	154	yellow ~green	66.7	2.833	0.933	*0.329*	2.863	0.987	*0.345*
GH	GH07	721	H1M 2	M	C	m	170	~orange/yell	71.7	2.740	0.897	*0.327*	2.837	0.937	*0.330*
GH	GH07	721	H1M 5	M	C	m	84	orange	71.0	2.613	1.430	*0.547*	3.250	1.403	*0.432*
GH	GH07	722	H2B 1	M	F	b	106	golden/salmon	69.0	2.367	0.727	*0.307*	2.513	0.757	*0.301*
GH	GH07	722	H2B 3	M	F	b	79	gold	64.3	3.013	0.967	*0.321*	3.013	0.800	*0.265*
GH	GH07	722	H2M 3	M	F	m	232	carrot org	69.3	2.098	0.790	*0.377*	2.445	0.873	*0.357*
GH	GH07	723	H3B 7	M	C	b	193	y-o	67.7	2.103	0.883	*0.420*	2.907	1.013	*0.349*
GH	GH07	723	H3M 4	M	C	m	202	golden	61.0	2.400	1.153	*0.481*	2.627	0.923	*0.352*
GH	GH07	723	H3M 7	M	C	m	206	yellow	64.0	3.003	0.980	*0.326*	2.973	1.010	*0.340*
GH	GH07	723	H3M 8	M	C	m	221	dark orange	74.3	2.537	0.850	*0.335*	2.810	1.170	*0.416*
GH	GH07	713	L3B 3	M	F	b	206	yellow	60.5	2.803	0.860	*0.307*	2.817	0.798	*0.283*
GH	GH07	713	L3B 5	M	F	b	100	gold	65.7	2.500	0.940	*0.376*	2.490	0.727	*0.292*
GH	GH07	713	L3B 8	M	F	b	128	yellow	66.7	2.630	0.975	*0.371*	2.255	0.600	*0.266*
GH	GH07	713	L3M 2	M	F	m	140	y/o	66.3	2.340	0.933	*0.399*	3.147	1.103	*0.351*
GH	GH07	713	L3M 5	M	F	m	77	yellow	68.3	3.160	0.993	*0.314*	2.813	0.743	*0.264*
GH	GH07	713	L3M 6	M	F	m	93	yellow	72.3	2.477	0.870	*0.351*	2.170	0.590	*0.272*
GH	GH07	714	L4B 1	M	C	b	80	yellow	77.7	2.603	0.917	*0.352*	2.383	0.730	*0.306*
GH	GH07	714	L4B 5	M	C	b	90	yellow	74.0	2.840	0.920	*0.324*	2.783	0.747	*0.268*
GH	GH07	714	L4B 6	M	C	b	126	yellow	73.3	2.650	0.877	*0.331*	2.313	0.657	*0.284*
GH	GH07	714	L4B 7	M	C	b	107	yellow	79.7	2.647	0.817	*0.309*	2.547	0.717	*0.281*
GH	GH07	714	L4M 5	M	C	m	206	yellow	75.8	3.100	1.057	*0.341*	2.787	0.757	*0.272*
GH	GH16	1621	H1B 1	M	C	b	93	golden	73.3	2.627	0.910	*0.346*	2.703	0.973	*0.360*
GH	GH16	1621	H1B 4	M	C	b	136	yell w/ orng tip	72.0	2.873	0.943	*0.328*	3.003	1.070	*0.356*
GH	GH16	1621	H1B 8	M	C	b	459	yellow	72.7	2.420	0.770	*0.318*	2.827	0.993	*0.351*
GH	GH16	1621	H1M 1	M	C	m	290	yellow	70.0	2.188	0.760	*0.347*	2.383	0.920	*0.386*
GH	GH16	1621	H1M 3	M	C	m	72	yellow	72.3	2.153	0.850	*0.395*	2.470	0.887	*0.359*
GH	GH16	1621	H1M 5	M	C	m	111	salmon	63.3	3.163	1.180	*0.373*	3.210	1.103	*0.344*
GH	GH16	1621	H1M 7	M	C	m	74	yellow	74.7	2.433	1.037	*0.426*	2.743	1.050	*0.383*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
GH	GH16	1623	H3B 1	M	C	b	198	golden	74.7	1.893	0.617	*0.326*	2.557	0.813	*0.318*
GH	GH16	1623	H3B 2	M	C	b	158	yellow org tip	74.3	2.700	0.973	*0.360*	2.563	0.970	*0.378*
GH	GH16	1623	H3B 2	M	C	b	247	yellow/gold	70.3	2.353	0.810	*0.344*	2.743	0.993	*0.362*
GH	GH16	1623	H3B 5	M	C	b	186	soft orange	72.3	2.720	0.667	*0.245*	3.163	1.043	*0.330*
GH	GH16	1623	H3B 6	M	C	b	255	Y/O	66.0	2.917	1.147	*0.393*	3.487	1.147	*0.329*
GH	GH16	1623	H3B 8	M	C	b	199	dark orange	58.3	1.773	0.853	*0.481*	2.963	1.220	*0.412*
GH	GH16	1623	H3M 1b	M	C	m	216	soft org/yellow	70.7	2.340	0.757	*0.323*	2.443	0.823	*0.337*
GH	GH16	1623	H3M 2	M	C	m	162	yellow w/ orng edge	70.3	3.000	0.763	*0.254*	3.213	1.133	*0.353*
GH	GH16	1623	H3M 6	M	C	m	160	salmon	65.0	2.373	1.090	*0.459*	2.570	0.977	*0.380*
GH	GH16	1623	H3M 8	M	C	m	187	yellow org tip	72.3	2.790	0.933	*0.335*	3.130	0.983	*0.314*
GH	GH16	1624	H4B 4	M	F	b	93	orange	70.3	2.037	0.837	*0.411*	2.730	1.053	*0.386*
GH	GH16	1624	H4B 7	M	F	b	132	O/Y	65.3	2.480	0.660	*0.266*	2.760	0.810	*0.293*
GH	GH16	1624	H4B 8	M	F	b	149	soft orange-yell	74.0	2.297	0.627	*0.273*	2.803	0.897	*0.320*
GH	GH16	1624	H4M 2	M	F	m	78	soft orange	72.0	2.703	1.140	*0.422*	2.853	0.893	*0.313*
GH	GH16	1624	H4M 7	M	F	m	65	red	58.7	2.810	1.323	*0.471*	2.920	1.200	*0.411*
GH	GH16	1624	H4M 8	M	F	m	93	ORANGE	60.3	2.603	1.240	*0.476*	2.923	1.123	*0.384*
GH	GH16	1611	L1B 1	M	C	b	104	yellow	72.0	2.883	1.007	*0.349*	2.907	0.683	*0.235*
GH	GH16	1611	L1B 3	M	C	b	282	golden	75.7	2.507	0.660	*0.263*	2.613	0.737	*0.282*
GH	GH16	1611	L1B 3b	M	C	b	191	golden	84.0	2.970	0.850	*0.286*	2.727	0.913	*0.335*
GH	GH16	1611	L1B 7	M	C	b	114	yellow	69.0	2.643	0.897	*0.339*	2.430	0.653	*0.269*
GH	GH16	1611	L1M 1	M	C	m	169	yellow	72.3	2.657	0.790	*0.297*	2.610	0.740	*0.284*
GH	GH16	1611	L1M 5	M	C	m	230	gold	71.5	2.670	0.630	*0.236*	2.453	0.750	*0.306*
GH	GH16	1612	L2B 3	M	F	b	163	gold	74.8	2.723	0.997	*0.366*	2.657	0.847	*0.319*
GH	GH16	1612	L2B 4	M	F	b	154	yellow	74.5	2.470	0.717	*0.290*	2.450	0.713	*0.291*
GH	GH16	1614	L4B 4	M	F	b	140	yellow	69.3	2.873	1.013	*0.353*	2.980	0.730	*0.245*
GH	GH16	1614	L4B 7	M	F	b	85	yellow	74.3	2.983	1.070	*0.359*	2.740	0.737	*0.269*
GH	GH16	1614	L4B 8	M	F	b	128	~yellow/green	70.3	2.837	0.787	*0.277*	2.767	0.710	*0.257*
GH	GH16	1614	L4M 2b	M	F	m	136	gold	71.5	3.050	0.997	*0.327*	2.787	0.717	*0.257*
GH	GH16	1614	L4M 5	M	F	m	147	golden	68.3	2.237	0.847	*0.379*	2.283	0.610	*0.267*
GH	GH16	1614	L4M 6	M	F	m	109	yellow	77.0	2.923	1.010	*0.345*	3.097	0.800	*0.258*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
GH	GH18	1821	H1B 1	M	C	b	221	orange	67.7	2.300	0.853	*0.371*	2.627	0.997	*0.379*
GH	GH18	1821	H1B 2	M	C	b	127	gold	71.3	2.930	0.857	*0.292*	3.253	1.083	*0.333*
GH	GH18	1821	H1B 3	M	C	b	135	golden	60.5	2.803	0.860	*0.307*	2.817	0.798	*0.283*
GH	GH18	1821	H1B 5	M	C	b	188	golden	71.3	3.297	1.037	*0.314*	3.443	1.177	*0.342*
GH	GH18	1821	H1M 1	M	C	m	221	y/o	68.3	2.540	0.810	*0.319*	3.257	1.160	*0.356*
GH	GH18	1821	H1M 3	M	C	m	106	salmon	67.0	2.663	0.987	*0.370*	3.273	1.043	*0.319*
GH	GH18	1821	H1M 3b	M	C	m	127	gold	70.0	2.867	0.830	*0.290*	3.343	1.083	*0.324*
GH	GH18	1821	H1M 7	M	C	m	148	salmon/yellow	71.3	2.267	0.877	*0.387*	2.693	0.857	*0.318*
GH	GH18	1822	H2B 1	M	F	b	155	soft red	66.3	1.690	1.013	*0.600*	2.863	1.173	*0.410*
GH	GH18	1822	H2B 7	M	F	b	140	orange	67.0	1.540	0.630	*0.409*	2.568	0.870	*0.339*
GH	GH18	1811	L1B 4	M	F	b	108	~yellow	69.3	2.477	0.710	*0.287*	2.310	0.663	*0.287*
GH	GH18	1811	L1B 5	M	F	b	149	green	54.7	2.800	0.827	*0.295*	2.250	0.567	*0.252*
GH	GH18	1811	L1B 6	M	F	b	156	~yellow	59.3	2.660	0.800	*0.301*	2.560	0.670	*0.262*
GH	GH18	1811	L1M 5	M	F	m	141	yellow	74.3	2.667	0.813	*0.305*	2.363	0.600	*0.254*
GH	GH18	1811	L1M 7	M	F	m	114	yellow	76.0	2.410	0.840	*0.349*	2.620	0.653	*0.249*
GH	GH18	1811	L1M 8	M	F	m	140	~gold/yell open	59.3	2.463	0.720	*0.292*	3.000	0.990	*0.330*
GH	GH18	1812	L2B 2	M	C	b	109	yellow	66.0	2.817	1.083	*0.385*	2.820	0.750	*0.266*
GH	GH18	1812	L2B 4	M	C	b	135	yellow	75.7	2.957	0.977	*0.330*	2.910	0.810	*0.278*
GH	GH18	1812	L2B 5	M	C	b	233	gold	73.3	3.117	1.097	*0.352*	2.620	0.720	*0.275*
GH	GH18	1812	L2B 6	M	C	b	107	~yellow/green	72.3	2.487	0.823	*0.331*	2.187	0.637	*0.291*
GH	GH18	1812	L2M 2	M	C	m	448	gold	75.7	2.825	0.907	*0.321*	2.593	0.743	*0.287*
GH	GH18	1812	L2M 6	M	C	m	184	yellow	71.3	2.677	0.913	*0.341*	2.640	0.640	*0.242*
GH	GH18	1814	L4B 1	M	C	b	160	yellow	67.8	3.073	0.920	*0.299*	2.993	0.713	*0.238*
GH	GH18	1814	L4B 3	M	C	b	103	yellow	70.3	2.820	0.717	*0.254*	2.630	0.777	*0.295*
GH	GH18	1814	L4M 3	M	C	m	233	gold	74.0	2.727	0.730	*0.268*	2.263	0.570	*0.252*
GH	GH18	1814	L4M 4	M	C	m	135	golden	72.0	3.375	1.193	*0.354*	3.257	1.008	*0.310*
GH	GH18	1814	L4M 7	M	C	m	241	yellow	71.0	3.140	0.870	*0.277*	2.997	0.807	*0.269*
GH	GH21	2112	L2B 6	S	S	b	100	yellow	70.7	3.033	0.873	*0.288*	2.777	0.777	*0.280*
GH	GH21	2112	L2B 8	S	S	b	506	yellow	72.7	2.813	1.010	*0.359*	2.577	0.690	*0.268*
GH	GH21	2112	L2M 3	S	S	m	121	yellow	74.3	2.787	0.870	*0.312*	2.370	0.607	*0.256*
GH	GH21	2112	L2M 5	S	S	m	105	yellow	70.7	3.047	1.103	*0.362*	2.653	0.650	*0.245*
GH	GH21	2112	L2M 7	S	S	m	71	yellow	73.3	3.047	0.977	*0.321*	2.633	0.697	*0.265*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
GH	GH21	2113	L3B 3	S	S	b	167	yellow	74.3	2.783	0.780	*0.280*	2.323	0.563	*0.242*
GH	GH21	2113	L3B 5	S	S	b	73	gold	72.7	2.860	1.027	*0.359*	2.823	0.787	*0.279*
GH	GH21	2113	L3B 6	S	S	b	92	yellow	75.7	2.697	1.020	*0.378*	2.603	0.693	*0.266*
GH	GH21	2113	L3B 7	S	S	b	275	~gold	73.5	2.890	1.000	*0.346*	2.893	0.733	*0.253*
GH	GH21	2113	L3M 6	S	S	m	121	yellow	75.7	2.350	0.907	*0.386*	2.580	0.743	*0.288*
GH	GH21	2113	L3M 7	S	S	m	109	yellow	68.7	2.530	0.890	*0.352*	2.550	0.670	*0.263*
GH	GH21	2113	L3M 8	S	S	m	128	yellow	74.0	2.407	0.827	*0.343*	2.647	0.543	*0.205*
GH	GH21	2114	L4B 3	S	S	b	392	grn-yell	75.8	1.970	0.685	*0.348*	2.473	0.800	*0.324*
GH	GH21	2114	L4M 5	S	S	m	185	yell/green	63.7	2.450	0.817	*0.333*	2.480	0.640	*0.258*
GH	GH21	2114	L4M 6	S	S	m	234	~yellow/green	61.3	2.647	0.763	*0.288*	2.470	0.617	*0.250*
GH	GH21	2114	L4M 8	S	S	m	128	yellow	67.3	2.237	0.623	*0.279*	2.387	0.660	*0.277*
GH	GH26	2612	L2B 2	SS	SS	b	100	yellow	60.3	2.333	0.993	*0.426*	2.397	0.630	*0.263*
GH	GH26	2612	L2B 5	SS	SS	b	78	yellow	72.3	3.110	0.987	*0.317*	2.653	0.720	*0.271*
GH	GH26	2612	L2M 1	SS	SS	m	165	gold	68.8	2.667	1.003	*0.376*	2.417	0.620	*0.257*
GH	GH26	2612	L2M 2	SS	SS	m	317	yellow	75.8	2.867	0.847	*0.295*	2.643	0.790	*0.299*
GH	GH26	2612	L2M 3	SS	SS	m	217	yellow	68.3	2.590	0.750	*0.290*	2.567	0.730	*0.284*
GH	GH29	2921	H1B 5	M	C	b	154	yellow	70.7	2.023	0.900	*0.445*	2.690	0.853	*0.317*
GH	GH29	2921	H1B 8	M	C	b	169	yellow	57.5	2.297	0.900	*0.392*	2.880	0.957	*0.332*
GH	GH29	2921	H1M 1	M	C	m	190	soft orange	58.0	2.250	1.020	*0.453*	3.300	1.260	*0.382*
GH	GH29	2921	H1M 2	M	C	m	269	salmon	67.7	2.303	0.843	*0.366*	3.310	1.390	*0.420*
GH	GH29	2921	H1M 5	M	C	m	230	gold	68.7	2.037	0.810	*0.398*	2.603	1.120	*0.430*
GH	GH29	2921	H1M 6	M	C	m	213	orange	58.7	2.477	0.917	*0.370*	2.813	1.157	*0.411*
GH	GH29	2922	H2B 6	M	F	b	135	yellow	70.0	1.990	0.520	*0.261*	2.920	0.900	*0.308*
GH	GH29	2922	H2B 8	M	F	b	380	golden	74.0	1.803	0.723	*0.401*	2.708	0.958	*0.354*
GH	GH29	2922	H2M 1	M	F	m	77	gold	67.7	2.180	0.843	*0.387*	2.987	1.103	*0.369*
GH	GH29	2922	H2M 2	M	F	m	288	gold	75.3	1.987	0.657	*0.331*	2.830	0.990	*0.350*
GH	GH29	2922	H2M 5	M	F	m	118	golden	68.7	2.130	0.810	*0.380*	2.557	0.877	*0.343*
GH	GH29	2923	H3B 4	M	C	b	144	yellow	74.0	2.870	1.253	*0.437*	3.177	1.197	*0.377*
GH	GH29	2923	H3B 7	M	C	b	212	soft yellow	57.7	2.280	1.140	*0.500*	2.860	1.140	*0.399*
GH	GH29	2923	H3M 1	M	C	m	103	yellow	68.3	2.673	1.187	*0.444*	2.933	0.950	*0.324*
GH	GH29	2923	H3M 2	M	C	m	128	yellow	67.0	2.380	0.990	*0.416*	2.670	0.833	*0.312*
GH	GH29	2923	H3M 3	M	C	m	90	gold	68.3	2.270	0.863	*0.380*	2.617	0.847	*0.324*
GH	GH29	2923	H3M 7	M	C	m	131	deep yellow	62.0	2.637	0.922	*0.347*	3.018	0.997	*0.330*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
GH	GH29	2912	L2B 7	M	F	b	85	yellow	71.7	3.347	1.007	*0.301*	3.017	0.857	*0.284*
GH	GH29	2912	L2B 8	M	F	b	401	yellow	73.7	2.390	0.693	*0.290*	2.357	0.650	*0.276*
GH	GH29	2912	L2M 1	M	F	m	97	yellow	70.0	2.860	0.870	*0.304*	2.537	0.647	*0.255*
GH	GH29	2912	L2M 2	M	F	m	103	yellow	72.0	2.207	0.793	*0.360*	2.343	0.610	*0.260*
GH	GH29	2912	L2M 3	M	F	m	189	salmon tips & yellow	62.7	2.648	1.293	*0.488*	2.890	0.960	*0.332*
GH	GH29	2912	L2M 4	M	F	m	213	gold	66.7	2.403	0.773	*0.322*	2.803	0.897	*0.320*
GH	GH29	2913	L3B 2	M	C	b	154	yellow	75.0	2.847	0.787	*0.276*	2.907	0.863	*0.297*
GH	GH29	2913	L3B 4	M	C	b	189	gold	75.3	3.423	1.067	*0.312*	2.900	0.710	*0.245*
GH	GH29	2913	L3B 5	M	C	b	154	yellow	73.3	3.093	1.105	*0.357*	2.835	0.728	*0.257*
GH	GH29	2913	L3M 4	M	C	m	167	gold	71.5	2.393	0.720	*0.301*	2.397	0.653	*0.273*
GH	GH29	2913	L3M 5	M	C	m	147	yellow	64.3	3.177	0.953	*0.300*	2.647	0.760	*0.287*
GH	GH29	2914	L4B 4	M	F	b	86	yellow	70.7	3.367	1.100	*0.327*	2.853	0.803	*0.282*
GH	GH29	2914	L4B 8	M	F	b	107	yellow	70.0	2.970	0.840	*0.283*	2.693	0.687	*0.255*
GH	GH29	2914	L4M 2	M	F	m	114	green	69.3	2.557	0.980	*0.383*	2.497	0.680	*0.272*
GH	GH29	2914	L4M 6	M	F	m	189	yellow	70.0	2.623	0.803	*0.306*	2.957	0.817	*0.276*
GH	GH29	2914	L4M 7	M	F	m	283	yellow	70.7	2.867	0.820	*0.286*	2.880	0.847	*0.294*
Off	GHNC	201	CAHI 10	Control	Off-site		106	dark yellow/gold	60.5	2.917	1.040	*0.357*	3.003	1.190	*0.396*
Off	GHNC	202	CAHI 16	Control	Off-site		154	Y/O	61.3	2.463	1.048	*0.425*	3.088	1.237	*0.401*
Off	GHNC	203	CAHI 6	Control	Off-site		119	orange	68.3	2.533	0.983	*0.388*	2.813	1.067	*0.379*
Off	GHNC	204	CAHI 8	Control	Off-site		137	dark org	66.8	1.743	0.733	*0.420*	2.325	0.868	*0.373*
Off	GHNC	205	CAHI1 10	Control	Off-site		108	yellow lt org tips	65.7	2.103	0.863	*0.410*	2.663	1.130	*0.424*
Off	GHNC	206	CAHI1 2	Control	Off-site		101	salmon	60.0	2.673	1.160	*0.434*	2.627	1.010	*0.385*
Off	GHNC	207	CAHI1 3	Control	Off-site		121	soft orange	70.0	1.918	0.985	*0.514*	2.310	0.920	*0.398*
Off	GHNC	208	CAHI2 6	Control	Off-site		151	orange	61.8	1.880	0.840	*0.447*	2.703	1.000	*0.370*
Off	GHNC	209	CAHI3 9	Control	Off-site		145	ORANGE	64.3	2.637	1.117	*0.424*	3.030	1.140	*0.376*
GH	GHSN2	222	H2M 4	S	S	m	323	orange	60.5	2.480	1.490	*0.601*	3.033	1.220	*0.402*
GH	GHSN2	222	H2M 5	S	S	m	135	carrot orange	66.7	2.450	1.337	*0.546*	3.077	1.327	*0.431*
GH	GHSN2	222	H2M 7	S	S	m	189	salmon	63.8	2.040	1.200	*0.588*	2.537	0.990	*0.390*
GH	GHSN2	222	H2M 8	S	S	m	239	salmon	64.0	2.473	1.347	*0.544*	2.923	1.150	*0.393*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
GH	GHSN2	223	H3B 4	S	S	b	189	yellow	63.3	2.937	1.413	*0.481*	3.433	1.310	*0.382*
GH	GHSN2	223	H3B 7	S	S	b	174	soft orange	57.3	2.233	1.010	*0.452*	2.853	1.117	*0.391*
GH	GHSN2	223	H3M 1	S	S	m	205	orange	64.0	2.700	1.190	*0.441*	3.273	1.270	*0.388*
GH	GHSN2	223	H3M 5	S	S	m	402	light orange	57.8	2.860	1.340	*0.469*	2.840	1.293	*0.455*
GH	GHSN2	223	H3M 6	S	S	m	394	salmon	68.8	2.102	0.898	*0.427*	2.993	1.203	*0.402*
GH	GHSN2	223	H3M 7	S	S	m	239	yellow	61.0	2.340	0.990	*0.423*	2.747	1.107	*0.403*
GH	GHSN2	224	H4B 4	S	S	b	179	yellow org edge	66.0	2.890	1.283	*0.444*	3.010	0.877	*0.291*
GH	GHSN2	224	H4B 5	S	S	b	158	golden	65.0	2.633	1.080	*0.410*	3.083	1.270	*0.412*
GH	GHSN2	224	H4B 6	S	S	b	200	soft orange	62.7	1.777	0.777	*0.437*	2.413	0.887	*0.367*
GH	GHSN2	224	H4M 2	S	S	m	228	orange	63.3	2.317	0.760	*0.328*	3.043	1.350	*0.444*
GH	GHSN2	224	H4M 4	S	S	m	156	bright orange	59.0	2.317	0.647	*0.279*	2.623	0.957	*0.365*
GH	GHSN2	224	H4M 6	S	S	m	194	salmon	44.3	2.387	1.037	*0.434*	2.493	1.030	*0.413*
GH	GHSN2	224	H4M 7	S	S	m	147	deep orange	59.3	2.323	0.853	*0.367*	2.650	1.013	*0.382*
Off	CALE1	101	Lev 1	Control	Off-site		106	yellow	74.3	3.603	1.120	*0.311*	3.023	0.913	*0.302*
Off	CALE1	102	Lev 2	Control	Off-site		120	yellow	68.7	3.493	1.037	*0.297*	2.503	0.767	*0.306*
Off	CALE1	103	Lev 3	Control	Off-site		95	yellow	75.7	2.793	1.017	*0.364*	2.920	0.833	*0.285*
Off	CALE1	104	Lev 4	Control	Off-site		102	yellow	70.7	3.067	1.077	*0.351*	2.827	0.670	*0.237*
Off	CALE2	105	Lev 1	Control	Off-site		112	gold	75.0	2.627	0.737	*0.280*	2.683	0.823	*0.307*
Off	CALE2	106	Lev 2	Control	Off-site		108	gold	74.3	2.263	0.810	*0.358*	2.283	0.717	*0.314*
Off	CALE3	107	Lev 1	Control	Off-site		97	yellow	70.3	3.408	0.885	*0.260*	2.867	0.753	*0.263*
Off	CALE3	108	Lev 2	Control	Off-site		122	yellow	75.7	3.197	1.147	*0.359*	2.657	0.767	*0.289*
Off	CALE3	109	Lev 3	Control	Off-site		104	yellow	78.0	2.620	0.520	*0.198*	2.748	0.820	*0.298*
Off	Schmi	110	Lev 1	Control	Off-site			yellow	79.3	2.783	0.967	*0.347*	2.387	0.720	*0.302*
Off	Schmi	111	Lev 2	Control	Off-site			yellow	78.0	2.780	0.685	*0.246*	2.570	0.843	*0.328*
Off	Schmi	112	Lev 3	Control	Off-site			yellow	78.0	2.710	0.738	*0.272*	2.253	0.648	*0.287*
Off	CALE0	113	RC080811	Control	Off-site		1910	yellow	74.3	2.828	0.768	*0.272*	2.498	0.688	*0.275*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
WR	WR06	621	H1B 3	SS	SS	b	194	yell w/ org tips	72.8	2.467	0.513	*0.208*	2.700	0.843	*0.312*
WR	WR06	621	H1B 4	SS	SS	b	224	yellow	72.3	2.373	0.893	*0.376*	2.513	0.930	*0.370*
WR	WR06	621	H1M 1	SS	SS	m	215	Y/O	75.3	2.690	0.967	*0.359*	2.567	0.800	*0.312*
WR	WR06	621	H1M 5	SS	SS	m	371	salmon	69.8	2.307	0.870	*0.377*	2.627	0.950	*0.362*
WR	WR06	621	H1M 7	SS	SS	m	231	Y/O	67.7	2.370	0.633	*0.267*	2.447	0.880	*0.360*
WR	WR06	622	H2B 4	SS	SS	b	105	orange	76.0	2.363	0.920	*0.389*	2.570	1.070	*0.416*
WR	WR06	622	H2M 1	SS	SS	m	103	orange	69.5	2.123	0.757	*0.356*	2.587	0.947	*0.366*
WR	WR06	622	H2M 4	SS	SS	m	138	orange	64.7	2.290	0.577	*0.252*	2.703	1.097	*0.406*
WR	WR06	624	H4B 1	SS	SS	b	115	soft orng/salm	76.0	2.297	0.710	*0.309*	2.537	0.847	*0.334*
WR	WR06	624	H4B 8	SS	SS	b	331	orange	72.7	2.540	0.990	*0.390*	2.987	0.943	*0.316*
WR	WR06	624	H4M 1	SS	SS	m	272	gold	71.8	2.270	0.913	*0.402*	2.410	0.847	*0.351*
WR	WR06	624	H4M 2	SS	SS	m	123	yellow	68.5	2.170	0.680	*0.313*	2.813	0.980	*0.348*
WR	WR06	624	H4B 3	SS	SS	b	104	yellow	74.5	2.203	0.940	*0.427*	2.613	0.900	*0.344*
WR	WR10	1021	H1B 3	M	F	b	182	orange	59.7	2.290	0.750	*0.328*	2.447	0.647	*0.264*
WR	WR10	1021	H1B 4	M	F	b	119	orange	65.3	1.850	0.723	*0.391*	2.320	1.023	*0.441*
WR	WR10	1021	H1B 6	M	F	b	338	gold	69.7	2.257	0.677	*0.300*	2.580	0.927	*0.359*
WR	WR10	1021	H1B 7	M	F	b	261	orange	63.0	2.257	0.860	*0.381*	2.537	1.107	*0.436*
WR	WR10	1021	H1B 8	M	F	b	138	yell w/orng tips	65.0	2.737	0.913	*0.334*	2.830	1.007	*0.356*
WR	WR10	1021	H1M 2	M	F	m	114	golden	62.0	2.757	0.770	*0.279*	2.787	0.897	*0.322*
WR	WR10	1021	H1M 5	M	F	m	93	yellow	61.0	2.370	0.747	*0.315*	2.850	0.943	*0.331*
WR	WR10	1021	H1M 6	M	F	m	119	yellow	67.7	2.183	0.873	*0.400*	2.730	0.960	*0.352*
WR	WR10	1024	H4M 2	M	C	m	105	yellow	72.7	2.683	0.980	*0.365*	3.070	0.963	*0.314*
WR	WR10	1024	H4M 5	M	C	m	118	yellow	59.0	2.680	1.033	*0.386*	2.737	0.983	*0.359*
WR	WR10	1024	H4M 6	M	C	m	148	golden	72.3	2.673	0.903	*0.338*	2.827	0.917	*0.324*
WR	WR10	1024	H4M 8	M	C	m	127	golden	69.0	2.537	0.997	*0.393*	2.673	0.900	*0.337*
WR	WR10	1011	L1B 1	M	F	b	155	golden	72.0	2.822	0.842	*0.298*	2.698	0.623	*0.231*
WR	WR10	1011	L1B 8	M	F	b	106	~gold	73.0	3.070	0.927	*0.302*	2.717	0.617	*0.227*
WR	WR10	1011	L1M 1	M	F	m	147	yellow	75.3	2.723	0.790	*0.290*	2.797	0.697	*0.249*
WR	WR10	1011	L1M 4	M	F	m	363	yellow	74.5	2.693	0.870	*0.323*	2.970	0.857	*0.288*
WR	WR10	1011	L1M 8	M	F	m	97	yellow	66.5	3.030	0.927	*0.306*	3.003	0.773	*0.257*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
WR	WR10	1012	L2B 2	M	C	b	280	dark org	70.0	2.110	0.883	*0.419*	2.347	0.747	*0.318*
WR	WR10	1012	L2B 5	M	C	b	157	golden	72.7	2.787	1.053	*0.378*	2.250	0.583	*0.259*
WR	WR10	1012	L2M 3	M	C	m	220	yellow	67.7	2.367	1.127	*0.476*	3.000	1.130	*0.377*
WR	WR10	1012	L2M 4	M	C	m	208	yellow	75.3	2.073	0.890	*0.429*	2.270	0.573	*0.253*
WR	WR10	1012	L2M 8	M	C	m	208	yellow	62.3	3.113	0.973	*0.313*	3.293	1.067	*0.324*
WR	WR10	1013	L3B 1	M	F	b	115	gold	71.3	2.615	0.973	*0.372*	2.453	0.675	*0.275*
WR	WR10	1013	L3M 1	M	F	m	225	golden	71.3	3.083	1.057	*0.343*	2.850	1.150	*0.404*
WR	WR10	1013	L3M 4	M	F	m	92	gold	72.8	2.670	0.880	*0.330*	2.600	0.660	*0.254*
WR	WR10	1013	L3M 5	M	F	m	140	gold	72.8	2.883	0.957	*0.332*	2.573	0.670	*0.260*
WR	WR14	1411	L1B 3	S	S	b	80	yellow	69.3	3.647	1.087	*0.298*	3.147	0.857	*0.272*
WR	WR14	1411	L1B 4	S	S	b	85	yellow	75.7	3.373	0.960	*0.285*	3.047	0.867	*0.284*
WR	WR14	1411	L1B 5	S	S	b	93	yellow	79.0	2.627	0.770	*0.293*	2.530	0.653	*0.258*
WR	WR14	1411	L1M 2	S	S	m	93	yellow	75.3	2.693	0.723	*0.269*	2.677	0.743	*0.278*
WR	WR14	1412	L2B 1	S	S	b	95	yellow	72.0	2.477	0.817	*0.330*	2.550	0.633	*0.248*
WR	WR14	1412	L2B 2	S	S	b	76	yellow	64.3	2.900	1.080	*0.372*	2.797	0.683	*0.244*
WR	WR14	1412	L2B 7	S	S	b	83	yellow	72.7	2.777	1.063	*0.383*	2.563	0.750	*0.293*
WR	WR14	1412	L2M 3	S	S	m	142	yellow ~green	70.7	2.743	0.960	*0.350*	2.257	0.560	*0.248*
WR	WR14	1413	L3B 1	S	S	b	66	yellow	74.3	2.843	0.753	*0.265*	2.523	0.707	*0.280*
WR	WR14	1413	L3B 6	S	S	b	105	yellow	77.3	2.110	0.687	*0.325*	2.637	0.833	*0.316*
WR	WR14	1413	L3B 8	S	S	b	92	yellow	70.3	2.817	0.683	*0.243*	2.637	0.707	*0.268*
WR	WR14	1413	L3M 5	S	S	m	86	yellow	75.0	2.670	0.580	*0.217*	2.527	0.753	*0.298*
WR	WR14	1413	L3M 6	S	S	m	318	pale gold	72.0	2.630	0.917	*0.349*	2.287	0.740	*0.324*
WR	WR16	16521	H1B 3	M	F	b	161	gold	72.0	2.555	0.858	*0.336*	3.058	1.088	*0.356*
WR	WR16	16521	H1B 4	M	F	b	175	~dark orange	65.7	2.827	1.083	*0.383*	3.280	1.340	*0.409*
WR	WR16	16521	H1B 8	M	F	b	150	yellow	70.7	2.313	0.773	*0.334*	3.333	1.183	*0.355*
WR	WR16	16521	H1M 1	M	F	m	111	soft orange	61.3	2.447	1.067	*0.436*	3.153	1.203	*0.382*
WR	WR16	16521	H1M 8	M	F	m	397	salmon	67.8	2.225	0.873	*0.392*	2.938	1.130	*0.385*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
WR	WR16	16522	H2B 7	M	C	b	205	soft orange	77.3	2.637	0.820	*0.311*	2.873	0.830	*0.289*
WR	WR16	16522	H2B 8	M	C	b	179	orng tip/yellow	69.7	2.250	0.800	*0.356*	2.660	0.847	*0.318*
WR	WR16	16522	H2M 2	M	C	m	151	yellow orng-tip	68.3	2.600	0.913	*0.351*	2.830	0.963	*0.340*
WR	WR16	16522	H2M 5	M	C	m	147	yellow	70.0	2.700	1.033	*0.383*	2.873	0.967	*0.336*
WR	WR16	16522	H2M 7	M	C	m	220	yellow	72.7	2.583	0.970	*0.375*	2.937	1.000	*0.341*
WR	WR16	16522	H2M 8	M	C	m	169	golden	69.0	2.248	0.758	*0.337*	2.480	0.897	*0.362*
WR	WR16	16523	H3B 3	M	F	b	126	deep orng edges	54.0	2.180	0.887	*0.407*	2.697	0.980	*0.363*
WR	WR16	16523	H3B 6	M	F	b	180	carrot org	66.3	2.283	0.910	*0.399*	2.683	0.933	*0.348*
WR	WR16	16523	H3M 3	M	F	m	152	ORANGE	66.3	2.373	0.747	*0.315*	3.363	1.227	*0.365*
WR	WR16	16523	H3M 4	M	F	m	132	orange	58.7	2.333	0.860	*0.369*	2.987	1.117	*0.374*
WR	WR16	16513	L3B 8	M	C	b	385	yellow	74.0	3.623	1.080	*0.298*	3.167	0.853	*0.269*
WR	WR16	16513	L3M 1	M	C	m	148	gold	75.7	2.580	1.017	*0.394*	2.410	0.677	*0.281*
WR	WR16	16513	L3M 5	M	C	m	112	yellow	72.5	3.043	0.980	*0.322*	2.690	0.713	*0.265*
WR	WR16	16513	L3M 6	M	C	m	197	yellow	68.3	2.723	0.853	*0.313*	2.747	0.793	*0.289*
WR	WR16	16514	L4B 1	M	F	b	94	yellow	72.3	2.950	1.287	*0.436*	2.930	0.733	*0.250*
WR	WR16	16514	L4B 4	M	F	b	220	gold	75.9	3.070	1.147	*0.374*	2.760	0.640	*0.232*
WR	WR16	16514	L4B 8	M	F	b	154	yellow	71.7	2.977	0.873	*0.293*	2.883	0.813	*0.282*
WR	WR16	16514	L4M 1	M	F	m	94	yellow	74.0	3.447	0.800	*0.232*	3.057	0.827	*0.270*
WR	WR16	16514	L4M 2	M	F	m	175	yellow	68.0	3.170	1.040	*0.328*	2.690	0.760	*0.283*
WR	WR16	16514	L4M 5	M	F	m	162	yellow	70.3	2.420	0.873	*0.361*	2.630	1.053	*0.401*
WR	WR16	16514	L4M 7	M	F	m	139	YELLOW	65.7	3.103	0.857	*0.276*	3.270	1.073	*0.328*
WR	WR22	2221	H1B 1	M	F	b	226	orange	59.3	2.783	1.270	*0.456*	2.740	1.147	*0.418*
WR	WR22	2221	H1B 3	M	F	b	110	yellow	73.5	2.650	1.070	*0.406*	2.822	0.858	*0.304*
WR	WR22	2221	H1B 8cot	M	F	b	310	salmon	64.7	2.687	1.127	*0.419*	2.970	1.220	*0.411*
WR	WR22	2221	H1M 4	M	F	m	242	yellow	71.7	2.650	1.050	*0.396*	2.850	0.937	*0.329*
WR	WR22	2221	H1M 5	M	F	m	198	golden	63.7	2.340	0.750	*0.321*	2.600	0.783	*0.301*
WR	WR22	2221	H1M 6	M	F	m	155	yellow	70.0	2.507	0.587	*0.234*	2.513	0.860	*0.342*
WR	WR22	2222	H2B 6	M	C	b	126	yell w/ soft orng	61.3	2.297	0.947	*0.412*	2.720	0.960	*0.353*
WR	WR22	2224	H4B 8	M	C	b	134	Y/O	67.0	1.940	0.600	*0.309*	2.383	0.820	*0.344*
WR	WR22	2224	H4M 1	M	C	m	121	yellow	74.3	2.230	0.983	*0.441*	2.907	1.157	*0.398*
WR	WR22	2224	H4M 6	M	C	m	132	y/o	69.7	2.683	0.967	*0.360*	2.467	0.733	*0.297*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
WR	WR22	2224	H4M 7	M	C	m	132	y/o	68.3	2.313	0.747	*0.323*	2.840	0.970	*0.342*
WR	WR22	2224	H4M 8	M	C	m	195	Y/O	62.3	2.463	1.040	*0.422*	3.040	1.037	*0.341*
WR	WR22	2211	L1B 3	M	C	b	237	yellow	71.3	2.250	0.720	*0.320*	2.208	0.543	*0.246*
WR	WR22	2211	L1B 4	M	C	b	117	yellow	72.3	2.640	0.590	*0.223*	2.633	0.807	*0.306*
WR	WR22	2211	L1M 1	M	C	m	161	yellow	75.0	2.427	0.617	*0.254*	2.380	0.637	*0.268*
WR	WR22	2211	L1M 6	M	C	m	120	gold	74.3	2.783	0.727	*0.261*	2.473	0.673	*0.272*
WR	WR22	2213	L3B 4	M	C	b	120	yellow	75.3	2.700	0.837	*0.310*	2.910	0.857	*0.294*
WR	WR22	2213	L3M 2	M	C	m	106	yellow	68.7	1.940	0.700	*0.361*	2.493	0.800	*0.321*
WR	WR22	2213	L3M 3	M	C	m	93	yellow	64.0	2.587	0.723	*0.280*	2.760	0.600	*0.217*
WR	WR22	2213	L3M 7	M	C	m	125	yellow	68.7	3.193	1.090	*0.341*	3.000	0.790	*0.263*
WR	WR22	2213	L3M 8	M	C	m	111	yellow	66.7	2.427	0.700	*0.288*	2.710	0.800	*0.295*
WR	WR22	2214	L4B 5	M	F	b	138	yellow	70.0	3.050	0.943	*0.309*	2.713	0.613	*0.226*
WR	WR22	2214	L4B 7	M	F	b	141	golden	69.3	2.425	0.875	*0.361*	2.125	0.600	*0.282*
WR	WR22	2214	L4M 1	M	F	m	208	yellow	73.0	2.713	0.973	*0.359*	2.720	0.683	*0.251*
WR	WR22	2214	L4M 4b	M	F	m	134	yellow	73.0	2.747	0.757	*0.275*	2.837	0.780	*0.275*
WR	WR22	2214	L4M 7	M	F	m	209	yellow	71.3	2.847	1.080	*0.379*	2.607	0.693	*0.266*
WR	WR26	26521	H1B 4	M	F	b	112	salmon	63.0	2.540	0.963	*0.379*	2.930	0.993	*0.339*
WR	WR26	26521	H1M 1	M	F	m	143	gold	67.0	2.387	0.660	*0.277*	2.817	0.903	*0.321*
WR	WR26	26521	H1M 3	M	F	m	168	salmon tips	66.3	2.403	0.837	*0.348*	2.907	0.943	*0.325*
WR	WR26	26521	H1M 4	M	F	m	237	light salmon	69.5	2.237	0.987	*0.441*	2.657	0.947	*0.356*
WR	WR26	26521	H1M 6	M	F	m	349	yell w/ org tip	66.7	2.243	0.987	*0.440*	2.243	0.593	*0.264*
WR	WR26	26522	H2B 3	M	C	b	109	salmon	58.7	2.320	0.937	*0.404*	2.773	0.987	*0.356*
WR	WR26	26522	H2B 6	M	C	b	162	light salmon	65.8	1.808	0.693	*0.383*	2.588	0.863	*0.333*
WR	WR26	26522	H2M/T 4	M	C	m	179	yellow	64.3	2.295	0.717	*0.312*	2.827	0.937	*0.331*
WR	WR26	26524	H4B 3	M	F	b	199	golden yellow	64.3	2.327	0.913	*0.393*	2.873	0.920	*0.320*
WR	WR26	26524	H4B 8	M	F	b	90	yellow	63.0	2.693	1.060	*0.394*	2.973	0.987	*0.332*
WR	WR26	26524	H4M 1	M	F	m	150	yellow	71.3	3.187	1.053	*0.331*	2.733	1.063	*0.389*
WR	WR26	26524	H4M 3	M	F	m	121	yellow	64.7	2.327	0.913	*0.393*	2.873	0.920	*0.320*
WR	WR26	26524	H4M 6	M	F	m	508	~gold	75.3	2.197	0.890	*0.405*	2.557	0.943	*0.369*
WR	WR26	26524	H4M 7	M	F	m	150	yellow	65.7	2.717	0.897	*0.330*	2.543	0.847	*0.333*
WR	WR26	26511	L1B 3	M	F	b	134	yellow	78.0	2.790	0.640	*0.229*	2.563	0.700	*0.273*
WR	WR26	26511	L1B 6	M	F	b	145	yellow	64.3	2.453	0.733	*0.299*	2.667	0.727	*0.273*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
WR	WR26	26511	L1B 8	M	F	b	107	yellow	66.7	2.943	0.623	*0.212*	2.610	0.577	*0.221*
WR	WR26	26511	L1M 1	M	F	m	106	yellow	69.7	2.747	0.820	*0.299*	2.467	0.717	*0.291*
WR	WR26	26511	L1M 4	M	F	m	188	yellow	73.7	2.477	0.740	*0.299*	2.500	0.663	*0.265*
WR	WR26	26511	L1M 7	M	F	m	464	gold	74.7	2.047	0.683	*0.334*	2.267	0.607	*0.268*
WR	WR26	26513	L3B 2	M	C	b	352	gold	74.8	2.607	0.903	*0.347*	2.680	0.757	*0.282*
WR	WR26	26513	L3B 3	M	C	b	178	yellow	79.0	2.397	0.903	*0.377*	2.483	0.673	*0.271*
WR	WR26	26513	L3B 5	M	C	b	107	yellow	55.3	2.360	0.617	*0.261*	2.913	0.887	*0.304*
WR	WR26	26513	L3M 2	M	C	m	171	yellow	74.3	2.430	1.103	*0.454*	3.197	1.053	*0.330*
WR	WR26	26513	L3M 3	M	C	m	325	gold	74.0	2.360	0.913	*0.387*	2.480	0.678	*0.273*
WR	WR26	26513	L3M 5	M	C	m	142	burnt yellow	68.0	2.828	1.093	*0.386*	2.510	0.693	*0.276*
WR	WR32	3222	H2B 1	M	C	b	128	YELLOW	53.3	2.593	0.963	*0.371*	3.047	0.933	*0.306*
WR	WR32	3222	H2B 4	M	C	b	92	soft orange	64.3	2.503	0.730	*0.292*	2.683	0.907	*0.338*
WR	WR32	3222	H2B 7	M	C	b	72	salmon	67.3	2.880	0.833	*0.289*	3.237	1.003	*0.310*
WR	WR32	3222	H2M 4	M	C	m	140	yellow	63.0	2.227	0.770	*0.346*	2.380	0.773	*0.325*
WR	WR32	3222	H2M 5	M	C	m	79	yellow	69.3	2.600	0.997	*0.383*	2.667	0.847	*0.318*
WR	WR32	3222	H2M 8	M	C	m	87	yellow	47.0	3.067	0.917	*0.299*	3.003	0.967	*0.322*
WR	WR32	3223	H3B 6	M	F	b	107	salmon	59.3	2.073	0.378	*0.182*	2.298	0.865	*0.376*
WR	WR32	3223	H3M 4	M	F	m	380	soft orange	69.7	2.250	1.037	*0.461*	2.733	0.913	*0.334*
WR	WR32	3211	L1B 1	M	C	b	90	yellow	70.0	2.927	0.880	*0.301*	2.937	0.937	*0.319*
WR	WR32	3211	L1B 4	M	C	b	98	yellow	69.7	2.873	1.087	*0.378*	2.607	0.793	*0.304*
WR	WR32	3211	L1B 5	M	C	b	98	yellow	78.7	2.650	0.710	*0.268*	2.627	0.730	*0.278*
WR	WR32	3211	L1B 7	M	C	b	100	yellow	73.0	2.513	0.970	*0.386*	2.347	0.710	*0.303*
WR	WR32	3211	L1B 8	M	C	b	84	yellow	71.7	3.003	0.940	*0.313*	2.683	0.703	*0.262*
WR	WR32	3211	L1M 1	M	C	m	142	yellow	61.0	2.930	1.060	*0.362*	2.967	0.857	*0.289*
WR	WR32	3211	L1M 2	M	C	m	133	yellow, soft	69.7	2.490	0.757	*0.304*	2.650	0.750	*0.283*
WR	WR32	3211	L1M 3	M	C	m	232	yellow	76.0	2.660	1.023	*0.385*	2.883	0.837	*0.290*

Site	Plot	Parent	#	Context	Distance	Capsule	Age	color	Galea-angle	Bract-length	Lobe-length	*Lobe-Brt ratio*	Galea-length	Beak-length	*Bk-G ratio*
WR	WR32	3213	L3B 2	M	C	b	407	yellow	70.5	3.197	0.997	*0.312*	2.850	0.710	*0.249*
WR	WR32	3213	L3B 4	M	C	b	64	gold	74.7	2.353	0.683	*0.290*	2.917	0.877	*0.301*
WR	WR32	3213	L3B 6	M	C	b	234	~yellow/green	64.0	3.033	0.930	*0.307*	2.460	0.577	*0.234*
WR	WR32	3213	L3B 7	M	C	b	108	~yellow/green	71.0	1.983	0.543	*0.274*	2.630	0.870	*0.331*
WR	WR32	3213	L3M 1	M	C	m	126	yellow	74.0	2.430	0.893	*0.368*	2.947	0.987	*0.335*
WR	WR32	3213	L3M 5	M	C	m	98	yellow	74.3	2.773	1.017	*0.367*	2.947	0.740	*0.251*
WR	WR32	3213	L3M 8	M	C	m	118	yellow	69.0	2.307	0.797	*0.345*	2.827	0.890	*0.315*
WR	WR32	3214	L4B 3	M	F	b	123	yellow	70.0	2.260	0.797	*0.353*	3.110	0.973	*0.313*
WR	WR32	3214	L4B 4	M	F	b	119	yellow	71.7	2.400	1.050	*0.438*	2.277	0.593	*0.261*
WR	WR32	3214	L4B 5	M	F	b	126	yell w/org edge	75.0	2.870	0.857	*0.298*	3.273	1.237	*0.378*
WR	WR32	3214	L4B 7	M	F	b	161	yellow	71.8	2.913	1.113	*0.382*	3.363	1.137	*0.338*
WR	WR32	3214	L4M 4	M	F	m	321	gold	74.7	2.577	0.827	*0.321*	2.510	0.697	*0.278*
WR	WR32	3214	L4M 8	M	F	m	90	yellow	70.0	2.500	0.810	*0.324*	2.470	0.573	*0.232*

- “cot” notation indicates a triplet cotyledon trait at the seedling stage of F1 plants
- M/T in plant number indicate seed capsule was taken from middle but close to the top of the stem.
- Distances are closer than 30 cm to other species- “C”, further than 30 cm but within same plot- “F”, in a semi-solo plot & closer than 20 meters- “SS”, in solo plot, further than 40 meters- “S”, and off-site- “off-site”.

- Contexts are either controls from off-site, solo-species (S), semi-solo (SS), or mixed-species (M).
- Ages are in days, from time of first planting.

Pollen Viabilities

Parent plants	control	site	Plot	parent Sp	parent #	Capsule	context	Distance	Tube germ	Aceto carmine
CAHI1 2	1	Off	2	2	206		control	Off-site	0.55	0.62
CAHI1 10	1	Off	2	2	205		control	Off-site	0.58	0.92
CAHI3 9	1	Off	2	2	209		control	Off-site	0.71	0.94
CAHI1 3	1	Off	2	2	207		control	Off-site	0.47	0.94
CAHI 6	1	Off	2	2	203		control	Off-site	0.59	0.95
CAHI2 6	1	Off	2	2	208		control	Off-site	0.49	0.97
CAHI 8	1	Off	2	2	204		control	Off-site	0.59	0.95
CAHI 16	1	Off	2	2	202		control	Off-site	0.60	0.96
CAHI 10	1	Off	2	2	201		control	Off-site	0.22	0.95
H2M 4	1	GH	2.1	2	222	M	solo	solo	0.29	0.84
H2M 5	1	GH	2.1	2	222	M	solo	solo	0.30	0.98
H2M 8	1	GH	2.1	2	222	M	solo	solo	0.43	0.58
H3B 4	1	GH	2.1	2	223	B	solo	solo	0.68	0.90
H3B 7	1	GH	2.1	2	223	B	solo	solo	0.62	0.94
H3M 1	1	GH	2.1	2	223	M	solo	solo	0.56	0.81
H3M 5	1	GH	2.1	2	223	M	solo	solo	0.42	0.93
H3M 6	1	GH	2.1	2	223	M	solo	solo	0.45	0.87
H3M 7	1	GH	2.1	2	223	M	solo	solo	0.26	0.83
H4B 4	1	GH	2.1	2	224	B	solo	solo	0.35	0.36
H4B 5	1	GH	2.1	2	224	B	solo	solo	0.48	0.95
H4B 6	1	GH	2.1	2	224	B	solo	solo	0.60	0.89
H4M 2	1	GH	2.1	2	224	M	solo	solo	0.33	0.61
H4M 4	1	GH	2.1	2	224	M	solo	solo	0.51	0.95
H4M 6	1	GH	2.1	2	224	M	solo	solo	0.68	0.95
H4M 7	1	GH	2.1	2	224	M	solo	solo	0.55	0.72
H2M 7	1	GH	2.1	2	222	M	solo	solo	0.42	0.96
H1B 3	2	GH	3	2	321	B	Mix	F	0.46	0.69
H1B 4	2	GH	3	2	321	B	Mix	F	0.53	0.89
H1B 7	2	GH	3	2	321	B	Mix	F	0.53	0.73
H1M 3	2	GH	3	2	321	M	Mix	F	0.13	0.77
H1M 4	2	GH	3	2	321	M	Mix	F	0.14	0.62

H1M 8	2	GH	3	2	321	M	Mix	F	0.38	0.60
H3B 8	2	GH	3	2	323	B	Mix	C	0.23	0.95
H4B 4	2	GH	3	2	324	B	Mix	F	0.34	0.85
H4B 6	2	GH	3	2	324	B	Mix	F	0.35	0.91
H4M 2	2	GH	3	2	324	M	Mix	F	0.42	0.79
H4M 6	2	GH	3	2	324	M	Mix	F	0.38	0.94
H4M 7	2	GH	3	2	324	M	Mix	F	0.02	0.89
H4M 8	2	GH	3	2	324	M	Mix	F	0.01	0.83
H1B 1b	2	GH	7	2	721	B	Mix	C	0.34	0.83
H1B 4	2	GH	7	2	721	B	Mix	C	0.53	0.97
H1B 6	2	GH	7	2	721	B	Mix	C	0.27	0.95
H1M 2	2	GH	7	2	721	M	Mix	C	0.56	0.96
H1M 5	2	GH	7	2	721	M	Mix	C	0.31	0.90
H2B 1	2	GH	7	2	722	B	Mix	F	0.04	0.77
H2B 3	2	GH	7	2	722	B	Mix	F	0.25	0.92
H2M 3	2	GH	7	2	722	M	Mix	F	0.19	0.87
H3B 7	2	GH	7	2	723	B	Mix	F	0.60	0.90
H3M 4	2	GH	7	2	723	M	Mix	F	0.42	0.71
H3M 7	2	GH	7	2	723	M	Mix	F	0.24	0.75
H3M 8	2	GH	7	2	723	M	Mix	F	0.44	0.89
H1B 1	2	GH	16	2	1621	B	Mix	C	0.28	0.73
H1B 4	2	GH	16	2	1621	B	Mix	C	0.61	0.75
H1B 8	2	GH	16	2	1621	B	Mix	C	0.44	0.67
H1M 1	2	GH	16	2	1621	M	Mix	C	0.39	0.96
H1M 3	2	GH	16	2	1621	M	Mix	C	0.13	0.65
H1M 5	2	GH	16	2	1621	M	Mix	C	0.34	0.75
H1M 7	2	GH	16	2	1621	M	Mix	C	0.07	0.32
H3B 1	2	GH	16	2	1623	B	Mix	C	0.33	0.95
H3B 2	2	GH	16	2	1623	B	Mix	C	0.33	0.59
H3B 5	2	GH	16	2	1623	B	Mix	C	0.42	0.87
H3B 6	2	GH	16	2	1623	B	Mix	C	0.30	0.71
H3B 8	2	GH	16	2	1623	B	Mix	C	0.78	0.98
H3M 1b	2	GH	16	2	1623	M	Mix	C	0.40	0.91
H3M 2	2	GH	16	2	1623	M	Mix	C	0.63	0.61
H3M 6	2	GH	16	2	1623	M	Mix	C	0.16	0.95
H3M 8	2	GH	16	2	1623	M	Mix	C	0.28	0.70
H4B 4	2	GH	16	2	1624	B	Mix	F	0.01	0.24
H4B 7	2	GH	16	2	1624	B	Mix	F	0.37	0.30
H4B 8	2	GH	16	2	1624	B	Mix	F	0.29	0.74
H4M 2	2	GH	16	2	1624	M	Mix	F	0.15	0.77
H4M 7	2	GH	16	2	1624	M	Mix	F	0.46	0.90

Parent plants	control	site	Plot	parent Sp	parent #	Capsule	context	Distance	Tube germ	Aceto carmine
H4M 8	2	GH	16	2	1624	M	Mix	F	0.29	0.94
H1B 1	2	GH	18	2	1821	B	Mix	C	0.08	0.81
H1B 2	2	GH	18	2	1821	B	Mix	C	0.16	0.35
H1B 3	2	GH	18	2	1821	B	Mix	C	0.32	0.72
H1B 5	2	GH	18	2	1821	B	Mix	C	0.32	0.45
H1M 1	2	GH	18	2	1821	M	Mix	C	0.22	0.60
H1M 3	2	GH	18	2	1821	M	Mix	C	0.29	0.67
H1M 3b	2	GH	18	2	1821	M	Mix	C	0.30	0.46
H1M 5	2	GH	18	2	1821	M	Mix	C	0.06	0.82
H1M 7	2	GH	18	2	1821	M	Mix	C	0.00	0.85
H2B 1	2	GH	18	2	1822	B	Mix	F	0.06	0.42
H2B 7	2	GH	18	2	1822	B	Mix	F	0.24	0.76
H1B 5	2	GH	29	2	2921	B	Mix	C	0.19	0.73
H1B 8	2	GH	29	2	2921	B	Mix	C	0.29	0.76
H1M 1	2	GH	29	2	2921	M	Mix	C	0.36	0.88
H1M 2	2	GH	29	2	2921	M	Mix	C	0.53	0.97
H1M 5	2	GH	29	2	2921	M	Mix	C	0.44	0.73
H1M 6	2	GH	29	2	2921	M	Mix	C	0.61	0.84
H2B 6	2	GH	29	2	2922	B	Mix	F	0.20	0.72
H2B 8	2	GH	29	2	2922	B	Mix	F	0.01	0.50
H2M 1	2	GH	29	2	2922	M	Mix	F	0.33	0.75
H2M 2	2	GH	29	2	2922	M	Mix	F	0.21	0.80
H2M 5	2	GH	29	2	2922	M	Mix	F	0.19	0.85
H3B 4	2	GH	29	2	2923	B	Mix	C	0.29	0.96
H3B 7	2	GH	29	2	2923	B	Mix	C	0.43	0.96
H3M 1	2	GH	29	2	2923	M	Mix	C	0.44	0.95
H3M 2	2	GH	29	2	2923	M	Mix	C	0.06	0.95
H3M 3	2	GH	29	2	2923	M	Mix	C	0.06	0.76
H3M 7	2	GH	29	2	2923	M	Mix	C	0.01	0.25
Lev 1	1	Off	1	1	110		control	Off-site	0.03	0.81
Lev 4	1	Off	1	1	111		control	Off-site	0.04	0.23
Lev 2	1	Off	1	1	112		control	Off-site	0.16	0.59
CALE08 RC-0811	1	Off	1	1	113		control	Off-site	0.29	0.95
Lev 1	1	Off	1	1	101		control	Off-site	0.37	0.94
Lev 2	1	Off	1	1	102		control	Off-site	0.30	0.95

Lev 3	1	Off	1	1	103		control	Off-site	0.31	0.94
Lev 3	1	Off	1	1	104		control	Off-site	0.35	0.93
Lev 1	1	Off	1	1	105		control	Off-site	0.24	0.52
Lev 2	1	Off	1	1	106		control	Off-site	0.00	0.07
Lev 3	1	Off	1	1	107		control	Off-site	0.01	0.71
Lev 2	1	Off	1	1	108		control	Off-site	0.02	0.86
Lev 1	1	Off	1	1	109		control	Off-site	0.01	0.24
L1B 1	2	GH	3	1	311	B	mix	F	0.36	0.92
L1B 2	2	GH	3	1	311	B	mix	F	0.44	0.96
L1B 3	2	GH	3	1	311	B	mix	F	0.08	0.83
L1B 5	2	GH	3	1	311	B	mix	F	0.54	0.91
L1B 6	2	GH	3	1	311	B	mix	F	0.33	0.97
L1B 8	2	GH	3	1	311	B	mix	F	0.29	0.93
L1M 2	2	GH	3	1	311	M	mix	F	0.03	0.55
L4B 1b	2	GH	3	1	314	B	mix	C	0.25	0.96
L4B 3	2	GH	3	1	314	B	mix	C	0.46	0.94
L4M 2cot	2	GH	3	1	314	M	mix	C	0.18	0.94
L4M 7	2	GH	3	1	314	M	mix	C	0.20	0.93
L4M 8	2	GH	3	1	314	M	mix	C	0.37	0.95
L3B 3	2	GH	7	1	713	B	mix	F	0.34	0.84
L3B 5	2	GH	7	1	713	B	mix	F	0.30	0.96
L3B 8	2	GH	7	1	713	B	mix	F	0.16	0.95
L3M 2	2	GH	7	1	713	M	mix	F	0.32	0.77
L3M 5	2	GH	7	1	713	M	mix	F	0.37	0.95
L3M 6	2	GH	7	1	713	M	mix	F	0.36	0.91
L4B 1	2	GH	7	1	714	B	mix	C	0.39	0.96
L4B 5	2	GH	7	1	714	B	mix	C	0.51	0.91
L4B 6	2	GH	7	1	714	B	mix	C	0.48	0.99
L4B 7	2	GH	7	1	714	B	mix	C	0.31	0.97
L4M 5	2	GH	7	1	714	M	mix	C	0.45	0.98
L1B 1	2	GH	16	1	1611	B	mix	C	0.48	0.94
L1B 3	2	GH	16	1	1611	B	mix	C	0.59	0.96
L1B 3b	2	GH	16	1	1611	B	mix	C	0.02	0.96
L1B 7	2	GH	16	1	1611	B	mix	C	0.20	0.53
L1M 1	2	GH	16	1	1611	M	mix	C	0.49	0.97
L1M 5	2	GH	16	1	1611	M	mix	C	0.55	0.99

Parent plants	control	site	Plot	parent Sp	parent #	Capsule	context	Distance	Tube germ	Aceto carmine
L2B 3	2	GH	16	1	1612	B	mix	F	0.37	0.96
L2B 4	2	GH	16	1	1612	B	mix	F	0.46	0.96
L4B 4	2	GH	16	1	1614	B	mix	F	0.39	0.98
L4B 7	2	GH	16	1	1614	B	mix	F	0.23	0.91
L4B 8	2	GH	16	1	1614	B	mix	F	0.35	0.88
L4M 2b	2	GH	16	1	1614	M	mix	F	0.01	0.89
L4M 5	2	GH	16	1	1614	M	mix	F	0.22	0.95
L4M 6	2	GH	16	1	1614	M	mix	F	0.23	0.98
L1B 4	2	GH	18	1	1811	B	mix	F	0.17	0.80
L1B 5	2	GH	18	1	1811	B	mix	F	0.01	0.92
L1B 6	2	GH	18	1	1811	B	mix	F	0.20	0.90
L1M 5	2	GH	18	1	1811	M	mix	F	0.46	0.96
L1M 7	2	GH	18	1	1811	M	mix	F	0.55	0.94
L1M 8	2	GH	18	1	1811	M	mix	F	0.40	0.93
L2B 2	2	GH	18	1	1812	B	mix	C	0.60	0.98
L2B 4	2	GH	18	1	1812	B	mix	C	0.22	0.68
L2B 5	2	GH	18	1	1812	B	mix	C	0.45	0.93
L2B 6	2	GH	18	1	1812	B	mix	C	0.32	0.84
L2M 2	2	GH	18	1	1812	M	mix	C	0.37	0.95
L2M 6	2	GH	18	1	1812	M	mix	C	0.43	0.93
L4B 1	2	GH	18	1	1814	B	mix	C	0.48	0.99
L4B 3	2	GH	18	1	1814	B	mix	C	0.45	0.97
L4M 4	2	GH	18	1	1814	M	mix	C	0.43	0.83
L4M 7	2	GH	18	1	1814	M	mix	C	0.52	0.98
L2M 3	1	GH	21	1	2112	B	solo	solo	0.04	0.75
L2B 6	1	GH	21	1	2112	B	solo	solo	0.41	0.96
L2B 8	1	GH	21	1	2112	B	solo	solo	0.19	0.99
L2M 5	1	GH	21	1	2112	M	solo	solo	0.30	0.92
L2M 7	1	GH	21	1	2112	M	solo	solo	0.49	0.93
L3B 3	1	GH	21	1	2113	B	solo	solo	0.03	0.94
L3B 5	1	GH	21	1	2113	B	solo	solo	0.28	0.59
L3B 6	1	GH	21	1	2113	B	solo	solo	0.22	0.94
L3B 7	1	GH	21	1	2113	B	solo	solo	0.55	0.97
L3M 6	1	GH	21	1	2113	M	solo	solo	0.40	0.96
L3M 7	1	GH	21	1	2113	M	solo	solo	0.29	0.96
L3M 8	1	GH	21	1	2113	M	solo	solo	0.34	0.60
L4B 3	1	GH	21	1	2114	B	solo	solo	0.23	0.96
L4M 5	1	GH	21	1	2114	M	solo	solo	0.31	0.97
L4M 6	1	GH	21	1	2114	M	solo	solo	0.18	0.82

L4M 8	1	GH	21	1	2114	M	solo	solo	0.34	0.94
L2B 2	2	GH	26	1	2612	B	SS	SS	0.20	0.90
L2B 5	2	GH	26	1	2612	B	SS	SS	0.02	0.46
L2M 1	2	GH	26	1	2612	T	SS	SS	0.55	0.97
L2M 2	2	GH	26	1	2612	T	SS	SS	0.50	0.98
L2M 3	2	GH	26	1	2612	T	SS	SS	0.58	0.94
L2B 7	2	GH	29	1	2912	B	mix	F	0.24	0.96
L2B 8	2	GH	29	1	2912	B	mix	F	0.31	0.97
L2M 1	2	GH	29	1	2912	M	mix	F	0.01	0.97
L2M 2	2	GH	29	1	2912	M	mix	F	0.13	0.86
L2M 3	2	GH	29	1	2912	M	mix	F	0.14	0.43
L2M 4	2	GH	29	1	2912	M	mix	F	0.46	0.80
L3B 2	2	GH	29	1	2913	B	mix	C	0.27	0.51
L3B 4	2	GH	29	1	2913	B	mix	C	0.00	0.21
L3B 5	2	GH	29	1	2913	B	mix	C	0.28	0.96
L3M 4	2	GH	29	1	2913	M	mix	C	0.17	0.61
L3M 5	2	GH	29	1	2913	M	mix	C	0.09	0.57
L4B 4	2	GH	29	1	2914	B	mix	F	0.46	0.95
L4B 8	2	GH	29	1	2914	B	mix	F	0.32	0.95
L4M 2	2	GH	29	1	2914	M	mix	F	0.00	0.95
L4M 6	2	GH	29	1	2914	M	mix	F	0.43	0.97
L4M 7	2	GH	29	1	2914	M	mix	F	0.26	0.97
H1B 3	2	WR	6	2	621	B	SS	SS	0.50	0.92
H1B 4	2	WR	6	2	621	B	SS	SS	0.34	0.93
H1M 1	2	WR	6	2	621	M	SS	SS	0.28	0.68
H1M 5	2	WR	6	2	621	M	SS	SS	0.19	0.90
H1M 7	2	WR	6	2	621	M	SS	SS	0.01	0.94
H2B 4	2	WR	6	2	622	B	SS	SS	0.45	0.97
H2M 1	2	WR	6	2	622	M	SS	SS	0.37	0.96
H2M 4	2	WR	6	2	622	M	SS	SS	0.26	0.70
H4B 1	2	WR	6	2	624	B	SS	SS	0.40	0.90
H4B 3	2	WR	6	2	624	B	SS	SS	0.13	0.28
H4B 8	2	WR	6	2	624	B	SS	SS	0.10	0.29
H4M 1	2	WR	6	2	624	M	SS	SS	0.02	0.56
H4M 2	2	WR	6	2	624	M	SS	SS	0.14	0.22
H1B 3	2	WR	10	2	1021	B	Mix	F	0.02	0.46
H1B 4	2	WR	10	2	1021	B	Mix	F	0.04	0.48
H1B 6	2	WR	10	2	1021	B	Mix	F	0.36	0.91
H1B 7	2	WR	10	2	1021	B	Mix	F	0.41	0.69
H1B 8	2	WR	10	2	1021	B	Mix	F	0.27	0.85
H1M 2	2	WR	10	2	1021	M	Mix	F	0.51	0.67

Parent plants	control	site	Plot	parent Sp	parent #	Capsule	context	Distance	Tube germ	Aceto carmine
H1M 5	2	WR	10	2	1021	M	Mix	F	0.68	0.96
H1M 6	2	WR	10	2	1021	M	Mix	F	0.03	0.41
H4M 2	2	WR	10	2	1024	M	Mix	C	0.30	0.67
H4M 5	2	WR	10	2	1024	M	Mix	C	0.25	0.77
H4M 6	2	WR	10	2	1024	M	Mix	C	0.52	0.84
H4M 8	2	WR	10	2	1024	M	Mix	C	0.57	0.72
H1B 3	2	WR	16.5	2	16521	B	Mix	F	0.13	0.88
H1B 4	2	WR	16.5	2	16521	B	Mix	F	0.36	0.35
H1B 8	2	WR	16.5	2	16521	B	Mix	F	0.51	0.92
H1M 1	2	WR	16.5	2	16521	M	Mix	F	0.04	0.55
H1M 8	2	WR	16.5	2	16521	M	Mix	F	0.61	0.96
H2B 7	2	WR	16.5	2	16522	B	Mix	C	0.20	0.72
H2B 8	2	WR	16.5	2	16522	B	Mix	C	0.21	0.80
H2M 2	2	WR	16.5	2	16522	M	Mix	C	0.15	0.78
H2M 5	2	WR	16.5	2	16522	M	Mix	C	0.34	0.93
H2M 7	2	WR	16.5	2	16522	M	Mix	C	0.57	0.95
H2M 8	2	WR	16.5	2	16522	M	Mix	C	0.63	0.91
H3B 3	2	WR	16.5	2	16523	B	Mix	F	0.46	0.86
H3B 6	2	WR	16.5	2	16523	B	Mix	F	0.44	0.94
H3M 4	2	WR	16.5	2	16523	M	Mix	F	0.00	0.24
H3M 7	2	WR	16.5	2	16523	M	Mix	F	0.52	0.93
H1B 1	2	WR	22	2	2221	B	Mix	F	0.44	0.57
H1B 3	2	WR	22	2	2221	B	Mix	F	0.15	0.66
H1B 8cot	2	WR	22	2	2221	B	Mix	F	0.26	0.84
H1M 4	2	WR	22	2	2221	M	Mix	F	0.06	0.32
H1M 5	2	WR	22	2	2221	M	Mix	F	0.36	0.71
H1M 6	2	WR	22	2	2221	M	Mix	F	0.17	0.69
H2B 6	2	WR	22	2	2222	B	Mix	F	0.49	0.98
H4B 8	2	WR	22	2	2224	B	Mix	C	0.01	0.30
H4M 1	2	WR	22	2	2224	M	Mix	C	0.73	0.94
H4M 6	2	WR	22	2	2224	M	Mix	C	0.50	0.89
H4M 7	2	WR	22	2	2224	M	Mix	C	0.29	0.42
H4M 8	2	WR	22	2	2224	M	Mix	C	0.29	0.90
H1B 4	2	WR	26.5	2	26521	B	Mix	F	0.59	0.82
H1M 1	2	WR	26.5	2	26521	M	Mix	F	0.49	0.97
H1M 3	2	WR	26.5	2	26521	M	Mix	F	0.04	0.82
H1M 4	2	WR	26.5	2	26521	M	Mix	F	0.34	0.82
H1M 6	2	WR	26.5	2	26521	M	Mix	F	0.03	0.76
H2B 3	2	WR	26.5	2	26522	B	Mix	C	0.28	0.91

H2B 6	2	WR	26.5	2	26522	B	Mix	C	0.05	0.57
H2M 4	2	WR	26.5	2	26522	M	Mix	C	0.23	0.31
H4B 3	2	WR	26.5	2	26524	B	Mix	F	0.35	0.73
H4B 8	2	WR	26.5	2	26524	B	Mix	F	0.44	0.96
H4M 1	2	WR	26.5	2	26524	M	Mix	F	0.27	0.54
H4M 3	2	WR	26.5	2	26524	M	Mix	F	0.39	0.92
H4M 6	2	WR	26.5	2	26524	M	Mix	F	0.18	0.67
H4M 7	2	WR	26.5	2	26524	M	Mix	F	0.25	0.95
H2B 1	2	WR	32	2	3222	B	Mix	C	0.28	0.94
H2B 4	2	WR	32	2	3222	B	Mix	C	0.17	0.62
H2B 7	2	WR	32	2	3222	B	Mix	C	0.32	0.86
H2M 4	2	WR	32	2	3222	M	Mix	C	0.02	0.64
H2M 5	2	WR	32	2	3222	M	Mix	C	0.02	0.30
H2M 8	2	WR	32	2	3222	M	Mix	C	0.24	0.53
H3B 6	2	WR	32	2	3223	B	Mix	F	0.15	0.78
H3M 4	2	WR	32	2	3223	M	Mix	F	0.42	0.85
L1B 1	2	WR	10	1	1011	B	Mix	F	0.30	0.87
L1B 8	2	WR	10	1	1011	B	Mix	F	0.50	0.93
L1M 1	2	WR	10	1	1011	M	Mix	F	0.34	0.74
L1M 4	2	WR	10	1	1011	M	Mix	F	0.36	0.84
L1M 8	2	WR	10	1	1011	M	Mix	F	0.21	0.87
L2B 2	2	WR	10	1	1012	B	Mix	C	0.36	0.93
L2B 5	2	WR	10	1	1012	B	Mix	C	0.11	0.91
L2M 3	2	WR	10	1	1012	M	Mix	C	0.02	0.30
L2M 4	2	WR	10	1	1012	M	Mix	C	0.16	0.74
L2M 8	2	WR	10	1	1012	M	Mix	C	0.53	0.70
L3B 1	2	WR	10	1	1013	B	Mix	F	0.43	0.90
L3M 1	2	WR	10	1	1013	M	Mix	F	0.01	0.21
L3M 4	2	WR	10	1	1013	M	Mix	F	0.34	0.91
L3M 5	2	WR	10	1	1013	M	Mix	F	0.29	0.98
L1B 3	1	WR	14	1	1411	B	solo	solo	0.25	0.97
L1B 4	1	WR	14	1	1411	B	solo	solo	0.42	0.97
L1B 5	1	WR	14	1	1411	B	solo	solo	0.38	0.96
L1M 2	1	WR	14	1	1411	M	solo	solo	0.29	0.84
L2B 1	1	WR	14	1	1412	B	solo	solo	0.36	0.96
L2B 2	1	WR	14	1	1412	B	solo	solo	0.23	0.97
L2B 7	1	WR	14	1	1412	B	solo	solo	0.23	0.97
L2M 3	1	WR	14	1	1412	M	solo	solo	0.53	0.97
L3B 1	1	WR	14	1	1413	B	solo	solo	0.00	0.00
L3B 6	1	WR	14	1	1413	B	solo	solo	0.17	0.87
L3B 8	1	WR	14	1	1413	B	solo	solo	0.04	0.19

Parent plants	control	site	Plot	parent Sp	parent #	Capsule	context	Distance	Tube germ	Aceto carmine
L3M 5	1	WR	14	1	1413	M	solo	solo	0.28	0.88
L3M 6	1	WR	14	1	1413	M	solo	solo	0.00	0.11
L3B 8	2	WR	16.5	1	16513	B	Mix	C	0.47	0.98
L3M 1	2	WR	16.5	1	16513	M	Mix	C	0.19	0.95
L3M 5	2	WR	16.5	1	16513	M	Mix	C	0.42	0.98
L3M 6	2	WR	16.5	1	16513	M	Mix	C	0.30	0.93
L4B 1	2	WR	16.5	1	16514	B	Mix	F	0.33	0.47
L4B 4	2	WR	16.5	1	16514	B	Mix	F	0.31	0.59
L4B 8	2	WR	16.5	1	16514	B	Mix	F	0.27	0.97
L4M 1	2	WR	16.5	1	16514	M	Mix	F	0.48	0.97
L4M 2	2	WR	16.5	1	16514	M	Mix	F	0.24	0.97
L4M 5	2	WR	16.5	1	16514	M	Mix	F	0.20	0.85
L4M 7	2	WR	16.5	1	16514	M	Mix	F	0.27	0.60
L1B 3	2	WR	22	1	2211	B	Mix	C	0.01	0.94
L1B 4	2	WR	22	1	2211	B	Mix	C	0.16	0.96
L1M 1	2	WR	22	1	2211	M	Mix	C	0.51	0.96
L1M 6	2	WR	22	1	2211	M	Mix	C	0.40	0.98
L3B 4	2	WR	22	1	2213	B	Mix	C	0.50	0.98
L32 2	2	WR	22	1	2213	M	Mix	C	0.02	0.30
L32 3	2	WR	22	1	2213	M	Mix	C	0.06	0.96
L32 7	2	WR	22	1	2213	M	Mix	C	0.24	0.96
L32 8	2	WR	22	1	2213	M	Mix	C	0.30	0.95
L4B 5	2	WR	22	1	2214	B	Mix	F	0.33	0.98
L4B 7	2	WR	22	1	2214	B	Mix	F	0.63	0.98
L42 1	2	WR	22	1	2214	M	Mix	F	0.51	0.97
L42 4b	2	WR	22	1	2214	M	Mix	F	0.46	0.98
L42 7	2	WR	22	1	2214	M	Mix	F	0.14	0.94
L1B 3	2	WR	26.5	1	26511	B	Mix	F	0.08	0.88
L1B 6	2	WR	26.5	1	26511	B	Mix	F	0.02	0.65
L1B 8	2	WR	26.5	1	26511	M	Mix	F	0.53	0.57
L1M 1	2	WR	26.5	1	26511	M	Mix	F	0.22	0.84
L1M 4	2	WR	26.5	1	26511	M	Mix	F	0.00	0.65
L1M 7	2	WR	26.5	1	26511	M	Mix	F	0.00	0.00
L3B 2	2	WR	26.5	1	26513	B	Mix	C	0.19	0.97
L3B 3	2	WR	26.5	1	26513	B	Mix	C	0.29	0.95
L3B 5	2	WR	26.5	1	26513	B	Mix	C	0.05	0.61
L3M 2	2	WR	26.5	1	26513	M	Mix	C	0.53	0.89
L3M 3	2	WR	26.5	1	26513	M	Mix	C	0.41	0.95
L3M 5	2	WR	26.5	1	26513	M	Mix	C	0.00	0.95

L1B 1	2	WR	32	1	3211	B	Mix	C	0.37	0.95
L1B 4	2	WR	32	1	3211	B	Mix	C	0.15	0.75
L1B 5	2	WR	32	1	3211	B	Mix	C	0.34	0.94
L1B 7	2	WR	32	1	3211	B	Mix	C	0.08	0.98
L1B 8	2	WR	32	1	3211	B	Mix	C	0.25	0.97
L1M 1	2	WR	32	1	3211	M	Mix	C	0.71	0.98
L1M 2	2	WR	32	1	3211	M	Mix	C	0.30	0.82
L1M 3	2	WR	32	1	3211	M	Mix	C	0.47	0.97
L3B 2	2	WR	32	1	3213	B	Mix	C	0.36	0.96
L3B 4	2	WR	32	1	3213	B	Mix	C	0.18	0.76
L3B 6	2	WR	32	1	3213	B	Mix	C	0.28	0.89
L3B 7	2	WR	32	1	3213	B	Mix	C	0.24	0.46
L3M 1	2	WR	32	1	3213	M	Mix	C	0.26	0.92
L3M 5	2	WR	32	1	3213	M	Mix	C	0.37	0.94
L3M 8	2	WR	32	1	3213	M	Mix	C	0.24	0.83
L4B 3	2	WR	32	1	3214	B	Mix	F	0.01	0.37
L4B 4	2	WR	32	1	3214	B	Mix	F	0.05	0.65
L4B 5	2	WR	32	1	3214	B	Mix	F	0.22	0.70
L4B 7	2	WR	32	1	3214	B	Mix	F	0.24	0.71
L4M 4	2	WR	32	1	3214	M	Mix	F	0.00	0.59
L4M 8	2	WR	32	1	3214	M	Mix	F	0.00	0.11