Ecological genetics of *Penstemon* in the Great Basin, U.S.A.

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THESIS

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To my husband, Brian Kramer, whose unconditional love, support, and sense of humor were beyond invaluable throughout the course of this thesis.

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1.1 INTRODUCTION

At a time of unprecedented alteration and destruction of habitat worldwide (Vitousek et al., 1997), ecological restoration provides hope for the survival of the species and ecosystems that sustain life on the planet. However, the practice of ecological restoration is based upon the still emerging science of restoration ecology, and humans are far from being able to replicate or re-create the world's diverse and dynamic ecosystems. One important component of ecological restoration that has received increasing attention from both scientists and practitioners addresses genetic considerations in ecological restoration. Here, I discuss key components of this topic, ultimately incorporating examples in the following three chapters of research on the ecological genetics of *Penstemon* and relating them to ecological restoration in the Great Basin region of the western United States.

1.2 What is ecological restoration?

Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed (SER, 2004). The specific objectives and methods of ecological restoration vary greatly by habitat and location, but are increasingly united by the common goals of creating dynamic communities that are self-sustaining, resilient, and capable of providing ecosystem services (Allen, Covington, and Falk, 1997; Hobbs and Harris, 2001; Choi, 2004). A range of disciplines inform the science of restoration ecology, ranging from population to community ecology, biology, genetics, geography and geology. In turn, restoration ecology informs the practice of ecological restoration at the population, species, community, and ecosystem level.

Ecological restoration is now being carried out in many countries and almost every ecosystem around the world (MEA, 2005) as a means of conserving biodiversity, improving human well-being, and sequestering carbon to help mitigate climate change. While the practice of ecological restoration has an increasingly cohesive body of theory to base its actions upon (Harris et al., 2006), a number of critical components are still based on incomplete

information. One such component that has received increasing attention from both scientists and practitioners is how the genetic composition of source material impacts the short and long-term success of a restoration (Millar and Libby, 1989; Fenster and Dudash, 1994; Knapp and Dyer, 1997; Montalvo et al., 1997; Havens, 1998; Lesica and Allendorf, 1999; Humphrey and Schupp, 2002; Hufford and Mazer, 2003; McKay et al., 2005). Fortunately, answers to basic questions regarding important genetic considerations in ecological restoration can be found by utilizing theories and tools from a number of well-established disciplines and applying them to specific restoration situations.

1.3 What is ecological genetics?

One such discipline is ecological genetics, which examines the origin of genetic variation within and among populations, its relationship with the environment, and its change over time (Lowe, Harris, and Ashton, 2004). Ecological genetics can help ecological restoration meet its broad objectives on a species-by-species basis, providing guidance for establishing populations that are not only genetically diverse, but also genetically appropriate for long-term restoration success (Fenster and Dudash, 1994; Knapp and Dyer, 1997; Hufford and Mazer, 2003; Rice and Emery, 2003; Rogers and Montalvo, 2004; McKay et al., 2005).

1.4 What ecological genetics tells us about natural plant populations

Many plant species commonly used in restoration activities are widely distributed throughout a heterogeneous environment, with varying degrees of isolation among populations (Hufford and Mazer, 2003). Ecological genetics identifies the extent to which this heterogeneity and isolation drives population differentiation that matters to restoration practitioners, and can be used in a restoration context to identify appropriate seed sources that maximize restoration success while minimizing restoration costs (Johnson et al., 2004). Population differentiation is a product of four key evolutionary processes: genetic drift, natural selection, mutation and gene flow (Hedrick, 2005). The interaction of these processes drives differences in allele frequencies and, potentially, differences in traits among populations over time (Endler, 1973). The resulting extent and degree of variation among populations depends on a balance between processes driving local differentiation and those homogenizing

populations (Slatkin, 1987; Garcia-Ramos and Kirkpatrick, 1997). Genetic drift, mutation, and natural selection will drive population differentiation, while gene flow opposes these divisive forces via the movement of gametes or individuals among populations.

Genetic drift in small, isolated populations will lead to the random loss and fixation of alleles among populations due only to chance, with a potential end result of complete differentiation. As shown by Wright (1951), an average of one migrant between two populations per generation can counteract the diversifying effects of genetic drift. Natural selection drives generational changes via selection for alleles that confer fitness advantages to parents and their offspring in a given environment (Hedrick, 2005). Natural selection in populations exposed to different environmental conditions will drive changes in the frequency of alleles (and the traits they influence) that confer fitness advantages in each given environment, leading to local adaptation (Linhart and Grant, 1996). Importantly, the interaction of mutation, drift, and selection, in the absence of gene flow, can lead to different populations finding different genetic solutions to the same selective pressure (Cohan, 1984). Gene flow can counteract these processes, slowing or even inhibiting local adaptation via an influx of genes not adapted to local conditions (Garcia-Ramos and Kirkpatrick, 1997). However, as first demonstrated by Endler (1973) and more recently by Kittelson and Maron (2001), sufficiently strong selection pressures can lead to local adaptation, and adaptive population differentiation, even as gene flow acts to homogenize populations.

Gene flow within and among populations of a plant species is determined by its geographic distribution, as well as its life history and breeding, mating, and dispersal systems (Loveless and Hamrick, 1984; Schoen and Brown, 1991; Hamrick and Godt, 1996; Richards, 1997; Manel et al., 2003). Generally, the highest level of gene flow among populations will occur in species that are long-lived, obligate outcrossing, and pollinated and dispersed either by wind (Dow and Ashley, 1998), or insects or animals that travel large distances within a foraging bout (Nason and Hamrick, 1997). This can eliminate the genetically isolating effects of geography, with populations showing little to no detectable among-population differentiation, at least in neutral genetic markers or

traits. At the opposite end of the spectrum, the lowest level of gene flow and highest degree of among-population differentiation is expected from annual, primarily selfing species with gravity dispersed seeds, such as *Bromus tectorum* (Ramakrishnan et al., 2004) .

Significant differences in patterns of gene flow exist even among otherwise similar species, such as members of the genus *Penstemon* (Plantaginaceae). Most *Penstemon* species are long-lived perennial forbs that are generally outcrossing but often capable of some level of self-pollination (Zorn-Arnold and Howe, 2007). Seeds are gravity-dispersed, so gene flow is predominantly via pollen movement. Pollinators include an array of insects, primarily bees, and hummingbirds, and can generally be predicted by their pollination syndrome (Thomson et al., 2000; Wilson et al., 2004; Wilson et al., 2006). Through their influences on pollen flow, pollinators may play a critical role in determining gene flow and population differentiation in *Penstemon* species. As the largest genus endemic to North America, with over 270 species (Lodewick and Lodewick, 1999), many species are currently utilized in ecological restoration efforts and restoration ecology experiments (Howe et al., 2006), and many more are candidates for increasing use in restoration efforts in the western United States because of their resistance to disturbance (including cattle grazing and fire) and contribution to increased community diversity by providing resources for a wide range of pollinators.

1.5 The role of ecological genetics in ecological restoration

The framework of ecological genetics can be used to understand how genetic diversity is structured within and among populations, allowing restoration practitioners to work with this structure to create self-sustaining populations. One of the first genetic assumptions in ecological restoration is that genetic diversity contributes to population persistence (Huenneke, 1991). A number of studies have supported this assumption, from findings of increased extinction probability (Newman and Pilson, 1997) to decreases in population growth and individual fitness (Williams, 2001; Reed and Frankham, 2003) with reduced population genetic diversity. Genetic diversity is important not only for individual species persistence, but also contributes to the diversity of the entire community, from other plant species (Booth and Grime, 2003) to arthropods (Bangert et al., 2005; Wimp et al.,

2005; Bangert et al., 2006) and even ecosystem processes and recovery (Schweitzer et al., 2004; Reusch et al., 2005; Whitham et al., 2006). Additionally, there is no question that genetic diversity is a necessary component for microevolutionary processes that will allow species to adapt to a changing environment over the short and long-term (Linhart and Grant, 1996; Boulding and Hay, 2001; Etterson, 2004a, b), and there is a growing base of literature (Ashley et al., 2003; Rice and Emery, 2003; Harris et al., 2006) encouraging stronger incorporation of evolutionary processes in ecological restoration practices, particularly in the face of global climate change.

A second key assumption is that plant populations are uniquely adapted to their local conditions, and therefore the most appropriate material for a restoration site is from a local source (Millar and Libby, 1989). This is supported by over 200 years of ecological genetics research on plant species (Langlet, 1971). Some of the first and most thorough studies to identify and explain local adaptation were performed on widespread forbs occupying a range of climates in western North America. The use of common garden and reciprocal transplant studies demonstrated that plants at different elevations in the Sierra Nevada mountain range were adapted to their local conditions and performed poorly or died when grown away from those conditions (Clausen, Keck, and Hiesey, 1940a, b; Clausen, Keck, and Hiesey, 1941; Clausen, Keck, and Hiesey, 1947, 1948; Clausen and Hiesey, 1958; Nunez-Farfan and Schlichting, 2001).

From these early studies, research on a range of species in numerous habitats continues to demonstrate patterns of local adaptation that need to be considered in ecological restoration efforts. Adaptation to soil conditions (McNeilly and Antonovics, 1968; McNeilly and Bradshaw, 1968; Feist and Parker, 2001), winter temperatures and winter length (Balduman et al., 1999), water availability and flood tolerance (Dudley, 1996a, b; Fenster, 1997), and many other biotic and abiotic factors indicate that local populations often perform poorly when transplanted away from their home site (Joshi et al., 2001; Montalvo and Ellstrand, 2001). For some species and some situations, this assumption does not prove true, as some plants may not be optimally adapted to their local conditions, phenotypic plasticity may allow successful growth in a range of conditions (Bender, Baskin, and Baskin, 2002), and some small isolated populations may have inappropriately low levels of genetic

diversity (Menges and Dolan, 1988; Ouborg, Vergeer, and Mix, 2006). However, given the collective results of decades of study, it is understood that great care should be taken in ensuring that the conditions of a source site match those of the restoration site so maladaptation will not lead to failure of the restoration.

The consequences of a source-site mismatch may be difficult to predict, as local adaptation often occurs for more cryptic selection events, such as rare climatic extremes (Lesica and Allendorf, 1999). An example comes from the U.S. Forest Service, where Douglas fir (*Pseudotsuga menziesii*) trees from numerous seed sources from throughout the range of the species were planted in Suislaw National Forest, Oregon in 1915. Trees from all sources performed well until 1955, when an unusual and prolonged cold spell seriously damaged or killed trees from off-site sources while causing only minor damage to trees from local sources (Millar and Libby, 1989; Johnson et al., 2004). Losses such as this led the forestry community to research and delineate appropriate transfer zones for commercially valuable tree species, beginning in the 1960's and continuing today. Such examples illustrate why genetic considerations are exceedingly important in restoration efforts.

Economic realities make the use of local seed sources for ecological restoration challenging, particularly for regions where restoration is needed on a large scale, as in the Great Basin region of western North America (BLM, 2000). Complicating matters, conditions at the restoration site may no longer match those of any local source populations (e.g. anthropogenic disturbance or invasive species may have altered soil conditions), making it difficult to match local sources with restoration conditions (Lesica and Allendorf, 1999; Wilkinson, 2001). And given that species do not have uniform or necessarily predictable population genetic structure, other important questions surround the definition and actual determination of "local" sources of different species in different habitats and restoration scenarios (Lesica and Allendorf, 1999; McKay et al., 2005; Bussell et al., 2006).

A final genetic consideration in ecological restoration involves a potential for outbreeding depression (exhibited as a reduction in offspring fitness relative to parents) to occur if genetically inappropriate transplants interbreed with native conspecifics, particularly in areas where restoration occurs on the scale of hundreds of

kilometers. Outbreeding depression requires at least one generation of successful establishment and reproduction of a non-local population in a restored site that is near or contains natural populations of the same species. These factors make the detection and reporting of negative effects due to outbreeding depression very unlikely. Until recently, outbreeding depression has been a cryptic source of restoration failure, but it is an important consideration in any restoration, given the potential for large and long-lasting effects on nearby native conspecific populations (Edmands and Timmerman, 2003). Indeed, a recent review by Edmands (2007) suggests that the negative effects of outbreeding depression on population persistence may be on par with the risks from inbreeding depression. Through careful selection of appropriate transplant material, it is possible to maximize the long-term health of a restored population and minimize both the risks of outbreeding depression (as exhibited in Montalvo and Ellstrand 2001) and genetic contamination of native populations via hybridization (Knapp and Dyer 1997).

1.6 A particular need for ecological genetics to guide restoration in the Great Basin

Perhaps nowhere is the need for ecological genetics research to guide large scale ecological restoration greater than in the Great Basin region. Scientifically-based, economical and large-scale ecological restoration is a pressing need throughout the region, where invasion by exotic plant species and efforts to restore habitat degraded by invasion is occurring on a massive scale (BLM, 2000). Cheatgrass (*Bromus tectorum*), a non-native invasive annual grass, is widely recognized as a key contributor to the rapid degradation of habitat in the Great Basin over the last century (BLM, 2000; Booth, Caldwell, and Stark, 2003), and has been labeled as "perhaps the most significant plant invasion in North America" (D'Antonio and Vitousek, 1992). It has numerous negative effects on the plant communities it invades, ranging from reduced species abundance to widespread and nearly wholesale replacement of native sagebrush and bunchgrass communities (Levine et al., 2003). In fact, it now dominates over 50% of the native sagebrush steppe in this expansive ecosystem, or about 63 million ha, particularly at low elevations (Brussard, Charlet, and Dobkin, 1999).

Much the land in the Great Basin is publicly owned and managed by the U.S. federal government. Efforts to minimize impacts from the spread of cheatgrass have focused on reintroducing native plant species after

wildfires, in an attempt to minimize erosion (Beyers, 2004) and stop the cycle of fire that it initiates (BLM, 2000). Seeding of natives after wildfires is occurring on an increasingly large scale using a limited selection of native grass and sagebrush seeds, but not enough is known about the genetic structure of most plant species in this region to allow for informed decisions regarding the choice of source material for restoration sites.

The potential for unique and unpredictable patterns of population genetic structure in widespread plant species often used in restoration efforts is highly likely in the Great Basin, which comprises over 390,000 km^2 and contains over 150 distinct mountain ranges (Grayson, 1993) isolated from one another by vast arid valleys. Rising an average of 1,750 meters above valley floors, these mountain ranges create cooler and more mesic conditions and consequently hold a majority of the region's 3,000+ vascular plant species (Grayson, 1993). It is unclear how much this rugged mountainous terrain isolates plant populations, but for some species these mountains function as sky islands (DeChaine and Martin, 2005). And rapidly-changing climatic conditions are only amplifying isolation among populations on different mountain ranges. An average global temperature increase of 0.74ºC over the last century (Solomon et al., 2007) in combination with a brief historical period of cooling (the Little Ice Age AD 1300-1850) has driven montane habitat in the Great Basin up some 180 m in the last 150 years (Munroe, 2003). This, in combination with the effects of rapidly-encroaching invasive species, may be leading to ever-increasing isolation of native plant populations on different mountain ranges, smaller population sizes, and increased extinction risk.

The Great Basin lies in the rainshadow of the Sierra Nevada Mountains, producing a significant east-west precipitation gradient that combines with a north-south temperature gradient to create a large-scale mosaic of environmental heterogeneity throughout the region. Even within mountain ranges, changes in temperature and precipitation imposed by rapid increases in elevation interact with differences in slope and aspect to create a virtually infinite array of microclimates within a relatively small spatial scale (Petersen, 1994). In combination, this environmental heterogeneity and varying degrees of genetic isolation in the Great Basin undoubtedly impacts plant populations, and these factors will be critically important to the success of any ecological restoration efforts.

The following ecological genetics studies on three *Penstemon* species found throughout the Great Basin region provide much-needed information to guide ecological restoration efforts here. Results of these studies have restoration implications both for my study species as well as many other perennial forbs with comparable life history characteristics distributed similarly throughout the region.

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2. LANDSCAPE GENETICS IN THE GREAT BASIN: INFLUENCES OF DISTANCE, TOPOGRAPHY AND POLLINATORS

2.1 INTRODUCTION

The population genetic structure of a plant species is a key parameter in its evolutionary biology, and largely a function of its population history, geographic distribution, and life-history traits which influence gene flow (Hamrick and Godt, 1996; Duminil et al., 2007). Populations that become genetically isolated in space or time may follow different evolutionary trajectories due to a combination of mutation, genetic drift and/or natural selection (Slatkin, 1985). Extreme isolation can lead to evolutionary radiation, as demonstrated in oceanic islands (Emerson, 2002; Dunbar-Co, Wieczorek, and Morden, 2008) and extreme habitat, such as high-elevation mountaintops [e.g. the Andes (Hughes and Eastwood, 2006), rock outcrop 'inselbergs' (Barbara et al., 2007) or the edaphically diverse habitat of southern Africa's karoo region (Ellis, Weis, and Gaut, 2006)]. Radiations in these situations are largely due to the isolating effects of geography and ecology which limit gene flow between populations, and a diversity of ecological opportunities for adaptive differentiation.

In few continental regions of the world is isolation more extreme than the Great Basin region of the western United States. Comprising over 390,000 km^2, the Great Basin contains over 150 distinct mountain ranges (Grayson, 1993) isolated from one another by vast arid valleys. Rising an average of 1,750 meters above valley floors, these mountain ranges create cooler and more mesic conditions and consequently hold a majority of the region's 3,000+ vascular plant species (Grayson, 1993). It is unclear how much this rugged mountainous terrain isolates plant populations, but for some species these mountains function as sky islands (DeChaine and Martin, 2005). Gene flow among populations on different mountain ranges appears to be quite high in some species, such as birds and the wind-pollinated trees whose seeds they disperse (Johnson, 1975; Wells, 1983; Hamrick, Schnabel, and Wells, 1994; Jorgensen, Hamrick, and Wells, 2002), while in other species it is more restricted (Floyd, Van Vuren, and May, 2005). However, at least one continental evolutionary radiation has been

documented in this region (in *Penstemon* Mitchell), so I expect that some groups of species may be more isolated by the topography of the Great Basin than others. *Penstemon* (Plantaginaceae) is America's largest endemic genus of plants (Lodewick and Lodewick, 1999), and a recent phylogenetic study (Wolfe et al., 2006) has detailed rapid continental evolutionary radiation centered in the western United States (including the Great Basin region) during the Pleistocene Era. This radiation has been attributed to divergence through adaptation to pollinators (Wilson et al., 2004; Wilson et al., 2006), and ecological niches made available by retreating glaciers (Wolfe et al., 2006), but the region's topography may have facilitated divergence by restricting gene flow between populations.

This study focuses on three species: *Penstemon deustus* Douglas ex Lindl. var. *pedicellatus* M.E. Jones, *Penstemon pachyphyllus* A. Gray ex Rydb. var. *congestus* (M.E. Jones) N.H. Holmgren, and *P. rostriflorus* (Kellogg). All three species are common and widespread throughout the western United States, including the Great Basin region (Kartesz 1999), occurring almost exclusively in sagebrush-steppe habitat at a range of mid to high-elevations on mountain ranges. However, *P. deustus* and *P. rostriflorus* generally occur on rockier microsites and have a broader distribution in the Great Basin than does *P. pachyphyllus*. They also share most life-history traits thought to affect population genetic structure. They are all long-lived herbaceous perennial forbs that produce numerous, structurally similar protandrous flowers borne on multiple flowering stalks. They have overlapping bloom times, with *P. deustus* and *P. pachyphyllus* generally blooming earlier in the season than *P. rostriflorus*. The species are not known to reproduce clonally, and have mixed mating systems with at least some degree of self-compatibility (A. Kramer Chapter 4). Seeds produced by all three species have no obvious dispersal mechanism and are presumably gravity dispersed, although they differ in size (*P. deustus* = 0.130mg $\pm$ 0.028; *P. pachyphyllus* = 1.411mg $\pm$ 0.472; *P. rostriflorus* = 0.343mg $\pm$ 0.095; A. Kramer unpub.). Because seeds are gravity dispersed, gene flow between populations will be predominantly via pollen movement by pollinators. All three study species are animal pollinated, so gene flow will be determined by the movement of pollinators within mountain ranges (and the steep elevation and habitat gradients they impose), and among mountain ranges which requires crossing arid valleys largely devoid of floral resources.

The primary difference among my three study species is their pollination syndrome. The concept of pollination syndromes stems from the observation that pollinators can generally be clustered into functional groups with similar behaviors with respect to floral visitation. Such functional groups exert similar selection pressures on the plants they visit, generating correlations among traits of similarly visited flowers (Fenster et al., 2004). For example, plants with red flowers, long narrow corolla tubes and exserted anthers tend to be attractive to, and most efficiently pollinated by, hummingbirds. Given its diverse array of floral morphology, *Penstemon* has been the subject of many studies on pollination syndromes (Thomson et al., 2000; Castellanos, Wilson, and Thomson, 2003; Castellanos, Wilson, and Thomson, 2004; Wilson et al., 2004; Castellanos et al., 2006; Wilson et al., 2006; Wilson et al., 2007; Thomson and Wilson, 2008). *Penstemon deustus* has a bee syndrome, producing small white flowers visited by small bees and bumblebees (Wilson et al. 2004), while *P. pachyphyllus* also has a bee syndrome but produces large purple flowers generally visited by larger bees (pers. obs.) and *P. rostriflorus* has a hummingbird syndrome, producing red tubular flowers (Thomson et al., 2000; Wilson et al., 2004) visited primarily by the many hummingbird species found in the western United States.

Not all pollinators are equal in their ability to connect plant populations through pollen movement (Fenster, 1991; Hughes et al., 2007). The direction, distance and frequency of pollinator-mediated gene flow among plant populations will be largely a function of the pollinator's life history and behavior. Hummingbirds and some bumblebees may have similar foraging behaviors within plant populations (Waser, 1982), but that bee species vary considerably in their foraging behavior and pollination efficiency (Cresswell et al., 1995; Dieringer and Cabrera, 2002). Additionally, hummingbirds and nectaring bumblebees remove pollen from certain *Penstemon* flowers equally well, while pollen-collecting bumblebees are more effective than either nectaring birds or bees. In general, birds are expected to be more effective than bees at connecting distant populations through successful long-distance pollination. Graves & Schrader (2008) suggest that hummingbirds are responsible for connecting populations of *Dirca occidentalis* separated by as much as 38 km in California, while Walther-Hellwig and Frankl (2000) note that of three species of bumblebees studied in the U.K., only one was recorded

flying more than 1.5 km between floral resources (*Bombus terrestris*). However, little specific information on the relative ability of different bird and bee pollinators to connect similarly isolated plant populations is available, despite its importance to understanding the genetic structure of plant populations and evolution of species.

In this study, I examine the interacting effects of distance, topography and pollination syndrome on population genetic differentiation in three *Penstemon* species found throughout the Great Basin using microsatellite DNA analysis. I ask whether the region's arid valleys are effective barriers to gene flow by constraining pollinator movement among mountain ranges. I expect that gene flow within mountain ranges will be more prevalent than among mountain ranges, but if not, I will identify potential barriers to pollinator movement and therefore among-population gene flow within mountain ranges. If differences in population genetic structure are identified among my three otherwise similar study species, I will assess whether these differences may be due to the different behavior of each species' primary pollinators.

2.2 MATERIALS AND METHODS

2.2.1 Study Sites

Seven populations of *P. pachyphyllus* and eight populations of both *P. deustus* and *P. rostriflorus* were sampled (Figure 2.1, Table I). Each population had more than 100 adult plants. For each species, populations were located on at least three distinct mountain ranges covering the full extent of its distribution in the Great Basin floristic region (Cronquist et al., 1972). When possible, two distinct populations separated by at least 2 km in geographic distance and 300 meters in elevation were located on each mountain range. While this was not possible for all three species on all mountain ranges, sampling represents at least two within-mountain range population comparisons where populations are separated by at least 300 m elevation. Population codes identify species, mountain range, and relative elevation (e.g. Pd-CH identifies *P. deustus* on the central mountain range at a high elevation; Pd-CL is its population pair at a low elevation). Voucher specimens for all species and sites were deposited at the Nancy Poole Rich Herbarium (Chicago Botanic Garden) and the Great Basin Herbarium (Utah State University).

2.2.2 Collections

In summer of 2003, five to ten grams of fresh leaf tissue (dried in silica gel) were collected from at least 32 haphazardly located individuals at each study site, spanning the range of the population and avoiding sampling from immediately adjacent plants. GPS coordinates were recorded for each individual, and all leaf collections were maintained separately in dry conditions until extractions were performed. Two additional populations for *P. deustus* were identified in 2006 (see Table I), with collections and extractions made following the same protocol as 2003 collections.

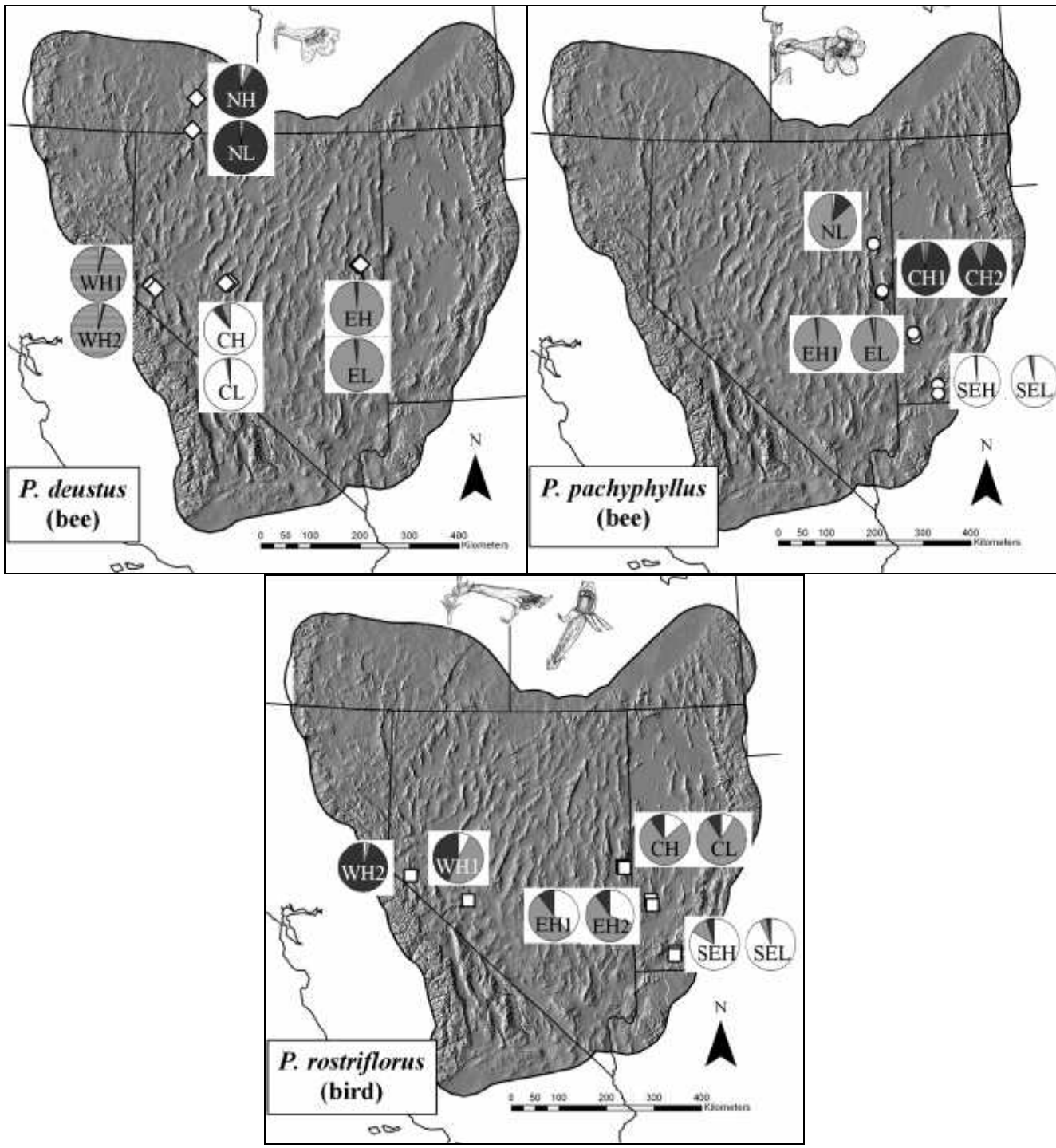

Figure 2.1. Study locations for 3 *Penstemon* species in the Great Basin. Pie charts depict the number and admixture of genetic clusters identified by Bayesian cluster analysis, as detailed in Table VII and Figure 2.5.

TABLE I
DETAILED STUDY SITE INFORMATION

Population	Mountain Range	State	Latitude	Longitude	Elevation (m)	Approx size (# plants)
P. deustus						
Pd-CH	Central (Desatoya)	NV	39.254	-117.681	2025	100-150
Pd-CL[a]	Central (Desatoya)	NV	39.240	-117.778	1909	400-500
Pd-EH	East (Schell Creek)	NV	39.557	-114.640	2649	200-300
Pd-EL[a]	East (Schell Creek)	NV	39.566	-114.586	2022	200-300
Pd-NH	North (Steens)	OR	42.629	-118.530	1793	200-300
Pd-NL	North (Steens)	OR	42.046	-118.620	1368	200-300
Pd-WH1	West (Pine Nut)	NV	39.176	-119.527	1861	150-200
Pd-WH2	West (Pine Nut)	NV	39.115	-119.424	1834	150-200
P. pachyphyllus						
Pp-SEH	Southeast (Zion NP)	UT	37.341	-113.077	2122	300-400
Pp-SEL	Southeast (Zion NP)	UT	37.173	-113.083	1119	300-400
Pp-EH1	East (Wah Wah)	UT	38.325	-113.590	2560	150-200
Pp-EL	East (Wah Wah)	UT	38.337	-113.609	2216	300-400
Pp-CH1	Central (Snake)	NV	39.109	-114.347	2323	1000+
Pp-CH2	Central (Snake)	NV	39.148	-114.330	2227	300-400
Pp-NL	North (Antelope)	NV	40.036	-114.510	1995	300-400
P. rostriflorus						
Pr-SEH	Southeast (Zion NP)	UT	37.345	-113.080	2092	200-300
Pr-SEL	Southeast (Zion NP)	UT	37.292	-113.096	1632	100-150
Pr-EH1	East (Wah Wah)	UT	38.354	-113.608	2510	100-150
Pr-EH2	East (Wah Wah)	UT	38.256	-113.581	2455	150-200
Pr-CH	Central (Snake)	NV	39.023	-114.270	2768	150-200
Pr-CL	Central (Snake)	NV	38.991	-114.220	2147	100-150
Pr-WH1	West (Pilot)	NV	38.392	-118.025	1919	100-150
Pr-WH2	West (Pine Nut)	NV	38.847	-119.438	1678	200-300

[a] All collections made in 2003 except Pd-CL and Pd-EL, collected in 2006.

2.2.3 Molecular data

Total genomic DNA was extracted from silica-dried leaf material following a CTAB method (modified from Doyle and Doyle (1987). Genotypes were obtained for seven polymorphic nuclear (dinucleotide repeat) microsatellite loci developed on *P. rostriflorus* (Pen02, Pen04, Pen05, Pen18, Pen23, Pen24, Pen25, detailed in Kramer and Fant (2007) as well as an eighth locus (Pen06) developed following the same protocol (Table II). DNA from all individuals were amplified using polymerase chain reaction (PCR) at all eight loci using fluorescently-tagged forward primers (WellRed D2, D3 or D4, Sigma-Proligo, following methods described in Kramer and Fant (2007). Genotypes were scored using a CEQ 8000 Genetic Analysis System and CEQ Fragment Analysis software (Beckman Coulter).

TABLE II
SUMMARY DETAILS FOR EIGHT *PENSTEMON* MICROSATELLITE LOCI

Locus	Repeat	GenBank	Primer sequences (5'-3')	Size range (bp)
Pen02	$(TC)_{14}(CA)_{13}$	DQ917423	F: TTCTATGCTTCGTTAACCCAAAA R: GGTCGTATTGGTCCTTTCCA	163-245
Pen04	$(TC)_{22}$	DQ917425	F: GATGGAAAATGTGCCAGGAC R: CTCTGCGGTGCATGAAAGTA	209-287
Pen05	$(TC)_{25}$	DQ917426	F: CAGATAGGGTGGAGGGGCTA R: CAACCCAATCTGGTCGATCT	159-245
Pen06	$(TG)_9(GA)_{12}$		F: TGTTGACAGTTTTAATTGAAAGGAA R: GAGGCCAGAAATGTTCCAAA	185-253
Pen18	$(CT)_{20}(CA)_{20}$	DQ917428	F: CTCATGATGATTGTGCGGATA R: ACAACTCTCGCACTCTCACG	530-616
Pen23	$(GA)_{21}$	DQ917430	F: TGGTCTGATTTCAGGAAAAGC R: TGCTCAAGACGATAATAAAAGTGC	148-206
Pen24	$(GT)_9(GA)_{22}$	EF203408	F: TCAAATTGAGAAAATGAGTGAAAGTC R: ATATGGTGGGACCTTTCGTG	145-225
Pen25	$(CT)_{29}$	DQ917431	F: GATGATCACCCAAGTTGCTT R: CCTAATGCACGAGGCAAACT	120-176

2.2.4 Analysis

Microsatellite genoptype data were formatted for analysis using CREATE (Coombs, Letcher, and Nislow, 2008). Descriptive parameters (A = total number alleles; H_O = observed heterozygosity; H_E = expected heterozygosity) were calculated, and Weir and Cockerham's (1984) estimates of Wright's F statistic F_{IS} (f; within population inbreeding coefficient) were estimated for all loci using FSTAT 2.9.3 (Goudet, 1995). Descriptive parameters for each population of each species were calculated in GDA (Lewis and Zaykin, 2001), including: P, proportion of polymorphic loci; n, mean sample size; A, mean number of alleles per locus; Ap, total number of private alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; f (Wier and Cockerham's estimate of the within-population inbreeding coefficient, F_{IS}). Pairwise comparisons of population differentiation in F_{ST} (θ) were also calculated for all populations in each species and globally in FSTAT; global F_{ST} estimates were tested for significant departures from the null hypothesis of panmixia by jack-knifing across loci. Departures from Hardy Weinberg Equilibrium (HWE) were tested for each species using exact tests in GENEPOP (Raymond and Rousset, 1995) for each locus and population, as well as globally.

To compare broad differences among species in sampling as well as genetic diversity and differentiation, an analysis of variance (ANOVA; JMP IN 5.1) was performed to test for significant species-level differences in expected heterozygosity, departure from HWE (F_{IS} as measured by f), pairwise F_{ST} comparisons (as measured by θ) and pairwise F_{ST} comparisons standardized by dividing by pairwise geographic distance. Analyses of pairwise comparisons were performed including and excluding within-mountain range comparisons to identify differences at the among-mountain range level. As in Jorgensen et al. (2002), if significant species-level variation was indicated by the ANOVA, Tukey-Kramer tests identified significant differences among species (JMP IN 5.1).

Several approaches were used to examine the population genetic structure of each species. To partition genetic variation among mountain ranges, among populations, and within populations for each species, analysis of molecular variance (AMOVA) was carried out in GENALEX (Peakall and Smouse, 2006); significance for each F-statistic was tested through 1,000 permutations. The relationship between linearized genetic (F_{ST} /(1- F_{ST})) and geographic distances (ln km), or isolation by distance (Slatkin, 1993) was measured by evaluating the Spearman rank correlation coefficients between genetic and geographic distance values for all pairwise population comparisons and using nonparametric Mantel (1967) tests (10^4 permutations) in GENEPOP. Nei's unbiased estimate of genetic distance (Nei, 1978) was used for unweighted pair-group clustering based on arithmetic averages (UPGMA), performed in TFPGA (Miller, 1997) to provide a graphical representation of genetic distance data and relationships within and among mountain ranges for each species.

The Bayesian clustering analysis software STRUCTURE v2.2 (Pritchard, Stephens, and Donnely, 2000; Falush, Stephens, and Pritchard, 2007) was used to provide insight into patterns of gene flow (admixture, or Q) and true subdivision (number of conceptual populations, or K) in each study species. This method uses individual multi-locus genotypes to test for the presence of population structure without a priori assignment of individual plants to populations. It does so by introducing population structure and finding population groupings in the least possible disequilibrium (HWE and linkage disequilibrium) using a Markov-Chain Monte Carlo method. For each species, I carried out 20 independent runs using a burn-in period of 10,000 and collected data for 10,000 iterations

for K = 1 to 11 (optimal parameters described in Evanno, Regnaut, and Goudet 2005). The most likely value of K was assessed using the rate of change in the log probability of data between following K values (ΔK) as detailed in Evanno, Regnaut, and Goudet (2005). For each species, average and individual admixture proportions (Q) were recorded for each study population by identified genetic cluster for the selected value of K.

2.3 RESULTS

2.3.1 Descriptive statistics of loci

All eight microsatellite primer pairs consistently amplified products and were polymorphic in at least one study species (Table III). The Pen25 locus did not consistently amplify in *P. deustus* and *P. pachyphyllus*, while Pen24 showed highly significant deviations from HWE (heterozygote deficiency) in all populations of *P. rostriflorus*, suggesting that null alleles may be present in this species. Gene diversity per locus and population was not significantly different between Pen24 for *P. deustus* and *P. pachyphyllus* and Pen 25 for *P. rostriflorus* (ANOVA; $F_{2, 20} = 2.18$, $P = 0.1393$). Thus, one locus per species was excluded from further analyses, as shown in Table III. Remaining loci were highly polymorphic. Significant deviations from Hardy-Weinberg expectations were identified at 4 of 7 loci in *P. deustus*, 3 of 7 loci in *P. pachyphyllus*, and 1 of 7 loci in *P. rostriflorus*, all in the direction of heterozygote deficiency.

TABLE III

SUMMARY STATISTICS FOR THREE *PENSTEMON* SPECIES BY LOCUS

	P. deustus						***P. pachyphyllus***						***P. rostriflorus***					
Locus	Number plants	Allele size (bp)	Number alleles	H_E[a]	H_O[b]	f[c]	Number plants	Allele size (bp)	Number alleles	H_E[a]	H_O[b]	f[c]	Number plants	Allele size (bp)	Number alleles	H_E[a]	H_O[b]	f[c]
Pen02	256	163-245	36	0.953	0.816	**0.074****	224	167-219	23	0.933	0.777	**0.054****	255	167-223	27	0.916	0.855	0.009
Pen04	255	221-287	30	0.939	0.840	**0.059****	224	209-263	25	0.936	0.830	**0.079****	254	217-265	22	0.894	0.886	-0.034
Pen05	255	159-217	23	0.857	0.646	0.024	222	159-197	7	0.226	0.149	-0.012	256	171-245	32	0.933	0.875	0.022
Pen06	255	185-243	27	0.942	0.827	0.001	214	189-227	20	0.776	0.626	-0.103	246	207-253	22	0.842	0.769	0.006
Pen18	253	536-616	33	0.904	0.775	-0.022	221	530-566	16	0.827	0.466	0.100*	251	548-600	26	0.917	0.786	**0.089****
Pen23	255	148-206	24	0.584	0.405	**0.170****	224	156-196	14	0.730	0.518	0.057	255	156-188	15	0.672	0.568	0.026
Pen24	256	145-225	38	0.952	0.770	**0.135****	223	129-197	26	0.692	0.776	0.023	-	-	-	-	-	-
Pen25	-	-	-	-	-	-	-	-	-	-	-	-	255	120-176	26	0.903	0.871	-0.009

[a] Expected heterozygosity.

[b] Observed heterozygosity.

[c] Weir and Cockerham's estimate of the within-population inbreeding coefficient (f).

* $P < 0.05$

** $P < 0.01$

2.3.2 Descriptive statistics of species and populations

When all loci were combined, each species displayed different patterns of among population diversity and heterozygosity (Table IV). All *P. deustus* populations harbored private alleles, but one high elevation population, Pd-NH, contained many more private alleles than any other *P. deustus* population (23 vs. 1-8). Populations of *P. pachyphyllus* and *P. rostriflorus* also harbored private alleles, ranging from 0-12 in *P. pachyphyllus*, and 0-7 in *P. rostriflorus*. ANOVA tests revealed that gene diversity (H_E) was significantly greater in *P. deustus* and *P. rostriflorus* than *P. pachyphyllus* ($F_{2, 20} = 15.73$, $P < 0.0001$). There was a trend for heterozygote deficiency in populations of *P. deustus*, intermediate levels in *P. pachyphyllus* and lower levels in *P. rostriflorus*, but ANOVA did not detect significant differences between species ($F_{2, 20} = 1.85$, $P = 0.1834$). Population-level inbreeding shows the largest and most significant inbreeding in 4 of the 5 high-elevation *P. deustus* populations, but not in any of the low-elevation populations, suggesting different patterns of pollination at higher elevation sites. A similar but less striking pattern is seen in *P. pachyphyllus*, with 2 of the 4 high-elevation populations demonstrating significant inbreeding but none of the low-elevation sites. Inbreeding was lowest and least significant in *P. rostriflorus*, with no obvious pattern.

TABLE IV
PER-POPULATION SUMMARY STATISTICS FOR THREE *PENSTEMON* SPECIES

Population	Number plants	Mean sample size	Proportion polymorphic loci	Mean alleles per locus	Private alleles	H_E[a]	H_O[b]	f[c]
P. deustus								
Pd-CH	32	31.9	1.000	14.7	8	0.807	0.736	**0.101*****
Pd-CL	32	31.9	1.000	10.7	4	0.730	0.736	-0.009
Pd-EH	32	32.0	1.000	7.9	1	0.701	0.674	0.039
Pd-EL	32	31.9	1.000	9.6	5	0.694	0.693	0.001
Pd-NH	32	31.7	1.000	14.7	23	0.812	0.744	**0.085*****
Pd-NL	32	31.9	1.000	11.0	8	0.771	0.740	0.041
Pd-WH1	32	31.9	1.000	12.4	1	0.847	0.758	**0.107*****
Pd-WH2	32	32.0	1.000	11.3	3	0.795	0.723	**0.092*****
Overall	*32*	*31.9*	*1.000*	*11.5*	*7*	*0.771*	*0.726*	*0.060*
P. pachyphyllus								
Pp-CH1	32	32.0	1.000	7.6	9	0.530	0.518	0.023
Pp-CH2	32	31.7	0.857	8.6	7	0.519	0.474	**0.087****
Pp-NL	32	31.7	0.857	6.3	7	0.608	0.563	0.076
Pp-EH1	32	31.0	0.857	8.3	0	0.617	0.560	**0.094****
Pp-EL	32	31.9	0.857	9.7	12	0.642	0.620	0.064
Pp-SEL	32	31.7	1.000	9.4	7	0.772	0.761	0.015
Pp-SEH	32	31.9	1.000	7.3	1	0.612	0.647	-0.058
Overall	*32*	*31.7*	*0.918*	*8.2*	*6*	*0.614*	*0.592*	*0.037*
P. rostriflorus								
Pr-CH	32	31.9	1.000	11.3	3	0.797	0.816	-0.025
Pr-CL	32	31.3	1.000	13.0	4	0.825	0.804	**0.026***
Pr-EH1	32	32.0	1.000	16.1	5	0.866	0.835	**0.036***
Pr-EH2	32	32.0	1.000	14.6	1	0.870	0.879	-0.012
Pr-SEH	32	31.9	1.000	16.4	7	0.906	0.870	**0.041***
Pr-SEL	32	31.9	1.000	12.3	0	0.799	0.776	0.030
Pr-WH1	32	30.7	1.000	12.6	4	0.779	0.786	0.009
Pr-WH2	32	31.6	1.000	7.9	2	0.665	0.645	0.030
Overall	32	*31.6*	*1.000*	*13.0*	*3*	*0.813*	*0.801*	*0.015*

[a] Expected heterozygosity
[b] Observed heterozygosity
[c] Weir and Cockerham's estimate of the within-population inbreeding coefficient
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

2.3.3 Population genetic structure

All three study species demonstrate both high levels of diversity within populations and significant population genetic differentiation within and among mountain ranges (Tables V & VI). AMOVA revealed the greatest proportion of genetic variance for all species at the 'within populations' level. A higher proportion of the genetic variance resided at this level for *P. rostriflorus* than *P. deustus* or *P. pachyphyllus*. *Penstemon*

rostriflorus has less overall variation partitioned among populations or mountain ranges, and mountain ranges are more distinct for *P. pachyphyllus* than the other two species, with significantly more genetic variation partitioned among mountain ranges than among populations.

TABLE V
RESULTS OF AMOVA FOR EACH *PENSTEMON* SPECIES

Species	Level of partitioning	Variation	F-statistic	P
P. deustus	Among mountain ranges	7%	$F_{RT} = 0.070$	<0.001
	Among populations	7%	$F_{SR} = 0.078$	<0.001
	Within populations	86%	$F_{ST} = 0.142$	<0.001
P. pachyphyllus	Among mountain ranges	15%	$F_{RT} = 0.146$	<0.001
	Among populations	9%	$F_{SR} = 0.102$	<0.001
	Within populations	77%	$F_{ST} = 0.234$	<0.001
P. rostriflorus	Among mountain ranges	4%	$F_{RT} = 0.039$	<0.001
	Among populations	3%	$F_{SR} = 0.035$	<0.001
	Within populations	93%	$F_{ST} = 0.074$	<0.001

Global estimates of F_{ST} (as measured by θ) identified the greatest structure in *P. pachyphyllus* ($\theta = 0.221$, $P < 0.001$), with *P. deustus* ($\theta = 0.134$, $P < 0.001$) and *P. rostriflorus* ($\theta = 0.071$, $P < 0.001$) demonstrating lower but still significant structure. Results of ANOVA on all pairwise F_{ST} comparisons (Table VI, Figure 2.2) confirmed these differences, regardless of whether within-mountain comparisons were included ($F_{2, 74} = 36.48$, $P < 0.0001$) or excluded ($F_{2, 64} = 53.94$, $P < 0.0001$). Individual F_{ST} estimates between populations on *different mountain ranges* were significantly higher in *P. pachyphyllus* than *P. deustus*, while *P. rostriflorus* was significantly lower than both ($F_{2, 64} = 53.96$, $p < 0.0001$). *P. pachyphyllus* populations were most genetically differentiated despite being geographically closer (176 km) than *P. deustus* (335 km) or *P. rostriflorus* (291 km) populations ($F_{2, 64} = 8.11$, $p = 0.0007$). This greater genetic differentiation over shorter distances in *P. pachyphyllus* is demonstrated by the significant ANOVA on pairwise F_{ST} divided by geographic distance among mountains ($F_{2, 64} = 84.16$, $P < 0.0001$). The same test including within population comparisons was not significant ($F_{2, 74} = 2.35$, $p = 0.1009$).

Individual F_{ST} estimates between population pairs *on the same mountain range* were generally greatest in *P. deustus*, intermediate in *P. pachyphyllus*, and lowest in *P. rostriflorus* (all pairwise P values < 0.001 except the comparison between Pr-EH1 and EH2, where $\theta = 0.0062$, $p = 0.027$). These within-mountain comparisons were usually genetically more similar than among-mountain comparisons, especially for *P. pachyphyllus* and *P. deustus*. For *P. rostriflorus*, this was also true for the within-mountain comparison between populations at similar elevations (Pr-EH1 and EH2), but the other two within-mountain range comparisons were actually greater than some among-mountain range comparisons.

TABLE VI

PAIRWISE GENETIC[a] AND GEOGRAPHIC[b] DISTANCES FOR EACH POPULATION

P. deustus								
	Pd-CH	Pd-CL	Pd-EH	Pd-EL	Pd-NH	Pd-NL	Pd-WH1	Pd-WH2
Pd-CH		8.5	265.0	269.0	381.0	320.0	160.0	152.0
Pd-CL	**0.0570**		237.0	277.0	382.0	319.0	151.0	143.0
Pd-EH	0.1290	0.1639		5.0	472.0	435.0	421.0	415.0
Pd-EL	0.1389	0.1797	**0.0647**		475.0	438.0	427.0	421.0
Pd-NH	0.0944	0.1406	0.1029	0.0797		65.0	393.0	397.0
Pd-NL	0.0917	0.1363	0.1301	0.1315	**0.1048**		328.0	332.0
Pd-WH1	0.0869	0.1362	0.1708	0.1857	0.1268	0.1424		11.0
Pd-WH2	0.1210	0.1493	0.2033	0.2160	0.1571	0.1811	**0.0794**	
P. pachyphyllus								
	Pp-SEL	Pp-SEH	Pp-EH1	Pp-EL	Pp-CH2	Pp-CH1	Pp-NL	
Pp-SEL		19.0	135.0	137.0	245.0	242.0	341.0	
Pp-SEH	**0.1963**		118	120	229	226	324.0	
Pp-EH1	0.2326	0.2866		2.1	112	109	206.0	
Pp-EL	0.2114	0.2661	**0.0433**		110	107	204.0	
Pp-CH2	0.2860	0.3521	0.1799	0.2102		4.6	100.0	
Pp-CH1	0.2805	0.3538	0.1688	0.1974	**0.0264**		104.0	
Pp-NL	0.2254	0.2937	0.1727	0.1731	0.1453	0.1347		
P. rostriflorus								
	Pr-SEL	Pr-SEH	Pr-EH1	Pr-EH2	Pr-CH	Pr-CL	Pr-WH1	Pr-WH2
Pr-SEL		5.6	125.0	107.0	218.0	213.0	450.0	581.0
Pr-SEH	**0.0597**		121.0	103.0	213.0	208.0	450.0	581.0
Pr-EH1	0.0694	0.0496		19.0	94.0	88.0	386.0	501.0
Pr-EH2	0.0690	0.0432	**0.0062***		113.0	115.0	389.0	524.0
Pr-CH	0.0982	0.0855	0.0223	0.0206		5.4	334.0	448.0
Pr-CL	0.0984	0.0616	0.0263	0.0258	**0.0358**		338.0	452.0
Pr-WH1	0.1328	0.1053	0.0392	0.0442	0.0420	0.0527		132.0
Pr-WH2	0.1901	0.1581	0.0962	0.0955	0.0899	0.0941	0.0662	

Bolded values identify pairwise comparisons within the same mountain range.

[a] Reported as pairwise F_{ST} values for each population comparison (on lower left matrix).

[b] Reported as geographic distance (km) between each population (on upper right matrix).

[*] All pairwise F_{ST} comparisons significant at $P < 0.001$ except this comparison, significant at $P < 0.027$.

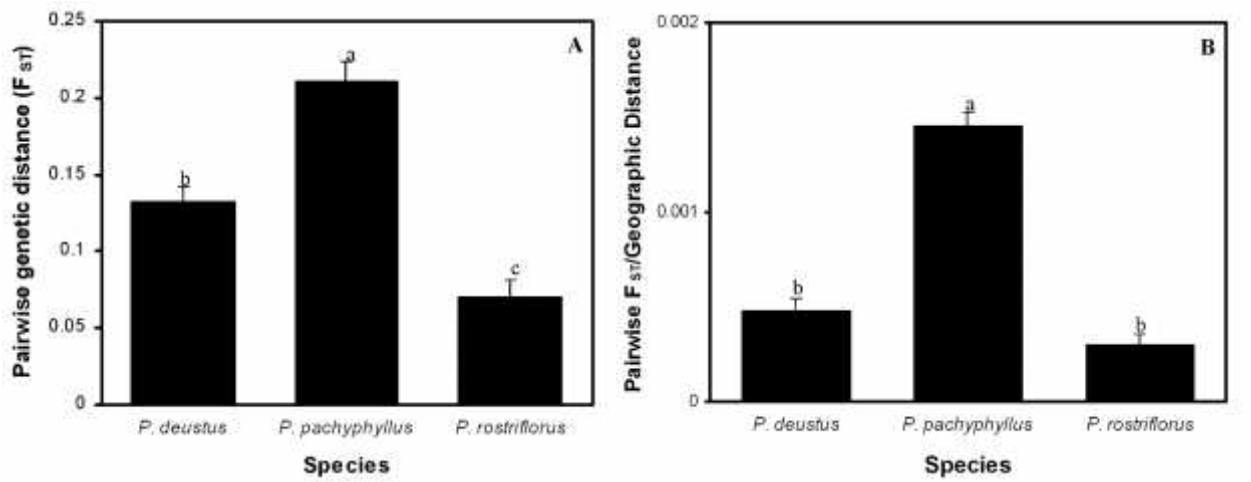

Figure 2.2. ANOVA and Tukey-Kramer results for significant differences by species in (A) pairwise genetic distances (F_{ST}), and (B) pairwise F_{ST}/geographic distance excluding within-mountain comparisons.

Mantel tests revealed a strong pattern of isolation-by-distance in all three study species (Figure 2.3). Correlation coefficients (r^2) between genetic and geographic distance ranged from 0.47 (*P. pachyphyllus*, P = 0.0059) to 0.33 (*P. deustus*, P = 0.0427) and 0.30 (*P. rostriflorus*, P = 0.0003). Figure 2.3 shows that *P. pachyphyllus* has a higher degree of genetic differentiation over a shorter distance than that observed in *P. deustus*, with *P. rostriflorus* having the least differentiation. UPGMA results (Figure 2.4) demonstrate similar patterns but provide additional resolution regarding genetic distances between populations on the same mountain range and on increasingly distant mountain ranges. *Penstemon pachyphyllus* has the largest genetic distances between populations, but populations located on the same mountain range always grouped together (Pp-CH1 and CH2, EH1 and EL, and SEH and SEL). Genetic differences among *P. deustus* populations were lower than for *P. pachyphyllus*, and again populations on the same mountain ranges clustered together with the exception of Pd-NL and NH, on the same mountain range but located 65 km apart. The UPGMA tree for *P. rostriflorus* was the shallowest, a result of relatively low genetic distances between all population pairs. While populations sampled within the same mountain range largely clustered together (Pr-EH1 and EH2, Pr-SEL and SEH), the high-elevation population CH clustered more closely to the high elevation populations on a mountain range over 100km away (EH1 and EH2) than the low elevation population on the same mountain range only 5.4 km away.

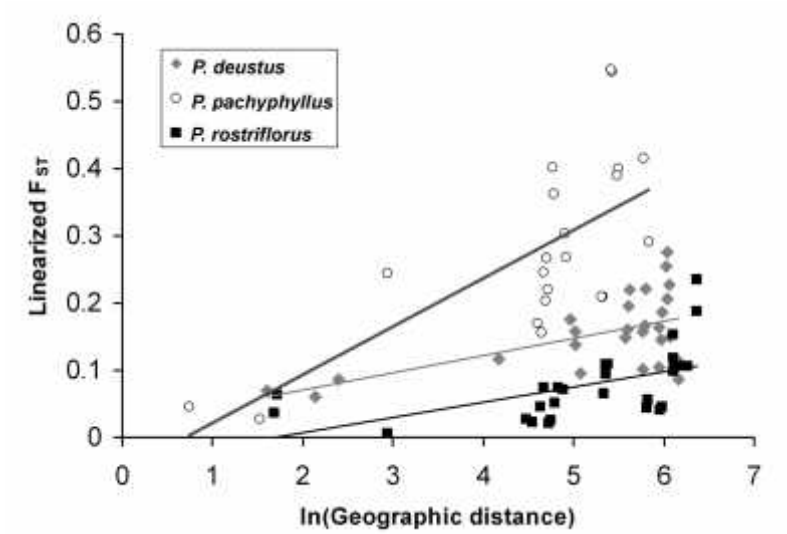

Figure 2.3. Genetic distance (linearized F_{ST} [or F_{ST} /(1- F_{ST})]) versus log-transformed geographic distance (originally measured in kilometers) for pairwise comparisons of populations of *P. deustus*, *P. pachyphyllus* and *P. rostriflorus*.

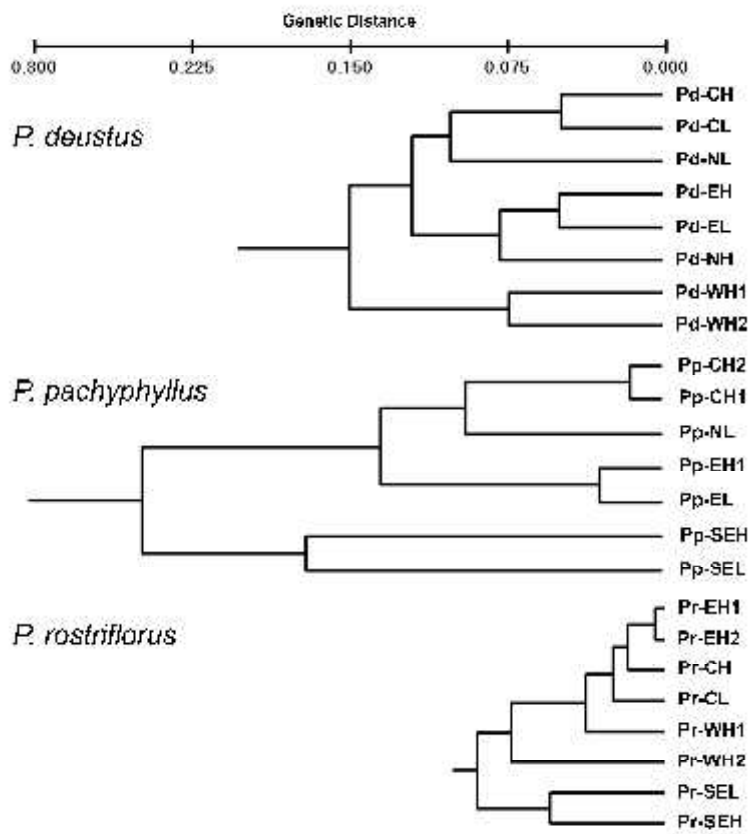

Figure 2.4. UPGMA clustering using Nei's unbiased estimate of genetic distance (1978) for each species.

Bayesian analysis performed by Structure was used to infer spatial population structure and estimate the number of genetic clusters (K), or populations, into which the genotypic data could be grouped. Structure results confirmed pronounced genetic structure in all three species, with generally more structure in *P. deustus* and *P. pachyphyllus* than *P. rostriflorus*. The modal value of the distribution of the true K identified a major peak at ΔK=4 with a smaller secondary peak at ΔK=7 for both *P. deustus* and *P. pachyphyllus*, while in *P. rostriflorus* the major peak was at ΔK=3 and minor peak at ΔK=5. Table VII details the average admixture proportion among identified 'genetic clusters' for each population of each species; these results are graphically depicted in Figure 2.5. In *P. deustus* and *P. pachyphyllus* the first major peak (K=4) clustered samples by mountain ranges, with very little admixture between populations for either species, while K=7 clustered each population sampled in *P. pachyphyllus* and one less than the number of populations sampled in *P. deustus* (Pd-EH and EL were clustered into one population). In *P. rostriflorus*, K values did not correspond to number of mountain ranges nor number of populations sampled, and all populations had considerably more admixture among genetic clusters than the other two species.

TABLE VII

AVERAGE ADMIXTURE PROPORTION FOR EACH SAMPLED POPULATION

	Clusters identified at major peak				Clusters identified at minor peak						
Population	I	II	III	IV	I	II	III	IV	V	VI	VII
P. deustus											
Pd-CH	**0.881**[a]	0.015	0.050	0.055	**0.801**	0.083	0.014	0.028	0.014	0.045	0.016
Pd-CL	**0.970**	0.013	0.009	0.008	0.027	**0.922**	0.016	0.009	0.009	0.011	0.007
Pd-EH	0.006	**0.980**	0.008	0.006	0.007	0.005	**0.958**	0.010	0.008	0.006	0.005
Pd-EL	0.006	**0.977**	0.010	0.007	0.008	0.005	**0.955**	0.009	0.010	0.006	0.007
Pd-NH	0.036	0.045	**0.904**	0.015	0.013	0.024	0.021	**0.918**	0.008	0.008	0.007
Pd-NL	0.013	0.014	**0.966**	0.007	0.018	0.007	0.013	0.018	**0.932**	0.006	0.006
Pd-WH1	0.019	0.015	0.012	**0.953**	0.014	0.024	0.016	0.011	0.013	**0.884**	0.039
Pd-WH2	0.020	0.010	0.008	**0.962**	0.017	0.026	0.008	0.008	0.005	0.087	**0.849**
P. pachyphyllus											
Pp-SEH	**0.981**	0.007	0.006	0.006	**0.939**	0.015	0.011	0.013	0.009	0.007	0.006
Pp-SEL	**0.960**	0.010	0.010	0.021	0.026	**0.925**	0.008	0.009	0.008	0.010	0.015
Pp-EH1	0.008	**0.968**	0.017	0.008	0.007	0.007	**0.743**	**0.198**	0.020	0.016	0.009
Pp-EL	0.005	**0.962**	0.014	0.018	0.005	0.007	**0.110**	**0.826**	0.019	0.015	0.018
Pp-CH2	0.009	0.032	**0.880**	0.080	0.007	0.014	0.002	0.024	**0.539**	**0.352**	0.044
Pp-CH1	0.006	0.022	**0.934**	0.038	0.005	0.011	0.037	0.018	**0.344**	**0.559**	0.027
Pp-NL	0.016	0.010	**0.115**	**0.859**	0.013	0.018	0.012	0.010	0.067	**0.100**	**0.781**
P. rostriflorus											
Pr-SEL	**0.929**	0.042	0.029		**0.808**	**0.118**	0.025	0.032	0.018		
Pr-SEH	**0.831**	**0.119**	0.051		0.006	**0.844**	0.049	0.023	0.024		
Pr-EH1	**0.382**	**0.511**	**0.106**		0.073	**0.403**	**0.179**	**0.284**	0.061		
Pr-EH2	**0.303**	**0.592**	**0.105**		0.070	**0.287**	**0.337**	**0.255**	0.051		
Pr-CH	0.084	**0.825**	0.092		0.052	0.040	**0.576**	**0.274**	0.058		
Pr-CL	**0.132**	**0.770**	0.098		0.035	**0.170**	**0.542**	**0.191**	0.062		
Pr-WH1	0.081	**0.485**	**0.434**		0.024	0.042	0.044	**0.802**	0.087		
Pr-WH2	0.021	0.030	**0.950**		0.013	0.014	0.014	0.062	**0.897**		

[a] Bolded text identifies genetic clusters with $\geq$ 10% representation in each population (values $\geq$ 0.1).

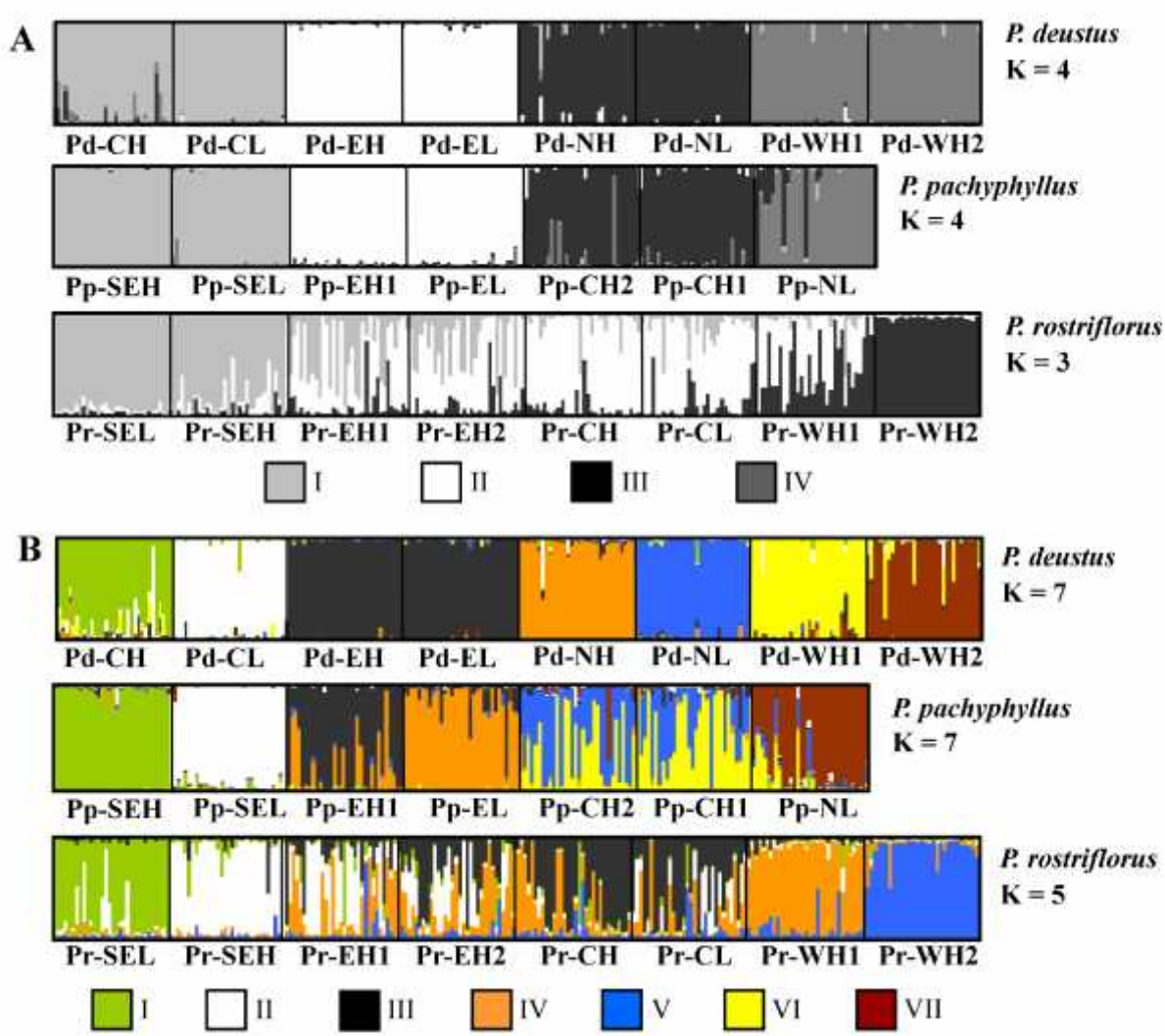

Figure 2.5. Identified genetic clusters and Bayesian admixture proportions (Q) depicted for individual plants and populations of all three *Penstemon* species. Results shown for models that reveal structure at two levels: (A) *P. deustus* K = 4; *P. pachyphyllus* K = 4; *P. rostriflorus* K = 3), and (B) *P. deustus* K = 7; *P. pachyphyllus* K = 7; *P. rostriflorus* K = 5).

2.4 DISCUSSION

Gene flow maintains species cohesion; in its absence populations isolated from one another will be free to follow different evolutionary trajectories that may ultimately lead to the formation of new species (Ellstrand, 1992; Morjan and Rieseberg, 2004). For plants, gene flow via the dispersal of seeds and pollen is therefore a critical component of current population genetic structure and future evolutionary potential (Kramer et al., 2008). Landscape effects can either deter or enhance gene flow, and are thus equally important considerations in

understanding the evolutionary context of a species. My results depict the Great Basin as a landscape capable of significantly isolating plant populations that occupy its montane islands, providing opportunities for evolutionary divergence and potential speciation. Further, my examination of three otherwise similar *Penstemon* species with different pollination syndromes demonstrates that pollinators can have significant effects on the evolutionary trajectory of a species via effects on population genetic structure.

I found significant population genetic differentiation in all three *Penstemon* species, which in nearly every case was greater than that reported for any co-occurring plant or animal species studied to date in the Great Basin region. Populations of eight wind-pollinated, bird-dispersed conifer species that are similarly distributed and sampled as my study species demonstrated levels of genetic differentiation almost always lower than my findings for *Penstemon* [average Gst ranging from 0.033 for pinyon pine to 0.169 for bristlecone pine (Hamrick, Schnabel, and Wells, 1994; Jorgensen, Hamrick, and Wells, 2002)]. Marmots, terrestrial mammals often confined to high-elevation alpine habitat on many of the same mountaintops, exhibit lower levels of genetic differentiation than the *Penstemon* species studied here [Fst from 0.05 – 0.23, average 0.13; (Floyd, Van Vuren, and May, 2005)]. In contrast, all of my analyses investigating population genetic structure indicated that neutral genetic diversity among *Penstemon* populations in the mountainous terrain of the Great Basin is highly structured both among populations and mountain ranges.

Such strong genetic differentiation is all the more surprising in light of the geological and climatalogical history of the Great Basin. The proposed evolutionary radiation that led to the current species diversity in the genus *Penstemon* occurred during the glacial advances and retreats of the Pleistocene (beginning ca. 1.8 mya), ending during the Wisconsin stage glacial maximum some 10,000 years ago in the late Pleistocene (Wells, 1983; Wolfe et al., 2006). At this time, the Great Basin was not glaciated, but its climate was substantially cooler and wetter than it is today. Most plants and animals were distributed up to 500 m lower than they are now, allowing a much larger and more contiguous network of habitat to exist throughout the region (Brown, 1971; Wells, 1983; Grayson, 1993). Following this period the warmer, drier climates of the Holocene have driven a majority of the

region's plants and animals to their current higher-elevation refugia on mountain ranges. Thus, the genetically isolated *Penstemon* populations found in the Great Basin today are direct descendants of once-continuous populations found throughout the region just 10,000 years ago.

Genetic isolation among populations on different mountain ranges is likely increasing more rapidly now than at any point in the past 10,000 years, given today's rapidly-changing climate. An average global temperature increase of 0.74ºC over the last century (Solomon et al., 2007) in combination with a brief historical period of cooling (the Little Ice Age, AD 1300-1850) has driven montane habitat in the Great Basin up some 180 m in the last 150 years (Munroe, 2003). This, in combination with rapid habitat degradation due to wildfires and invasive species now common throughout the region, will contribute to continued rapid increases in isolation among *Penstemon* populations.

While genetic diversity among *Penstemon* populations is significantly structured among mountain ranges, the three species are not equally isolated by the mountainous terrain they occupy. Genetic differentiation among mountain ranges was highest in *P. pachyphyllus* (large bee pollination syndrome), intermediate in *P. deustus* (small bee pollination syndrome), and lowest in *P. rostriflorus* (hummingbird pollination syndrome). This trend was supported by every analysis conducted, including ANOVA tests of pairwise F_{ST} comparisons (Figure 2.2 C and D), UPGMA clustering using genetic distance (Figure 2.4) and Bayesian clustering analyses (Figure 2.5A). These results demonstrate the important but often overlooked impact of pollinators on the evolutionary trajectory of a species. Given that these three *Penstemon* species share most life history traits and because it is highly unlikely that their gravity-dispersed seeds contribute significantly to gene flow among populations on different mountain ranges, differences in primary pollinators for each species are the most likely explanation for the striking differences in population connectivity. This study suggests that pollination syndromes does not just summarize the floral architecture and functional pollinator group of a species (Thomson et al., 2000), but actually determines its population genetic structure.

With far less genetic structure than its bee-syndrome counterparts, results from *P. rostriflorus* indicate that hummingbirds are better than bees at maintaining species cohesion between populations separated over much greater distances. This is often suggested but rarely substantiated in other studies (e.g. Graves & Schrader 2008). Bird-mediated long-distance (>5 km) gene flow between populations in fragmented agricultural habitat was recently documented in the sunbird-pollinated *Calothamnus quadrifidus* (Byrne et al., 2007), but the specific distances and terrain bird-pollination may be capable of bridging between populations is largely unknown. Additionally, the territoriality of some bird species (hummingbirds) may actually limit long-distance pollen movement in certain scenarios (Parra et al. 1993). Nine hummingbird species are recorded in the Great Basin region, including Broad-billed (*Cynanthus latirostris*), Magnificent (*Eugenes fulgens*), Black-chinned (*Archilochus alexandri*), Anna's (*Calypte anna*), Costa's (*C. costae*), Calliope (*Stellula calliope*), Broad-tailed (*Selasphorus platycercus*), Rufous (*S. rufus*), and Allen's (*S. sasin*) hummingbirds (Johnsgard, 1983). My study reveals that at least some of these species are capable of moving pollen over large distances across the Great Basin's inhospitable arid valleys. Results of Bayesian cluster analyses (Table VII and Figure 2.5) indicate hummingbird-assisted admixture between populations separated by at least 19 km within mountain ranges (e.g. Pr-EH1 and EH2) and over 100 km between mountain ranges, particularly when populations are at high elevations (e.g. Pr-SEH and EH1). A recent study of two *Streptocarpus* species in South Africa also reported significantly lower genetic differentiation in a primarily sunbird-pollinated species than a fly-pollinated species (Hughes et al. 2007), providing another case where bird pollination maintains greater species cohesion than insect pollination. Further studies are needed to test whether this is a consistent pattern of bird and insect pollination systems.

These findings are of particular interest given the ongoing study of evolutionary shifts between bee and hummingbird pollination syndromes (Cronk and Ojeda, 2008; Thomson and Wilson, 2008). In *Penstemon*, hummingbird pollination syndromes have arisen independently from a bee syndrome at least 10 and as many as 21 times, and all of these shifts have been unidirectional (Wilson et al., 2007). Similar scenarios of multiple independent origins of bird from bee syndromes have been detected in other genera, including *Mimulus* [2 times

(Beardsley, Yen, and Olmstead, 2003)]; *Erythrina* [4 times (Bruneau, 1997)] and *Costus* [7 times (Kay et al., 2005)]. My findings help explain these multiple independent and unidirectional shifts from bee to bird pollination syndromes. Once a bird pollination syndrome has arisen, reversion back to a bee syndrome or towards another syndrome may be more difficult because of higher gene flow among populations.

Both species with bee pollination syndromes (*P. deustus* and *P. pachyphyllus*) had highly significant population genetic divergence, and Bayesian clustering analysis independently identified each sampled mountain range as a separate genetic cluster, with little or no admixture between mountain ranges (Figure 2.5A). I conclude that bees either avoid crossing the Great Basin's arid valley floors or, if they do, they are ineffective at transferring pollen across these expanses. Many bees groom pollen from their bodies at regular intervals, so even if they fly long distances they may not effect long-distance pollination (Wilson et al., 2004). While all bee pollinators appear to be equally ineffective at mediating long-distance gene flow between Great Basin mountain ranges, bees pollinating *P. pachyphyllus* appear more effective at moving pollen between populations on the same mountain range (Figure 2.5B) than those pollinating *P. deustus*. I can only speculate on the specific differences in bee pollinators between these two species, but numerous bee visitors to *P. deustus* have been documented, including *Osmia* species, *Anthophora* and similar (small) nectar-collecting bees, as well as larger nectar-collecting *Bombus* species (Wilson et al., 2004). Visitors to *P. pachyphyllus* flowers have not been well-documented, but flowers are comparable to those of the well-studied *P. strictus*, so I expect a similar range of visitors. This includes the same functional groups as *P. deustus*, but extends to a broader range of generally larger bee and wasp visitors (Wilson et al., 2004). The different pollinators observed for each species may effect long-distance pollen flow over different scales (e.g. larger bee pollinators of *P. pachyphyllus* move pollen more effectively between distant populations on the same mountain range than smaller bee pollinators of *P. deustus*). However, the observed difference in admixture between *P. pachyphyllus* and *P. deustus* for populations on the same mountain range may be in part a reflection of my study design. The two within-mountain population comparisons which showed high admixture in *P. pachyphyllus* were separated by under 5 km, while all comparisons for *P. deustus*

were separated by over 5 km. Resolving this question will require incorporating additional populations on each mountain range at a variety of geographic distances and elevational differences.

These results can be used to guide ecological restoration efforts for my study species and those that share similar characteristics found throughout the unique landscape of the Great Basin region. Findings of high genetic diversity and significant population genetic structure in all three species support ongoing efforts to bank seeds of multiple populations to store genetic diversity for future restoration and research efforts (DeBolt and Spurrier, 2004). However, these findings of significant genetic structure also caution against broad-scale movement and mixing of populations for restoration purposes. Yet given the greater among-population gene flow identified in the hummingbird pollinated *P. rostriflorus*, the large scale movement of seeds to restore populations of hummingbird-pollinated species may pose less risk to the success of a restoration than bee-pollinated species. This recommendation does not take into account the fact that adaptive population divergence can still occur even in the presence of gene flow if selection on heritable quantitative traits is strong enough (Endler, 1973). This means that the broad movement of seeds for either bee *or* hummingbird-pollinated species may lead to restoration failure if seeds are not adapted to conditions at the restoration site. To address this issue and help guide successful restoration efforts, additional research is necessary to investigate adaptive divergence in quantitative traits that may influence the success or failure of a restoration.

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3. QUANTITATIVE AND MOLECULAR GENETIC DIVERGENCE WITHIN AND AMONG SKY ISLANDS IN THE GREAT BASIN: A COMPARISON AMONG *PENSTEMON* SPECIES

3.1 INTRODUCTION

The populations of a species widely distributed throughout an environmentally heterogeneous landscape will be subject to adaptive divergence due to natural selection. If these populations are connected by gene flow, species cohesion can be maintained and divergence constrained to varying degrees depending on both the amount of gene flow and the strength of selection (Endler, 1973; Slatkin, 1985; Hendry, Day, and Taylor, 2001; Hendry and Taylor, 2004). However, if these populations are genetically isolated from one another, divergence due to the interacting effects of natural selection, mutation and genetic drift will be more likely (Ehrlich and Raven, 1969; GarciaRamos and Kirkpatrick, 1997; Rieseberg, Church, and Morjan, 2004). Understanding how these forces interact to determine adaptive population divergence is critical to many issues in evolution, ecology and conservation (Hendry, Day, and Taylor, 2001), yet few studies are carried out to provide this broader perspective. An unprecedented growth in molecular genetic methods (such as neutral genetic markers like microsatellites) over the past two decades has provided insight into the movement of genes between populations for an ever-growing array of plant and animal species. These studies have demonstrated that among-population gene flow varies greatly depending upon the life history of a species and the landscape it occupies (Hamrick and Godt, 1996), but because they do not detect potentially adaptive genetic differentiation (Merila and Crnokrak, 2001; McKay and Latta, 2002; Bekessy et al., 2003), these studies alone have limited application to questions regarding natural selection and adaptation.

The presence and structure of potentially adaptive genetic diversity can be identified in common garden studies. By growing plants collected from multiple populations under uniform conditions, common garden studies eliminate environmentally-driven differences in phenotypes to reveal underlying genetic differences

among populations that are the product of adaptive and random population differentiation (Galloway and Fenster, 2000). Despite being labor, time and cost-intensive (Holderegger, Kamm, and Gugerli, 2006), these techniques have been used for nearly a century to understand the adaptive genetic divergence of plant populations, beginning with the work of Turesson (1922) and Clausen et al. (Clausen, Keck, and Hiesey, 1940; Clausen, Keck, and Hiesey, 1941; Clausen, Keck, and Hiesey, 1947). More recent studies have shown that, when used in concert with neutral genetic markers, common garden studies can help determine the relative importance of gene flow, genetic drift and natural selection in the process of population differentiation (Knapp and Rice, 1998; Leinonen et al., 2008). Numerous studies on plants have identified a number of traits under divergent adaptive selection (Bonnin, Prosperi, and Olivieri, 1996; McKay et al., 2001; Steinger et al., 2002; Volis et al., 2005). Reciprocal transplant studies (Montalvo and Ellstrand, 2000; Jones, Hayes, and Sackville Hamilton, 2001; Proffitt et al., 2005; Bischoff et al., 2006) have also demonstrated that plants often exhibit a 'home site advantage', with greater fitness when grown in their 'home' versus 'away' conditions. However, it is still unclear how environmental heterogeneity and gene flow are likely to interact to produce or restrict adaptive population divergence.

In few regions of the world are environmental heterogeneity and potential genetic isolation more extreme than in the Great Basin region of the western United States. Comprising over 390,000 km^2, the Great Basin contains over 150 distinct mountain ranges (Grayson, 1993) separated from one another by vast arid valleys. These mountain ranges, which rise an average of 1,750 meters above the valley floors, contain cooler, more mesic habitats where a majority of the vascular plant species native to the region are nearly exclusively found (Grayson, 1993). It is unclear how much these arid valleys act to isolate the plant populations on different mountain ranges, but for some species it is likely that these mountains function as sky islands (DeChaine and Martin, 2005). Lying in the rainshadow of the Sierra Nevada Mountains, a significant east-west precipitation gradient combines with a north-south temperature gradient to create a large-scale mosaic of environmental heterogeneity throughout the Great Basin. Even within mountain ranges, changes in temperature and precipitation imposed by rapid increases in elevation interact with differences in slope and aspect to create a virtually infinite array of microclimates within a relatively small scale (Petersen, 1994). In combination, these factors make the Great

Basin ideal for examining the divergence of plant populations in response to changing levels of environmental heterogeneity and varying degrees of genetic isolation.

In this study, I compare the relationship between gene flow and quantitative population divergence in three *Penstemon* species distributed throughout Great Basin region. *Penstemon* is North America's largest endemic genus (over 270 species) and the result of a recent and rapid evolutionary radiation centered in the western United States, including the Great Basin (Wolfe et al., 2006). Rapid speciation in *Penstemon* is largely attributed to evolutionary adaptations to pollinator specialization (Wilson et al., 2004; Wilson et al., 2006), and adaptation to the ecological niches created during multiple historical glacial advances and retreats in the region. My study species are a product of, and exemplify the extremes of, this floral radiation, including *P. deustus* Douglas ex Lindl. var. *pedicellatus* M.E. Jones, *P. pachyphyllus* A. Gray ex Rydb. var. *congestus* (M.E. Jones) N.H. Holmgren, and *P. rostriflorus* (Kellogg). All three species are animal-pollinated perennial forbs with mixed mating systems (A. Kramer pers. obs., Chapter 4) and gravity-dispersed seeds. They are all common and widespread throughout the western United States, including the Great Basin region (Kartesz, 1999), occurring almost exclusively in sagebrush-steppe habitat at a range of mid to high-elevations on mountain ranges. A study of neutral population genetic diversity and differentiation within and among Great Basin mountain ranges (as identified by microsatellite markers) revealed population genetic differentiation that is correlated with the pollination syndrome of each species (A. Kramer Chapter 2). *Penstemon pachyphyllus* (large purple flowers and bee pollination syndrome) exhibited extremely high levels of population differentiation between mountain ranges (F_{ST} = 0.221), while *P. deustus* (small white flowers and bee pollination syndrome) demonstrated significant but somewhat lower differentiation (F_{ST} = 0.134) and *P. rostriflorus* (red flowers and hummingbird pollination syndrome) demonstrated the least (F_{ST} = 0.071). Hence, over similar geographic landscapes higher gene flow is likely to be a greater constraint to population divergence in bird pollinated species compared with species pollinated by bees.

Here, I compare potential adaptive quantitative and neutral genetic divergence between study populations for all three species located on Great Basin sky islands/mountain ranges. Because each species has different levels of

gene flow between populations, I expect that quantitative divergence by mountain range will be greater in *P. pachyphyllus* than *P. deustus* and *P. rostriflorus*, and greater in *P. deustus* than *P. rostriflorus*. Because selection may drive adaptive divergence in different traits depending upon species and situation (Hendry, Day, and Taylor, 2001), I identify potential divergence in a range of traits, including growth, vegetative structure, flowering phenology and floral morphology. For this, nearly every population utilized in the above-mentioned microsatellite study (A. Kramer Chapter 2) was also grown in a series of common garden experiments, and quantitative trait data was used along with climatic data for each site to ask whether population divergence in potentially adaptive quantitative traits is restricted by gene flow, associated with climate (and therefore likely adaptive) differences, or some combination of both factors.

3.2 METHODS

3.2.1 Study populations and common garden sites

When possible, two distinct populations separated by at least 300m in elevation were located on each of at least 2 mountain ranges for each study species, covering their full range in the Great Basin floristic region (Cronquist et al., 1972). While this was not possible for all mountain ranges or species, between six and eight study populations (each with over 100 plants) were identified for each species representing extreme geographic and climatic differences (Table VIII and Figure 3.1). Population codes identify species, mountain range, and relative elevation (e.g. Pr-CH identifies *P. rostriflorus* on the central mountain range at a high elevation; Pr-CL is its population pair at a low elevation). From August to October 2003, seed was collected from up to 10% of ripe seed capsules from fifty haphazardly located individual plants at each study site (avoiding sampling plants within 1m of each other), cleaned and stored at room temperature and 20% relative humidity. In all, seed was collected from 22 sites between August and October 2003, and maintained in uniform storage conditions until studies were initiated. Two common garden sites were established within the Great Basin floristic region at: 1) Utah Botanical Center (Vitousek et al.) in Kaysville, UT and 2) Boise State University (BSU) in Boise, ID (see Table I). These sites varied considerably in climatic characteristics and soil composition. For example, UBC had greater

precipitation and clay alluvial soil, while BSU had drier conditions in combination with fine sandy soil. These differences allowed me to identify plastic responses of quantitative traits to different growing conditions.

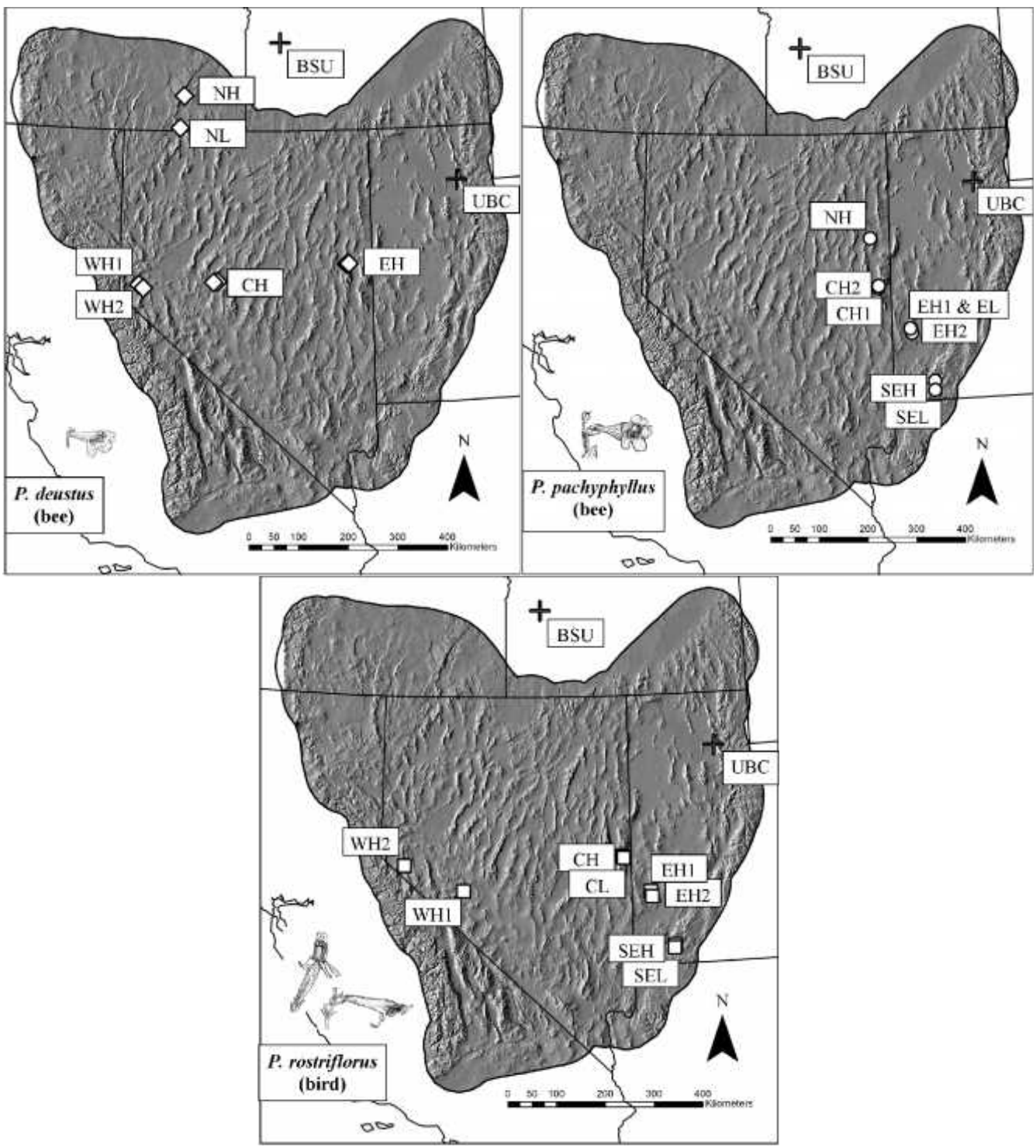

Figure 3.1. Site map showing collection and common garden sites for each species. Outline depicts the Great Basin geological region, bordered on the west by the Sierra Nevada mountain range and on the east by the Wasatch mountain range. Relief shows the region's many mountain ranges.

TABLE VIII

DETAILED STUDY SITE INFORMATION

Population	Mountain Range	State	Lat.	Long.	Elevation (m)	Approx size (# plants)	Temperature [a]	Precipitation [b]	PCA Axis 1 [c]	PCA Axis 2 [d]
Penstemon deustus										
Pd-CH	Desatoya Mountains	NV	39.254	-117.681	2025	100-150	5.9 (-10.9, 28.1)	252 (16, 28)	-13.71	-3.99
Pd-EH	Schell Creek Range	NV	39.557	-114.640	2649	200-300	3.9 (-13.2, 25.8)	429 (31, 44)	7.92	-12.63
Pd-NH	Steens Mountains	OR	42.629	-118.530	1793	200-300	6.3 (-9.5, 28.0)	299 (11, 33)	-10.45	2.87
Pd-NL	Steens Mountains	OR	42.046	-118.620	1368	200-300	8.5 (-8.1, 31.4)	235 (8, 28)	-16.67	4.31
Pd-WH1	Pine Nut Mountains	NV	39.176	-119.527	1861	150-200	8.6 (-8.1, 30.6)	264 (11, 39)	-4.87	5.76
Pd-WH2	Pine Nut Mountains	NV	39.115	-119.424	1834	150-200	7.6 (-8.7, 29.1)	293 (12, 42)	-1.45	5.74
Penstemon pachyphyllus										
Pp-SEH	Zion National Park	UT	37.341	-113.077	2122	300-400	7.8 (-9.4, 28.6)	435 (15, 50)	7.87	5.27
Pp-SEL	Zion National Park	UT	37.173	-113.083	1119	300-400	15.3 (-2.9, 36.8)	316 (9, 43)	-1.74	10.26
Pp-EH1	Wah Wah Mountains	UT	38.325	-113.590	2560	150-200	5.0 (-12.2, 26.6)	451 (22, 53)	13.37	-1.05
Pp-EH2	Wah Wah Mountains	UT	38.254	-113.575	2430	300-400	5.7 (-11.8, 27.5)	417 (20, 48)	7.71	-0.82
Pp-EL	Wah Wah Mountains	UT	38.337	-113.609	2216	300-400	6.3 (-11.6, 28.4)	384 (19, 44)	3.32	-1.23
Pp-CH1	Snake Range	NV	39.109	-114.347	2323	1000+	6.8 (-11.2, 29.5)	350 (23, 37)	-2.20	-7.45
Pp-CH2	Snake Range	NV	39.148	-114.330	2227	300-400	6.3 (-11.5, 28.9)	366 (25, 39)	0.54	-8.67
Pp-NL	Antelope Range	NV	40.036	-114.510	1995	300-400	6.9 (-11.3, 29.9)	294 (19, 35)	-5.71	-4.12
Penstemon rostriflorus										
Pr-SEH	Zion National Park	UT	37.345	-113.080	2092	200-300	8.3 (-8.9, 29.1)	424 (15, 49)	6.85	5.04
Pr-SEL	Zion National Park	UT	37.292	-113.096	1632	100-150	10.9 (-6.7, 31.9)	390 (12, 47)	3.65	7.82
Pr-EH1	Wah Wah Mountains	UT	38.354	-113.608	2510	100-150	5.1 (-12.1, 26.7)	446 (22, 52)	12.39	-1.37
Pr-EH2	Wah Wah Mountains	UT	38.256	-113.581	2455	150-200	5.7 (-11.8, 27.5)	417 (20, 48)	7.71	-0.82
Pr-CH	Great Basin National Park	NV	39.023	-114.270	2768	150-200	3.9 (-12.9, 25.4)	475 (30, 53)	16.39	-8.57
Pr-CL	Great Basin National Park	NV	38.991	-114.220	2147	100-150	7.4 (-10.6, 30.2)	347 (22, 38)	-1.65	-6.02
Pr-WH1	Pilot Mountains	NV	38.392	-118.025	1919	100-150	8.2 (-9.4, 30.4)	161 (10, 17)	-26.87	-2.05
Pr-WH2	Pine Nut Mountains	NV	38.847	-119.438	1678	200-300	8.1 (-8.6, 29.7)	282 (12, 42)	-1.57	5.78
Common Garden										
BSU	Boise State University	ID	43.598	-116.162	830	-	10.6 (-5.5, 32.8)	330 (8, 42)	-2.60	10.43
UBC	Utah Botanical Center	UT	41.022	-111.935	1399	-	10.5 (-7.0, 32.9)	502 (19, 63)	22.07	7.34

[a] Shown as mean annual temperature (minimum temperature, maximum temperature) in ºC.

[b] Shown as mean annual precipitation (minimum precipitation driest month, maximum precipitation wettest month) in millimeters.

[c] First Principal Components Analyses (PCA) axis, explaining 72% of climatic variation among sites; strongly influenced by precipitation.

[d] Second PCA axis explaining an additional 24% of climatic variation among sites; strongly influence by temperature and precipitation seasonality.

3.2.2 Quantitative trait measures

In winter 2003, seeds from each of 40 maternal lines per population were germinated and grown under common greenhouse conditions. In May 2004, at least one seedling from each of 30 maternal lines per population (when available; see Table X) was planted in a randomized design by species into each common garden site. Supplemental irrigation was added as needed to keep plants alive, and weeds were controlled by hand. In addition to monitoring survival, quantitative trait data on each species at both common garden sites were collected from spring 2004 to fall 2006. Many of the traits I chose were highly heritable in a previous study on *Penstemon centranthifolius* (Mitchell and Shaw, 1993). My measures are grouped into four categories:

1. **Growth**: three measures of growth were recorded for all 3 species at each common garden site. Total (dm^3 / 16 months): a volume measure of change in plant size from spring 2004 through fall 2005. For more detailed analysis of growth patterns, this was divided into two components: Winter (dm^3 / 9 months), recording growth from Sept 2004 – May 2005, and Summer (dm^3 / 4 months) recording growth from May – Aug. 2005.

2. **Vegetation structure**: included Leaf area (cm^2), determined by multiplying the length and width of the 5th leaf from the base of a haphazardly selected flowering stalk (*P. deustus* and *P. rostriflorus*) or haphazardly chosen from the basal rosette (*P. pachyphyllus*) for all plants. In summer 2005, Stem length (cm) was measured on an arbitrarily chosen flowering stem, and measured from the soil surface to the stem tip. On that same stem, three additional measures were taken: Flower height (cm) was measured from the soil surface to the first whorl of flowers, Internode (cm) space between the 3rd and 4th flower whorls, and Stem diameter (mm) the at the stem base was measured with electronic calipers. Unique measures were additionally taken for *P. deustus* (Dentation, or average number of teeth on one side of the same leaf measured for leaf area above) and *P. pachyphyllus* (Peduncle length (cm), a measure of the stalk supporting the inflorescence at the 3rd whorl on the arbitrarily selected stem).

3. **Flower/fruit phenology**: 2-3 measures were recorded in May (for *P. deustus* and *P. pachyphyllus* flowers) or July (*P. rostriflorus* flowers, and *P. deustus* and *P. pachyphyllus* and *P. rostriflorus* fruit) 2005. Percent flowering (% of flowers open or just finished relative to all potential flowers on the stem) was recorded for all species, as was the Number of fruit produced. For *P. rostriflorus*, Percent flowers finished was also recorded, as very few fruit had begun to form.

4. **Floral shape**: three flowers per maternal line per population were collected shortly after opening and placed directly in glass vials with 70% EtOH. Three flower measures were then recorded with digital calipers in the laboratory in winter 2006: Corolla length (mm), Flower opening (mm; measured as diameter or circumference), and Anther exsertion (mm). Note:

measures were collected from all species at both common garden sites, except *P. rostriflorus*, which was only collected in sufficient numbers from UBC due to low flower production at this site.

An additional lab-based common garden study was initiated at Chicago Botanic Garden (CBG) in 2004 to determine population-level differences in seed germination requirements. Using seeds from 2003 collections, two seed mixes were created for detailed study. First, for each study population, a bulk collection with equal founder representation was created by mixing an equal number of seeds from a random subset of 25 of the 50 original maternal lines collected per population. Bulked seed created for each population was divided into 10 replicates of 50 seeds each. Second, for three populations of *P. pachyphyllus* and 4 of *P. rostriflorus*, 10 maternal lines per population were selected for individual-level assessment of seed germination requirements. Seed for each maternal line was divided into six replicates of 30-50 seeds, for a total of 180-300 seeds per individual included in the study. All seeds were washed in 0.25% sodium hypochloride (bleach) solution for one minute and rinsed twice in deionized water before being placed into a 5.5 cm petri dish with two layers of No5 Whatmann filter paper and dampened with deionized water. Replicates were placed directly in cold stratification (CS = 8 hours at 10ºC with light, 16 hrs at 4ºC in the dark) and regularly monitored for 20 weeks, with any germinants (radicle emergence greater than 1mm) recorded and discarded following Meyer et al. (1995). Filter paper was kept moist throughout the study with regular application of deionized water. Average days to germination were determined for each population or maternal line using combined results from all replicates.

3.2.3 Site characterization

All study populations and both common garden sites were characterized by 19 bioclimatic parameters (Hijmans et al., 2005), using the BIOCLIM GIS dataset (downloaded October 2007) and ArcMap GIS software (ESRI). The BIOCLIM dataset is a climatic model based upon input from weather stations around the world that allows a very high resolution (1 km grid) of global weather data via a series of algorithms. As described in Hijmans et al. (2005), BIOCLIM is currently the best dataset for reliable predictions of climatic parameters in mountainous areas where few weather stations are established (e.g. the Great Basin). Data collected for all

BIOCLIM parameters at each study population was standardized (transformed into standard-deviation units, or z-scores), and principal components analysis (PCA) was performed to generate two principal axes accounting for the majority of variance in BIOCLIM variables (Ellison et al., 2004). PCA results were used to calculate pairwise climate distances between all study populations and common garden sites for each PCA axis. Differences between sites were also characterized by pairwise geographic distance (straight-line geographic distances (km) measured between all sites using ArcMap (ESRI)). This, combined with pairwise genetic distances (measured as Fst or θ) reported in Chapter 2, ultimately allowed us to quantify the relationships between quantitative differences and climatic, geographic and genetic distances.

3.2.4 Data analysis

JMP IN statistical analysis software (SAS Institute, 2004) was used for all analyses and, where necessary, quantitative trait measures were square root or log transformed to meet assumptions of normality prior to analysis; all results were reverse-transformed prior to visualization on tables and figures. To identify differences in survival for each species in the field-based common garden sites after four growing seasons, nominal logistic regression was performed and a likelihood ratio test used to determine significance of 1) species, common garden, and their interaction, as well as 2) common garden, study population, and their interaction for each species separately. Percent survival for each population at each common garden site was also regressed on two potential predictors of survival: climatic distance (PCA1 and PCA2) and geographic distance (km) between each study population and the common garden site.

All quantitative characters were tested for pairwise correlations with plant size (volume at end of first growing season). For field-based common garden work at BSU and UBC, a two-way analysis of variance (ANOVA) by study population, common garden site, and their interaction, with plant size used as a covariate to control for differences associated with size, was used to identify significant factors which affected all my measured quantitative traits for each species. For all significant traits, Tukey-Kramer HSD tests were used to identify study populations which were most similar and those which differed significantly from each other. For

lab-based work at CBG, a one-way ANOVA by study population was used to identify significant differences in seed germination requirements for each species. Tukey-Kramer HSD tests were likewise used to identify study populations which differed significantly in days to germination. Euclidean distance in days to germination was calculated between each population pair and used to calculate unweighted pair-group clustering based on arithmetic averages (UPGMA) using PHYLIP (Felsenstein, 2004), to provide a graphical representation of quantitative genetic distance data and relationships within and among mountain ranges for each species.

For all common garden study results, the variance of measured traits was partitioned among multiple levels of organization via nested ANOVA using coefficients of expected mean squares. This methodology is modified from Venable and Burquez (1989) and Bonnin et al. (1996). A nested ANOVA model was used that split variance components for each measured trait into common garden site (σ^2_{CG}), mountain range (σ^2_{MTN}) and population nested within mountain range (σ^2_{BP}). As there was considerable difference in plant size among the two common gardens, plant size was added as a covariant ($cov_{(size)}$) to control for differences due to plant size. The remainder of variance was contained within populations arising among maternal lines of the same population (σ^2_{WP}). Total variance was calculated as the sum of all components ($\sigma^2_T = \sigma^2_{CG}+\sigma^2_{MTN}+\sigma^2_{BP}+ \sigma^2_{WP}+cov_{(size)}$). Each variance component was divided by total variance to calculate proportion of variance attributed to common garden sites ($\sigma^2_{CG}/ \sigma^2_T$), among mountain ranges ($\sigma^2_{MTN}/ \sigma^2_T$), among populations ($\sigma^2_{BP}/ \sigma^2_T$), within populations ($\sigma^2_{WP}/ \sigma^2_T$) and covariance with plant size ($cov_{(size)}/\sigma^2_T$). For floral shape traits, measures included within-plant replication, allowing us to partition variance at the within-plant level (σ^2_F).

To identify climatic measures as predictors of potentially adaptive quantitative trait variation, standardized trait measures were regressed on both climatic axes, PCA1 and PCA2. To identify relationships between geographic distance and PCA1 and PCA2 climatic distances, Mantel (1967) randomization tests were performed in GENEPOP (Raymond and Rousset, 1995). This test randomizes one matrix 10,000 times while keeping the other constant to determine the significance of each relationship. Results are presented as the number of randomizations resulting in a relationship as large as the tested relationship, e.g. if the observed regression was

larger than at least 95% of randomly generated regressions, the observed regression was considered statistically significant ($P < 0.05$).

Lab-based common garden studies at CBG provided detailed information on individual maternal line variation for days to germination, allowing the calculation of broad-sense heritability of days to germination for each population, following Lynch and Walsh (1998) and Edmands & Harrison (2003). One-way ANOVA was performed for each population, and σ^2_F (variance among maternal families of half-sibs) and σ^2_T (total variance) were obtained by equating observed mean squares (MS) with their expectations. Thus, $\sigma^2_F = (MS_F - MS_{ERROR})$/family size and $\sigma^2_T = (\sigma^2_{F+} MS_{ERROR})$ and heritability = $4\ \sigma^2_F / \sigma^2_T$. A value of 1 for this measure indicates no within-family variance for the trait, and therefore complete genetic control (note, however that potential maternal effects cannot be ruled out with this design). Additionally, Q_{ST} was calculated between each pair of populations by performing a nested ANOVA, with individuals nested within families within populations. Following Spitze (1993), the genetic variance distributed between populations (σ^2_{GB}) and the genetic variance within populations (σ^2_{GW}, which is actually 4 times the mean σ^2_F calculated as above) was used to calculate Q_{ST} as $\sigma^2_{GB}/(\sigma^2_{GB} + 2\sigma^2_{GW})$. For all tests, coefficients for expected mean square were adjusted for unequal sample sizes caused by different germination levels between families and populations. Studies partitioning genetic variation in quantitative traits within and among populations (Q_{ST}) and neutral genetic variation identified by molecular DNA markers (F_{ST}) have detected numerous traits under selection (Spitze, 1993). Effectively, traits not under selection are hypothesized to have $Q_{ST} = F_{ST}$, while those under divergent selection will show $Q_{ST} > F_{ST}$ and those under convergent selection $Q_{ST} < F_{ST}$.

3.3 RESULTS

The BIOCLIM data revealed considerable differences in climatic conditions among my study populations (Figure 3.2). Climatic data were well summarized by the first two principal component axes (Table IX), which collectively accounted for 96% of the variation among all 24 study populations and two common garden sites. The first PCA axis was strongly correlated with longitude ($r^2 = 0.50$, $P < 0.0001$) and largely reflected

precipitation differences among sites due to differences in elevation and increasing distance from the rainshadow effect of the Sierra Nevada Mountains (rainier high elevation sites on the eastern portion of the Great Basin had higher values for this axis). The second PCA axis was strongly correlated with elevation ($r^2 = 0.56$, $P < 0.0001$) and largely reflected temperature differences, temperature extremes and precipitation seasonality among sites (warm, lower elevation sites with greater precipitation seasonality had higher values for this axis). In combination, these axes summarize the differences associated with changing elevation within a mountain range. Higher elevation sites, which are generally cooler with more precipitation and fewer extremes and seasonality, have greater values on PCA1 and lower values on PCA2 (Figure 3.2). Populations on the same mountain range were often, but not always, the most similar to each other (Pr-CH and Pr-CL are the most extreme example on PCA1), and populations from the southeastern region were most similar to common garden climates. Along PCA1, BSU was in the mid-range of my collection sites, while UBC was to the far right, representing greater precipitation than at collection sites. Both common gardens represent the high end of the PCA2, with warm temperatures, temperature fluctuations and temperature and precipitation seasonality.

TABLE IX

RESULTS [a] OF PRINCIPAL COMPONENTS ANALYSIS ON BIOCLIMATIC VARIABLES

BIOCLIM Variable	PCA1	PCA2
Annual mean temperature (BIOCLIM1)	-0.015	0.119
Mean diurnal range (BIOCLIM2)	-0.052	-0.065
Isothermality (BIOCLIM3)	-0.044	-0.033
Temperature seasonality (BIOCLIM4)	0.034	0.027
Max temperature of warmest week (BIOCLIM5)	-0.025	0.102
Min temperature of coldest week (BIOCLIM6)	-0.017	0.132
Temperature annual range (BIOCLIM7)	-0.019	-0.073
Mean temperature wettest quarter (BIOCLIM8)	-0.040	-0.019
Mean temperature driest quarter (BIOCLIM9)	-0.001	0.103
Mean temperature warmest quarter (BIOCLIM10)	-0.008	0.110
Mean temperature coldest quarter (BIOCLIM11)	-0.025	0.120
Annual precipitation (BIOCLIM12)	0.087	-0.015
Precipitation wettest week (BIOCLIM13)	0.918	0.334
Precipitation driest week (BIOCLIM14)	0.343	-0.873
Precipitation seasonality (BIOCLIM15)	-0.001	0.135
Precipitation wettest quarter (BIOCLIM16)	0.086	0.034
Precipitation driest quarter (BIOCLIM17)	0.069	-0.088
Precipitation warmest quarter (BIOCLIM18)	0.066	-0.067
Precipitation coldest quarter (BIOCLIM19)	0.074	0.065
Proportion of variance explained	**0.717**	**0.960[b]**

[a] Values shown are loadings of each variable on each of the first two axes.

[b] The first two axes accounted for 96% of the variance in the data.

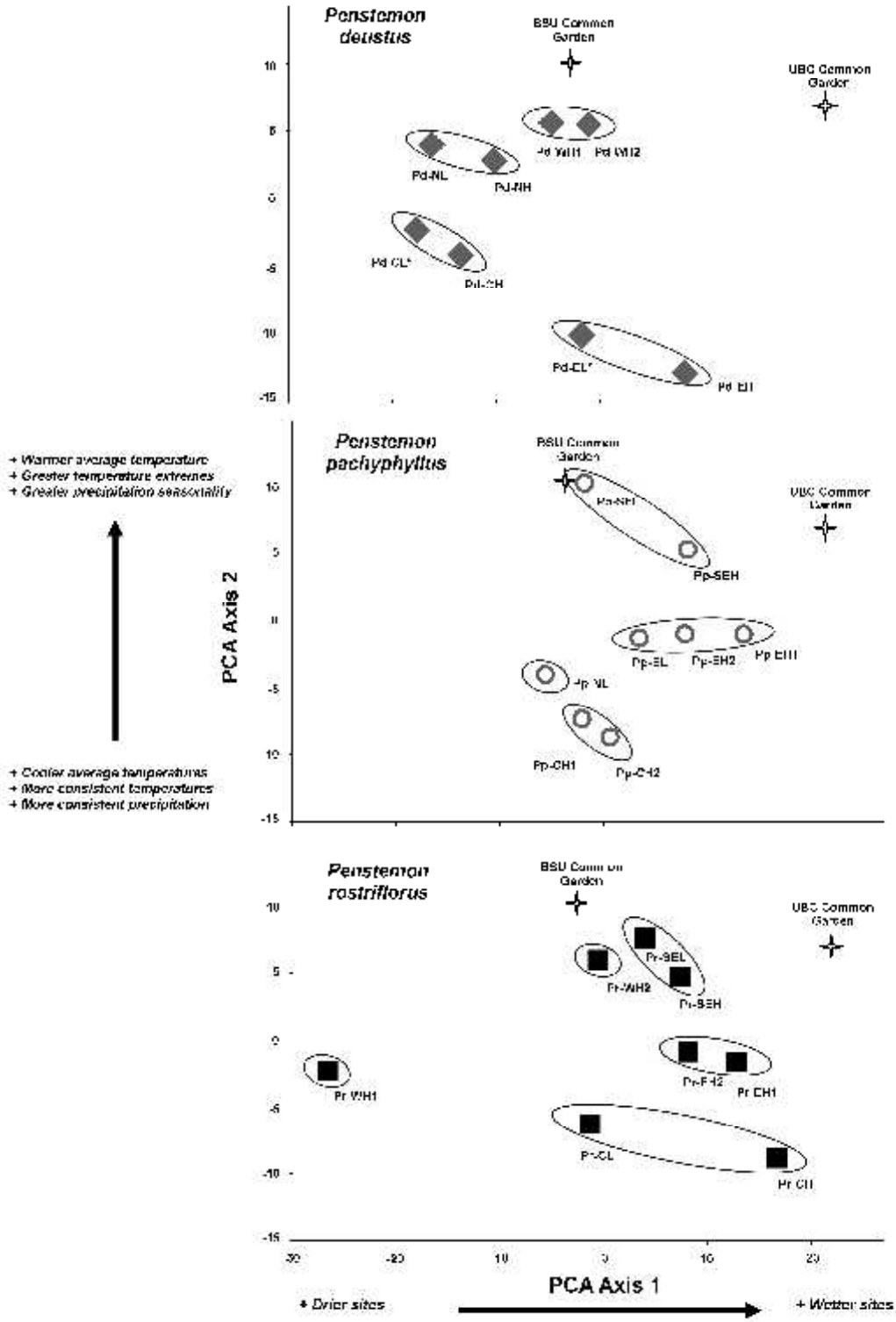

Figure 3.2: Climatic differences at collection and common garden sites, graphed by the results of a principle component analysis on 19 BIOCLIM parameters. Each site is represented by a grey diamond (*P. deustus*), open circle (*P. pachyphyllus*), black square (*P. rostriflorus*), or white cross (common garden). Circles depict populations located on the same mountain range. *Denotes two populations of *P. deustus* that were used in Chapter 2 but not in this common garden study.

Mantel tests of geographic and climatic (PCA1 and PCA2) distances revealed a range of relationships between study populations for each species. Geographically close populations were not always climatically close (e.g. Pr-CH is geographically closest to Pr-CL, but climatically closest to a population on another mountain range; Pr-EH1). This was the intent of the original population sampling design, and was true for all tests between geographic distance and PCA1, and in *P. rostriflorus* was also true for PCA2. However, geographic distance between population pairs significantly predicted climatic distances in PCA2 for *P. deustus* (P = 0.0278) and for *P. pachyphyllus* (P = 0.0032), which means that any quantitative divergence that is correlated to PCA2 may be either adaptive (natural selection for increasingly dissimilar climatic values) or random (assuming geographically distant populations are increasingly dissimilar due to genetic drift). To distinguish between the effects of these two processes on divergence, it is therefore useful to include results from neutral genetic markers, as reported in Chapter 2, which demonstrated very different patterns of gene flow within and between populations and mountain ranges in these study species.

3.3.1 Common garden survival

There was a significant effect of species (χ^2= 50.12, P = 0.0000), common garden (χ^2 = 11.33, P = 0.0008), and their interaction (χ^2 = 16.68, P = 0.0002), on plant survival over 4 years of my field-based common garden study. Percent survival by species, common garden and study population is summarized in Table X.

TABLE X
SUMMARY OF COMMON GARDEN PLANTING AND SURVIVORSHIP [a]

	Collection Site	Boise State University planted	Boise State University Survival	Utah Botanical Center planted	Utah Botanical Center Survival
Penstemon deustus	Pd-CH	32	63%	34	29%
	Pd-EH	33	73%	29	48%
	Pd-NH	30	83%	31	58%
	Pd-NL	31	81%	33	58%
	Pd-WH1	35	80%	34	50%
	Pd-WH2	27	74%	30	53%
	TOTAL	*188*	*76%*	*191*	*49%*
Penstemon pachyphyllus	Pp-CH1	24	4%	30	27%
	Pp-CH2	15	7%	27	26%
	Pp-NL	25	16%	30	10%
	Pp-EH1	5	40%	12	33%
	Pp-EH2	5	40%	10	50%
	Pp-EL[b]	0	-	3	67%
	Pp-SEH	32	72%	32	78%
	Pp-SEL	33	76%	32	69%
	TOTAL	*139*	42%	*176*	43%
Penstemon rostriflorus	Pr-CH	17	71%	27	67%
	Pr-CL[b]	3	0%	4	100%
	Pr-EH1	21	67%	23	43%
	Pr-EH2	29	62%	32	63%
	Pr-SEH	32	84%	32	84%
	Pr-SEL	31	94%	32	84%
	Pr-WH1	31	68%	32	63%
	Pr-WH2	32	41%	34	44%
	TOTAL	*196*	*68%*	*216*	*65%*
	TOTAL	523		583	

[a] Survivorship from spring 2004 to fall 2006 at each common garden site.
[b] Pp-EL and Pr-CL excluded from all common garden analyses.

Only *P. deustus* showed a significant difference in survival by common garden site (greater survival at BSU; $\chi^2 = 28.62$, P = 0.0000; see Table X). Survival was generally higher at BSU than UBC, likely due to the greater natural rainfall and higher water-retention of the clay soils at UBC, which caused many plants to rot by the end of the study. All three species exhibited significant population-level difference in survival at each common garden, some of which was explained by the climatic distance between study population and common garden site (geographic distance did not significantly predict percent survival for any study species). Survival of *P. deustus* was significantly different at each common garden site, with significantly different survival by population ($\chi^2 = 11.47$, P = 0.0427). However, there was no significant interaction between common garden and study population.

Eastern and central populations of *P. deustus* generally had lower survival at both common garden sites, and percent survival of study populations was significantly predicted by climatic distance between the study population and common garden site (plants from sites most climatically similar to common garden climates had higher percent survival there (r^2=0.61, F=15.6, P < 0.0027). In *P. pachyphyllus* survival was significantly different by population (χ^2 = 114.02, P = 0.000), Eastern and Central populations generally had lower survival at both common garden sites, and percent survival of study populations was significantly predicted by climatic distance between each study population and common garden site (r^2=0.195, F=4.9, P < 0.0466). *P. rostriflorus* survival was high at both common garden sites, but there was nonetheless a significant difference in survival by population (χ^2 = 47.14, P = 0.000). However, percent survival was not significantly predicted by climatic or geographic distance between study population and common garden site (r^2=0.025, F=0.30, P < 0.5875).

3.3.2 Differences in quantitative traits by common garden, species and study population

The different growing conditions at each common garden produced highly significant differences in plant size and consequently there was a significant difference between common garden sites in most traits measured for all three species. A pairwise comparison found significant correlation for plant size (volume) and many of the measured traits (Table XI), identifying many highly significant (p < 0.001) correlations between plant size and growth and vegetation structure characters. Exceptions included leaf dentation, winter growth and number of fruit (*P. deustus*) and peduncle size (*P. pachyphyllus*), suggesting these traits are not size-dependent. Fruit number showed a highly significant correlation with plant size in *P. rostriflorus*, which is expected as fruit number is at least in part a resource-dependent character. Phenological measurements that were also explained by plant size (percent flowering in *P. deustus* an *P. rostriflorus* and percent flowers finished in *P. rostriflorus*) may be a reflection of plant resources, as well (healthier plants can flower more, longer), or more indirectly due to differences in environmental cues related to plant growth at each common garden (BSU has a milder winter than UBC). Floral measures showed the weakest correlation to plant size. A weak but significant correlation was found between plant size and all *P. deustus* floral characters, only corolla length for *P. rostriflorus*, and no correlation between floral shape and plant size was found in *P. pachyphyllus*.

TABLE XI

PAIRWISE CORRELATION AND SIGNIFICANCE OF QUANTITATIVE TRAITS AND PLANT SIZE

		Penstemon deustus		*Penstemon pachyphyllus*		*Penstemon rostriflorus*	
		r^2	P value	r^2	P value	r^2	P value
Growth	Total	0.57	***	0.02	*	0.50	***
	Winter	-0.06	ns	-0.46	***	0.43	***
	Summer	0.65	***	0.87	***	0.45	***
Vegetation structure	Leaf area	0.19	**	0.77	***	0.30	***
	Stem length	0.26	***	0.18	*	0.19	***
	Flower height	0.28	***	0.22	***	0.10	*
	Internode	0.22	***	0.21	*	0.19	***
	Stem diameter	0.26	***	0.22	**	0.00	ns
	Dentation	-0.04	ns	-	-	-	-
	Peduncle	-	-	0.14	ns	-	-
Flower/Fruit Phenology	% flowers finished	-	-	-	-	-0.18	***
	Number fruit	-0.04	ns	0.13	ns	0.20	***
	Percent flowering	-0.13	*	0.02	ns	0.41	***
Floral Shape	Corolla length	0.07	*	0.00	ns	0.16	**
	Flower opening	0.02	*	0.03	ns	0.00	ns
	Anther exsertion	0.11	*	0.01	ns	-0.01	ns

$^{*}p < 0.05$
$^{**}p < 0.01$
$^{***}p < 0.0001$

Many measured quantitative traits showed significant differences ($P < 0.0001$) by common garden and population for each species (Table XII, XIII), and many traits that differed significantly by common garden were strongly correlated to plant size. In *P. deustus* there was variation by study population, predominately in summer growth, with Pd-NL and Pd-WH1 showing considerably more growth in the summer than other populations. For all other measurements, *P. deustus* showed significant among-population differences, no interaction between common garden and study population, and a weak or insignificant correlation to plant size. The largest difference between study populations for most measurements was in Pd-NL, which was generally larger and faster growing

than all other populations, including Pd-NH, located on the same mountain range that was generally the smallest and slowest growing population.

In *P. pachyphyllus,* I identified a significant difference in total and winter growth by study population and an interaction between common garden and population for summer growth. This interaction was attributable to both Pp-SEL and Pp-SEH producing significantly less summer growth at UBC than at BSU. All vegetation structure measures showed significant variation by study population, while leaf area, stem height, flower height, stem diameter and peduncle size all showed a significant correlation to plant size. Internode length was the only measurement which showed no variation by common garden or interaction between common garden and study population. In *P. pachyphyllus* there was a significant interaction between common garden and study population in leaf area, where Pp-EH1 and Pp-EH2 produced comparatively larger leaves at UBC than at BSU, and in stem diameter and flower height, where Pp-SEH, Pp-SEL and Pp-NL produced thinner and smaller flower stems at UBC compared with BSU. Percent flowering and all floral shape traits showed significant variation by study population, with no correlation to plant size, or significant interaction between study population and common garden location. There was, however, a significant difference in floral shape traits by common garden location.

In *P. rostriflorus* a large proportion of the variance in growth was explained by common garden and plant size, although there was a significant difference in total growth by study population, and weak interaction for total, winter and summer growth. This interaction was a result of both Pr-CH and Pr-SEH performing comparatively better at BSU than at UBC. *P. rostriflorus* showed a similar breakdown as *P. pachyphyllus* for vegetation structure measurements, with all populations showing significant variation by study population. Leaf area, stem height, flower height and stem diameter, as well as number of fruit, significantly correlated to plant size. Vegetative and floral stem length also showed a significant difference by common garden and, as was the case with *P. pachyphyllus*, leaf area and flower height showed a significant interaction between common garden and study population. This interaction was attributable to both Eastern populations (Pr-EH1 and EH2) and both Western populations (Pr-WH1 and WH2) producing larger leaves and taller flower stems at UBC. Phenological

measurements, including percent flowering and percent flowers finished, showed a significant difference by study population and common garden, suggesting both an environmental and genetic cue to flowering. There was also a significant interaction in percent flowers finished and a weak correlation to plant size; this was predominantly due to Pr-WH2 which was the latest flowering population studied (Table XIII). Floral traits showed a significant common garden and study population interaction. For anther exsertion, which is the only floral trait to show no correlation to plant size, the interaction was attributed to both Eastern populations (Pr-EH1 and EH2) producing shorter exsertion at UBC. The interaction for both corolla length and flower opening, along with correlation with plant size, was attributable to Pr-WH2, which produced much longer corollas and wider floral openings at UBC.

TABLE XII

SIGNIFICANCE OF MAIN, INTERACTION AND COVARIANCE EFFECTS ON QUANTITATIVE TRAITS

		Penstemon deustus				***Penstemon pachyphyllus***				***Penstemon rostriflorus***			
		CG [a]	Pop[b]	CGxPop[c]	Size[d] (COV)	CG [a]	Pop[b]	CGxPop[c]	Size[d] (COV)	CG [a]	Pop[b]	CGxPop[c]	Size[d] (COV)
Growth	Total	<0.0001	<0.0001	ns	<0.0001	<0.0001	0.003	ns	<0.0001	<0.0001	<0.0001	0.02	<0.0001
	Winter	<0.0001	ns	ns	<0.0001	0.0005	0.0005	ns	<0.0001	<0.0001	ns	0.03	<0.0001
	Summer	<0.0001	<0.0001	ns	<0.0001	0.0002	ns	<0.0001	<0.0001	<0.0001	ns	0.02	<0.0001
Vegetation structure	Leaf area	<0.0001	0.0004	ns	ns	<0.0001	0.007	0.008	<0.0001	ns	<0.0001	0.006	0.003
	Stem length	0.0007	<0.0001	ns	0.01	0.01	0.01	ns	0.003	0.005	<0.0001	ns	<0.0001
	Flower height	<0.0001	0.04	ns	0.0002	ns	0.0002	0.01	0.002	0.02	<0.0001	0.02	0.006
	Internode	0.0003	<0.0001	ns	ns	ns	0.05	ns	ns	ns	0.01	ns	ns
	Stem diameter	<0.0001	0.02	ns	ns	ns	0.001	0.04	0.002	ns	<0.0001	ns	0.008
	Dentation	0.003	<0.0001	ns	ns	-	-	-		-	-	-	
	Peduncle	-	-	-		ns	<0.0001	ns	0.02	-	-	-	
Flower/Fruit Phenology	% flowering	ns	<0.0001	ns	ns	ns	<0.0001	ns	ns	<0.0001	0.0003	ns	ns
	Number fruit	0.006	0.0003	ns	ns	ns	ns	ns	ns	ns	<0.0001	ns	0.004
	% flowers finished	-	-	-		-	-	-	-	<0.0001	<0.0001	0.001	0.01
Floral Shape	Corolla length	ns	<0.0001	ns	ns	0.02	<0.0001	ns	ns	-	<0.0001	<0.0001	0.009
	Flower opening	0.0004	<0.0001	ns	ns	0.006	<0.0001	ns	ns	-	<0.0001	0.007	0.02
	Anther exsertion	ns	<0.0001	ns	ns	0.006	<0.0001	ns	ns	-	<0.0001	<0.0001	ns

[a] CG = Common garden effect.
[b] Pop = Original collection population effect.
[c] CGxPop = Interaction effect between common garden and original collection location.
[d] Size (COV) = Covariance by plant size.

TABLE XIII

MEAN VALUES BY POPULATION AND SPECIES FOR MEASURED TRAITS

	Growth			Vegetation Structure						Flower/fruit phenology		Floral shape			Seeds
Population	Total	Winter	Summer	Leaf area	Stem length	Flower height	Internode	Stem Dia.	Add'l measure[b]	Percent flower	Number fruit	Corolla length	Flower opening	Anther exsertion	Days to germ.
Penstemon deustus															
Pd-CH	10.2 bc	4.02 a	0.22 c	1.3 bc	23.2 bc	8.6 ab	1.6 abc	1.6 ab	2.9 c	9.6 ab	36.4 a	10.3 a	3.75 ab	1.65 bc	90.4 a
Pd-EH	15.2 bc	4.40 a	0.31 c	1.6 ab	22.9 bc	9.8 a	1.5 bc	1.5 ab	2.9 c	12.0 a	36.7 a	9.8 b	3.87 a	1.73 ab	87.1 a
Pd-NH	11.1 bc	3.62 a	0.39 bc	1.2 c	20.9 c	8.4 ab	1.4 c	1.5 b	3.7 bc	2.7 cd	24.9 b	10.2 a	3.52 b	1.45 cd	92.3 a
Pd-NL	27.7 a	3.71 a	1.39 a	1.7 a	31.4 a	10.3 a	1.9 a	1.7 ab	6.2 a	0.3 d	33.8 ab	10.2 ab	3.95 a	1.88 a	62.3 c
Pd-WH1	17.6 b	6.42 a	0.82 ab	1.4 abc	25.5 b	9.1 ab	1.7 ab	1.7 a	5.3 ab	4.3 bc	41.9 a	8.7 c	3.60 b	1.46 d	59.9 c
Pd-WH2	9.20 c	3.45 a	0.34 c	1.6 abc	20.9 c	7.4 b	1.5 bc	1.6 ab	5.8 a	5.7 abc	37.0 a	9.0 c	3.58 b	1.45 cd	70.2 b
Penstemon pachyphyllus															
Pp-SEH	0.7 ab	0.11 bc	0.60 a	11.2 a	51.2 ab	21.9 ab	2.9 a	4.7 b	0.7 c	0.2 c	35.7 a	15.1 b	6.65 c	1.81 bc	70.4 c
Pp-SEL	0.5 b	0.001 c	0.47 abc	9.3 a	59.6 a	26.1 a	3.1 a	5.5 ab	0.4 c	0.1 c	37.2 a	14.3 c	6.75 bc	2.36 a	55.1 d
Pp-EH1	0.6 ab	-0.04 bc	0.61 ab	2.2 abc	47.0 ab	18.7 ab	3.3 a	6.0 ab	0.6 bc	6.3 b	42.3 a	15.2 bc	7.66 a	1.93 abc	79.8 bc
Pp-EH2	0.8 ab	0.42 abc	0.41 abc	2.5 a	60.0 a	23.7 ab	3.2 a	6.7 a	0.6 bc	5.4 b	54.8 a	15.0 bc	7.46 ab	2.36 ab	97.9 ab
Pp-CH1	1.0 a	0.47 a	0.48 abc	2.1 ab	52.0 ab	20.8 ab	3.4 a	5.4 ab	1.3 ab	13.4 ab	43.4 a	16.2 a	7.91 a	1.94 bc	89.6 ab
Pp-CH2	0.6 ab	0.28 abc	0.32 c	1.6 c	48.8 ab	17.3 b	3.5 a	5.2 ab	1.6 a	22.7 a	37.3 a	15.9 ab	7.25 abc	1.58 c	85.6 b
Pp-NL	0.6 ab	0.31 ab	0.36 bc	1.7 bc	45.0 b	17.8 b	2.8 a	4.9 b	1.2 ab	11.8 ab	36.2 a	15.5 ab	7.51 a	1.98 abc	99.9 a
Penstemon rostriflorus															
Pr-SEH	127.4 a	0.01 a	4.41 a	2.8 a	69.6 ab	39.4 a	3.9 ab	3.3 a	41.7 c	11.4 a	22.3 a	18.7 cd	4.07 bc	7.20 d	59.7 e
Pr-SEL	89.3 abc	0.01 a	5.79 a	2.8 a	58.6 cd	28.2 cd	4.1 ab	3.1 ab	71.0 ab	6.7 abc	21.1 ab	19.7 b	4.05 c	7.51 cd	63.2 e
Pr-EH1	57.4 c	0.01 a	3.9 a	1.2 bc	60.2 bc	32.5 bc	4.5 a	2.7 c	71.3 ab	5.0 bc	10.4 c	18.7 cd	3.99 cd	7.97 abc	92.8 bc
Pr-EH2	93.0 abc	0.01 a	4.3 a	1.3 b	71.0 ab	33.7 ab	4.2 ab	2.6 c	74.4 ab	4.1 c	11.6 c	18.4 d	3.67 d	7.97 abc	89.9 c
Pr-CH	65.3 bc	0.01 a	4.08 a	1.3 b	49.2 d	27.0 d	4.0 ab	2.6 c	82.7 a	4.1 bc	10.8c	19.3 bc	4.23 abc	7.81 bcd	108.2 a
Pr-CL[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.0 ab
Pr-WH1	103.2 ab	0.01 a	3.08 a	1.3 b	74.4 a	38.5 a	4.2 ab	2.9 bc	54.3 bc	8.1 abc	10.0 bc	19.1 bcd	4.44 a	8.64 a	81.6 d
Pr-WH2	77.5 bc	0.01 a	3.59 a	1.0 c	66.4 abc	34.3 ab	3.7 b	2.6 c	26.0 d	9.1 ab	7.1 c	20.8 a	4.42 ab	8.35 ab	78.1 d

[a] Too few plants in field-based common gardens; excluded from all but lab-based common garden analyses.

[b] Dentation (*P. deustus*), Peduncle (*P. pachyphyllus*), %Flowers finished (*P. rostriflorus*).

TABLE XIV

VARIATION IN TRAITS PARTITIONED AT MULTIPLE LEVELS (EXPRESSED AS A PERCENTAGE)

	Growth			**Vegetation Structure**						**Flower/fruit phenology**		**Floral shape**			**Seeds**	**Genetic diversity**
Level of partitioning	Total	Winter	Summer	Leaf area	Stem length	Flower height	Internode	Stem Diameter	Add'l measure[b]	Percent flower	Number fruit	Corolla length	Flower opening	Anther exsertion	Days to germinate	Neutral markers
Penstemon deustus																
within plants[a]												1	5	2		
within populations	1	0	1	3	2	5	5	2	3	6	7	**3** ***	**15** ***	**4** ***	1	**87*****
among populations	**7** **	1	**6** **	**28** **	**69** ***	**21** *	**51** ***	**7** *	**36** ***	**14** *	**38** *	**7** *	**51** **	**23** ***	**52*****	**9*****
among mountains	1	0	**4** *	5	**7** *	8	3	3	**38** ***	**75** ***	**32** **	**89** ***	**25** *	**23** ***	**47*****	**4*****
between gardens	**71** ***	**92** ***	**59** ***	**63** ***	**12** *	**54** **	9	**81** ***	**14** *	5	23	0	3	**45** ***		
plant size (COV)	**20** ***	**6** *	**30** ***	1	8	11	33	6	9	0	0	1	2	4		
Penstemon pachyphyllus																
within plants[a]												2	3	5		
within populations	**2** *	4	0	1	12	12	17	12	6	**3** **	22	**10** ***	**15** ***	**17** ***	1	**77*****
among populations	3	**12** *	0	1	32	**39** **	14	42	**14** *	**4** *	17	**22** *	4	**49** **	**14*****	**9*****
among mountains	**10** **	**17** *	1	**4** *	13	**32** *	39	31	**79** ***	**88** ***	40	**46** ***	**55** ***	**28** *	**85*****	**15*****
between gardens	**52** ***	**64** ***	**10** ***	**34** ***	19	6	8	8	0	0	11	19	14	5		
plant size (COV)	**33** ***	3	**88** ***	**60** ***	25	12	21	8	2	**5** *	11	4	12	1		
Penstemon rostriflorus																
within plants[a]												6	2	0		
within populations	0	1	0	1	2	2	3	4	2	1	4	**10** ***	**17** *	**12** ***	1	**93*****
among populations	**2** *	1	1	0	**15** **	**39** ***	2	6	**11** **	2	5	**30** *	19	9	**3***	**3*****
among mountains	**1** *	1	1	**46** ***	**25** ***	**32** ***	**7** *	**51** ***	**34** ***	**3** **	**63** ***	**56** ***	**51** *	**74** ***	**96*****	**4*****
between gardens	**79** ***	**84** ***	**83** ***	**53** ***	**54** ***	**24** ***	**88** ***	**22** *	**51** ***	**95****	11	2	5	2		
plant size (COV)	**17** ***	**13** ***	**15** ***	0	4	2	0	**18** *	3	0	**18***	1	7	3		

[a] Partitioning only possible for floral shape traits.

[b] Dentation (*P. deustus*), Peduncle (*P. pachyphyllus*), %Flowers finished (*P. rostriflorus*).

* $p < 0.05$

** $p < 0.01$

*** $p < 0.0001$

3.3.3 Partitioning quantitative traits

The nested ANOVAs to partition variance in quantitative traits among common garden sites, mountain ranges, and study populations were significant ($p < 0.0001$) for all traits and species (Table XIV), with the exception of four traits in *P. pachyphyllus* (stem length, internode, stem diameter and number of fruit produced). The proportion of variation explained by common garden is an indication of traits with a strong environmental component or plasticity, while there is a strong genetic basis for variation attributed to mountain range and population. For all three species, common garden accounted for the largest and most significant proportion of the variation measured in growth traits, which was also significantly correlated to plant size (Table XII). However, it is noteworthy that most remaining variation was significantly partitioned by study population in *P. deustus*, and by mountain range in *P. pachyphyllus*.

For all vegetative structure measurements in *P. deustus*, study population and common garden accounted for a significant amount of the variation seen for all characters, with the exception on internode length which showed no variation by common garden. Common garden accounted for the largest variation in leaf area, flower height and stem diameter, study population accounted for greatest variation in stem length and internode, and mountain range account for the most variation in dentation. In *P. pachyphyllus*, only leaf area, flower height and peduncle length showed any significant response, with most of the variation accounted for by common garden (and plant size), study population, and mountain range respectively. In *P. rostriflorus,* all characters had a significant response by common garden and mountain range, with only stem length and flower height showing a significant study population response.

Much of the variation in flowering phenology was partitioned by mountain range in all three species. However, flowering phenology in *P. rostriflorus* showed more plasticity between common garden sites than the other two species. All measured floral traits, with the exception of anther exsertion in *P. deustus*, showed very little variation associated with common garden or plant size. Most of the variation in these traits was partitioned by mountain range, followed by among population and finally within population. Partitioning of variation in seed

germination requirements was significant in all three species, with different degrees of partitioning among populations and mountain ranges by species. Variance in *P. deustus* was nearly equally partitioned between mountain ranges and populations (52% versus 43%, respectively), while much more variance was partitioned by mountain range (85%) than population (15%) in *P. pachyphyllus*. Nearly all variance in *P. rostriflorus* was partitioned by mountain range (96%).

UPGMA results on days to germination (Figure 3.3), one of the most striking and significant traits measured here, provide additional details on quantitative divergence between populations and mountain ranges. Where the UPGMA using Nei's unbiased genetic distance showed divergence in neutral traits that was very different between species (*P. pachyphyllus* much greater than *P. deustus*, with *P. rostriflorus* lowest), UPGMA of days to germination showed generally equivalent divergence between populations in all three species. In fact, *P. rostriflorus* had slightly greater branch lengths than *P. deustus*, providing additional evidence for adaptive population divergence in the presence of gene flow for this hummingbird pollinated species. This comparison also identifies populations on different mountain ranges that have similar (convergent) seed germination requirements (e.g. particularly for *P. deustus*, where all high elevation populations grouped together and separately from warmer or low elevation populations) despite the fact that differences in neutral genetic diversity group them much more uniformly by mountain range.

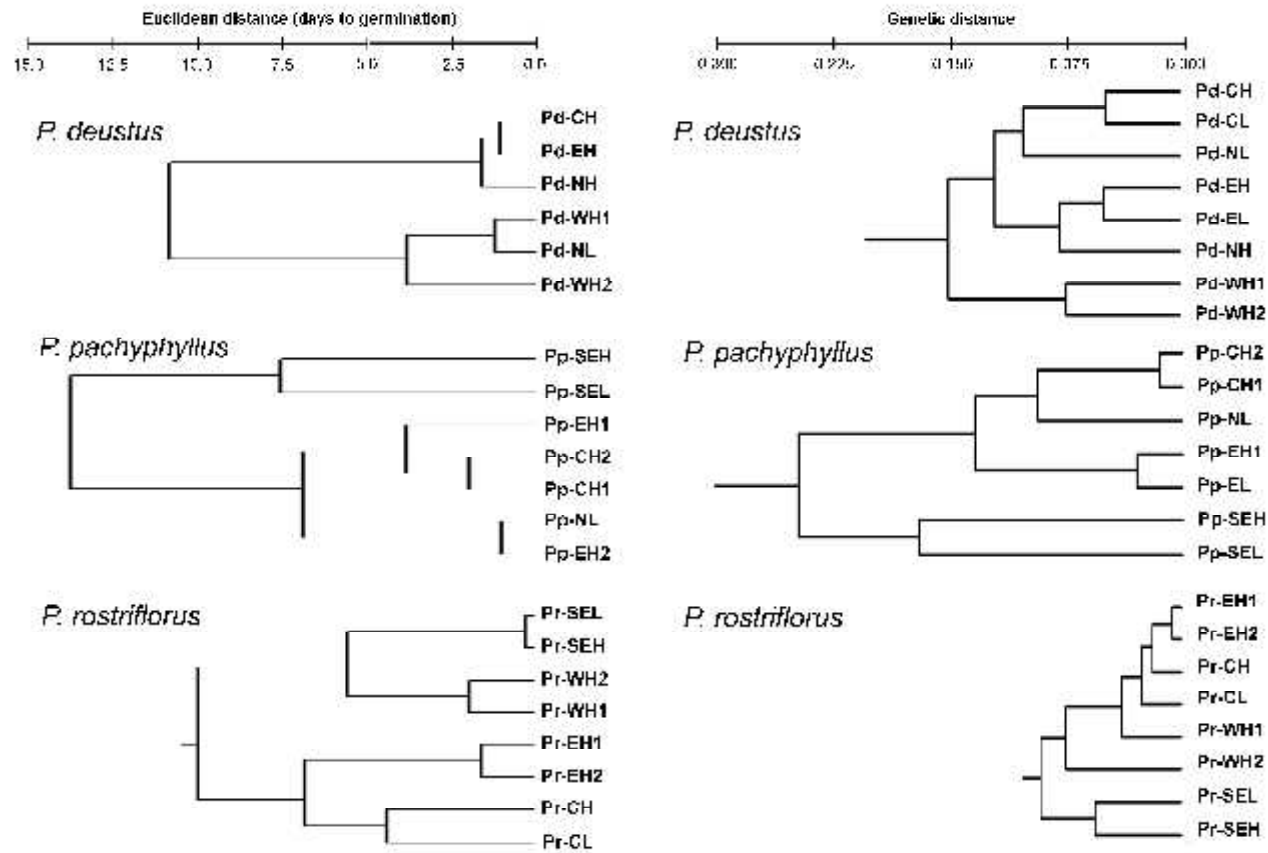

Figure 3.3: UPGMA clustering for each species using Euclidean distance in days to germination (left) and Nei's unbiased genetic distance (right).

3.3.4 Climatic measures as predictors of measured traits

Many significant relationships between climatic and quantitative measures were identified (Table XV), consistent with a hypothesis that natural selection driven by climatic differences has an important role in shaping the quantitative variation in these *Penstemon* species, but to varying degrees depending on trait and species. PCA1, dominated by precipitation, explained less variation in measured traits for each species than did PCA2, dominated by temperature and precipitation extremes. In *P. deustus*, PCA2 provided the most significant tests, including on dentation (14%) and days to germination (13%). In *P. pachyphyllus*, PCA2 again significantly explained 23% of variation in peduncle length, 11% of corolla length, 11% of mouth diameter, 42% of percent flowering and 21% of days to germination. In *P. rostriflorus* slightly less variation was explained by PCA2, but percent flowers finished (7%), leaf area (11%), and days to germination (22%) were all highly significant.

TABLE XV
REGRESSION OF QUANTITATIVE MEASURES ON CLIMATIC PREDICTOR VARIABLES

		Penstemon deustus				***Penstemon pachyphyllus***				***Penstemon rostriflorus***			
		PCA 1		PCA 2		PCA 1		PCA 2		PCA 1		PCA 2	
	Trait	P value	r^2	P value	r^2	P value	r^2	P value	r^2	P value	r^2	P value	r^2
Growth	Total	0.0202	*0.02*	ns	-	ns	-	0.0185	*0.02*	0.0169	*0.02*	0.0105	*0.02*
	Winter	ns	-	ns	-	0.0426	*0.01*	<0.0001	*0.09*	ns	-	ns	-
	Summer	0.0019	*0.03*	<0.0001	*0.05*	<0.0001	*0.06*	0.0103	*0.02*	ns	-	ns	-
Vegetation structure	Leaf Area	ns	-	ns	-	<0.0001	*0.06*	<0.0001	*0.07*	0.0289	*0.01*	<0.0001	*0.11*
	Stem length	<0.0001	*0.06*	0.0341	*0.02*	ns	-	0.0278	*0.03*	<0.0001	*0.08*	ns	-
	Flower ht.	ns	-	ns	-	ns	-	<0.0001	*0.08*	<0.0001	*0.10*	ns	-
	Internode	0.0039	*0.03*	0.045	*0.01*	ns	-	ns	-	ns	-	ns	-
	Stem Dia.	ns	-	0.0132	*0.02*	ns	-	ns	-	ns	-	<0.0001	*0.05*
	Dentation	0.0206	*0.02*	<0.0001	*0.14*	-	-	-	-	-	-	-	-
	Peduncle	-	-	-	-	0.0043	*0.04*	<0.0001	*0.23*	-	-	-	-
Flower/Fruit Phenology	% flowers finished	-	-	-	-	-	-	-	-	0.0005	*0.03*	<0.0001	*0.07*
	Percent flower	<0.0001	*0.10*	<0.0001	*0.10*	0.0019	*0.05*	<0.0001	*0.42*	0.0254	*0.01*	0.0021	*0.03*
	Number fruit	ns	-	ns	-	ns	-	ns	-	ns	-	0.0002	*0.04*
Floral Shape	Corolla length	<0.0001	*0.03*	<0.0001	*0.08*	ns	-	<0.0001	*0.11*	ns	-	<0.0001	*0.04*
	Mouth diameter	ns	-	<0.0001	*0.02*	0.0224	*0.01*	<0.0001	*0.11*	<0.0001	*0.03*	ns	-
	Anther exsertion	ns	-	<0.0001	*0.03*	ns	-	0.0004	*0.03*	<0.0001	*0.04*	0.0225	*0.01*
Seeds	Days to germ.	0.0093	*0.01*	<0.0001	*0.13*	ns	-	<0.0001	*0.21*	ns	-	<0.0001	*0.22*

3.3.5 Heritability and Q_{ST} in seed germination requirements

Each species had differing heritability for seed germination requirements among populations. In *P. pachyphyllus,* heritability was generally low among all three populations, at 0.118 $\pm$ 0.094. Generally low values of Q_{ST} were also found in this species; pairwise Q_{ST} values are presented in Table XVI along with equivalent F_{ST} values determined with microsatellite markers and explained in Chapter 2 for comparison. All populations of *P. rostriflorus* had higher heritability than *P. pachyphyllus* populations, averaging 0.510 $\pm$ 0.081 (Table XVI), and Q_{ST} values in this species were both higher and lower than those found for *P. pachyphyllus,* ranging from -0.003 to 0.6095.

TABLE XVI

HERITABILITY AND PAIRWISE COMPARISONS OF Q_{ST} FOR SEED GERMINATION REQUIREMENTS (BELOW) AND F_{ST} FOR MICROSATELLITES (ABOVE) FOR EACH SPECIES AND POPULATION

Penstemon pachyphyllus				
Heritability	Population	Pp-SEH	Pp-CH2	Pp-NL
0.104	Pp-SEH	-	0.3521	0.2937
0.227	Pp-CH2	0.1209	-	0.1453
0.024	Pp-NL	0.2399	0.0323	-
Avg =0.118 ± 0.094				

Penstemon rostriflorus					
Heritability	Population	Pr-SEL	Pr-CH	Pr-CL	Pr-WH2
0.592	Pr-SEL	-	0.0982	0.0984	0.1901
0.535	Pr-CH	0.5517	-	0.0358	0.0899
0.679	Pr-CL	0.2345	0.1068	-	0.0941
0.236	Pr-WH2	-0.003	0.6095	0.2856	-
Avg = 0.510 ± 0.081					

3.4 DISCUSSION

3.4.1 Quantitative divergence and climate

My common garden experiment revealed significant, genetically-based quantitative divergence among populations of *Penstemon deustus, P. pachyphyllus,* and *P. rostriflorus* occupying the environmentally heterogeneous terrain of the Great Basin. Differences in every trait measured in common environments, including growth, vegetative and floral structure and flowering phenology traits, were significantly explained by provenance (Table XII). Study populations for each species occupied a similar range of climatic extremes throughout the region, and much of the divergence in quantitative traits revealed by this study is associated with climatic differences within and among the sky islands of the Great Basin (Table XV). Taken together, my results provide strong evidence for adaptive population divergence driven by natural selection in these widely distributed *Penstemon* species.

Seed germination requirements provided some of the most striking and consistent patterns of divergence among populations for all three study species. This divergence was significantly explained by climatic differences (Table XV). Seeds from colder, high elevation sites required more days in cold stratification before germinating; seeds appear to be timing germination to maximize the likelihood of encountering favorable growing

conditions (spring snowmelt). This trend was found for all three species, and is supported by previous work with *Penstemon* in western North America (Meyer and Kitchen, 1994; Meyer, Kitchen, and Carlson, 1995). Given the population divergence in seed germination requirements for all three species, is it important to consider my results on the heritability of this trait, as natural selection can only drive adaptive divergence if a trait is heritable. Here, I demonstrate that seed germination timing is indeed heritable in *P. rostriflorus* and, to a lesser extent, *P. pachyphyllus* (Table XVI).

Significant population divergence explained by climatic differences was also identified in flowering phenology traits for all three species. This relationship was strongest in *P. pachyphyllus,* where 42% of the variation in flowering phenology was explained by average temperature, temperature extremes and precipitation seasonality (dominant variable loadings on PCA axis 2) at study sites (Table VIII). In general, plants from colder, higher-elevation sites with more consistent temperature and precipitation flowered earlier in warm common garden settings than those from warm sites. This occurred despite the fact that, in their natural environments, populations from warmer low-elevation sites flowered earlier than those at high elevations (A. Kramer, pers. obs.). Many potential mechanisms may trigger flowering in plants, ranging from reaching a critical size threshold or developmental stage to being exposed to some threshold of hot or cold temperatures, amount of precipitation, or even day length (Domínguez and Dirzo, 1995; Diekmann, 1996; Fenner, 1998). In many *Clarkia* species, populations from low elevations tend to have faster growth rates and flower earlier than those from high elevations, presumably in order to avoid the more extreme mid-season drought conditions often present at lower elevations (Jonas and Geber, 1999). While the specific mechanism triggering flowering is unknown in *Penstemon,* my results suggest that temperature plays an important role but, unlike in *Clarkia,* plants from high elevations grow and flower faster under warm conditions than plants from low elevations.

One striking exception to this pattern was found in *P. rostriflorus.* Consistent with natural populations of all three species, the low elevation population on the southeast mountain range (Pr-SEL) flowered many weeks before the high elevation population (Pr-SEH). However, unlike all other species and populations, when grown in

common garden experiments, plants from the low-elevation population (Pr-SEL) continued to flower before those from the high-elevation (Pr-SEH, particularly for percent flowers finished; Table XIII). Therefore, flowering may be triggered by different mechanisms in populations of *P. rostriflorus*.

3.4.2 Quantitative and molecular divergence

Previous investigations of neutral genetic diversity in these three species identified very different opportunities for evolutionary process to drive population divergence. *P. rostriflorus,* with the lowest F_{ST} values and greatest admixture in genetic clusters identified through Bayesian cluster analysis, showed the most genetic cohesion among populations throughout the Great Basin (A. Kramer Chapter 2). This is presumably because hummingbirds, which predominantly pollinate the species, are capable of moving pollen over great distances of rather inhospitable terrain. Thus, I expect genetic drift to play a relatively small role in population divergence of either molecular or quantitative traits in *P. rostriflorus,* and know that only quantitative traits under sufficiently strong selection will be capable of adaptive population divergence (Endler, 1973; GarciaRamos and Kirkpatrick, 1997; Kittelson and Maron, 2001). The same is not true of the bee-pollinated *P. deustus* or *P. pachyphyllus,* where microsatellite markers showed that populations are much more genetically isolated from each other. Therefore, I expect genetic drift to play a greater role in population divergence for both species, and note that the interaction of genetic drift with natural selection in isolated populations occupying this heterogeneous landscape may lead to otherwise similar populations finding different genetic solutions to the same selective pressure (Cohan, 1984). These broad expectations were not borne out in my results, largely because *P. rostriflorus* demonstrated strong population divergence in a number of quantitative traits despite higher levels of gene flow.

For many quantitative traits, including seed germination requirements and flowering phenology, the expectation that higher among-population gene flow in *P. rostriflorus* would constrain population divergence was incorrect. Because divergence in these traits was strongly correlated with average temperature, temperature extremes and precipitation seasonality, it appears that that these traits were under sufficient selection that population divergence occurred even in the presence of gene flow. Given the population divergence in seed

germination requirements for all three study species, is it useful to examine the heritability of this trait. Heritability by species was 0.118 $\pm$ 0.094 for *P. pachyphyllus* and 0.510 $\pm$ 0.081 for *P. rostriflorus* (Table XVI). Low heritability in *P. pachyphyllus* is due either to little variation in seed germination requirements within populations and/or because there is not a strong genetic basis for seed germination requirements as measured here. Comparatively higher values in *P. rostriflorus* reflect high levels of variation within populations (but much less than is present between populations) and a strong genetic basis for this variation.

As calculated here, Q_{ST} measures are expected to be equivalent to F_{ST} measures for traits with additive effects that are not under selection (McKay and Latta, 2002). In *P. pachyphyllus*, all measures of Q_{ST} are lower than for F_{ST}, indicating either stabilizing or fluctuating selection (Edmands and Harrison, 2003), while in *P. rostriflorus*, nearly all measures of Q_{ST} are higher than for F_{ST}, indicating divergent selection. A notable exception is the Pr-SEL and Pr-WH2 comparison (Q_{ST} = -0.0003, F_{ST} = 0.1901), indicating stabilizing selection as with *P. pachyphyllus*. This is interesting because these populations are extremely geographically far apart (581km) but very similar climatically (see Figure 3.2).

Results of UPGMA tests for seed germination provide additional details on quantitative divergence in relationship to neutral genetic divergence between populations and mountain ranges. Where the UPGMA for Nei's unbiased genetic distance showed divergence in neutral traits that was very different between species (*P. pachyphyllus* much greater than *P. deustus*, with *P. rostriflorus* lowest), UPGMA for days to germination showed generally equivalent divergence between populations in all three species. For *P. rostriflorus* in particular, these results provide additional evidence for adaptive population divergence in the presence of gene flow. This comparison also identifies populations on different mountain ranges that have similar (convergent) seed germination requirements. For example, in *P. deustus*, all high elevation populations grouped together and separately from warmer or low elevation populations, despite the fact that analyses of neutral genetic diversity group them much more uniformly by mountain range.

3.4.3 Quantitative trait plasticity

My use of two common garden sites with different growing environments revealed different levels of plasticity in all measured traits. Floral traits consistently showed the least plasticity, as evidenced by little variation partitioned by common garden or plant size (Table XIV). This supports numerous studies that have found vegetative traits to be more plastic than floral traits (Bradshaw, 1965; Frazee and Marquis, 1994). Many other traits, however, showed significant plasticity. Not surprisingly, this was most true for growth measures in all three species. However, vegetative and flowering phenology traits also showed consistently high plasticity in *P. rostriflorus*. Generally greater levels of plasticity in traits for the hummingbird-pollinated *P. rostriflorus* as compared to the other two bee-pollinated species may be a product of higher levels of among-population gene flow identified by microsatellite markers. As demonstrated by Sultan and Spencer (2002), trait plasticity is favored when among-site gene flow is allowed in an environmentally heterogeneous metapopulation. My results support the findings of this study, and broadly indicate the many ways that taxa-level differences in dispersal and migration rates play an important role in determining patterns of adaptive population differentiation.

3.4.4 Implications for ecological restoration

These results can be used to guide ecological restoration efforts for my study species and those that share similar characteristics found throughout the unique landscape of the Great Basin region. Findings of high genetic diversity and significant population structure in quantitative traits correlated to climate caution against broad scale movement of populations for restoration purposes in all three species. Of great significance to restoration practitioners is the finding of adaptive divergence in seed germination requirements for all three species. This means that climate at the seed source and restoration site must be closely matched to ensure that seeds do not germinate during unfavorable conditions, leading to immediate restoration failure. In all three species, population divergence in other traits, particularly floral morphology traits, suggests that populations may be adapted to local biological conditions as well as local climatic conditions. Additional studies will be necessary to identify whether this divergence in floral morphology is the result of adaptation to different pollinator communities or some other factor or suite of factors. If this is the case, efforts to identify which seed source is

most appropriate for a given restoration site will require matching not only climate but also pollinator community to ensure both short-term survival and long-term reproduction of a restored population. Finally, species-level differences identified in phenotypic plasticity are noteworthy for restoration practitioners, as they suggest that the movement of seeds to restore populations of hummingbird-pollinated species may be more successful over larger geographic and climatic distances than for bee-pollinated species, given the generally greater plasticity identified for many traits in the hummingbird-pollinated *P. rostriflorus*. Similar studies incorporating additional bee and hummingbird-pollinated species in the Great Basin and other regions will be necessary to understand if this finding has broader applicability when making decisions about seed sources for successful ecological restoration practice.

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4. INBREEDING AND OUTBREEDING DEPRESSION IN CROSSES SPANNING GEOGRAPHIC AND GENETIC DISTANCES: A COMPARISON OF TWO *PENSTEMON* SPECIES

4.1 INTRODUCTION

The fitness of a plant is a product of the genetic makeup of its parents and the environment in which it grows. If parents are genetically too similar, offspring may exhibit a decrease in fitness relative to their parents, particularly in sexually reproducing and primarily outcrossing species (Husband and Schemske, 1996). This fitness decline, also known as inbreeding depression, is usually due to within-locus (dominance) effects that expose recessive deleterious alleles in homozygous offspring. As such, inbreeding depression effects are often most pronounced in the first generation of a cross, but may only be apparent in certain situations, such as stressful environments (Dudash, 1990; Keller and Waller, 2002). Plants with offspring displaying inbreeding depression may also display hybrid vigor when parents are genetically more dissimilar, as heterozygosity can mask recessive deleterious alleles and result in heterosis (Charlesworth and Charlesworth, 1987; Keller and Waller, 2002). However, if parents are genetically too dissimilar, a decline in fitness may result (Waser, Price, and Shaw, 2000). This decrease in fitness, known as hybrid breakdown or outbreeding depression, is often more difficult to detect, as the genetic mechanisms underlying it are complex. Outbreeding depression has received much less attention than inbreeding depression (Edmands, 2007), but it is believed to be largely the result of the breakup of favorable epistatic (among-loci) interactions (Lynch, 1991).

The complexities of outbreeding depression are generally explained by the action of two mechanisms; dilution and hybrid breakdown, described by Lynch (1991) and outlined in Hufford and Mazer (2003). A dilution mechanism occurs when mating between parents adapted to different local conditions results in offspring ill-suited for either parental environment due to the dilution of locally adapted genes. In this scenario, declines in fitness of offspring are predicted in the first generation if parents are adapted to different local conditions and if their hybrid offspring are then grown in either parental environment. This mechanism can be tested by growing

the progeny of known crosses between adaptively divergent parents in a reciprocal transplant study and assessing their relative fitness by treatment. Strong evidence for this mechanism of outbreeding depression was found when two varieties of *Lotus scoparius* were experimentally hybridized (Montalvo and Ellstrand, 2001), but surprisingly few studies have utilized these techniques to identify outbreeding depression through a dilution mechanism.

Alternatively, a hybrid breakdown mechanism involves the disruption of beneficial epistatic interactions among loci (Fenster, Galloway, and Chao, 1997; Hufford and Mazer, 2003), and rests on the assumption that genetically isolated populations can have unique co-adapted gene complexes resulting from the accumulation of both neutral and advantageous mutations over many generations. Outbreeding depression following this mechanism occurs when hybridization between parental populations disrupts co-adapted gene complexes through segregation and recombination over one to many generations, leading to fitness decreases in progeny (Lynch, 1991; Edmands, 2007). Unlike inbreeding depression or outbreeding depression via dilution, the effects of disrupting co-adapted gene complexes are usually not detected immediately (but see Galloway and Etterson 2005 for an example of negative nuclear and cytoplasmic genetic interactions contributing to outbreeding depression in the first generation). In fact, no treatment effect, or even an increase in hybrid fitness (heterosis), may be found in first generation progeny of an inter-population cross that ultimately displays outbreeding depression in following generations (Fenster and Galloway, 2000; Bailey and McCauley, 2006; Johansen-Morris and Latta, 2006). Indeed, as indicated in a recent review of the subject by Edmands (2007), intentional crosses to avoid inbreeding depression may generate hybrid vigor in the first generation but ultimately lead to fitness losses via outbreeding depression in subsequent generations.

The potential for inbreeding and outbreeding depression in offspring depends upon the genetic similarity of parents. For this, it is useful to consider how the differentiating forces of genetic drift, mutation, and divergent selection interact with the homogenizing forces of gene flow and stabilizing selection to drive genetic divergence in natural populations (Slatkin, 1987; GarciaRamos and Kirkpatrick, 1997). All of these factors vary depending upon the historical and current distribution, density, and life history of the species, its populations, and the

individuals under consideration, making predictions regarding the degree of genetic similarity between two given individuals quite difficult. For sessile organisms such as plants, genetic similarity is often related to geographic separation as some function of the distribution, mating and dispersal systems of that species (Hamrick and Godt, 1996; Richards, 1997). For example, when comparing genetic similarity of populations for two species with gravity dispersed seeds, I demonstrated in Chapter 2 that populations of a species pollinated by hummingbirds were genetically more similar than those of a species pollinated by bees, despite the fact that geographic distance between populations for each species was equivalent. For these two species, I also found that the degree of genetic similarity between populations depends upon which measure of genetic diversity is used (A. Kramer Chapter 3). While divergence of neutral genetic diversity differed significantly between the two study species (attributed to pollinator behavior), divergence of potentially adaptive genetic diversity was in many cases quite similar (e.g. divergence in seed germination requirements) and unrelated to patterns of neutral divergence.

These complex patterns of genetic differentiation are largely a product of the region in which these studies were carried out: the Great Basin region of the western United States. Comprising over 390,000 km^2, the Great Basin contains over 150 distinct mountain ranges (Grayson, 1993) isolated from one another by vast arid valleys. These mountain ranges rise an average of 1,750 meters above valley floors, and a majority of vascular plant species native to the region are nearly exclusive residents of the cooler, more mesic montane habitats (Grayson, 1993). This rugged mountainous terrain isolates plant populations to varying degrees; for some species like the bee pollinated *P. pachyphyllus* referenced above, these mountains function as sky islands (DeChaine and Martin, 2005), with little genetic movement between mountain islands. Lying in the rainshadow of the Sierra Nevada Mountains, a significant east-west precipitation gradient combines with a north-south temperature gradient to create a large-scale mosaic of environmental heterogeneity throughout the Great Basin. Even within mountain ranges, changes in temperature and precipitation imposed by rapid increases in elevation interact with differences in slope and aspect to create a virtually infinite array of microclimates on a relatively small scale (Petersen, 1994). In combination, these factors make the Great Basin an ideal setting to address the links between genetic similarity and outbreeding depression, as it has created a range of genetic divergence between plant

populations in response to changing levels of environmental heterogeneity and varying degrees of genetic isolation. I capitalize on this situation to test how different types and degrees of genetic divergence between populations may affect the relative effects of inbreeding and outbreeding depression on hybrid offspring fitness.

Specifically, I use experimental crosses to produce offspring with known parents of decreasing genetic similarity. To determine the role of inbreeding and outbreeding depression (via the hybrid breakdown genetic mechanism) in decreasing fitness via fruit and seed production, I track the success of all attempted crosses. Because previous studies have shown that seed germination requirements are divergent between populations (A. Kramer Chapter 3), likely due to local adaptation to winter conditions, I perform a simulated reciprocal transplant study with seeds produced through experimental crosses to detect potential outbreeding depression through a dilution mechanism. Finally, because inbreeding and outbreeding depression may be detected in the early stages of seedling growth, particularly in stressful environments (Armbruster and Reed, 2005; Galloway and Etterson, 2005), I germinate and grow a subset of experimental crosses in greenhouse conditions with and without competition. I expect that *P. pachyphyllus*, the species with higher neutral genetic differentiation (A. Kramer Chapter 2), will demonstrate outbreeding depression via the hybrid breakdown mechanism over shorter crossing distances than *P. rostriflorus*. However, that each species may be equally likely to demonstrate outbreeding depression via a dilution mechanism, as they both have similar adaptive genetic differences in seed germination requirements (A. Kramer Chapter 3).

4.2 METHODS

4.2.1 Study species

In this study, I compare the relationship between genetic similarity and progeny fitness in two *Penstemon* species found in the Great Basin region. *Penstemon* is North America's largest endemic genus (over 270 species) and the result of a recent and rapid evolutionary radiation centered in the western United States, including the Great Basin (Wolfe et al., 2006). Rapid speciation in *Penstemon* is largely attributed to evolutionary adaptations to pollinator specialization (Wilson et al., 2004; Wilson et al., 2006), and the creation of ecological niches during

multiple historical glacial advances and retreats in the region. Our study species are a product of and exemplify the extremes of this radiation, including *Penstemon pachyphyllus* A. Gray ex Rydb. var. *congestus* (M.E. Jones) N.H. Holmgren, and *P. rostriflorus* (Kellogg). Both species are animal-pollinated perennial forbs with mixed mating systems and gravity-dispersed seeds. They are common and widespread throughout the western United States, including the Great Basin region (Kartesz, 1999), occurring almost exclusively in sagebrush-steppe habitat at a range of mid to high-elevations on mountain ranges.

The neutral genetic diversity and differentiation within and among populations of these species on Great Basin mountain ranges (as identified by microsatellite markers) was described in Chapter 2. This work revealed population genetic differentiation that is largely a function of each species' pollination syndrome. That is, *P. pachyphyllus* (large purple flowers and bee pollination syndrome) exhibited extremely high levels of population differentiation between mountain ranges ($F_{ST} = 0.221$), while *P. rostriflorus* (red flowers and hummingbird pollination syndrome) demonstrated much lower differentiation ($F_{ST} = 0.071$). Hence, over similar geographic landscapes, populations are genetically more similar over greater distances in *P. rostriflorus* than *P. pachyphyllus*, setting the expectation that hybrid breakdown may be more likely in distant crosses of *P. pachyphyllus*.

Quantitative divergence in these same species and populations was studied in Chapter 3, and in many cases results identified patterns of genetic divergence that were equivalent in both *P. pachyphyllus* and *P. rostriflorus* over similar scales. This was particularly true for divergence in seed germination requirements, which are likely adaptive (they are correlated with climatic conditions at collection sites, particularly winter length; see Figure 4.1). These results supported earlier studies of *Penstemon* species in this region (Meyer, Kitchen, and Carlson, 1995), and demonstrated that seed germination traits are heritable in both species (0.118 ± 0.094 in *P. pachyphyllus*, 0.510 ± 0.081 in *P. rostriflorus*) and that the seed germination requirements of different *P. rostriflorus* populations appear to be locally adapted despite inter-population gene flow (A. Kramer Chapter 2).

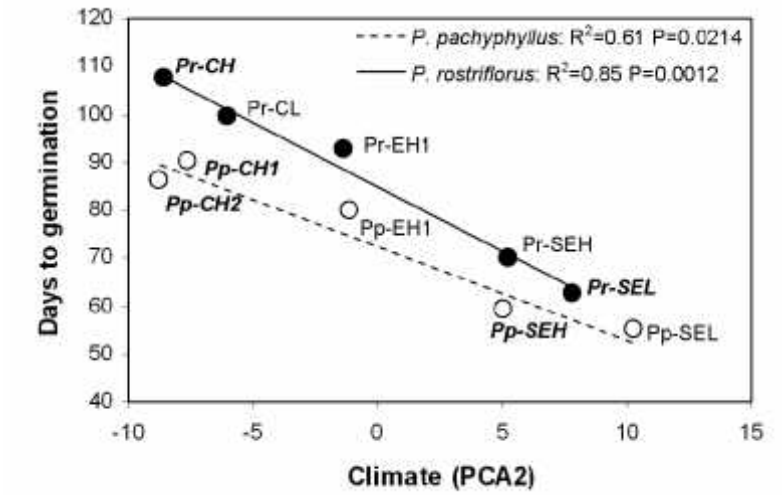

Figure 4.1. Climate significantly predicts mean days to germination in both study species. Focal populations are shown in bold italics. Climate is shown as PCA2, which corresponds with increasing temperatures and precipitation seasonality. Filled circles represent *P. rostriflorus* populations, open circles represent *P. pachyphyllus* populations.

4.2.2 Study populations

Five populations of both *P. pachyphyllus* and *P. rostriflorus* were chosen for additional study; while populations of each species do not co-occur, they are found on the same mountain ranges and collectively represent similar geographic distances (Table XVII, Figure 4.2). For *P. pachyphyllus*, three focal populations were chosen to receive pollen from increasingly distant populations; two high elevation populations on the same mountain range in the central Great Basin (Pp-CH1 and Pp-CH2) and another at a high elevation in the southeast (Pp-SEH). For *P. rostriflorus*, only two focal populations were used; one high elevation population on a mountain range in the central Great Basin (Pr-CH) and a lower elevation population in the southeast Great Basin (Pr-SEL). Remaining populations were located on mountain ranges at increasing distances from the focal populations; 10 km (within the same mountain range of the focal population), 100 km (equidistant between both focal populations) and 200 km (cross between the two focal populations).

TABLE XVII
STUDY POPULATIONS[a] FOR EACH SPECIES

Population	Mountain Range	State	Lat.	Long.	Elevation (m)	Approx size (# plants)	Climate[a]	Days to germination
Penstemon pachyphyllus								
Pp-SEH	Southeast (Zion NP)	UT	37.341	-113.077	2122	300-400	5.27	70
Pp-SEL	Southeast (Zion NP)	UT	37.173	-113.083	1119	300-400	10.26	55
Pp-EH1	East (Wah Wah)	UT	38.325	-113.590	2560	150-200	-1.05	80
Pp-CH1	Central (Snake)	NV	39.109	-114.347	2323	1000+	-7.45	90
Pp-CH2	Central (Snake)	NV	39.148	-114.330	2227	300-400	-8.67	86
Penstemon rostriflorus								
Pr-SEH	Southeast (Zion NP)	UT	37.345	-113.080	2092	200-300	5.04	60
Pr-SEL	Southeast (Zion NP)	UT	37.292	-113.096	1632	100-150	7.82	63
Pr-EH1	East (Wah Wah)	UT	38.354	-113.608	2510	100-150	-1.37	93
Pr-CH	Central (Snake)	NV	39.023	-114.270	2768	150-200	-8.57	108
Pr-CL	Central (Snake)	NV	38.991	-114.220	2147	100-150	-6.02	100

Focal (maternal) populations are shown in grey.
[a] Climatic differences between sites described by a Principal Components Analysis in Chapter 3; shown are values for axis 2, as these values are significantly correlated with days to germination.

Partitioning of neutral genetic diversity within and among these populations and mountain ranges was described in Chapter 2, and the interaction of genetic distance with geographic and climatic distance between populations on population divergence in morphological (quantitative) traits was described in Chapter 3. Plants of all populations established in common gardens at Utah Botanical Center (Vitousek et al.) for Chapter 3 were used for crosses here. An additional common garden was established for *P. pachyphyllus*, as many plants at the original common gardens had died by the time this study was initiated. For this, seeds remaining from original collections of *P. pachyphyllus* populations used to establish common gardens in Chapter 3 were germinated and grown for two years in a randomized plot in an elevated sand bed at Chicago Botanic Garden (CBG).

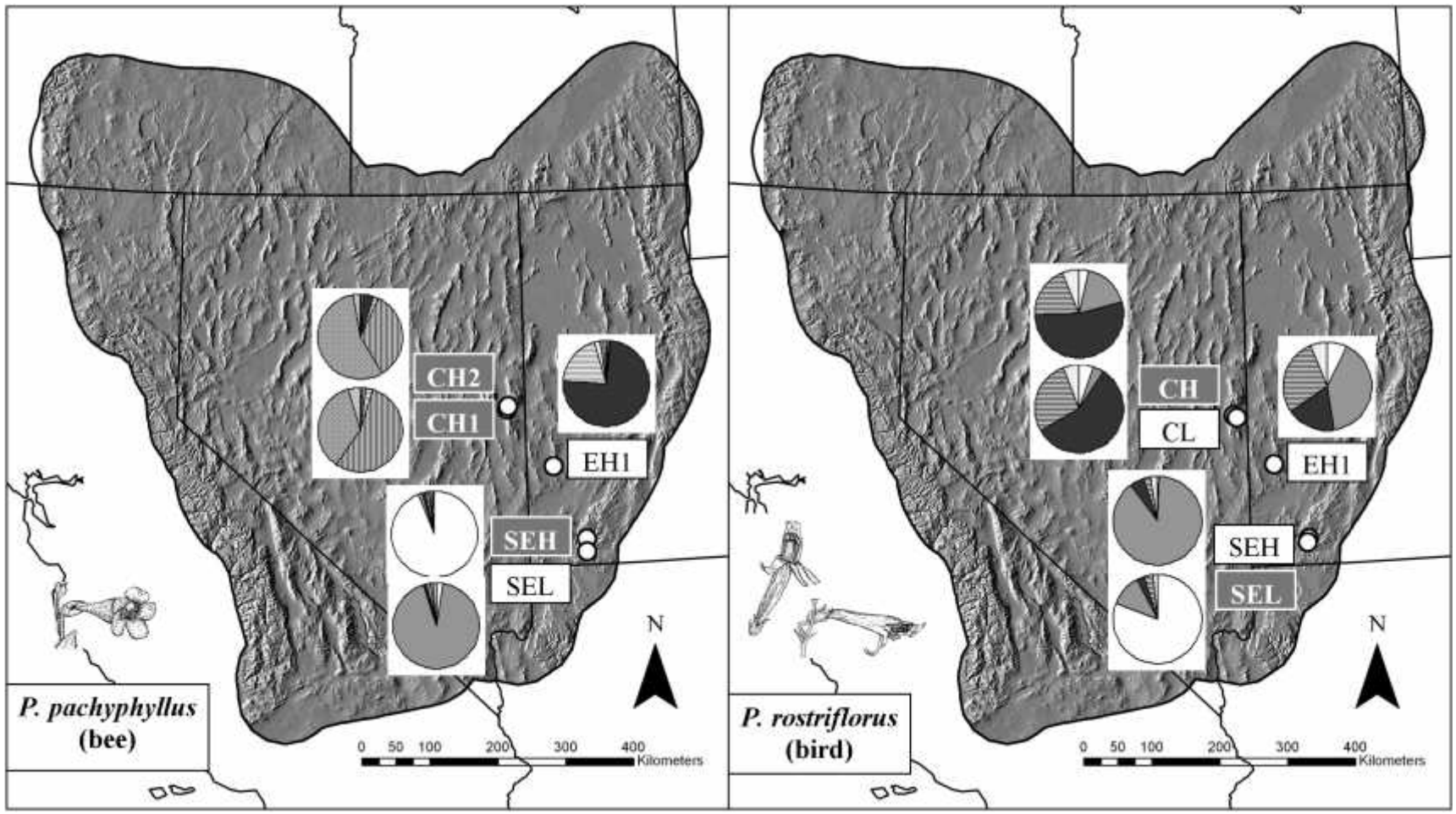

Figure 4.2. Five study populations for each species. Focal populations for each species are shown in grey (for *P. pachyphyllus*: SEH, CH1 and CH2, for *P. rostriflorus*: SEL and CH). Pie charts depict clusters of neutral genetic diversity identified through the STRUCTURE Bayesian analysis with microsatellite DNA markers. In general, more mixing between mountain ranges and populations is evident in *P. rostriflorus* than *P. pachyphyllus*.

4.2.3 Crossing design

Crosses were made for *P. pachyphyllus* at CBG, and for *P. rostriflorus* at UBC. For each species and each focal population, 5 cross treatments were imposed on at least 7 different maternal plants using pollen from donor plants and populations, resulting in: 1) geitanogamous selfing (0 km); 2) within-population (0.01 km); 3) within mountain range (10 km); 4) intermediate mountain range (100 km); and 5) between focal populations (200 km). See Table XVIII for additional details. Each population used for cross treatments had at least 5 plants from which pollen was collected and randomly assigned to maternal plants for each focal population. I avoided potential bi-parental inbreeding in my within-population treatment by selecting pollen donors whose mothers had been at least 10 m apart in the original study population.

Penstemon inflorescences are borne on cymes, and flowers are protandrous, with stigmas becoming receptive (visibly bending) 1-2 days after anthers dehisce. Crosses were made on *P. pachyphyllus* at CBG in May and June 2007. *P. rostriflorus* crosses were all performed at UBC in June 2007. For all crosses, flowers on each maternal plant that had not yet opened were emasculated and a thin colored wire identifying the eventual cross treatment was randomly drawn and tied around the pedicel of each flower. At the same time, undehisced anthers from all treatment populations were collected and stored in microcentrifuge tubes and stored in cool dry temperatures overnight. For *P. rostriflorus*, fine-meshed pollinator exclusion bags were placed over stems after flowers were emasculated to exclude any potential pollinators. These bags were not used on *P. pachyphyllus*, as no other *Penstemon* plants were flowering nearby, no pollinators were observed visiting flowers, and all pollen was removed from flowering common garden plants prior to dehiscence. On the following day, individual pollen donors (using collected anthers that dehisced overnight in each microcentrifuge tube) for each population treatment were randomly drawn and applied to receptive anthers on emasculated and tagged flowers using a q-tip.

TABLE XVIII

CROSS TREATMENTS FOR EACH MATERNAL (FOCAL) POPULATION, SHOWING GEOGRAPHIC DISTANCE (KM) AND GENETIC DIFFERENTIATION (IN PARENTHESIS; F_{ST}) BETWEEN EACH PAIR

Penstemon pachyphyllus	Paternal population				
Maternal (focal) population	Pp-SEL	Pp-SEH	Pp-EH1	Pp-CH1	Pp-CH2
Pp-SEH	10 km (0.1963)	0 km 0.01 km	100 km (0.2866)		200 km (0.3521)
Pp-CH1		200 km (0.3538)	100 km (0.1688)	0 km 0.01 km	10 km (0.0264)
Pp-CH2		200 km (0.3521)	100 km (0.1799)	10 km (0.0264)	0 km 0.01 km

Penstemon rostriflorus	Paternal population				
Maternal (focal) population	Pr-SEL	Pr-SEH	Pr-EH1	Pr-CH	Pr-CL
Pr-SEL	0 km 0.01 km	10 km (0.0597)	100 km (0.0694)	200 km (0.0982)	
Pr-CH	200 km (0.0982)		100 km (0.0223)	0 km 0.01 km	10 km (0.0358)

For *P. pachyphyllus*, whenever possible at least one cross set (e.g. a full set of 5 treatment crosses/plant and a control, where flowers were emasculated but received no pollen) was made on maternal plants for each focal population on each of 3-5 days. For *P. rostriflorus*, at least 3 cross sets and controls per plant were made on one day only.

Fruit was harvested immediately in August and September upon ripening (brown, dried, and just cracked open at the top of the capsule), maintained separately and dried to 15% relative humidity. Each fruit was then weighed, and seeds produced were counted and weighed to obtain an average seed weight for each fruit. Cleaned seed was maintained separately in manila folders at 15% relative humidity until January 2008, when bulked seed lots for each treatment with equal founder (maternal line) representation were created for use in seed germination and greenhouse trials.

Two primary sets of analyses were performed on the outcome of all crosses to detect potential inbreeding depression, hybrid vigor, and outbreeding depression. First, the effects of cross treatment, focal population, and their interaction on the production of fruit and the production of fruit with seeds was assessed with nominal

logistic and likelihood ratio tests. Second, two-way Analysis of Variance (ANOVA) tests were used to determine the effect of cross treatment, focal population, and their interaction on four response variables/fitness measures; 1) fruit weight, 2) total seed weight, 3) average seeds per fruit and 4) average seed weight using JMP IN (SAS 2004; this program used for analyses throughout unless otherwise noted). All variables were square root or log transformed prior to analysis to meet assumptions of normality. Any significant cross treatment effects were examined with Tukey-Kramer HSD tests to identify specific treatments resulting in lower fitness values than the within-population treatment (e.g. inbreeding depression if found in the 0 km treatment, outbreeding depression if found in the 10, 100, or 200 km treatments) or in higher fitness values than the within-population treatment (e.g. heterosis). Because all crosses were performed in a common environment, any detected outbreeding depression can be attributed to a hybrid breakdown genetic mechanism (e.g. the break-up of favorable epistatic interactions) rather than a dilution mechanism.

4.2.4 Seed germination

Significant divergence in seed germination requirements has been identified for both species and focal populations (A. Kramer Chapter 3) , with chilling requirements for germination highly correlated with climatic conditions (particularly winter-length) at each site (reported on Table XVII, Figure 4.1, and described in Chapter 3). This presumably adaptive divergence presents an excellent opportunity to test for outbreeding depression via a dilution mechanism. Here, I used a series of seed germination trials aimed at confirming the genetic basis of seed germination requirements in my study species while testing for outbreeding depression via a dilution mechanism in experimentally created hybrid progeny through the use of simulated reciprocal transplant studies.

For F_1 seed produced from experimental crosses for each species, focal population and cross treatment, a bulk collection was created with equal founder representation from all maternal lines. To sterilize seed surfaces prior to germination trials, all seeds were washed in 0.25% sodium hypochloride (bleach) solution for one minute and rinsed twice in deionized water. Seeds were then divided into sets of 10 seeds and placed into three to five 3.5 cm petri dishes per treatment with two layers of No5 Whatmann filter paper and dampened with deionized

water. Replicates were placed directly in cold stratification (CS = 8 hours at 10ºC with light, 16 hrs at 4ºC in the dark) and regularly monitored for 20 weeks, with any germinants (radicle emergence greater than 1mm) recorded and discarded following Meyer, Kitchen, and Carlson (1995). After 8 weeks (*P. rostriflorus*) and 13 weeks (*P. pachyphyllus*), two additional sets of three to five petri dishes for all treatments were prepared as above and placed haphazardly with the previous petri dishes in cold stratification. These conditions were intended to simulate optimal germination conditions at each focal population, thus forming the basis for a simulated reciprocal transplant study of seed germination requirements. Filter paper was kept moist throughout the study with regular application of deionized water. All petri dishes were removed from cold stratification on the same day and placed in warm stratification (WS = 12 hrs at 10^{o}C in the dark, 12 hrs at 20^{o}C with light) for an additional 4 weeks to simulate spring conditions, with regular checks for germination and watering maintained as before. At the end of the study, all seeds remaining viable (e.g. firm when slight pressure was applied with forceps) were counted as an estimate of whether ungerminated seed was dead or capable of forming a seed bank. Average days to germination were determined for each cross and cold stratification treatment using combined results from all replicates.

To determine if seed germination requirements as measured here are genetically controlled and additive, predictions for days to germination for each cross treatment were established using mid-parent values calculated from 2004 germination trials, and results of F_1 days to germination were regressed on these predictions. If this trait is genetically controlled and additive, mid-parent values for days to germination will accurately predict values in F_1 progeny. To analyze the outcome of the simulated reciprocal transplant study for each species, two-way Analysis of Variance (ANOVA) tests were used to identify the effect of cross treatment, focal population, and their interaction on percent germination for each species and cold stratification treatment. If local adaptation for seed germination requirements is confirmed, I expect progeny from within-population crosses (0.01 km) to outperform hybrid progeny (fitness measured as percent germination) between increasingly climatically dissimilar populations when subjected to conditions that approximate its specific winter conditions. If fitness (measured by percent germination) of increasingly distant crosses is lower than the mean within-population F1 at each site, I

cannot reject the hypothesis that a dilution mechanism is contributing to outbreeding depression in one or both study species.

4.2.5 Greenhouse study

With remaining seeds, a greenhouse study was utilized to identify any effects of cross treatments on seedling growth with and without competition as a measure of potential inbreeding and outbreeding depression. Not enough seeds from crosses remained for a full complement of all focal populations and cross treatment, so a subset from focal populations with sufficient seeds for each cross treatment was chosen. For *P. pachyphyllus*, 2,681 seeds produced from the CH1 focal population representing all cross treatments were used, and for *P. rostriflorus*, 965 seeds produced from Pr-CH at the 0 km (self), 0.01 km (within population) and 200 km cross treatments (Pr-CH crossed with SEL pollen) were used, as well as the reciprocal of the 200 km treatment (Pr-SEL crossed with CH pollen). These seeds were equally distributed and randomly planted into thirteen 512-cell greenhouse flats filled with Superfine Germinating Mix (Conrad Fafard, Agawam, MA, USA), and inner cells planted with a (randomly selected) *P. pachyphyllus* or *P. rostriflorus* seed from F1 crosses (sterilized with 0.25% bleach and rinsed prior to sowing, as above).

Half of all cells with seeds were randomly assigned a competition treatment, which involved placing three seeds of *Bromus tectorum* (harvested from the Great Basin in 2007) in the soil in a triangle pattern at the edge of each cell, with the *P. pachyphyllus* or *P. rostriflorus* seed in the middle. Flats were prepared in January, 2008, and once *Penstemon* and *Bromus* seeds had been sown accordingly, flats were evenly watered and placed in cold stratification (a refrigerator at about 5 ºC) for 20 weeks. Flats were checked and watered once a week or as needed to ensure moist soil conditions. After 20 weeks (Hoegh-Guldberg et al., 2008), flats were removed from cold stratification and placed in a greenhouse at Chicago Botanic Garden. Flats were randomly assigned a location on a single bench, and rotated along the bench in order every other day. Flats were watered every morning from the bottom until all cells had become equally saturated and maintained in the greenhouse for 4

weeks, at which point all germinated seedlings were harvested (above-ground biomass only), dried to 15% relative humidity, and weighed.

Nominal logistic regression was performed and likelihood ratio tests used to determine the effect of tray, cross treatment, competition, and their interaction on germination and survival through the study, and a three way ANOVA was used to test the effects of tray, cross treatment, competition, and their interaction on seedling weight.

4.3 RESULTS

4.3.1 Cross success

In all, 1,448 crosses were made on *P. pachyphyllus*, with a 58% percent success rate that produced 839 fruit. Of these fruits, only 701 contained seeds, with an average of 15 seeds/fruit (10340 seeds total). For *P. rostriflorus*, 576 crosses were made, producing 215 fruit (37% success rate). Of these fruits, only 118 contained seeds, with an average of 22 seeds/fruit (2,642 seeds total). See Figure 4.3 for additional details. No fruit was produced among controls for either species, indicating no unintentional pollination in emasculated flowers unless pollen was specifically applied as a treatment.

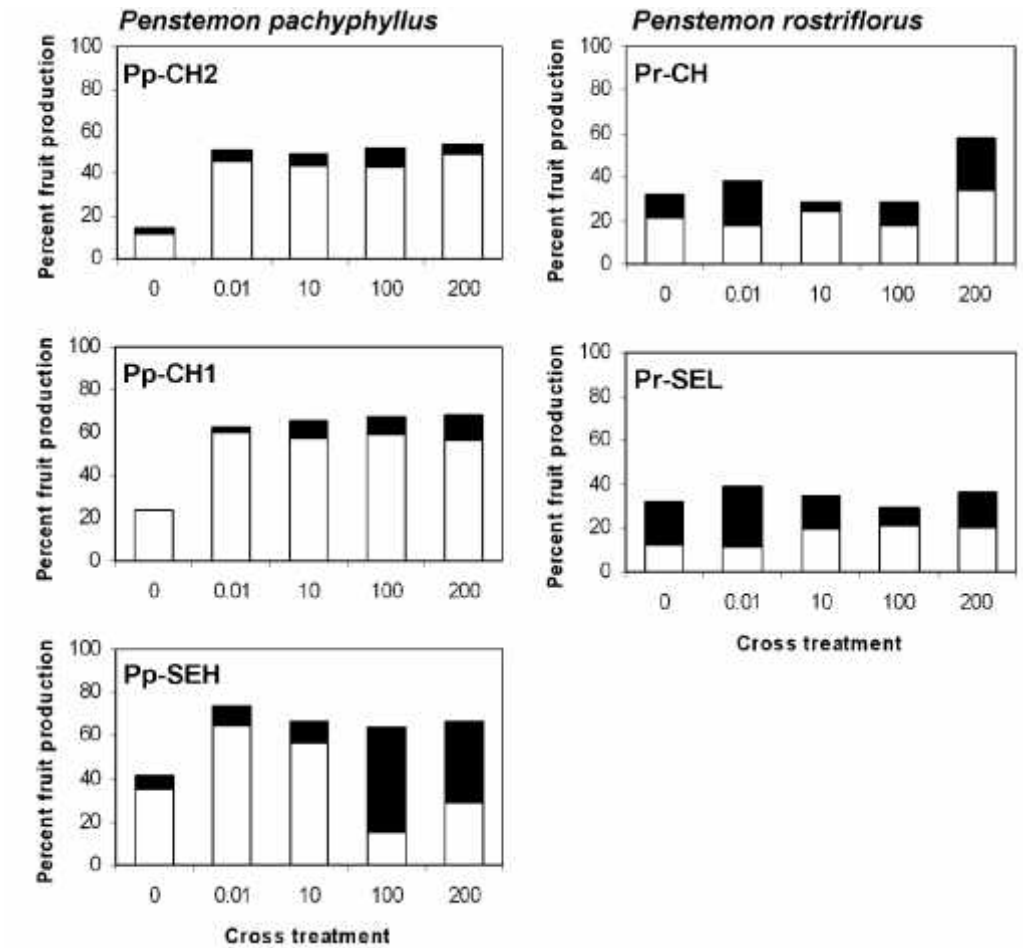

Figure 4.3. Results of each cross treatment for all three focal populations of *P. pachyphyllus* and two focal populations of *P. rostriflorus*. Black bars indicate percent of all crosses that produced fruit, while inset white bars indicate crosses that produced fruit containing seeds. Cross treatment 0 represents self-fertilization, while 0.01 (km) represents a within-population cross, 10 (km) a within-mountain range cross, 100 (km) a between mountain range cross, and 200 (km) a between focal population cross.

While cross success was low in *P. rostriflorus*, there was no effect of cross treatment, focal population, or their interaction on the production of fruit (DF = 10, $\chi^2 = 16.64$, P = 0.0827) or the production of fruit with seeds (DF = 10, $\chi^2 = 13.16$, P = 0.2150). This was not the case for *P. pachyphyllus*, where the same model resulted in highly significant effects for both measures of cross success (fruit production: DF = 9, $\chi^2 = 69.01$, P = 0.0001, and production of fruit with seeds DF = 9, $\chi^2 = 94.89$, P = 0.0001). Likelihood ratio tests identified significant focal population (DF = 1, $\chi^2 = 22.48$, P = 0.0001) and cross treatment effects (DF = 4, $\chi^2 = 23.06$, P = 0.0000) in *P.*

pachyphyllus fruit production; fruit production was always lowest in self-pollination treatments (0 km). For fruit with seed production, the focal population effect was not significant, but cross treatment (DF = 4, $\chi^2 = 52.17$, P = 0.0000) and the interaction of cross treatment and focal population was significant (DF = 4, $\chi^2 = 46.99$, P = 0.0000), again driven by very low success of the self-pollination treatment.

For the four cross fitness measures recorded, *P. rostriflorus* generally had significant effects by cross treatment, but no other effects were significant (Table XIX). However, every test for *P. pachyphyllus* cross success was significant ($P \leq 0.05$), and in many cases highly significant ($P < 0.0001$). As shown in Figure 4.4, self treatments (0 km) generally showed lower values than the within-population cross (0.01 km) in fitness measures in *P. pachyphyllus* and *P. rostriflorus*, except in average seed weight. Fitness declines in selfed progeny varied by progeny and focal population, but were greatest in the measure of seed count for Pp-CH1 (62% fitness decline) and total seed weight for Pr-CH (55% fitness decline). All other crosses showed distinctly different trends in each species. *P. pachyphyllus* showed steady declines in fitness measures with increasingly distant cross treatments, with the 100 and 200 km cross treatments lowest for the Pp-SEL focal populations (33% and 44% fitness declines in seed count, respectively) and the 200 km cross treatment lowest for the Pp-CH1 and CH2 treatments (24% and 29% fitness decline in average seed weight, respectively). Pp-CH1 and CH2 were quite similar in climate and close to each other, and they often behaved similarly. Pp-CH1 produced larger fruit and more seeds regardless of cross treatment. *P. rostriflorus* showed a trend towards increasing fitness measures with increasing cross distance.

TABLE XIX

EFFECT OF CROSS TREATMENT AND FOCAL POPULATION ON FOUR CROSS FITNESS MEASURES

	Fruit weight			Total seed weight			Number of seeds			Average seed weight		
Penstemon pachyphyllus	d.f.	*F*	P value	d.f.	*F*	P value	d.f.	*F*	P value	d.f.	*F*	P value
Cross treatment	4	*17.59*	**<.0001**	4	*18*	**<.0001**	4	*7.15*	**<.0001**	4	*59.88*	**<.0001**
Focal population	2	*11.72*	**<.0001**	2	*18.53*	**<.0001**	2	*23.96*	**<.0001**	2	*8.44*	**0.0002**
Cross treatment x focal population	8	*2.35*	**0.0167**	8	*2.75*	**0.0054**	8	*4.1*	**<.0001**	8	*2.53*	**0.0103**
Penstemon rostriflorus												
Cross treatment	4	*5.0967*	**0.0006**	4	*3.63*	**0.0081**	4	*3.66*	**0.0078**	4	*2.34*	0.0598
Focal population	1	*0.11*	ns	1	*0.13*	ns	1	*0.49*	ns	1	*0.24*	ns
Cross treatment x focal population	4	*0.53*	ns	4	*0.38*	ns	4	*0.52*	ns	4	*2.01*	ns

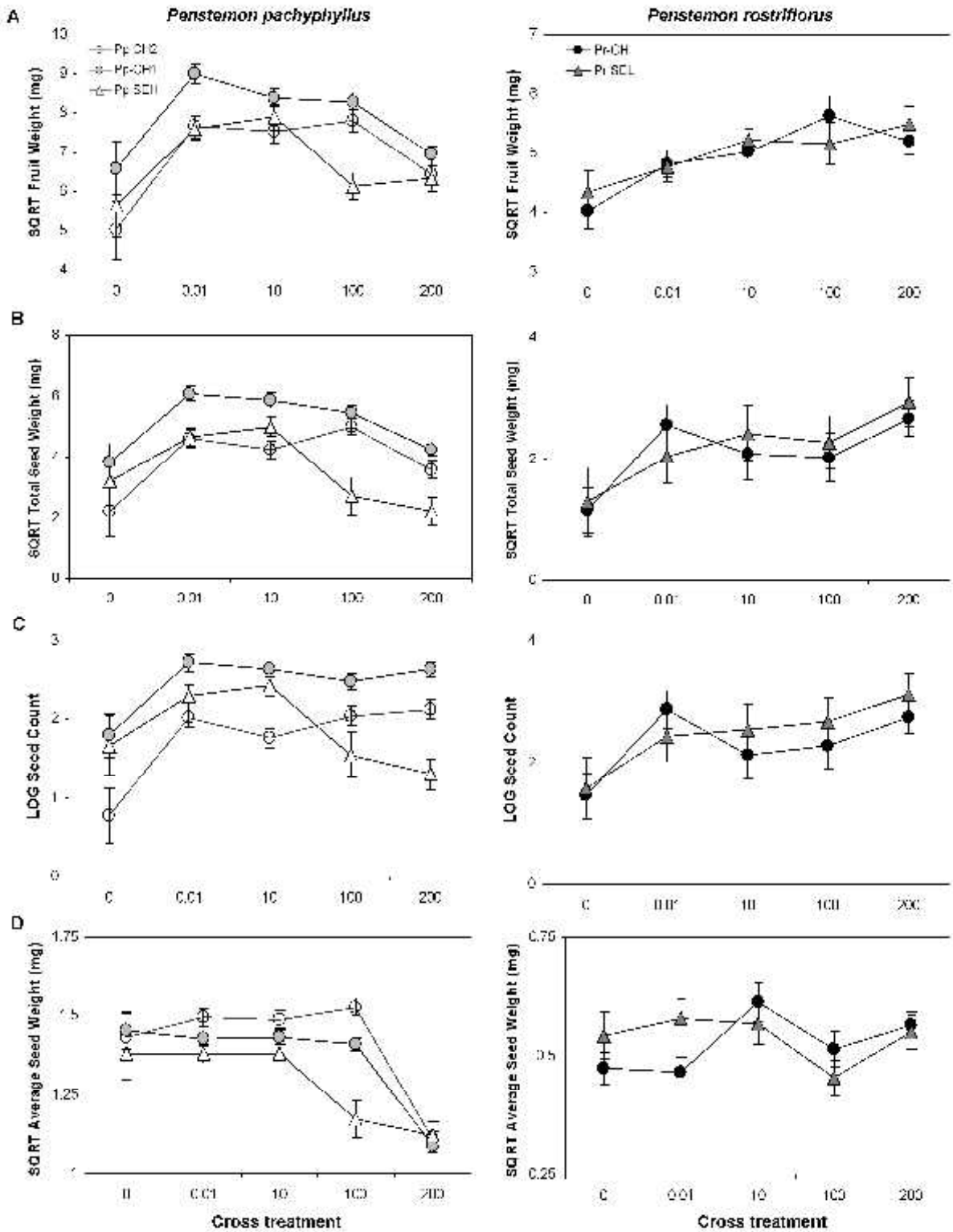

Figure 4.4. Least squares means and standard error bars showing cross success as a measure of fitness for A) fruit weight, B) total seed weight produced per fruit, C) average seed count/fruit, and D) average seed weight. Results are shown as least square means of square root or log transformed values for each focal population; bars represent one standard error. Crossing treatment 0 represents self-fertilization, while 0.01 (km) represents a within-population cross, 10 (km) a within-mountain range cross, 100 (km) a between mountain range cross, and 200 (km) a between focal population cross.

4.3.2 Seed germination

Seed germination requirements are indeed genetically controlled, but environment effects may have a greater influence in *P. pachyphyllus* than *P. rostriflorus*. Average days to germination for *P. pachyphyllus* populations was similar but almost always greater than that predicted by mid-parent days to germination (Figure 4.5), with the exception of the 10km cross in the Pp-CH2 focal population. In this cross treatment, fewer than 5% of all seeds germinated, all under the predicted mid-parent days to germination of 86 days. It is unclear why these seeds didn't germinate, as they were produced from multiple maternal and paternal combinations. Consequently, the regression of F1 and mid-parent values was not significant. For *P. rostriflorus*, however, mid-parent days to germination significantly explained over 87% of the variation in F1 days to germination ($y = 1.0161x - 9.3739$, $R^2 = 0.8787$, $P = 0.0018$), with all values slightly lower than expected. This suggests that reciprocal transplant tests that simulate optimal germination time for each focal population are an appropriate method for testing outbreeding depression via the dilution mechanism in crosses between increasingly genetically dissimilar parent populations.

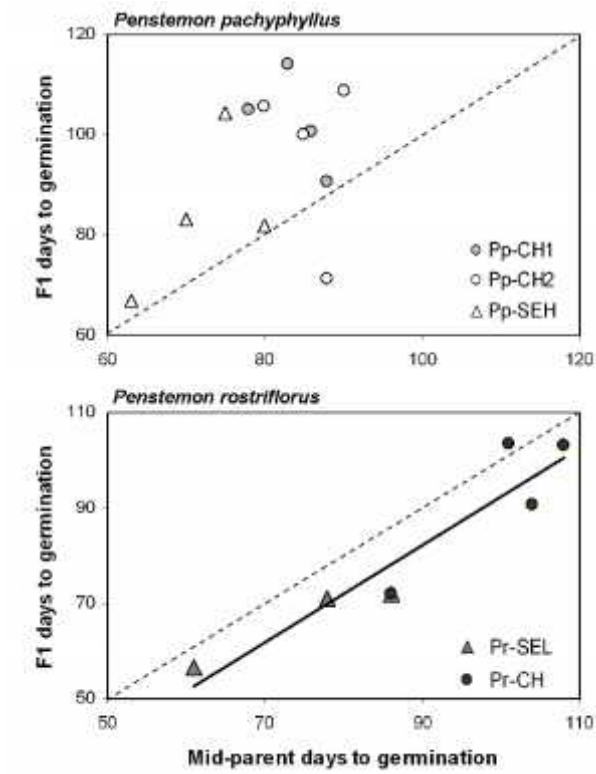

Figure 4.5. Regression of mid-parent days to germination on F1 days to germination for all species and focal populations. Dotted line depicts expectations if seed germination requirements are heritable and additive.

Simulated reciprocal transplant germination studies suggested that outbreeding depression via a dilution mechanism may be occurring in both species. The model of cross treatment, focal population and their interaction significantly explained percent germination for *P. pachyphyllus* in both cold stratification treatments (20 week 'long winter' and 7 week 'short winter' conditions; see Table XX). Differences between focal populations were most striking in the short winter conditions, which were intended to replicate optimal germination conditions for the Pp-SEH focal populations. While the same model was significant for *P. rostriflorus*, the only significant effect under long winter conditions was the interaction effect. This was because seed in all treatments germinated to similar high levels and, because I removed each seed after it germinated, I was not able to estimate the fitness consequences of early or late germination (note that the greenhouse study was able to approximate this condition). However, under short winter conditions all effects were significant, with greater percent germination in the Pr-SEL focal population treatments.

TABLE XX
CROSS TREATMENT EFFECTS ON PERCENT GERMINATION AS A FITNESS MEASURE

	Percent germination long winter condition[a]			Percent germination short winter condition[b]		
P. pachyphyllus	d.f.	*F*	P	d.f.	*F*	P
Cross treatment	3	*5.39*	**0.0026**	3	*10.95*	**<.0001**
Focal population	2	*67.44*	**<.0001**	2	*135.9*	**<.0001**
Cross treatment x focal population	6	*16.21*	**<.0001**	6	*16.68*	**<.0001**
P. rostriflorus						
Cross treatment	3	*2.18*	ns	3	*4.99*	**0.0063**
Focal population	1	*1.71*	ns	1	*19.46*	**0.0001**
Cross treatment x focal population	3	*13.45*	**0.0001**	3	*9*	**0.0002**

[a]20 weeks of cold stratification followed by warm stratification.
[b]12 (*P. rostriflorus*) or 7 (*P. pachyphyllus*) weeks of cold stratification followed by warm stratification.

As shown in Figure 4.6A, percent germination (a potential proxy for fitness) was greatest in Pp-SEH crosses, particularly in both within-mountain crosses (0.01 km and 10 km). Significant drops in germination were found at increasingly distant crosses. The 100 and 200 km treatments were crossed with populations that tend to require more time in cold stratification before germinating, particularly the Pp-CH2 focal population in the 200 km treatment. The Pp-CH1 and CH2 populations and cross treatments had significantly less germination in short winter conditions than Pp-SEH, including the 200 km treatment, which displayed slightly lower percent germination than its reciprocal cross. This could be evidence of a dilution mechanism to outbreeding depression, as the 200 km cross resulted in slightly lower germination than the more geographically similar crosses. However, a large increase in germination in the 10 km cross was recorded for the Pp-SEH focal population, likely due to even lower seed germination requirements in the population it was crossed to (Pp-SEL).

The long winter treatment (Figure 4.6B) was intended to simulate conditions at Pp-CH1 and Pp-CH2 and reveal possible outbreeding depression via a dilution mechanism at increasingly distant crosses. Percent germination went up only marginally in these two populations, and increasingly distant cross treatments led to

increased percent germination, contrary to expectations. Inspection of the percent of all seeds remaining viable shows that seeds either germinated or remained viable in all populations and cross treatments except the 100 and 200 km crosses on the Pp-SEH focal populations, which again germinated at a relatively low percentage or died.

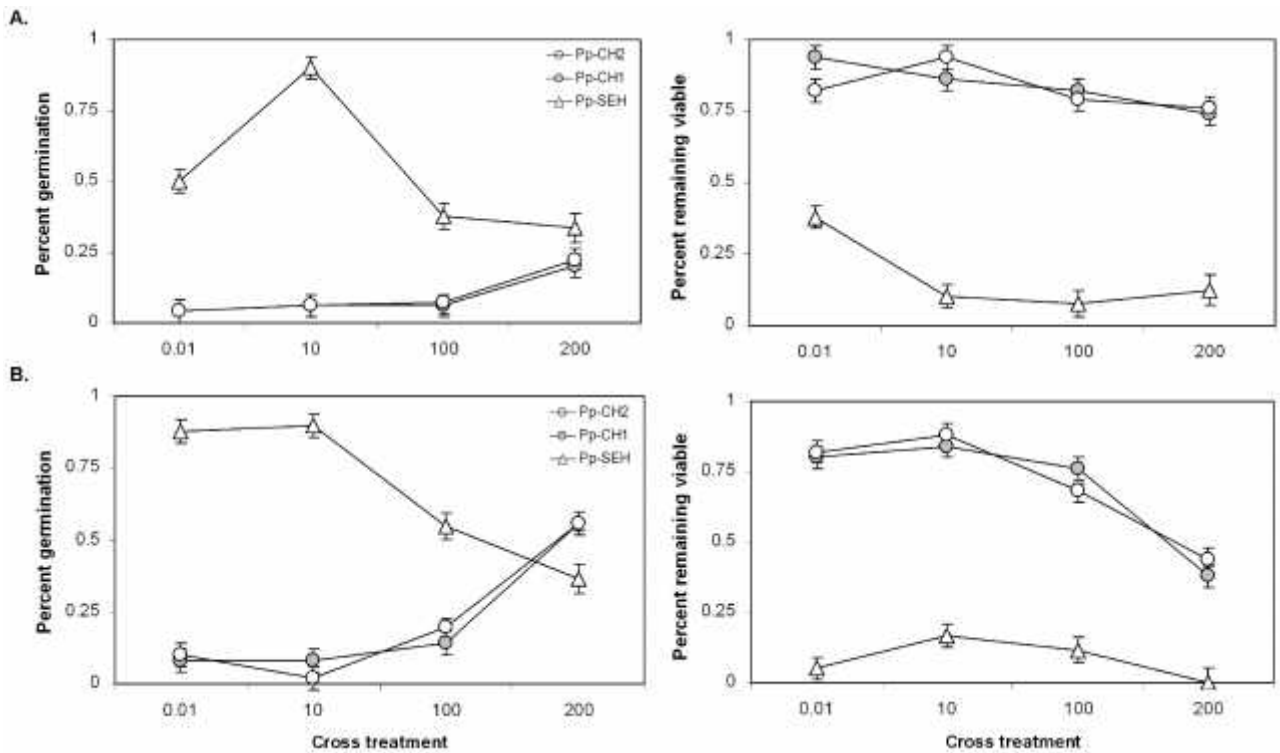

Figure 4.6. Least squares means and standard error bars showing percent of all *P. pachyphyllus* seeds that germinated (left) and percent of all seeds that remained viable at the end of the study (right) for each focal populations and cross treatment under A) short winter conditions equivalent to Pp-SEH, and B) under long winter conditions equivalent to Pp-CH1 and CH2.

Similar unexpected results were found for *P. rostriflorus*, and are shown in Figure 4.7. While Pr-SEL cross treatments generally follow expectations for outbreeding depression in increasingly distant crosses based upon optimal germination conditions at the site (short winter conditions, Figure 4.7A), as with Pp-SEL, results reflect an unexpectedly low percent germination in the within-population (0.01 km) cross in presumably optimal

conditions, and an unpredicted increase in percent germination in the within mountain-range (10 km) cross. Figure 4.7B reveals a nearly exact pattern to Figure 4.7A, with the only difference being length in cold stratification (12 versus 20 weeks). This again ran counter to expectations, as the 0.01 and 10 km cross treatments for Pr-CH were expected to exhibit high levels of germination in the long winter "optimal" conditions. Inspection of seeds remaining viable shows that all seeds either germinated or died in the long winter conditions, as did seeds from the Pr-SEL focal population in short winter conditions. The only seeds remaining viable at the end of the study were from the 10 and 100 km crosses on the Pr-CH focal population under non-optimal germination conditions (short winter length).

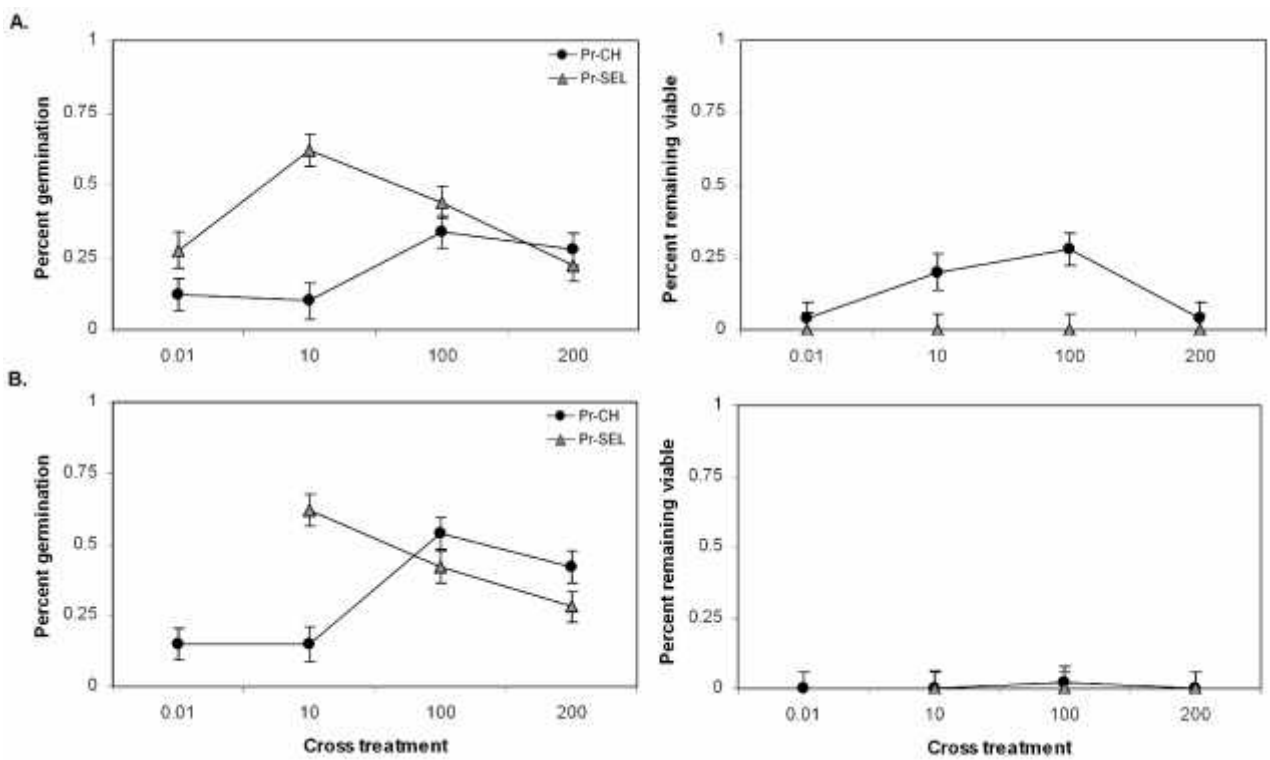

Figure 4.7. Least squares means and standard error bars showing percent of all *P. rostriflorus* seeds that germinated and percent of all seeds that remained viable at the end of the study (right) for each focal populations and cross treatment under A) short winter conditions equivalent to Pp-SEH, and B) under long winter conditions equivalent to Pp-CH1 and CH2.

4.3.3 Greenhouse study

Likelihood ratio tests (Table XXI) showed that germination and survival of *P. pachyphyllus* in the greenhouse study was not significantly explained by any factors, while in *P. rostriflorus* there were marginally significant tray effects (P = 0.0178) and a significant cross treatment effect (P = 0.0048), due primarily to a lower germination and survival rate in the 0 km (selfed) treatment. This provides only weak support for inbreeding depression in one population of *P. rostriflorus*. However, ANOVA results detected a slight tray effect on seedling weight in *P. pachyphyllus* but not *P. rostriflorus*, and most importantly, highly significant effects of cross treatment and competition treatment in both species (P < 0.0001). No interaction effects were significant, including the interaction of cross and competition treatments, meaning that in this case competition was not any more or less effective at revealing inbreeding or outbreeding depression in crosses between parents with extreme genetic similarity or increasing dissimilarity.

TABLE XXI
PERCENT GERMINATION AS A FITNESS MEASURE

		Survival			Seedling weight	
Penstemon pachyphyllus	d.f.	χ^2	P	d.f.	*F*	P
Tray	12	*18.821*	0.0930	11	*2.10*	**0.0181**
Cross treatment	4	*2.771*	0.5969	4	*72.28*	**<0.0001**
Competition treatment	1	*0.0835*	0.7726	1	*139.02*	**<0.0001**
Tray x Cross treatment	48	*16.505*	1.0000	48	*1.04*	0.3996
Tray x Competition treatment	12	*7.5873*	0.8165	12	*1.72*	0.0581
Cross treatment x Competition treatment	4	*2.1561*	0.7071	4	*0.23*	0.9208
Penstemon rostriflorus						
Tray	12	*24.423*	**0.0178**	7	*1.08*	0.3817
Cross treatment	2	*10.661*	**0.0048**	1	*31.83*	**<0.0001**
Competition treatment	1	*1.2106*	0.2712	1	*39.58*	**<0.0001**
Tray x Cross treatment	24	*19.173*	0.7427	19	*1.00*	0.4681
Tray x Competition treatment	12	*7.2774*	0.8387	12	*0.89*	0.5600
Cross treatment x Competition treatment	2	*0.6726*	0.7144	2	*1.72*	0.1820

Figure 4.8 shows the significant and universally negative effects of competition with *Bromus tectorum* on *Penstemon* seedling growth, regardless of cross treatment or species. It also shows a large and significant decrease in seedling weight at the most distant cross treatment (200 km) in *P. pachyphyllus* and a large and significant *increase* in seedling weight at the most distant cross treatment in *P. rostriflorus*. This increase was greater for the Pr-CH focal population than its reciprocal cross on the Pr-SEL population.

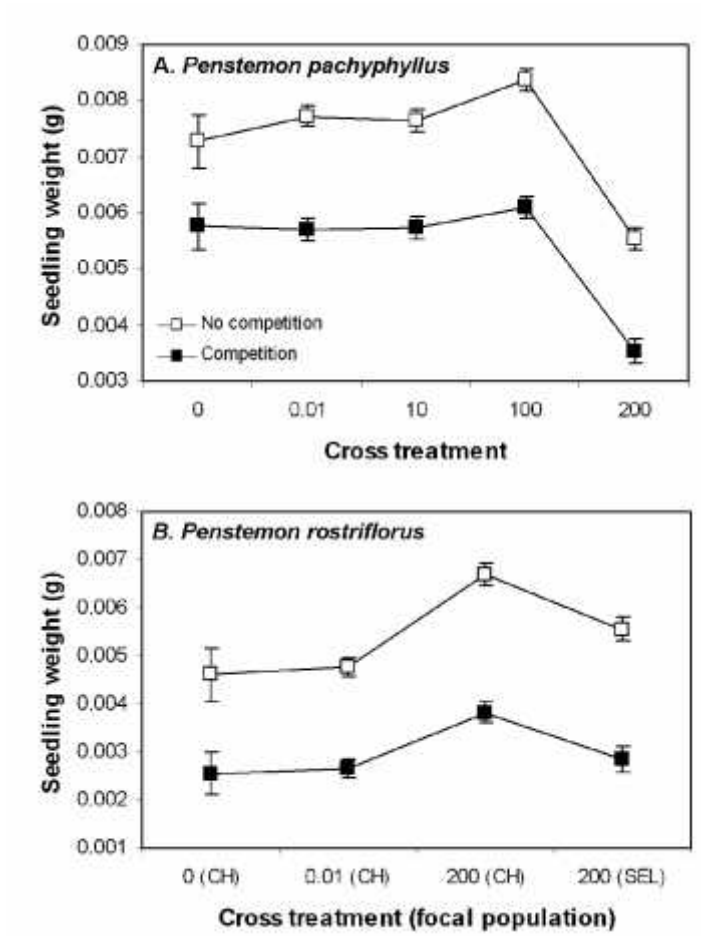

Figure 4.8. Least squares means and standard error showing effects of cross and competition treatments on greenhouse-grown F_1 progeny in A) focal population Pp-CH1 and 5 cross treatments for *P. pachyphyllus,* B) a combination of two focal populations (denoted in brackets by cross treatment) and 4 cross treatments for *P. rostriflorus*. Open squares denote plants grown with no competition; solid black squares denote plants grown in competition with *Bromus tectorum.*

4.4 DISCUSSION

This study of *Penstemon pachyphyllus* and *P. rostriflorus* occupying sky islands in the Great Basin revealed population-level divergence that significantly impacted fitness in inbred and outcrossed progeny. Considerable variation in fitness was observed among progeny resulting from selfing for different populations in both species. Furthermore, substantial variation in progeny fitness was documented for all measured traits, with a surprising demonstration of outbreeding depression in the first generation of crosses between genetically dissimilar parents. Specific fitness declines are a function of the maternal (focal) population and the geographic and genetic distance of the pollen donor, which in many cases can be explained by known patterns of genetic dissimilarity between populations identified in previous studies.

4.4.1 Inbreeding and outbreeding depression in cross success

Fitness declines, or inbreeding depression, have been detected in self-pollinated or nearest-neighbor crosses in numerous studies of plant species with an array of life history and dispersal backgrounds (Dudash, 1990; Waser and Price, 1991; Waser and Price, 1994; Fischer and Matthies, 1997; Byers, 1998; Bailey and McCauley, 2006). In this study, neither species was strictly self-incompatible, but self-pollination resulted in the production of fewer fruit and seeds than other cross treatments, regardless of focal population (Figure 4.3). Successful self-crosses, on average, resulted in smaller fruit and fewer seeds; three of four measures of cross success, including fruit weight, total seed weight per fruit, and seed count, revealed inbreeding depression in all three *P. pachyphyllus* focal populations (Figure 4.4). The fourth measure of cross success, average seed weight, showed no inbreeding depression effects; inbred plants produced smaller fruits with fewer seeds, but any seed that was produced was equivalent or larger in size compared to all other treatments. In this case, genetic similarity causes lower fitness, and indicates significant genetic load, or recessive deleterious alleles, in maternal plants used from all three focal populations. Within-population crosses (0.01 km) between more genetically dissimilar parents masked these lethal alleles and led to significant increases in fitness measures (or heterosis) for the same three measures. Additional fitness gains, or heterosis, were not identified beyond the within-population cross,

however, as the within-mountain cross (10 km) measures were never significantly different from within-population crosses.

Patterns of inbreeding depression in *P. rostriflorus* were generally less significant, trending towards greater fitness in progeny with increasingly dissimilar parents. The differences between *P. pachyphyllus* and *P. rostriflorus* may reflect a combination of differences in population size and the spatial structure of genetic variation within and among populations of each species. Results from microsatellite analysis showed that the hummingbird pollinators of *P. rostriflorus* are more effective at maintaining gene flow between populations over relatively large distances than the bee pollinators of *P. pachyphyllus*, but the within-population spatial genetic structure and history of inbreeding may also be quite different between species. Populations of *P. pachyphyllus* used in this study were at least an order of magnitude larger than *P. rostriflorus* populations, with fewer plants occupying a smaller space at a greater density in *P. rostriflorus* than *P. pachyphyllus.* These factors, and interactions with the behavior of primary pollinators for each species (bees for *P. pachyphyllus* versus hummingbirds for *P. rostriflorus)* may mean that *P. rostriflorus* is more often subjected to inbreeding which has purged more of its genetic load (Husband and Schemske, 1996; Byers and Waller, 1999) than *P. pachyphyllus*.

This study joins others that have identified both inbreeding and outbreeding depression, with some intermediate distance (e.g. optimal outcrossing distance) showing the greatest fitness (Waser and Price, 1989, 1994; Schierup and Christiansen, 1996; Fischer and Matthies, 1997; Waser, Price, and Shaw, 2000). Specifically, in *P. pachyphyllus* the highest fitness in measures of cross success were recorded for crosses made within-populations in this species. First generation progeny produced from parents that were increasingly geographically (and therefore genetically) dissimilar demonstrated outbreeding depression in all three focal populations of this species. This outbreeding depression, measured as a reduction in fitness from within-population crosses, was observed in multiple traits, including fruit weight, total seed weight per fruit, seed count and average seed weight, in three populations of *P. pachyphyllus*. As crosses were conducted in a common garden setting, the primary mechanism for this outbreeding depression is hybrid breakdown. This form of outbreeding depression is driven

by the loss of favorable epistatic interactions, and therefore expected to be more pronounced in second and following generations after recombination breaks up co-adapted gene complexes (Fenster and Galloway, 2000). However, the loss of favorable additive-by-additive epistatic interactions can occur in the first generation (Lynch, 1991), as is documented here. It is also possible in *P. pachyphyllus* that negative genetic interactions between nuclear and cytoplasmic genes in increasingly distant crosses are driving fitness declines in this first generation (Galloway and Fenster, 1999; Galloway and Fenster, 2001; Galloway and Etterson, 2005). Significant differences between traits like total seed weight and seed count in the 200 km cross treatment (which are reciprocal crosses between focal populations; Figure 4.4) indicate that negative nuclear and cytoplasmic interactions may contribute to observed outbreeding depression in this species.

Not all focal populations of *P. pachyphyllus* demonstrated similar patterns or levels of outbreeding depression with different cross treatments. Perhaps the most striking difference was in the 100 km cross treatment, where Pp-SEL performed significantly and consistently lower than either Pp-CH1 or CH2 (Figure 4.4). A number of other studies have identified similar disparities in study species and populations (Byers, 1998; Fenster and Galloway, 2000; Waser, Price, and Shaw, 2000; Bailey and McCauley, 2006), largely because geographic distance is not always an effective or consistent predictor of genetic dissimilarity. Given results of neutral genetic dissimilarity among populations identified in Chapter 2 and shown on Table XVIII, it is possible to pinpoint a potential cause of this disparity, as Pp-SEL and Pp-EH1 (used to generate the 100 km cross treatment) are more differentiated from each other (F_{ST} = 0.2866) than either Pp-CH1 or CH2 are from Pp-EH1 (F_{ST} = 0.1688 and 0.1799, respectively). Indeed, investigation of genetic dissimilarity between all cross populations of *P. pachyphyllus* (except between Pp-CH1 and CH2) was greater than for *P. rostriflorus* cross treatments. *P. rostriflorus* showed no strong or consistent signs of outbreeding depression, perhaps because its history of greater gene flow among populations has limited the formation of localized beneficial epistatic interactions.

Taken together, it is clear that populations of *P. pachyphyllus* responded significantly and differently to the different cross treatments, demonstrating both inbreeding depression in the selfed treatments and outbreeding depression in the distant crosses, while *P. rostriflorus* did not (Figure 4.4). Insight into the mechanisms which underlie outbreeding depression can be gained by comparing the very different outcomes of these studies between *P. pachyphyllus* and *P. rostriflorus*. While significant evidence for outbreeding depression was documented in *P. pachyphyllus*, little to none was found in *P. rostriflorus*, despite the fact that crosses were made between populations in a nearly identical array between populations and mountain ranges. The key difference between these two species appears to be the extent to which gene flow and genetic drift are acting to drive population divergence. Co-adapted gene complexes with additive-by-additive epistasis are present in *P. pachyphyllus*, likely facilitated by the relative isolation of populations on different mountain ranges driven by the fact that its bee pollinators do not effect among-mountain-range gene flow. *P. rostriflorus* populations, however, are much more connected though gene flow by their hummingbird pollinators, and therefore appear to have more limited opportunities for co-adapted gene complexes to form. It is, of course, possible that further study involving additional generations (e.g. additional recombination in hybrids) would reveal outbreeding depression caused by the loss of favorable epistatic interactions even in *P. rostriflorus*, as in Fenster and Galloway (2000). However, these immediate, first generation differences between species are striking, and provide further evidence of the significant influence pollination syndrome has on the evolutionary trajectory of a plant species.

4.4.2 Fitness measures in seed germination requirements

Seed germination trials confirmed that seed germination requirements (in terms of germination timing in days of chilling) are heritable and likely the result of additive genetic effects (Figure 4.5), particularly for *P. rostriflorus*. These results confirmed the validity of a reciprocal transplant study utilizing different simulated winter lengths to identify a potential dilution mechanism to outbreeding depression, but results from this experiment created more questions than it answered. While focal populations and cross treatments certainly responded differently to each other and different simulated winter lengths (Figures 6 & 7), resulting outcomes were neither predicted nor easily explainable.

One population (Pp-SEH) may have demonstrated slight outbreeding depression via a dilution mechanism with increasingly geographically and climatically dissimilar pollen donors (Figure 4.6A), as the most distant cross had the lowest percent germination. However, the mixed results of this portion of the study draw into question the utility of using percent germination as a measure of fitness. It is often used as a key trait to measure fitness (Fenster and Galloway, 2000; Galloway and Etterson, 2005), but this is usually undertaken in studies on annuals. As shown here, germination it is not always a useful measure in and of itself, particularly in a long-lived perennial that likely relies on seed banking to maintain positive demographic growth in a stressful, unpredictable and sometimes extreme habitat (Meyer and Kitchen, 1992, 1994). If ability to seed bank is a component of fitness here, the longer distance crosses (100 and 200 km) on the Pp-SEH focal population may be demonstrating even greater outbreeding depression, as seeds either germinated or died in these treatments, whereas viable seed remained at the end of the study for the 0.01 km (within-population) cross treatment. It is possible that seeds from these distant crosses were more susceptible to disease given their small size (Figure 4.4) or the negative genetic interactions that led to their small size, as in Goldberg et al. (2005).

4.4.3 Outbreeding depression in competitive greenhouse environments

In growing a selection of seeds produced through different cross treatments for each species in a common environment, the potential for nuclear and cytoplasmic interactions was identified in *P. rostriflorus,* and an additional level of outbreeding depression was detected in *P. pachyphyllus.* First, Figure 4.7 shows that seedlings produced via long-distance (200 km) crosses in which Pr-CH was the dam and Pr-SEL was the sire grew larger than when Pr-SEL was the dam and Pr-CH was the sire. Ideally, these plants would be grown out in each maternal environment over multiple seasons to identify the potential for outbreeding depression or continued disparities in progeny fitness given different maternal and paternal genetic contributions (Waser and Price, 1994). Second, additional outbreeding depression was detected in progeny of *P. pachyphyllus* plants from Pp-CH1 subjected to the 200 km cross treatment (crossed with Pp-SEH). These plants were significantly less fit than all other cross treatments, regardless of competition treatment (Figure 4.8). The seeds used for this cross treatment

were significantly smaller than for all other treatments (Figure 4.4), so the very small seedlings in this treatment may have been a direct result of this fact and the very little maternal provisioning such small seeds may have allowed. However, because no relationship was detected between seedling size and seed weight in a common garden study utilizing the same populations (A. Kramer, unpublished), it is likely that whatever negative genetic effects (via the hybrid breakdown mechanism) led to decreased seed size in this treatment also led to at least some of the size effects detected here.

4.4.4 Implications for restoration

Climate change is driving plants and animal species up the slopes of mountain ranges and poleward, creating both opportunities and challenges for restoration practitioners. Opportunities present themselves as invasive species, like cheatgrass, experience range contraction in regions that become climatically unsuitable (Bradley, 2009), opening up potentially vast acreage to restoration potential. Yet major challenges exist around how to undertake restoration that allows plant and animal communities to survive in the short-term and persist over the long-term as the climate changes. Increasingly, this is leading to discussions of assisted colonization (Hoegh-Guldberg et al., 2008) as a management tool to avoid species extinction and maintain diverse communities. My findings of significant outbreeding depression in the bee-pollinated *P. pachyphyllus* at both the 100 and 200 km cross distances for numerous fitness measures clearly demonstrate the negative impacts the inappropriate movement of seed may have on the long-term reproduction and survival of the restored population or any nearby intact populations if pollinators facilitate gene flow between them, or if seed mixtures are created from a number of broadly distributed populations. However, these results, combined with findings from Chapter 2 demonstrating very little gene flow between populations of bee-pollinated species separated by mountain ranges or by over 5 km on the same mountain range, suggest that the likelihood of gene flow between restored and intact populations will be low if they are sufficiently far from one another.

This is not the case for the hummingbird-pollinated *P. rostriflorus,* where no immediate outbreeding depression was identified in seed production or seedling growth measures, suggesting that the movement of seeds

as a restoration technique to help populations adapt to climate change may pose less risk. However, because common garden studies (Chapter 3) identified likely local adaptation in important traits like seed germination requirements in this species, it is possible that outbreeding depression would be detected in later life history stages, under different environmental conditions, or in following generations as recombination disrupts coadapted gene complexes. And because microsatellite markers showed higher levels of gene flow among populations separated by greater distances, the risk of pollinators effecting gene flow between restored and intact populations is also greater than for the bee-pollinated *P. pachyphyllus*. In order to develop true guiding principles to help direct restoration in a changing climate, more research is needed on these and similarly distributed and pollinated species. Ultimately, when assisted colonization is used as a restoration technique in the Great Basin or any landscape, it should be approached as a research opportunity, and long-term monitoring must be in place to detect any impacts on the short and long-term survival of the restored and any nearby intact populations.

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