EVOLUTION AND DYNAMICS OF HYBRIDIZATION IN
PENSTEMON SUBGENUS DASANTHERA (SCROPHULARIACEAE S.L.)

DISSERTATION

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ABSTRACT

*Penstemon* subgenus *Dasanthera* is a small group of 16 taxa distributed at high elevations in western North America. Hybridization is common in the subgenus when two or more species occur in sympathy. In this study, I examined evolutionary trends and the importance of hybridization and gene flow to the evolution of the subgenus. Phylogenetic relationships among members of *Penstemon* subgenus *Dasanthera* were assessed using ITS and *matK* sequence data, and inter-simple sequence repeat (ISSR) markers to elucidate biogeographic relationships and morphological trends in the subgenus. These data support previous hypotheses suggesting that the Cascade-Sierra Nevada lineage is derived from the northern Rocky Mountain lineage. Furthermore, there is a shift in growth form from deciduous perennials to evergreen, woody subshrubs concurrent with migration to the Cascade-Sierra Nevada mountains. Within the Cascade-Sierra lineage, *P. newberryi* and *P. rupicola* display a series of floral shifts that may represent adaptations for hummingbird pollination. The molecular data presented here also demonstrate the utility of dominant marker data for reconstructing phylogenetic relationships among closely-related species.

To examine the potential outcomes of hybridization in the subgenus, ISSR and morphological markers were used to assess hybrid zone structure and patterns of gene
flow between *Penstemon davidsonii* and *P. rupicola* on Wizard Island in Crater Lake National Park, Oregon (USA). On Wizard Island, a hybrid zone was found on the southwest portion of the island, in addition to unintrogressed populations of both species throughout the island. I surveyed three hybrid subpopulations from different localities in the hybrid zone to examine population structure throughout the hybrid zone. In each subpopulation, plants were categorized based on morphological characters diagnostic for *P. davidsonii* and *P. rupicola*. Using molecular data, the proportion of *P. davidsonii*-typical and *P. rupicola*-typical ISSR bands was calculated for each putative hybrid. Comparisons were made between hybrid categories and unintrogressed populations of *P. davidsonii* and *P. rupicola* using an approximation of Fisher's Exact Test to test for directionality of gene flow. These results indicate an asymmetrical pattern of gene flow and the potential for introgressive hybridization in these species.

The observed asymmetry in the pattern of gene flow on Wizard Island could be the result of either endogenous selection pressures, in the form of pre-pollination barriers (ethological isolation) or post pollination barriers (pollen-tube growth rate or seed-siring ability), or exogenous selection in the form of differential fitness of hybrids in hybrid zones. I tested for differences in both pollinator visitation rates and pollinator behavior during visitation, and differential seed siring ability for *P. davidsonii* and *P. rupicola* on the observed pattern of gene flow on Wizard Island. Although the same suites of pollinators visit both *P. davidsonii* and *P. rupicola*, the frequency of visitation varies between the two species. *Penstemon rupicola* is visited primarily by pollen-collecting insects, including syrphid flies and sweat bees, that probably do not serve as effective
pollinators, whereas *P. davidsonii* is visited primarily by leafcutter bees that may be good pollinators. From these studies, it is not clear how pollinator visitation affects gene flow in hybrid zones. Fruit set and seed set data demonstrate that *P. rupicola* shows a greater seed-siring ability relative to *P. davidsonii* for heterospecific crosses. However, seed germination rates were higher for *P. davidsonii* than for *P. rupicola* for all cross types, and highest for heterospecific crosses. These results suggest that *P. davidsonii* serves as a better maternal parent with regard to seed germination. The data presented suggest that the factors influencing hybrid zone structure on Wizard Island are complex, and probably include a combination of pre-pollination barriers, post-pollination barriers, and selection for fitness traits in the hybrid zone.
Dedicated to my family.
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CHAPTER 1

INTRODUCTION

Hybridization has long been noted as an important evolutionary process in plants at both the diploid and polyploid levels (Anderson 1948; Anderson 1949; Arnold 1997; Jackson 1976; Levin 1983; Lewis 1980; Soltis and Soltis 1993; Stebbins 1959; Stebbins 1969). Potential outcomes of hybridization at the diploid level include (1) hybrid speciation (Arnold 1993; Galiez and Gottlieb 1982; Rieseberg 1997; Sang et al. 1995; Wolfe and Elisens 1993), (2) introgression (Anderson 1949; Arnold 1997; dePamphilis and Wyatt 1990; Rieseberg et al. 2000; Wendel et al. 1991; Wolfe and Elisens 1995; Wolfe et al. 1998a), and (3) genetic assimilation of a rare taxon by a more common congener (Abernathy 1994; Leary et al. 1993; Levin et al. 1996; Liston et al. 1990; Rhymer and Simberloff 1996; Rieseberg 1991b).

Hybridization can have a profound influence in natural populations as a result of introgression, or leakage, of genetic material from one species into another. Introgression of fitness characters through hybrid zones may result in differential fitness of hybrids, allowing hybrid genotypes to occupy a wider range of habitats. Furthermore, such a scenario may have disastrous effects in situations where one species is rare or threatened.
(Levin et al. 1996; Rhymer and Simberloff 1996; Rieseberg 1991b; Rieseberg et al. 1990), or in hybrid zones involving invasive species (Albert et al. 1997; Anttila et al. 1998).

Hybrid zone structure is the result of both endogenous selection pressures, such as differences in mating system and genetic compatibility, and exogenous selection in the form of differential survivorship of hybrids based on environment-dependent selection (Arnold 1997). Mating system differences and hybrid fitness are of primary importance in determining hybrid zone structure and patterns of gene flow in hybrid zones. Studies of Louisiana iris hybrids have demonstrated that hybrids are often more fit than parental species in hybrid zones, suggesting that hybrids confer an evolutionary advantage in the hybrid zone (Arnold et al. 2001; Cruzan and Arnold 1993; Emins and Arnold 1997; Johnston et al. 2001a). Fitness advantages have also been noted in reproductive characters in hybrid zones involving rare and introduced species. Although numerical advantage is often considered to be of primary importance in determining hybrid zone structure (Arnold et al. 1993; Cruzan and Arnold 1994; Levin et al. 1996; Rieseberg and Gerber 1995), fitness advantages of the rare species have occur hybrid zones of an introduced, locally-rare species of Spartina (Anttila et al. 1998; Daehler and Strong 1997).

The potential value of hybridization and natural hybrid zones has recently been studied in the context of understanding the processes of adaptive evolution (Bradshaw et al. 1998; Hodges and Arnold 1994; Rieseberg 1998; Rieseberg et al. 2000; Rieseberg et al. 1996a; Rieseberg et al. 1999; Schemske and Bradshaw 1999). QTL-mapping studies
with the bumblebee-pollinated *Mimulus lewisii* and hummingbird-pollinated *M. cardinalis* demonstrated that pollinator visitation was dramatically affected by anthocyanin content, carotenoid pigmentation, nectar volume and content, and projected area, all of which are under relatively simple genetic control (Bradshaw et al. 1998; Bradshaw et al. 1995; Schemske and Bradshaw 1999). These studies show that population-level studies focusing on selection for adaptive traits can provide information for understanding the reinforcement of species barriers (Schemske and Bradshaw 1999). Genetic mapping of artificially-synthesized and homoploid hybrid species of *Helianthus* have demonstrated selection for similar chromosomal blocks in both synthesized hybrids and homoploid hybrid species (Kim and Rieseberg 1999; Rieseberg 1998; Rieseberg et al. 1996a). Such studies will be invaluable in understanding population-level processes that are potentially important, not only in understanding hybrid zone evolution, but also in teasing apart adaptive traits in divergent evolution.

Examining evolutionary relationships among organisms is an important precursor to studies of evolutionary processes in biology. Coupled with phylogenetic studies, hybrid zone evolution can contribute to knowledge of underlying processes contributing to biodiversity through understanding reproductive isolation and species barriers. The work presented here examines evolutionary relationships and the evolutionary importance of hybridization *Penstemon* subgenus *Dasanthera* (Raf.) Pennell, a small subgenus of closely-related hybridizing species. These data illustrate some of the potentially important morphological and ethological barriers to hybridization, and the importance of hybridization to the modern day diversity of *Penstemon* subg. *Dasanthera*. 
1.1. Study system.— *Penstemon* Mitchell is the largest genus of plants endemic to North America, with approximately 275 species, and the largest genus of the tribe Cheloneae. The original circumscription of *Penstemon* included several other genera, including *Keckiella*, *Nothochelone*, and *Pellennianthus*. These genera were segregated from *Penstemon* by Straw (1966; 1967), and Crosswhite and Kawano (1970) based on chromosome numbers and differences in nectary type. Whereas most members of the tribe have nectaries in the form of a hypogynous disk, *Penstemon* and *Chionophila* both have epistaminal nectaries. Further segregating *Penstemon* from *Chionophila* is the presence of unwinged, angular seeds. DNA sequence data further supported the segregation of these genera from *Penstemon* based on DNA sequence data (Wolfe et al. in press).

As currently circumscribed, *Penstemon* subgenus *Dasanthera* (Raf.) Pennell comprises 16 taxa distributed in western North America: *Penstemon barrettiae* Gray, *P. cardwellii* Howell, *P. davidsonii* Greene var. *davidsonii*, var. *menziesii* (Keck) Cronq., and var. *praeteritus* Cronq., *P. ellipticus* Coul. & Fish., *P. fruticosus* (Pursh) Greene var. *fruticosus*, var. *serratus* (Keck) Cronq. and var. *souleri* (Lindl.) Cronq., *P. lyalli* Gray, *P. montanus* Greene var. *montanus* and var. *idahoensis* (Keck) Cronq., *P. newberryi* Gray var. *newberryi*, var. *berryi* (Eastw.) Holmgren, and var. *sonomensis* (Eastw.) Holmgren and *P. rupicola* (Piper) Howell. Members of the subgenus are characterized by dense, lanate pubescence on the surface of the anthers. In most species, the anthers are held together in a single unit by the intertwining of these hairs. Furthermore, members of the subgenus have a dorsal keel extending the full length of the corolla. Species in the
subgenus are also characterized by a variable degree of woodiness. Three species, *P. ellipticus*, *P. lyallii*, and *P. montanus*, are deciduous perennials from either a woody caudex or woody rhizomes. In contrast, all other species in the subgenus are evergreen, woody subshrubs. Furthermore, the deciduous species are all restricted to the northern Rocky Mountains whereas the evergreen species are distributed primarily in the Cascade and Sierra Nevada mountain ranges (Every 1977). Previous studies based on variation in growth form, chromosome number, and flavonoid data (Every 1977; Straw 1966) have suggested that the northern Rocky Mountain species are basal within the subgenus.

Pollination syndrome also varies in the subgenus. Two species, *P. newberryi* and *P. rupicola*, have several differences in floral morphology that are hypothesized to be related to differences in pollination syndrome. Most species in the subgenus have purple or blue-purple flowers, anthers inserted within the corolla tube, and anther sacs facing one another. *Penstemon newberryi* and *P. rupicola* have pink to magenta flowers, anthers exserted from the corolla tube, and the anthers are oriented on a flat plane, all of which are associated with hummingbird pollination (Every 1977). In contrast, other members of the subgenus appear to be primarily bee-pollinated (Every 1977).

*Penstemon* subgenus *Dasanthera* has been considered an “old” lineage within *Penstemon* (Savile 1968), and therefore understanding the phylogenetic relationships within the subgenus and its relationship to the rest of *Penstemon* are of key importance in understanding the biogeographic relationships in *Penstemon* and character evolution within the subgenus.
Despite differences in habitat specificity and pollination syndrome, hybridization is common in the subgenus, especially among species in the Cascade/Sierra Nevada mountain species (Every 1977). In the Cascade Mountain Range, hybrid zones have been reported between *P. davidsonii* and *P. rupicola*, *P. davidsonii* and *P. cardwellii*, *P. davidsonii* and *P. fruticosus*, *P. davidsonii* and *P. newberryi*, *P. rupicola* and *P. cardwellii*, *P. rupicola* and *P. fruticosus*, *P. fruticosus* and *P. barrettiae*. Three-way hybrid zones involving *P. davidsonii*, *P. rupicola* and *P. fruticosus* have been reported from Mt. Adams in Washington and Mt. Hood in Oregon (Every 1977). Furthermore, based on morphology and flavonoid data, two taxa in the subgenus are hypothesized to be of hybrid origin between *P. davidsonii* var. *davidsonii* and *P. fruticosus* var. *fruticosus*: *P. davidsonii* var. *praeteritus* and *P. fruticosus* var. *serratus*. *Penstemon newberryi* var. *newberryi* has also been hypothesized to be an introgressant form of *P. newberryi* as a result of hybridization with *P. cardwellii* (Every 1977). Hybrid zones have also been reported between *P. lyallii* and *P. ellipticus*, and *P. ellipticus* and *P. fruticosus* in the Northern Rocky mountains, but hybrid zones are much less common among species in the Northern Rocky Mountains (pers. obs.)

The overall goals of this work were to examine the evolutionary patterns in *Penstemon* subg. *Dasanthera* in order to identify potentially important adaptive traits within the subgenus and to examine the evolutionary importance of hybridization in the subgenus. Chapter two examines evolutionary relationships and character evolution within *Penstemon* subgenus *Dasanthera* using both sequence data and inter-simple sequence repeat (ISSR) markers. By examining important morphological shifts, the
results presented here provide background information on evolutionary adaptations in the subgenus. Chapter three examines patterns of hybridization between *P. davidsonii* and *P. rupicola*, two species demonstrating differences in pollination syndrome, in Crater Lake National Park in order to assess the potential evolutionary outcomes of hybridization in this system. Chapter four examines two possible factors, pollinator visitation and seed siring ability of *P. davidsonii* and *P. rupicola*, and the effect of these factors on the observed asymmetry in the hybrid zone on Wizard Island. Together, this work provides the background for studies of adaptive trait evolution and factors promoting speciation in *Penstemon* subgenus *Dasanthera*. 
CHAPTER 2

PHYLOGENETIC RELATIONSHIPS AND MORPHOLOGICAL SHIFTS AMONG

PENSTEMON SUBG. DASANTHERA (SCROPHULARIACEAE S.L.)

2.2 INTRODUCTION

Reconstructing evolutionary relationships among closely-related species using molecular sequence data is often complicated by not having enough data to resolve relationships adequately. In plants, the internal transcribed spacer (ITS) region and quickly-evolving chloroplast introns and intergenic spacers have been used to examine species-level relationships with some success (Albach and Chase 2001; Baldwin et al. 1995; Demesure et al. 1995; Kim et al. 1996; Manos 1997; McDade and Moody 1999; Oxlman et al. 1997; Small et al. 1998). However, these regions are often insufficient for resolving relationships at the interspecific level (e.g., Small et al. 1998). A number of recent studies have used quickly-evolving introns of nuclear genes (e.g., alcohol dehydrogenase [Adh], granule bound starch synthase [GBSS-1, or waxy], glyceraldehyde 3-phosphate dehydrogenase [G3pdh], and glycerol-3-phosphate acyltransferase [Gpat]). These studies have demonstrated the utility of these regions at the intraspecific level.
(Evans et al. 2000; Mason-Gamer et al. 1998; Mathews et al. 2000; Olsen and Schaal 1999; Sang and Zhang 1999; Small et al. 1998; Tank and Sang 2001). Furthermore, the 18S-26S external transcribed spacer of the nuclear ribosomal DNA repeat has demonstrated higher levels of polymorphism than ITS sequence data (Andreasen and Baldwin 2001; Baldwin and Markos 1998; Clevinger and Panero 2000; Linder et al. 2000). Although these regions typically provide much more phylogenetically-informative data at the interspecific level, there is a large investment of time and money to characterize paralogous gene copies and design primers for regions with high levels of variation. Additionally, although these regions can potentially provide information about past hybridization events, they can be problematic with regard to lineage sorting and coalescence of gene trees. Therefore, sampling many individuals within each species may be necessary (Avise 1998).

Hypervariable dominant markers, including randomly amplified polymorphic DNA (RAPDs) and inter-simple sequence repeat (ISSR) markers, are typically used for applications involving one or a few species. Some of the applications of dominant markers are: (1) examining genetic variation at the intraspecific level (Esselman et al. 1999; Friar et al. 1996; Rieseberg and Gerber 1995; Schierenbeck et al. 1997; Stewart and Porter 1995; Tsumura et al. 1996), (2) identifying species-diagnostic markers (Arnold 1993; Bartish et al. 2000; Daehler and Strong 1997; Dawson et al. 1996; Martin and Cruzan 1999; Smith et al. 1996; Wolfe et al. 1998a), and (3) generic trait loci (Arcade et al. 2000; Blair et al. 1999; McGregor et al. 2000; Rafalski and Tingey 1993; Zietkiewicz et al. 1994). Relatively few studies that used data from dominant markers to
examine interspecific relationships (Borowsky et al. 1995; Federici et al. 1998; McArthur et al. 1998a; McArthur et al. 1998b; Nair et al. 1999; Pacak et al. 1998; Wolfe and Randle 2001; Zouhair et al. 2000).

Using data from dominant markers to examine evolutionary relationships can be complicated. First, several studies have suggested that data from dominant markers, such as RAPDs, are often not repeatable (Smith et al. 1994; Williams et al. 1993). Second, the homology of comigrating bands is often questionable when examining interspecific relationships, especially as taxonomic distance increases (Adams and Rieseberg 1998; Rieseberg 1996). Finally, data analysis is complicated by the anonymous nature of the bands, lack of information on heterozygosity, and high levels of polymorphism. As a result, most bands are polymorphic within species, making sampling strategy very important, and complicating analyses using cladistic methods (Wolfe and Liston 1998).

Although studies by Rieseberg (1996) and Adams and Rieseberg (1998) demonstrate that non-homology of comigrating bands does not have a great effect on the overall results or data interpretation based on ordination methods, no consensus exists on how dominant marker data should be treated. Ordination methods provide information about the distinctiveness of groups or species, but not about relative relationships among groups or species (Adams and Demeke 1993). Tree-based ordination methods, such as Neighbor-Joining (Saitou and Nei 1987) and UPGMA (Sneath and Sokal 1973) are often used to examine relative relationships among species, or clusters of individuals or populations. However, these methods may force hierarchy into non-hierarchical systems. Cladistic analyses have been used in some studies (Borowsky et al. 1995; Huang and Sun
2000; Wolfe and Randle 2001) but may also be inappropriate in cases where bands are polymorphic within species, and accounting for intraspecific polymorphisms is rarely addressed (however, see Wiens 1999).

In this study, we have examined the utility of ISSR markers for phylogeny reconstruction in *Penstemon* subg. *Dasanthera*, a small group of closely-related, hybridizing species. Subgenus *Dasanthera* is sister to the rest of *Penstemon* (Wolfe et al. in press). Therefore, understanding evolutionary and biogeographic relationships within the subgenus is important for understanding the evolution of the subgenus as a whole.

As originally circumscribed (Wettstein 1891), *Penstemon* included nine other species in three genera: *Nothochelone* (1 spp.), *Keckiella* (7 spp.), and *Pennelianthus* (1 spp.). These three genera were segregated from *Penstemon* based on chromosome number, nectary position, and seed characters (Crosswhite and Kawano 1970; Straw 1966). Segregation of these genera has been further supported by DNA sequence data (Wolfe et al. in press).

Currently, subgenus *Dasanthera* is circumscribed as nine species and seven varieties, distributed at high elevations in western North America (Every 1977; Straw 1966). Members of the subgenus are characterized by dense, lanate pubescence on the anther surface and a dorsal keel extending down the length of the corolla tube. Most members of this group are woody subshrubs, but some variation exists in growth form, from suffrutescent perennials with a woody caudex to evergreen woody subshrubs. Morphological characters such as leaf size, leaf shape, and surface waxes are variable within species and overlapping among species (Every 1977). In addition, hybridization is
common among many species pairs, especially among the western Cascade/Sierra taxa, further complicating species identification. The importance of hybridization in the evolution of the subgenus is highlighted by the hypothesized hybrid origin of *P. davidsonii* var. *praeteritus* and *P. fruticosus* var. *serratus*, with *P. davidsonii* var. *davidsonii* and *P. fruticosus* var. *fruticosus* as parental taxa, based on intermediate morphology and flavonoid data (Cronquist 1964; Every 1977). Furthermore, *P. newberryi* var. *berryi* apparently has combined morphological and chemical features of *P. rupicola* and *P. cardwellii* (Every 1977).

Within the subgenus, there are two primary centers of diversity: the northern Rocky Mountains and the Cascade and Sierra Nevada mountain ranges. Four taxa are restricted to the northern Rocky Mountains: *P. lyallii* Gray, *P. montanus* Greene var. *montanus* and *P. montanus* var. *idaheensis* (Keck) Cronq., and *P. ellipticus* Coulter & Fish., and eight taxa are found in the Cascade/Sierra mountains and west: *P. fruticosus* (Pursh.) Greene var. *fruticosus*, *P. fruticosus* var. *serratus* (Keck.) Cronq. and *P. fruticosus* var. *scouleri* (Lindl.) Cronq., *P. davidsonii* (Greene) var. *davidsonii*, *P. davidsonii* var. *menziesii* (Keck) Cronq., and *P. davidsonii* var. *praeteritus* Cronq., *P. cardwellii* Howell, *P. barrettiae* Gray, *P. rupicola* (Piper) Howell, and *P. newberryi* Gray var. *newberryi*, *P. newberryi* var. *berryi* (Eastw.) Holmgren, and *P. newberryi* var. *sonomensis* (Eastw.) Holmgren. Three of the Cascade/Sierra taxa are found in the intermountain regions of Oregon, Washington, and southern Canada: *P. fruticosus* (all varieties) and *P. davidsonii* var. *praeteritus*. The differences in growth form within the subgenus are correlated with distribution. All of the deciduous species are restricted to
the Northern Rocky Mountains, whereas the evergreen woody species are found in the
Cascade/Sierra mountains and one species (P. fruticosus) spans the range of both. Based
on leaf surface characters and flavonoids, Every (1977) hypothesized that the Rocky
Mountain species were primitive within the subgenus and that the woody habit is derived
in Penstemon, supporting the biogeographic hypotheses set forth by Straw (1966) that
suggest an origin of Penstemon in the northern Rocky mountains from Chionaphila
Benth.

In this study, we have used a combination of DNA sequence data and ISSR
markers to gain insight into evolutionary and biogeographic relationships in Penstemon
subg. Dasanthera, and we hope to provide a framework to study the nature of adaptive
characters in Penstemon subg. Dasanthera. The specific objectives of this study were to:
(1) investigate evolutionary relationships among species within Penstemon subgenus
Dasanthera using a combination of DNA sequence data and ISSR markers, (2) identify
key morphological transitions among members of subgenus Dasanthera, and (3) assess
the utility of dominant marker data for examining evolutionary relationships among a
closely-related species group.

2.2 MATERIALS AND METHODS

DNA was extracted from either fresh-frozen or silica gel dried leaf material using
a modified CTAB protocol (Wolfe and Randle 2001). All species in Penstemon subg.
Dasanthera and all available varieties were used in the DNA sequencing study. In
addition, six species of *Keckiella*, two species of *Chionophila*, *Nothochelone nemorosa*, two species of *Chelone*, and four species of *Penstemon* representing other subgenera were included as outgroups in these analyses. All available collections of *Penstemon* subg. *Dasanthera* were used for the ISSR survey (Table 2.1).

2.2.1 Sequence data.— PCR products were generated for both ITS and *matK* following Wolfe and Randle (2001) and Wolfe et al. (in press). PCR products were cleaned for sequencing using the Concert rapid PCR purification system (Gibco BRL), PEG precipitation of PCR products, or isolated from agarose gels using the Sephaglas kit (Pharmacia).

Di-deoxy termination sequencing was performed using both manual and automated techniques. Manual sequencing was performed using the dITP terminators of the USB DNA sequencing kit. Automated cycle sequencing reactions were performed using the Big Dye terminator chemistry (ABI) and reactions were run on an ABI 310 automated DNA sequencer. Double stranded sequences were generated for both ITS and *matK* regions. *MatK* sequencing protocol follows Wolfe et al. (in press).

Sequences were assembled using AssemblyLign (Oxford) or Sequencher (Gene Codes Corp.). Alignments were made using ClustalX with manual adjustments as necessary. Analyses were performed separately for ITS and *matK* data sets in addition to a combined data set. Phylogenetic analyses were performed using PAUP* 4.0b8 (Swofford 1999). Uninformative characters were excluded from all analyses. Trees were generated using the heuristic search option with 100 random addition sequences. Support for each node was assessed using 500 bootstrap (Felsenstein 1985) replicates (10 random
addition sequences per replicate, maxtrees set at 10,000) and Bremer support estimated by the converse constraints method (Bremer 1988; Bremer 1994). Congruence between data sets was assessed using the partition homogeneity test (Johnson and Soltis 1998) with 500 replicates, which is a modification of incongruence length difference (Mickevich and Farris 1981). To identify the source of incongruence, multiple tests were performed with single taxon deletions.

2.2.2 ISSR Data.— To test the utility of ISSR markers for inferring evolutionary relationships within Penstemon subgenus Dasanthera, we used data from five ISSR primers (Table 2.2). Taxon sampling for this study included as many representatives as possible for each species within the subgenus, and when possible, population-level sampling. However, sampling was limited for some species, especially the Rocky Mountain taxa (Table 2.1).

ISSR reactions were PCR-amplified in 25 μl reaction volumes following standard conditions as follows: 20 mM Tris-HCl pH 8.4; 50 mM KCl; 12.5 μM each dATP, dCTP, dGTP, and dTTP; 0.4 μM primer and 0.5 μl template DNA. Both MgCl₂ and Taq DNA polymerase concentration were optimized for each primer. Reaction conditions are summarized in Table 2.2. Thermal cycler conditions were as follows: initial denaturation at 94°C for 90 s, 35 cycles of 94°C for 45 s, annealing (temperatures specified in Table 2.2) for 45 s, 72°C extension for 90 s and a final extension cycle for 5 min. Reactions were run on 1.2% agarose gels in 1X Tris Acetate EDTA (TAE) buffer. Gels were run until bromphenol blue indicator dye migrated 10-11 cm, and stained with ethidium bromide; images were viewed under UV light and recorded digitally. Two
replicates were run for all reactions to test for repeatability of bands. Bands that were not present in both replicates were eliminated from the analysis. A binary matrix was constructed from presence-absence of all bands produced with five primers where each distinctively migrating band was scored as a unique locus. All individuals that had any missing data were excluded from the data set. Evolutionary relationships were assessed using both distance and parsimony criteria.

Pairwise similarities were computed between all individuals in the matrix using the Dice (1945) coefficient in a software package provided by V. Ford (UC Davis, unpublished; available from the author). Neighbor-Joining trees were generated using the Neighbor component of PHYLIP version 3.57c (Felsenstein 1993) with randomized input order. Analyses were conducted with all bands included, and with deletions of rare bands at frequencies equal to or less than 0.025, 0.050, 0.075, 0.100, 0.125, and 0.150 intervals to test the effects of rare bands on the clustering patterns in Neighbor-Joining trees. This is based on the observation that low frequency bands often show low levels of homology as revealed by Southern hybridization (Datwyler and Wolfe unpubl.). Additionally, individual taxon deletions were performed on individuals that moved among clusters in rare band deletions.

To test the utility of coding band frequencies as character states in cladistic analyses, data matrices were constructed using several different methods for coding polymorphic characters (Wiens 1999). Band frequencies were calculated for each species for each locus in the matrix, and were coded in several different ways: any instance, 0.10 intervals (10 character states, ordered and unordered), 0.25 intervals (4 character states,
ordered and unordered), and the method of Thiele (1993), with 30 character states. This method standardizes the observed frequencies based on the observed variation across each character and recodes character states along a scale based on the observed frequencies within each character. In all coding methods other than any instance coding, character states were used in analyses where they were considered both ordered and unordered character states.

2.3 RESULTS

The aligned sequence for ITS1, 5.8s, and ITS2 was 614 bp, with individual sequences varying between 594 (for *P. personatus*) and 604 (for *Keckiella breviflora, K. cordifolia*, and *P. montanus var. montanus*). Of 614 characters, 46 were phylogenetically informative. Sequence divergence within the ingroup (subg *Dasanthera*) ranged from 0% to 4.0% with a mean of 1.2% for pairwise comparisons. Within the Rocky Mountain taxa, sequence divergence averaged 2.4% whereas sequence divergence in the Cascade/Sierra taxa averaged 0.4%. Parsimony analysis yielded 592 equally parsimonious trees of 93 steps (Consistency Index [CI] 0.656, Retention Index [RI] 0.823; Fig. 2.1). The strict consensus of 592 trees collapses *Chelone, Nothochelone, Chionophila* and *Penstemon* into a monophyletic group. Within that clade, subgenus *Dasanthera* is monophyletic with the inclusion of *P. personatus*, the only representative of the monotypic subgenus *Cryptostemon*, which is endemic to Northern California. The Rocky Mountain taxa are paraphyletic (with the inclusion of *P. personatus*), coming out
in two clades sister to the Cascade/Sierra taxa. The Cascade/Sierra taxa emerge as a moderately-supported monophyletic clade (Bootstrap: 69%). However, there is no resolution in the consensus tree.

The aligned length of matK is 1813 bp, with sequences ranging from 1765 (in *Keckiella corymbosa*) to 1800 (in *Chionophila tweedyi*). Of these 1813 characters, 88 are parsimony-informative. Sequence divergence in the ingroup varied from 0% to 1.4%, with a mean of 0.3%. Within the Rocky Mountain taxa, sequence divergence averaged 0.9% and within the Cascade/Sierra taxa averaged 0.07%. Parsimony analysis yielded a single most parsimonious tree of 112 steps (CI 0.866, RI 0.964; Fig. 2.2). In this analysis, *Penstemon* forms a well-supported monophyletic lineage (Fig. 2.2) with *P. montanus* sister to the rest of *Penstemon*. The rest of subg. *Dasanthera* is monophyletic, and sister to the other five subgenera of *Penstemon*. *Penstemon personatus* is basal in this lineage with strong bootstrap support (100%). Within the *Dasanthera* clade, interspecific relationships are unresolved, with the exception of the clade including *P. ellipticus* and *P. lyallii*, and *P. fruticosus* var. *serratus*, which has moderate support (Bootstrap: 87%) and the clade that includes *P. cardwellii* and *P. rupicola* (Bootstrap: 61%).

Partition homogeneity tests (PHT) suggest significant heterogeneity between the ITS and matK data sets (p=0.007). Inclusion of all taxa in a combined analysis results in 1764 equally parsimonious trees of 215 steps (CI 0.735, RI 0.904; Fig. 2.3). In the strict consensus, *Penstemon* is monophyletic, with *P. montanus* sister to the rest of *Penstemon*. In the clade comprising the rest of *Penstemon*, subgenus *Dasanthera* forms a
monophyletic group sister to the other subgenera of Penstemon. *Penstemon personatus* once again comes out sister to all other species of *Penstemon*. Exclusion of both varieties of *P. montanus*, *P. personatus*, and all species of *Keckiella* results in homogeneity of the data set (p=0.21). The resulting topology is identical to that of the combined analysis (Fig. 2.3) with the exclusion of heterogeneous taxa.

Using five ISSR primers, 132 bands were scored as distinct loci. The number of loci produced per primer ranged from 15 ("Manny") to 38 (17902), with an average of 26.8 loci per primer. Of these, only one locus was fixed in all individuals. No diagnostic loci were found in this study. A diagnostic locus would be defined as a locus that is fixed in a species or group of species, and absent in all others. Most loci varied in band frequency among species.

When loci were recoded into cladistic characters based on band frequencies within species, tree topology was very sensitive to the coding method that was used (Fig. 2.4). Furthermore, CI and RI were low in almost all cases (Table 2.3; Fig. 2.4). Of all analyses, Thiele coding with ordered characters and 9.10 interval coding with ordered characters gave the greatest resolution, but CI and RI were low for both of these analyses (Table 2.3; Fig. 2.4).

Neighbor-joining trees based on pairwise similarity showed considerable sensitivity to sampling with deletion of rare loci (Fig. 2.5). With all loci included, *P. davidsonii*, *P. cardwellii*, and *P. fraticosus* all come out in multiple terminal clusters that change position with rare locus deletion (Fig. 2.5). In most analyses, *P. barretti*ae clusters with *P. cardwellii*, however, deleting loci of moderate frequency results in two
individuals of *P. fruticosus* clustering with *P. barretti*, or *P. barretti* clustering with *P. fruticosus*. Furthermore, three individuals, *P. davidsonii* X1, *P. davidsonii* X2, *P. fruticosus*-Warm Springs, move between *P. davidsonii* cluster B and *P. fruticosus* cluster A, and have a tendency to pull these clusters together in analyses.

Elimination of the five individuals that show aberrant clustering resulted in almost all species (except for the Rocky Mountain taxa) grouping together in terminal clusters (Fig. 2.6a). Furthermore, the topology of terminal clusters appeared to be more stable to rare locus deletion with these five individuals removed (Fig. 2.6 b,c). In all analyses, the Rocky Mountain taxa (*P. ellipticus*, *P. montanus*, and *P. lyallii*) came out interspersed in clusters of *P. fruticosus*. However, sampling was very limited for the Rocky Mountain taxa.

2.4 DISCUSSION

Hypotheses about biogeographic relationships among *Penstemon* subg. *Dasanthera* have suggested a Rocky Mountain origin of the subgenus from a Chionophila-like ancestor (Straw 1966), followed by migration and radiation in the Cascade/Sierra Nevada cordillera based on chromosome numbers and shifts in growth form in the subgenus. The data presented here further support the hypothesis of a Rocky Mountain origin of *Penstemon* based on both ITS and matK sequence data.

Although ITS and matK sequence data were inadequate for resolving relationships among most members of *Penstemon* subg. *Dasanthera*, these data were sufficient to
address biogeographic hypotheses regarding the origin of *Penstemon*. In both ITS and *matK* analyses, all members of the Cascade/Sierra group of subg. *Dasanthera* appear in the same clade, with no resolution among members of this group. In the ITS analysis, the Cascade/Sierra taxa form a monophyletic group, where the two varieties of *P. montanus* emerge in different clades, var. *montanus* is sister to *P. lyallii* and var. *idahoensis* is sister to *P. ellipticus* (Fig. 2.1). However, in the *matK* analysis, *P. ellipticus* and *P. lyallii* form a monophyletic group within the Cascade/Sierra clade, and *P. montanus* comes out as sister to the rest of *Penstemon* with moderate bootstrap support (Fig. 2.2). These differences in topology with regard to the varieties of *P. montanus* could be the result of either lineage sorting or the result of introgression. Hybridization has been reported among many different species pairs in *Penstemon* subg. *Dasanthera*. However, *P. montanus* is an exception in this regard. There are no known reports of hybridization of either variety of this species hybridizing with any other species, even though *P. montanus* overlaps in range with *P. fruticosus*. Although the Rocky Mountain species never appear as a monophyletic group, the Rocky Mountain taxa are always ancestral relative to the Cascade/Sierra taxa, suggesting a Rocky Mountain origin of *Penstemon* (Figs. 2.1, 2.2, and 2.3). This hypothesis is also supported by a shift in growth form from herbaceous perennial to long-lived evergreen subshrubs.

Our results from ISSR data indicate that ISSR markers are potentially useful for examining interspecific relationships among closely related species. However, these data also demonstrate the sensitivity of Neighbor-Joining algorithms to taxon sampling. The analyses presented here detected five individuals that affected clustering patterns. These
individuals may have been problematic because they represent hybrids or introgressants. *Penstemon fruticosus*-Warm springs was collected from a morphologically unique population of *P. fruticosus* which is the only known population of its kind. Morphologically, it resembles *P. fruticosus* var. *serratus*, but has succulent leaves similar to *P. davidsonii*. *Penstemon fruticosus* var. *serratus* and *P. davidsonii* var. *praeteritus* both have hypothesized hybrid origins with *P. davidsonii* var. *davidsonii* and *P. fruticosus* var. *fruticosus* as the parental species (Cronquist 1964; Every 1977).

Unfortunately, sampling from the Warm Springs population was limited, but these results indicate that this population warrants further study. The two individuals of *P. davidsonii* that cluster with *P. fruticosus*—Warm Springs were collected in or near hybrid populations, suggesting that introgression is likely in these individuals. *Penstemon barretti*ae also showed some instability in Neighbor-Joining analyses. This could simply be the result of sampling. However, *P. barretti*ae has been documented to hybridize with *P. fruticosus*, which could explain its behavior in these analyses. Wider sampling of this species is necessary to examine the possibility of introgression.

ISSR data suggest several ecological and morphological shifts in the Cascade/Sierra lineage. *Penstemon fruticosus*, *P. cardwellii*, *P. davidsonii*, and *P. barretti*ae all have purple flowers, and are similar in floral morphology with regard to flower color, orientation of the anthers, relative size of the corolla, and degree of anther exsertion. Although there are differences in floral size within and among species, these differences appear to be largely ecological and, based on purported hybridization, do not appear to have an effect on pollen transfer between species. The purple-flowered species
may be reproductively isolated from one another primarily by ecological and geographic barriers. *Penstemon fruticosus* is found east of the crest of the Cascade mountains, at high elevations in intermountain ranges, and in the Northern Rocky Mountains.

*Penstemon cardwellii* is found on the west side of the crest of the Cascade Mountains, into the Siskiyou mountains of Southern Oregon and Northern California, and high elevations in the Coast Range of Oregon, often on partially forested rock outcrops and disturbed roadcuts. *Penstemon davidsonii* is a high elevation species, found at or near the crest of the Cascade and Sierra Mountain Ranges and on the eastern slope, into the Olympic Mountains in Washington, and in a few intermountain areas in southeastern Oregon and northern Nevada. *Penstemon davidsonii* is found most often on exposed rock outcrops or lava flows. *Penstemon barrettiae* is endemic to the Columbia River Gorge, and is known from only a few populations, all of which are on basalt outcrops. Many species pairs have been observed in sympathy where hybridization is rampant. Therefore, the reproductive and genetic barriers in these species appear incomplete.

The most evident shift in morphology within the Cascade/Sierra lineage is associated with a shift in apparent pollination syndrome. *Penstemon newberryi* and *P. rupicola* are both pink- to red-flowered. These two species have distinct ranges: *P. rupicola* occupies the northern part of the range, from southern Oregon into northern Washington, whereas *P. newberryi* is found in the southern part of the range, from the southern Cascade Mountains to the southern Sierra Nevada Mountains. Based on ISSR data, these species are sister to one another, and derived from the purple-flowered species. Concurrent with shift in floral color, there have been shifts in anther orientation.
and anther exsertion. In the purple-flowered species, anthers are more or less inserted within the corolla and anther sutures face one another, held together by lanate hairs on the anther surface. In the pink-flowered species, anthers are exserted and oriented along a plane so that the sutures face downward toward the palate; thus, when the flower is moved at all pollen can easily be shaken from the anthers (pers. obs.). These three morphological changes are associated with a shift from pollination primarily by bees to pollination by a combination of bees and hummingbirds. Although isolated by floral morphology and pollination syndrome, *P. rupicola* and *P. newberryi* are known to hybridize with many of the purple-flowered species, indicating that ecological and genetic barriers in these species are incomplete.

*Penstemon newberryi* var. *berryi* differs from the two other varieties of this species in having magenta corollas that appear to be somewhat intermediate in color between the pink-flowered and purple-flowered species. It also differs from pink-flowered taxa in having anthers that are inserted and facing one another, similar to the purple-flowered species. Unfortunately, I was unable to sample this variety. Every (1977) suggested that *P. newberryi* var. *berryi* was involved in a complex hybrid zone between *P. rupicola* and *P. cardwellii*, both of which overlap in range with *P. newberryi* var. *berryi*. Based on morphology, further investigation is warranted on this variety.

2.4.1 Adaptive evolution in *Penstemon* subg. *Dasanthera*.— The shift in anther orientation observed in *P. rupicola* and *P. newberryi* appears to be correlated with a shift in pollination syndrome, and potentially the primary pollinators of these species (Datwyler unpubl.). Examining the important morphological shifts in the subgenus,
including characters such as flower color, nectar composition and volume, flower size, as well as the range of species may provide important clues to evolution within the subgenus as a whole. Detailed studies of the adaptive nature of these traits will help to elucidate differences in pollinator cues and important characters limiting interspecific hybridization within the group. Furthermore, understanding the adaptive nature of characters limiting interspecific hybridization will be important in understanding the speciation process in *Penstemon* subg. *Dasanthera*.

2.4.2. **Biogeography of Penstemon subg. Dasanthera.**— Phylogeny reconstruction based on ITS and *matK* sequence data reveals a Rocky Mountain origin of *Penstemon*, with radiation from the Northern Rocky Mountains from a *Chionophila*-like ancestor (reviewed in Wolfe et al. in press). These results further reveal an important shift in habit within the subgenus, from the plesiomorphic condition of suffrutescent perennials with a below-ground caudex (*P. iyallii*), to suffrutescent perennials with woody above-ground rhizomes (*P. ellipticus* and *P. montanus*), to long-lived, evergreen woody perennials (Cascade/Sierra clade). All three of these species are morphologically and geographically distinct. Only a few hybrid populations have been reported, and these are in areas of human disturbance (Every 1977).

The results presented here support the presence of two centers of diversity within the subgenus, in which the northern Rocky Mountain species have given rise to the Cascade/Sierra lineage. The northern Rocky Mountain group includes *P. iyallii*, *P. montanus*, and *P. ellipticus*. The Cascade/Sierra group includes *P. newberryi*, *P. rupicola*, *P. davidsonii*, *P. carowellii*, *P. barrettiae*, and *P. fruticosus*. *Penstemon*
fruticosus is interesting in this respect because the range of the species extends from the eastern slope of the Cascades into the northern Rocky Mountains, including high elevation mountains in the intermountain areas. Based on the topology of the ISSR trees, when rooted with P. lyalli, P. fruticosus may have evolved in the northern Rocky Mountains and migrated westward to the Cascade/Sierra mountains, possibly across the Columbia plateau during one of the glacial maxima when cooler conditions would have supported P. fruticosus at lower elevations. This hypothesis would suggest recent divergence within the Cascade and Sierra Nevada mountain ranges (within the last 150,000 years, Orr et al. 1992). A recent origin of the Cascade/Sierra lineage is also supported by low sequence divergence among the species within this group. Subgenus Dasanthera shows very low levels of sequence divergence relative to other subgenera of Penstemon. Low levels of sequence divergence in the subgenus may be explained in part by long generation time and the short growing season at high elevations within the subgenus relative to other species of Penstemon. Interfertility among species and frequent hybridization may also contribute to low levels of sequence divergence in this group.

2.4.3 Analysis of dominant-marker data.— Although levels of sequence divergence among species in subgenus Dasanthera are very low, ISSR markers appear to be useful for examining interspecific relationships in subg. Dasanthera, thereby suggesting the utility of dominant markers for examining interspecific relationships. However, several issues should be considered. Our results suggest that hybrids may have a profound influence on the topology of Neighbor-Joining trees. Furthermore, low
frequency, potentially non-homologous bands appear to add a negligible degree of noise to the data set. Adams and Rieseberg (1998) also found that erroneous homology assessments of dominant markers only affected the relative similarity values, but did not affect the overall relationships in ordination analyses. Therefore, elimination of rare bands appears to be unnecessary, even in studies examining interspecific relationships. We found that tree topology was more stable to rare band deletion when problematic taxa were removed. Ordination analyses may be useful for pinpointing potentially problematic taxa.

The results presented here point out the importance of sampling strategy when using dominant marker data to assess evolutionary relationships. Accounting for species-level variation is extremely important when using hypervariable markers. In this study, reduction of the data set to one or a few samples per cluster resulted in dramatically different clustering patterns among terminal clusters (data not shown). Although this may partially be an artifact of Neighbor-joining algorithms, it emphasizes importance of sampling the full range of intraspecific diversity.

When ISSR data were coded as cladistic characters based on the observed locus frequency within species, the resulting analyses varied greatly in topology based on the coding method that was used. Although some of the recoding methods gave a great deal of resolution, CI and RI were low for these analyses, suggesting high levels of homoplasy in the data sets. Notably, none of these recoding methods recovered topologies in which P. rupicola and P. newberryi were sisters, which was recovered in all Neighbor-Joining analyses.
The most appropriate use of ISSR data in cladistic analyses would be for loci that do not demonstrate intraspecific polymorphism (i.e., fixed within species), where loci could be recoded as present/absent within species in a method similar to Borowsky et al. (1995). Although a few studies have used data from dominant markers in cladistic analyses that have corroborated DNA sequence-based phylogenies (Federici et al. 1998; Wolfe and Randle 2001), it is likely that sufficient structure existed within the data set at the interspecific level to yield loci that were fixed within species or species groups, even with limited taxon sampling. These studies do not, however, test the utility of ISSR markers for examining relationships among closely-related species groups in which sequence data has been insufficient for resolving interspecific relationships. Consequently, the method of analysis that is appropriate for studies using polymorphic markers should depend on the structure of the data. For example, in data sets that have high levels of polymorphic loci and lack fixed loci at the species level, relationships among species may not be strictly divergent and therefore, similarity-based methods would be more appropriate than cladistic analysis. Furthermore, Wiens (1999) has demonstrated that Neighbor-Joining recovers the "correct" topology more often than parsimony criteria when considering polymorphic data. Although data from dominant markers are not an ideal source of characters for phylogenetic analysis, parsimony may indeed be appropriate for some types of analyses involving dominant markers. The appropriate treatment of data from dominant markers for cladistic analysis should be limited to loci that are fixed in species or clusters, allowing loci to be recoded as presence/absence characters within species for phylogenetic analysis.
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<sup>a</sup>=collections deposited at OS.  
<sup>b</sup>=collections deposited at OKL.  
<sup>c</sup>=collections deposited at UW.  
<sup>d</sup>=collections deposited at RM. Genbank sequences: first line indicates ITS accession, second line indicates matK accession. Specimens sequenced but not yet entered in genbank are labeled "not in genbank".
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Table 2.2. PCR amplification conditions for each ISSR primer surveyed.
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Table 2.3. Number of trees and support measures for cladistic analyses of ISSR methods under different coding strategies. Tree letter refers to trees shown in figure 2.4.
Figure 2.1. Strict consensus of 592 trees based on ITS sequence data for Penstemon subg. Dasanthera. Bootstrap values are given above branches and Bremer Support is shown below.
Figure 2.2. The single most parsimonious tree recovered using matK sequence data for *Penstemon* subg. Dasanthera. Bootstrap values are given above branches and Bremer Support is shown below.
Figure 2.3. Strict consensus of 1762 equally parsimonious trees based on combined ITS and matK sequence data for *Penstemon* subg. *Dasanthera*. Bootstrap values are given above branches and Bremer Support is shown below.
Figure 2.4. ISSR Band frequency data recoded as cladistic characters. A. Phylogeny based on combined sequence data. B. Any instance coding. C. 0.25 interval coding, unordered characters. D. 0.25 interval coding, ordered. E. 0.10 interval coding, unordered characters. F. 0.10 interval coding, ordered. G. Thiele coding (based on 30 characters), unordered. H. Thiele coding, ordered.
Figure 2.5. Effects of rare band deletion on tree topology with all individuals included. A. All bands included. B. Bands at frequency of less than 0.025 excluded. C. Bands at frequency of less than 0.05 excluded.
Figure 2.6. Effects of rare band deletion on tree topology with *P. barretti*ae, *P. fruticosus*-Warm Springs, and two problematic *P. davidsonii* individuals excluded. A. All Bands included. B. Bands at frequency of less than 0.025 excluded. C. Bands at frequency of less than 0.05 excluded.
CHAPTER 3

HYBRID ZONE STRUCTURE AND PATTERNS OF GENE FLOW BETWEEN

PENSTEMON DAVIDSONII AND P. RUPICOLA

3.1 INTRODUCTION

The importance of hybridization as a mechanism for evolutionary change has
been debated for years (Anderson 1949; Arnold 1997; Rieseberg 1995; Stebbins 1959).
Much of the argument stems from assertions as to whether intrinsic genetic factors or
extrinsic ecological factors are more important in determining the structure of hybrid
zones (Arnold 1997). Several models have been proposed to explain the observed
patterns of hybrid zone structure, and they differ in the importance placed on endogenous
versus exogenous selection pressures in hybrid zones and the degree of fitness that
hybridization confers relative to parental species (reviewed in Arnold 1997).

Hybrid zone structure is important when considering the consequences of natural
hybridization. Considering that hybrids are not always less fit than parental species
(Arnold and Hodges 1995), several potential outcomes of hybridization exist.
Hybridization can result in either homoploid or polyploid hybrid speciation (Arnold
1993; Galletz and Gottlieb 1982; Rieseberg 1997; Sang et al. 1995; Wolfe et al. 1998b),

Despite the apparent frequency of allopolyploid speciation (Jackson 1976; Lewis 1980; Soltis and Soltis 1993), homoploid hybrid speciation has seldom been documented (Arnold 1993; Arnold 1997; Brochmann et al. 2000; Buerkle et al. 2000). Because homoploid hybrid species tend to have narrow geographical distributions (Rieseberg 1997), the formation of localized or widespread hybrid zones is a common outcome of hybridization. Introgressive hybridization, whereby genetic material becomes incorporated into the gene pool of one of the parental species, has been well documented, both in plants and animals (e.g., Dawson et al. 1996; Dowling and Hoeh 1991; Nielsen and Siegismund 1999; Rieseberg 1995; Rieseberg et al. 2000; Rieseberg et al. 1999; Smith and Sytsma 1990; Stebbins 1959; Wolfe and Elisens 1993; Wolfe and Elisens 1994; Wolfe and Elisens 1995; Wolfe et al. 1998a). Introgressive hybridization may result in the genetic assimilation of rare species by more common congeners (reviewed in Huxel 1999; Levin et al. 1996; Rhymer and Simberloff 1996). Moreover, genetic assimilation has been implicated as a threat to the survival of many species of plants (Brochmann 1984; Liston et al. 1990; Rieseberg and Gerber 1995; Rieseberg et al. 1989).
and animals (Abernathy 1994; Ankney et al. 1987; Butler 1994; Leary et al. 1993; Rhymer et al. 1994).

With increasing interest in assessing the importance of hybridization, numerous studies have examined patterns and processes of hybridization. These studies have varied in focus from examining hybridization on a regional or geographic scale to examining population-level patterns of hybridization. Intrapopulational studies not only address questions related to patterns of gene flow, but can provide a framework for examining the importance of ecological and genetic factors in concert. Studies of intrapopulational patterns and ecological factors in localized hybrid zones may lead to a better understanding of the factors promoting hybridization under natural conditions, and may be invaluable in evaluating adaptive traits and key innovations in divergent evolution (Bradshaw et al. 1998; Hodges and Arnold 1994; Schemske and Bradshaw 1999).

A variety of molecular markers have been used to document hybridization and gene flow, including allozymes (Gallagher et al. 1997; Gaughier et al. 1999; Rieseberg et al. 1991; Wolfe and Elisens 1993), restriction-site variation of nuclear ribosomal DNA (Keim et al. 1989; Liston et al. 1990; Rieseberg 1991a; Sang et al. 1995; Wolfe and Elisens 1994), restriction-site variation of cpDNA (Cruzan and Arnold 1993; Rieseberg et al. 1991; Rieseberg et al. 1990; Wolfe and Elisens 1995), various PCR-based markers, including randomly amplified polymorphic DNA (RAPD Cruzan and Arnold 1993; Daehler and Strong 1997; Dawson et al. 1996; Martin and Cruzan 1999; Neuffer et al. 1999; Rieseberg and Gerber 1995) and, more recently, inter-simple sequence repeat (ISSR) markers (Wolfe et al. 1998a; Wolfe et al. 1998b). ISSR markers are similar to
RAPD markers in many respects, but have several advantages over RAPD markers. First, ISSR primers are typically much longer than RAPD primers (14-20 bp vs. 10 bp). Longer primers allow for a higher annealing temperature, reducing the potential for mispairing during PCR. Also, ISSR markers tend to show high levels of variation relative to RAPD markers (Esselman et al. 1999; Wolfe and Liston 1998). Only a few studies have utilized ISSR markers to examine patterns of hybridization and gene flow. Wolfe et al. (1998a; 1998b) used ISSR markers to examine hybrid speciation and patterns of gene flow in *Pectenom centranthifolius*, *P. spectabilis* and *P. clevelandii*. To date, however, no ISSR-based studies have directly addressed patterns of gene flow in hybrid populations.

Relatively few studies of hybrid zones have used both morphological and molecular markers to examine patterns of gene flow (however, see Carney et al. 2000; Hardig et al. 2000; Hodges and Arnold 1994; Kim and Rieseberg 1999; Martin and Cruzan 1999; Neuffer et al. 1999). Furthermore, no consensus exists on how to estimate gene flow using two marker types in concert. Although hybrid indices (Anderson 1949) are commonly used to examine patterns of gene flow, they are not without problems (see Rieseberg and Linder 1999). Recent applications of hybrid indices have used maximum likelihood estimates to distribute traits of parental species onto a linear scale (Carney et al. 2000; Hardig et al. 2000; Rieseberg et al. 1998). These estimates can also account for the presence of heterozygotes obtained from dominant marker data (i.e., RAPD, AFLP, ISSR). However, these estimates do not allow a direct comparison of molecular and morphological data in assessing hybrid zone evolution. In this study, we compare
morphological and molecular estimates of hybridization in concert using a modification of a hybrid index analysis, where molecular marker frequency for parental species is expressed as frequency is hybrid index categories based on morphology to make direct comparisons of morphological and molecular data in examining population structure. Furthermore, we use an approximation of Fisher's exact test (Raymond and Rousset 1995) to examine congruence of morphological and molecular assessments of hybridization.

Our focus in this study is on the hybrid zone structure and the patterns of gene flow between two species of Penstemon, P. davidsonii Greene var. davidsonii and P. rupicola (Piper) Howell using a combination of morphological and molecular (ISSR) data. Our study is centered on Wizard Island in Crater Lake National Park, Oregon, and several features make this system well suited for studies of hybridization. The recent formation of Wizard Island (less than 7000 years bp), provides an opportunity to study hybrid zone dynamics and the conservation implications of hybridization on a recently colonized island. Our findings may also be applied more generally to studies of colonization and population dynamics in both habitat and oceanic islands. Additionally, the situation on Wizard Island represents an example of natural hybridization in the absence of human disturbance. Overall, the specific objectives of this study are to examine: (1) the extent and location of hybrid zones on Wizard Island, (2) the structure of the hybrid zone on Wizard Island based on morphological and molecular markers, and (3) the pattern of gene flow between P. davidsonii and P. rupicola.
3.1.1 Study System.— *Penstemon* subg. *Dasanthera* is a small subgenus comprising of nine closely related species that occur at high elevations in the Northern Rocky, Cascade, and Sierra Mountain ranges of western North America. Although species in the subgenus tend to have distinct elevational ranges, hybridization is common when two species occur in sympatry (Every 1977). Hybridization is most frequent, but not limited to, areas of disturbed or intermediate habitat, such as road cuts or intermediate elevations throughout the Cascade Mountain range (Every 1977).

Subgenus *Dasanthera* is characterized by lanate hairs on the anther surfaces, a keeled dorsal ridge with two ventral ridges in the corolla, and, in most species, a persistent woody subshrub habit. The four taxa endemic to the Northern Rocky Mountains differ from the Cascade/Sierra taxa in being either suffrutescent perennials from a woody caudex or in possessing persistent woody rhizomes (Every 1977). Studies of the evolutionary relationships of *Penstemon* and Cheloneae (Wolfe et al. in press) show that subg. *Dasanthera* is sister to the rest of *Penstemon*. In addition, these studies suggest an origin of the subgenus in the Northern Rocky mountains, followed by migration to and radiation in the Cascade/Sierra mountain ranges.

*Penstemon davidsonii* is distributed at high elevations (generally above 1800 m) in the Cascade and Sierra Mountain ranges of western North America. Plants occur on open, well-drained soils, such as lava flows. Flowers are relatively small for the subgenus (corolla length 18–39 mm; mean 25.6 mm), purple in color, with stamens inserted (or barely exserted) within the corolla. Anthers are oriented with the sutures facing inward and the anther sacs are held together by the dense, lanate hairs on the
another surface. Leaves are entire (no serration on leaf margins) and lack glaucous waxes on the surface (Fig. 3.1a). In contrast, *P. rupicola* occurs on steep, often more mesic rock outcrops, generally at lower elevations than *P. davidsonii* (ca. sea level to 2200 m).

Plants are characterized by relatively large pink flowers (corolla length 24-39 mm; mean 32.1 mm) and exerted stamens that are oriented downward toward the palate (Fig. 3.1b). Leaves have serrate margins and a waxy, glaucous surface.

Preliminary results indicate that *P. davidsonii* and *P. rupicola* are not sister species (Datwyler and Wolfe, unpubl.). Reports of hybridization between these two species are limited to subalpine zones at or near the crest of the Cascade Mountain range. Extensive hybridization between these species has been reported in Crater Lake National Park in Southern Oregon, USA (Every 1977).

Crater Lake was formed approximately 8000 years bp following the collapse of Mt. Mazama. Subsequent volcanic activity 7000 years bp resulted in the formation of Wizard Island on the Western side of the lake. *Penstemon davidsonii* and *P. rupicola* occur both on the rim of the lake and on the island. Hybrids are known to occur at both locations. On Wizard Island, *P. rupicola* is found typically on the western part of the island on steep rock faces near lake level, whereas *P. davidsonii* is distributed on higher, exposed scree slopes. Hybrid zones are found near lake level in subalpine habitats (Fig. 3.2).
3.2 MATERIALS AND METHODS

3.2.1 Collection strategy.— Populations of P. davidsonii and P. rupicola were sampled in Crater Lake National Park and throughout the ranges of each species where morphological variation gave no evidence of hybridization. A total of six populations of P. davidsonii and six populations of P. rupicola were sampled (Table 3.1). On Wizard Island, three points along the hybrid zone were sampled (FB, WI, and WS; Table 3.1, Fig. 3.2). These subpopulations were chosen to represent a range of habitat types, from exposed rocky substrates, to forested subalpine slopes. Within each sampled subpopulation, all flowering individuals were mapped, leaves were collected for DNA isolation and one flower from each plant was collected and preserved for floral measurements (see morphological measurements below for details). Within a plant, no variation occurred in the floral characters that were measured; therefore these were considered representative of the plant. Incomplete floral data in sampled subpopulations reduced data analysis to six individuals from FB, 19 from WI, and 39 from WS.

3.2.2 Morphological measurements.— Four morphological traits, all of which are diagnostic characters separating P. davidsonii and P. rupicola (Table 3.2), were measured from unintrogressed populations of P. davidsonii and P. rupicola, and all hybrid plants were surveyed. Each measurement was qualitative, where zero represented P. davidsonii-like and two represented P. rupicola-like traits. Anther orientation demonstrated an intermediate morphology, which was assumed to represent an intermediate character state (Table 3.2). The intermediate state was given a score of one.
3.2.3 *Genetic Analyses.*— DNA was isolated using a modification of the CTAB protocol of Doyle and Doyle (1987). Reactions were scaled for small quantities of tissue and included a second high salt ethanol precipitation. Polymerase chain reaction was conducted in 25 μl reaction volumes following standard conditions as follows: 20 mM Tris-Cl pH 8.4; 50 mM KCl; 3 μM MgCl₂, 12.5 μM each dATP, dCTP, dGTP, and dTTP; 0.4 μM primer, and 0.25 U Taq DNA polymerase (Gibco BRL) and 0.5 μl template DNA. Thermal cycler conditions were as follows: initial denaturation at 94° for 90 sec., 35 cycles of 94° for 45 sec., annealing (temperatures specified in Table 3.3) for 45 sec., 72° extension for 90 sec. and a final extension cycle for 5 min. Reactions were run on 1.2% agarose gels with 1X Tris Acetate EDTA (TAE) buffer. Gels were stained in ethidium bromide and images were viewed under UV light and recorded digitally. Two replicates were run for all reactions to test for repeatability of bands, all of which were demonstrated to be repeatable for this analysis.

Thirty ISSR primers were surveyed for species-typical bands of *P. davidsonii* or *P. rupicola*. A species-typical band was defined as a band that occurs in high frequency in one species and was either absent or present at low frequency in the other species. Although none of the species-typical bands were determined to be diagnostic for species, we defined species-typical bands as those that were found with a difference in proportion of 0.75 between parental taxa. This difference in band proportion was chosen arbitrarily because it represents a natural division in band frequencies observed between parental taxa. For example, if a band occurs at a frequency of 0.78 in *P. davidsonii* and 0.02 in *P. rupicola*, it would be considered a *P. davidsonii*-typical band. Conversely, if a band is
fixed in *P. rupicola* and at a frequency of 0.20 in *P. davidsonii*, it would be considered a *P. rupicola*-typical band. Of the 30 primers surveyed, seven primers that demonstrated species-typical bands were chosen for this study (Table 3.3). For each individual, species-typical bands were scored as present/absent.

Populations of *P. davidsonii* and *P. rupicola* were tested for population differentiation using a modification of Fisher's exact test (Raymond and Rousset 1995), where R represents populations (or HI classes) and C represents loci (species-typical bands) to test for band-by-band and global (all species-typical bands) population differentiation. For all pairwise comparisons, 1000 dememorisation steps, 10 batches and 2000 permutations per batch were performed using TFPGA version 1.0 (Miller 1997).

Pearson correlation coefficients (Sokal and Rohlf 1995) were calculated for all possible pairwise comparisons of morphological and molecular markers evaluated. Calculations were done for each hybrid subpopulation, and for all three hybrid subpopulations together. Parental species were not included in these calculations so as not to inflate correlations (Grant 1979; Rieseberg and Ellstrand 1993). Calculations were conducted in SPSS version 10.0.

3.2.4 *Hybrid index design.*—Morphological characters were used to design a simple additive hybrid index (HI). To calculate the HI score for each individual, the sum of scores for all morphological characters was taken (see above). Hybrid index scores ranged from zero to eight, where a score of zero was identical in morphology (for the characters measured) to *P. davidsonii* and a score of eight was identical to *P. rupicola*.
Hybrid plants from all three subpopulations were categorized based on their HI score. For each HI category, we tested for differentiation among the three hybrid subpopulations surveyed using the modification of Fisher’s exact test described above. Pairwise comparisons demonstrated no significant differentiation within HI classes among the hybrid subpopulations (all $P \geq 0.90$; data not shown, available by request from the first author), so further analyses treat all individuals in each HI class together.

The genetic makeup of plants in each HI category was evaluated as the proportion of *P. davidsonii*-typical and *P. rupicola*-typical bands in each HI category (i.e., the total number of species-typical bands divided by the total number of bands scored for each individual). To test for directionality of gene flow, we compared the species-typical band frequencies in parental species to each HI category using the modification of Fisher’s exact test (described above). Two independent comparisons were made: (1) all hybrid classes versus *P. davidsonii* and (2) all hybrid classes versus *P. rupicola*. Bonferroni adjustments were made to correct for multiple comparisons.

Our null hypotheses were that species-typical band frequencies in HI categories similar to *P. davidsonii* (i.e., HI 0-1) would not differ from *P. davidsonii*, and species-typical band frequencies in HI categories similar to *P. rupicola* (i.e., HI 7-8) would not differ from *P. rupicola*. Deviations from the null hypothesis would indicate introgression of species-typical bands. We interpret this to indicate interspecific gene flow. Differences in the patterns of gene flow for *P. davidsonii*-like and *P. rupicola*-like hybrids suggest differences in directionality of gene flow.
3.3 RESULTS

3.3.1 Distribution of hybrids.— Initial surveys of Wizard Island were conducted in July 1997 to locate and estimate the extent of hybridization between *P. davidsonii* and *P. rupicola*. *Penstemon davidsonii* is abundant on the island, being distributed widely on the cinder cone and less abundantly in forested zones (Fig. 3.2). *Penstemon rupicola* is relatively restricted in distribution. Two apparently non-introgressed populations of *P. rupicola*, as indicated by morphology, are located on steep rock faces of the western shore (Fig. 3.2), yet hybrids are found in close proximity to these populations. A hybrid zone from lake-level (1883 m) to about 1920 m was identified based on a range of morphological variation of plants within this zone. The hybrid zone is limited primarily to the southwestern shore of the island (Fig. 3.2).

The degree of correlation between morphological and molecular markers varied considerably across the three hybrid subpopulations surveyed (Fig. 3.3). In comparisons of character coherence among all hybrids surveyed, 72% of the pairwise correlations are nominally significant (*p*<0.01). In the FB subpopulation, only 10% of pairwise correlations demonstrate nominally significant correlations. However, in WI, 24% of correlations, and in WS, 43% of correlations show nominally significant correlations (Fig. 3.3). Each subpopulation demonstrates lower degrees of pairwise correlations relative to overall correlations because of the smaller sample sizes in each subpopulation sample.
3.3.2 Species-typical bands in unintrogressed populations.— With seven primers, seven *P. rupicola*-typical markers and four *P. davidsonii*-typical markers were identified (Table 3.3). Mean frequencies of species-typical bands in unintrogressed populations of *P. davidsonii* and *P. rupicola* were high for conspecific bands and low for heterospecific bands (*P. davidsonii*: conspecific mean=0.86, heterospecific mean=0.06; *P. rupicola*: conspecific mean=0.94, heterospecific mean=0.07; Fig. 3.4). Although some variation existed in the mean proportion of species-typical bands in pure populations (Fig. 3.5), pairwise comparisons reveal no significant differences among populations (*P. davidsonii*: Table 3.4; *P. rupicola*: Table 3.5). Notably, populations of *P. rupicola* found in close proximity to the hybrid zone on Wizard Island show high frequencies of *P. rupicola* typical markers and complete absence of *P. davidsonii*-typical markers (Fig. 3.5a,b; e.g., IR).

Within the surveyed hybrid subpopulations, the number of individuals with *P. davidsonii*-like (HI categories 0-2), intermediate (HI categories 3-5), and *P. rupicola*-like (HI categories 6-8) morphologies varied among the subpopulations surveyed (Table 3.6). In the WI and WS subpopulations, significantly more *P. rupicola*-like hybrids were found (WI: $\chi^2_{0.001,2}=26.3$; WS: $\chi^2_{0.001,2}=24.6$) and in the FB subpopulation, more *P. davidsonii*-like hybrids were present although this difference was not significant (FB: $\chi^2_{0.10,2}=5.51$). The mean proportion of species-typical bands in each HI, as expected, shows a predominance of *P. rupicola*-typical bands and a decrease in *P. davidsonii*-typical bands in plants with a greater morphological likeness to *P. rupicola* (Fig. 3.6). However, *P. davidsonii*-like hybrids show a surprisingly high frequency of *P. rupicola*-typical bands
(HI classes 1 and 2; Fig. 3.6). Such a trend is not apparent in the *P. rupicola*-typical hybrids (HI classes 7-8) when examining the frequency of *P. davidsonii*-typical bands.

Tests of differentiation between each parental species and each HI category show different levels of differentiation among HI categories (Table 3.7). Overall comparisons of species-typical band frequencies of *P. davidsonii* and *P. rupicola* with each HI category indicate the HI categories that show significant deviation from expected species-typical band frequencies of parental species (Table 3.7). Comparisons of *P. davidsonii* with *P. davidsonii*-like hybrids (HI categories 0-1) indicate differentiation of both of these HI categories from the expected frequencies of species-typical bands. However, after Bonferroni adjustment for multiple comparisons, HI class 0 is not significant. In both HI class 0 and 1, these differences are attributable to greater than expected frequencies of three *P. rupicola*-typical bands and lower than expected frequency of one *P. davidsonii*-typical band (Fig. 3.6, Table 3.7). Additionally, intermediate HI classes 3 and 4 do not demonstrate significant differentiation from *P. davidsonii* after Bonferroni adjustment (Table 3.7). However, sampling in these HI classes was limited to four and two individuals, respectively.

In comparisons of *P. rupicola*-like hybrids (HI classes 7-8) to *P. rupicola*, HI class 8 demonstrates differentiation from *P. rupicola* before Bonferroni adjustment. It is important to note, however, that differentiation in HI class 8 is the result of lower than expected frequencies of *P. rupicola*-typical band frequencies for three bands. All other HI classes show significant differentiation from *P. rupicola* band frequencies after Bonferroni adjustment.
These results indicate that *P. davidsonii*-like hybrids demonstrate a higher than expected proportion of *P. rupicola*-typical bands, indicating an asymmetrical pattern of gene flow from *P. rupicola* into *P. davidsonii* (Fig. 3.6; Table 3.7).

3.4 DISCUSSION

In recent years, an increasing number of studies have used both morphological and molecular markers to study hybridization and gene flow in natural populations (e.g., Carney et al. 2000; Hardig et al. 2000; Hodges and Arnold 1994; Martin and Cruzan 1999; Neuffer et al. 1999; Rieseberg and Ellstrand 1993). In this study, we used a combination of morphological and molecular markers to examine the extent and distribution of hybrids, hybrid zone structure, and patterns of gene flow in a hybrid zone of *Penstemon* in Crater Lake National Park. Our surveys demonstrate that hybridization is limited to a narrow zone on the island, and apparently unintrogressed populations (based on morphological resemblance) of *P. davidsonii* and *P. rupicola* also occur on the island. Additionally, our results indicate a directional pattern of gene flow from *P. rupicola* into *P. davidsonii*, which may be explained by several factors. First, a unidirectional pattern of gene flow may be the result of differences in pollinator visitation between *P. davidsonii* and *P. rupicola*. Based on the observation that hybrids are present on Wizard Island, we would expect that similar suites of pollinators visit both species, but some pollinators may be expected to visit *P. rupicola* at higher frequency than *P. davidsonii*. Preliminary observations indicate that both species are visited by similar
suites of pollinators and that some of the pollinators, such as hummingbirds, may show differential rates of visitation (Datwyler and Wolfe unpubl.). For example, hummingbirds visit *P. rupicola* more often than *P. davidsonii*, but visit *P. davidsonii* infrequently, possibly facilitating deposition of heterospecific *P. rupicola* pollen onto its stigmas.

In addition to pollinator visitation rates, different pollinators may show different effectiveness of pollination for the two species. This could be related to the orientation of the anthers and the ability of different pollinators to remove pollen. Both species have two pairs of anthers at slightly different heights in the corolla (Fig. 3.1a,b). The anther sacs of *P. davidsonii* face one another, have sutures that face inward, and are held together by lanate hairs on the anther surfaces. These hairs also hold together the two pairs of anthers into one unit. This, in effect, may limit pollen removal from the anthers of *P. davidsonii* and may therefore require pollinators to "buzz" the anthers to remove pollen effectively. In contrast, *P. rupicola* anthers face downward, such that the sutures face the palate of the corolla. Additionally, the anthers are much less densely hairy, allowing pollen to be easily shaken from them. Because the mechanism of pollen presentation appears to be different for these two species, the amount of pollen deposited on potential pollinators may affect the effectiveness of cross-pollination.

Second, flower size may influence the efficiency of cross-pollination because *P. rupicola* has larger flowers than *P. davidsonii*. Therefore, differential gene flow may be related to differences in the distances that pollen tubes grow in heterospecific styles. Pollen from species with longer styles may produce pollen tubes better adapted to
growing down a short style than in species with shorter styles (Blakeslee 1945). If pollen tube growth rate limits gene flow, we would expect to see greater seed set in interspecific crosses where *P. davidsonii* serves as the maternal donor rather than in cross-pollinations where *P. rupicola* serves as the maternal donor. We are currently investigating this possibility.

Ecological tolerances, such as altitudinal preferences, water availability, light availability, and drought resistance, may also influence the observed pattern of gene flow. *Penstemon rupicola* often occurs at lower elevations than *P. davidsonii* and is found on relatively mesic rock outcrops or steep rock faces. In contrast, *P. davidsonii* is distributed in subalpine zones, most often on dry, exposed rock or scree, such as lava flows and high elevation granite outcrops in the southern part of its range. Although the hybrid zone on Wizard Island occurs in areas near pure populations of *P. rupicola*, hybrids are found in a variety of habitats spanning a range of ecological conditions, from dry, exposed rock outcrops to relatively mesic, wooded slopes. It is possible that elements of the *P. rupicola* genome may confer a selective advantage in habitats where hybrids are found. Rieseberg and Linder (1999) suggested that bias toward the molecular profile of one species could be the result of selection for certain genomic elements in hybrids. Based on genetic mapping in *Helianthus*, they demonstrated that selection for genetic “blocks” could lead to rapid elimination of certain portions of one parental genome. Our observation of a higher than expected proportion of *P. rupicola*-typical genetic markers in *P. davidsonii*-like hybrids is consistent with the possibility that elements of the *P. rupicola* genome confer a selective advantage in *P. davidsonii*-like
hybrids on Wizard Island. Selection for *P. rupicola* like elements could result from ecological adaptations in hybrid zones.

3.4.1 **Character coherence.**— Grant (1979) has observed that degrees of character coherence vary between hybrid swarms and among species owing to the life history of the organisms under study and the age of the hybrid zone. Character coherence can break down more rapidly in annual plants due to the number of recombination events that annuals undergo relative to long-lived perennials. Long-lived plants often overlap in generations, and also do not undergo as many generations per unit time, increasing the expected character coherence in perennials. We found a large degree of character coherence in hybrids from Wizard Island (Fig. 3.3). Because these are long-lived plants that have come into contact relatively recently (in the last 7000 years), the high degree of character coherence observed on Wizard Island might be expected. The high degree of character coherence observed in the WS subpopulation may suggest that *P. davidsonii* and *P. rupicola* may have come into contact more recently in this subpopulation relative to the other subpopulations.

3.4.2 **Hybridization as an evolutionary process.**— Although there are several documented examples of homoploid hybrid speciation, (Arnold 1993; Brochmann et al. 2000; Francisco-Ortega et al. 1996; Gallez and Gottlieb 1982; Rieseberg 1991a; Rieseberg et al. 1990; Sang et al. 1995; Wolfe et al. 1998a; Wolfe et al. 1998b), the conditions under which hybrid species are formed is quite restrictive. However, differentiating between homoploid hybrid speciation and introgressive hybridization is often quite difficult (Wolfe and Eliseas 1995). Models of homoploid hybrid speciation
stress the importance of intrinsic reproductive barriers in the evolution of a homoploid hybrid species in sympatry (reviewed in Rieseberg 1997). However, Charlesworth (1995) argued that hybrid speciation could take place when hybrid plants become spatially or ecologically isolated from their parents. The potential for homoploid hybrid speciation on Wizard Island seems unlikely given the limited zone of hybridization on Wizard Island, apparent interfertility of P. davidsonii and P. rupicola, maintenance of pure populations of both species on the island, and the observation that the same pollinators are often observed visiting both species.

Our results indicate directional gene flow from P. rupicola into P. davidsonii. We are currently investigating the potential role of pollination biology (pollinator visitation and efficiency of cross-pollination) in limiting bidirectional gene flow between P. rupicola and P. davidsonii. Numerous studies have demonstrated directional patterns of gene flow in hybrid zones. Two primary mechanisms have been hypothesized to explain apparent directionality of gene flow in hybrid zones. First, recent contact and migration of the hybrid zone has been invoked to explain the pattern of gene flow in the Piriqueta caroliniana complex in central Florida (Martin and Cruzan 1999), hybridization between the cyprinid fish species Notropis cornatus and N. chrysocephalus in the southeastern United States (Dowling and Hoeh 1991), and in chromosomal races of the Australian grasshopper Caledia captiva (Shaw et al. 1990). In each of these three cases, the directionality of gene flow appears to be a consequence of recent migration of the hybrid zone, leaving behind a genetic trail. Pollen-mediated gene flow has been invoked to explain patterns of gene flow in Aesculus (dePamphilis and Wyatt 1989; dePamphilis and
Wyatt 1990) and *Penstemon* (Wolfe and Elisens 1993; Wolfe and Elisens 1994; Wolfe and Elisens 1995). In both these cases, directionality of gene flow was attributed to hummingbird migration patterns. In the case of hybridization between *Aquilegia formosa* and *A. pubescens*, Hodges and Arnold (1994) demonstrated bidirectional introgression of molecular markers across both ecological and elevational transects, but strong selection for four of five morphological traits suggests limited morphological introgression of traits affecting pollinator visitation. In *Penstemon*, morphological traits appear to be under strong selection maintaining species integrity in unintrogressed populations on Wizard Island. However, in hybrid zones there appears to be selection for *P. rupicola*-like genetic elements. This could be the result of selection for ecological traits in the hybrid zone, although further investigation would be necessary to confirm this possibility.

Much attention has recently been given to the impact of hybridization in conservation biology. The system on Wizard Island provides an opportunity to examine the potential consequences of genetic assimilation in an environment free of human disturbance. The observations from this system may also be more widely applicable to systems where the genetic integrity of one species is threatened by a second species. On Wizard Island, *P. rupicola* is much less common than *P. davidsonii*. Based on sheer numbers, one might expect that *P. rupicola* would be in danger of assimilation by *P. davidsonii* because of the numerical advantage of *P. davidsonii* on Wizard Island. However, the observed frequencies of species-typical bands in the sampled population of *P. rupicola* on Wizard Island (IR; Fig. 4) demonstrates that there is little, if any, gene flow from *P. davidsonii* into these populations, or that there is selection against *P.*
davidsonii-typical genetic elements in this population. However, assimilation of P. davidsonii by P. rupicola is also possible if the fitness of P. rupicola is higher than that of P. davidsonii. In Sportina, the greater fitness of a rare, introduced species (S. alterniflora) has been identified as a potential threat to the more abundant native species, S. foliosa (Anttila et al. 1998). Given this, one might expect to see a high frequency of P. rupicola-typical bands in apparently unintrogressed populations of P. davidsonii, especially those in close proximity to P. rupicola populations. On Wizard Island, populations of P. davidsonii (WM, WT; Fig. 3.5) show relatively high frequencies of P. rupicola-typical bands. However, since the observations are within the range of data from other populations, only moderate levels of introgression are likely beyond the hybrid zone. For example, there may be selection for P. rupicola genetic elements in hybrid zones that may render hybrids more fit in these zones. In fact, plants with P. rupicola-like morphological characters are occasionally observed in otherwise unintrogressed populations of P. davidsonii, but the converse has never been observed (Datwyler unpubl.).

Hybridization is common between many species pairs in Penstemon subgenus Dasanthera throughout the Cascade and Sierra Mountain ranges in California, Oregon and Washington. Hybrid zones have been documented for P. davidsonii with P. rupicola, P. newberryi, and P. fruticosus (Every 1977). Penstemon rupicola hybridizes with P. davidsonii, P. cardwellii, and P. fruticosus (Every 1977). In addition to these hybrid combinations, many other species pairs are known to hybridize within the subgenus (Every 1977). Future studies of hybridization between different species pairs in
*Penstemon* subg. *Dasanthera* may help to elucidate important floral and genetic characteristics limiting gene flow between species.
<table>
<thead>
<tr>
<th>Species</th>
<th>Pop. Code</th>
<th>Population Location</th>
<th>Collection Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. davidsonii</em></td>
<td>CH</td>
<td>OR: Deschutes Co.</td>
<td>Datwyler 107</td>
</tr>
<tr>
<td><em>P. davidsonii</em></td>
<td>CM</td>
<td>OR: Lake Co.</td>
<td>Datwyler 83</td>
</tr>
<tr>
<td><em>P. davidsonii</em></td>
<td>MC</td>
<td>OR: Deschutes Co.</td>
<td>Datwyler 109</td>
</tr>
<tr>
<td><em>P. davidsonii</em></td>
<td>WM</td>
<td>OR: Crater Lake National Park; Wizard Island</td>
<td>Datwyler 94</td>
</tr>
<tr>
<td><em>P. davidsonii</em></td>
<td>WT</td>
<td>OR: Crater Lake National Park; Wizard Island</td>
<td>Datwyler 93</td>
</tr>
<tr>
<td><em>P. davidsonii</em></td>
<td>PS</td>
<td>OR: Crater Lake National Park; East rim</td>
<td>Datwyler 87</td>
</tr>
<tr>
<td><em>P. rupicola</em></td>
<td>SI</td>
<td>OR: Josephine Co.</td>
<td>Datwyler 14</td>
</tr>
<tr>
<td><em>P. rupicola</em></td>
<td>IM</td>
<td>OR: Lane Co.</td>
<td>Datwyler 103</td>
</tr>
<tr>
<td><em>P. rupicola</em></td>
<td>IR</td>
<td>OR: Crater Lake National Park; Wizard Island</td>
<td>Datwyler 96</td>
</tr>
<tr>
<td><em>P. rupicola</em></td>
<td>RD</td>
<td>OR: Douglas Co.</td>
<td>Datwyler 98</td>
</tr>
<tr>
<td><em>P. rupicola</em></td>
<td>CL</td>
<td>OR: Crater Lake National Park; Cleatwood Cove</td>
<td>Datwyler 86</td>
</tr>
<tr>
<td><em>P. rupicola</em></td>
<td>VF</td>
<td>OR: Crater Lake National Park; East Rim</td>
<td>Datwyler 84</td>
</tr>
<tr>
<td><em>P. davidsonii X rupicola</em></td>
<td>FB</td>
<td>OR: Crater Lake National Park; Wizard Island</td>
<td>Datwyler 106</td>
</tr>
<tr>
<td><em>P. davidsonii X rupicola</em></td>
<td>WI</td>
<td>OR: Crater Lake National Park; Wizard Island</td>
<td>Datwyler 89</td>
</tr>
<tr>
<td><em>P. davidsonii X rupicola</em></td>
<td>WS</td>
<td>OR: Crater Lake National Park; Wizard Island</td>
<td>Datwyler 95</td>
</tr>
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</table>

Table 3.1. Collection information for populations sampled in this study. All voucher specimens are deposited at OS.
<table>
<thead>
<tr>
<th>Character</th>
<th><em>P. davidsonii</em> state (Score=0)</th>
<th>Intermediate (Score=1)</th>
<th><em>P. rupicola</em> state (Score=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf surface</td>
<td>Glaucous</td>
<td></td>
<td>Not glaucous</td>
</tr>
<tr>
<td>Leaf serration</td>
<td>Serrate</td>
<td></td>
<td>No serration</td>
</tr>
<tr>
<td>Anther exsertion</td>
<td>Anthers inserted</td>
<td></td>
<td>Anthers exserted</td>
</tr>
<tr>
<td></td>
<td>within corolla throat</td>
<td></td>
<td>from corolla throat</td>
</tr>
<tr>
<td>Anther orientation</td>
<td>Sutures facing down</td>
<td>Anthers perpendicular to one another</td>
<td>Anthers facing one another</td>
</tr>
<tr>
<td></td>
<td>toward palate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2. Morphological differences between *Penstemon davidsonii* and *P. rupicola*.  

64
<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer Sequence</th>
<th>Annealing Temp (°C)</th>
<th>Marker Bands</th>
<th>Freq. <em>P.</em> <em>rupicola</em></th>
<th>Freq. <em>P.</em> <em>davidsonii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Becky</td>
<td>(CA)$_7$YC</td>
<td>47</td>
<td>B-2075R</td>
<td>1.000</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B-493R</td>
<td>0.853</td>
<td>0.040</td>
</tr>
<tr>
<td>Chris</td>
<td>(CA)$_7$YG</td>
<td>47</td>
<td>C-1141R</td>
<td>1.000</td>
<td>0.122</td>
</tr>
<tr>
<td>Terry</td>
<td>(GTG)$_6$RG</td>
<td>45</td>
<td>T-681R</td>
<td>0.947</td>
<td>0.041</td>
</tr>
<tr>
<td>Manny</td>
<td>(CAC)$_6$RC</td>
<td>45</td>
<td>M-541D</td>
<td>0.093</td>
<td>0.851</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M-282D</td>
<td>0.147</td>
<td>0.919</td>
</tr>
<tr>
<td>17902</td>
<td>(GT)$_6$AY</td>
<td>42</td>
<td>902-459R</td>
<td>0.960</td>
<td>0.072</td>
</tr>
<tr>
<td>AW-2</td>
<td>(GT)$_6$YC</td>
<td>42</td>
<td>AW2-439D</td>
<td>0.000</td>
<td>0.847</td>
</tr>
<tr>
<td>AW-3</td>
<td>(GT)$_6$RG</td>
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<td>AW3-1247D</td>
<td>0.000</td>
<td>0.781</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AW3-1217R</td>
<td>0.946</td>
<td>0.027</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>AW3-636R</td>
<td>0.986</td>
<td>0.164</td>
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Table 3.3. Summary of primers, PCR conditions, and species-typical bands used in this study.
<table>
<thead>
<tr>
<th></th>
<th>CH</th>
<th>CM</th>
<th>MC</th>
<th>PS</th>
<th>WM</th>
<th>WT</th>
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<tr>
<td>CH</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CM</td>
<td>18.58</td>
<td>--</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>25.53</td>
<td>9.88</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.27</td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS</td>
<td>31.15</td>
<td>15.74</td>
<td>6.36</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>0.83</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WM</td>
<td>7.84</td>
<td>22.12</td>
<td>18.12</td>
<td>15.83</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.45</td>
<td>0.70</td>
<td>0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>12.29</td>
<td>4.55</td>
<td>2.96</td>
<td>10.31</td>
<td>10.10</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>0.95</td>
<td>1.00</td>
<td>1.00</td>
<td>0.98</td>
<td>0.99</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.4. Pairwise comparisons of population differentiation for all populations of *P. davidsonii*. $\chi^2$ value for Fisher’s exact test over all primers on first line (d.f. = 22 for all comparisons); $P$-value on second line.
<table>
<thead>
<tr>
<th></th>
<th>CL</th>
<th>IM</th>
<th>IR</th>
<th>RD</th>
<th>SI</th>
<th>VF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>1.49</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>IR</td>
<td>6.02</td>
<td>4.49</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RD</td>
<td>11.62</td>
<td>11.83</td>
<td>4.56</td>
<td>--</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.96</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SI</td>
<td>4.30</td>
<td>8.28</td>
<td>14.88</td>
<td>13.28</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td>0.87</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VF</td>
<td>1.94</td>
<td>4.51</td>
<td>7.70</td>
<td>12.04</td>
<td>7.35</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.96</td>
<td>1.00</td>
<td></td>
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</table>

Table 3.5. Pairwise comparisons of population differentiation for all populations of *P. rupicola*. $\chi^2$ value for Fisher’s exact test over all primers on first line (d.f. = 22 for all comparisons); $P$-value on second line.
<table>
<thead>
<tr>
<th>Subpopulation</th>
<th><em>P. davidsonii</em>-like</th>
<th>Intermediate</th>
<th><em>P. rupicola</em>-like</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(HI classes 0-2)</td>
<td>(HI classes 3-5)</td>
<td>(HI classes 6-8)</td>
</tr>
<tr>
<td>FB</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>WI</td>
<td>3</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>WS</td>
<td>7</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13</strong></td>
<td><strong>11</strong></td>
<td><strong>40</strong></td>
</tr>
</tbody>
</table>

Table 3.6. Number of individuals displaying *P. davidsonii*-like, intermediate and *P. rupicola*-like morphologies in each hybrid subpopulation surveyed.
<table>
<thead>
<tr>
<th>HI Category</th>
<th>P. davidsonii</th>
<th>P. rupicola</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>36.76</td>
<td>188.26</td>
</tr>
<tr>
<td></td>
<td>0.0252</td>
<td>0.000*</td>
</tr>
<tr>
<td>1</td>
<td>53.07</td>
<td>73.96</td>
</tr>
<tr>
<td></td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>2</td>
<td>106.14</td>
<td>55.90</td>
</tr>
<tr>
<td></td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>3</td>
<td>39.34</td>
<td>126.58</td>
</tr>
<tr>
<td></td>
<td>0.013</td>
<td>0.000*</td>
</tr>
<tr>
<td>4</td>
<td>27.15</td>
<td>73.17</td>
</tr>
<tr>
<td></td>
<td>0.206</td>
<td>0.000*</td>
</tr>
<tr>
<td>5</td>
<td>143.43</td>
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<td>195.74</td>
<td>28.03</td>
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<td></td>
<td>0.000*</td>
<td>0.175</td>
</tr>
<tr>
<td>8</td>
<td>217.88</td>
<td>34.03</td>
</tr>
<tr>
<td></td>
<td>0.000*</td>
<td>0.049</td>
</tr>
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</table>

Table 3.7. Differentiation of each hybrid index category from parental species. Top line indicates \( \chi^2 \) differentiation value over all bands (all comparisons d.f.=22). Second line indicates p-value. * indicates significance at p=0.05 level after Bonferroni adjustment.
Figure 3.1. Floral and vegetative characters for Penstemon davidsonii and P. rupicola. A. *P. davidsonii*. Note insertion of anthers and orientation of anther sacs toward one another and entire leaf margin. B. *Penstemon rupicola*. Note the position and orientation of anthers and leaf serration. Illustrations by Andrea Foust.
Figure 3.2. Distribution of Penstemon on Wizard Island and sampling sites. Dots indicate unintrogressed populations surveyed; hatched boxes indicate hybrid subpopulations surveyed through the hybrid zone.
Figure 3.3. Histograms representing Pearson correlation coefficients for pairwise comparisons of all morphological and molecular markers surveyed. Open bars indicate non-significant correlations at $P = 0.01$; Hatched bars indicate significant correlations. 

A. All hybrid individuals considered together. B. FB hybrids only. C. WI hybrids only. D. WS hybrids only.
Figure 3.4. Mean frequency of species-typical bands in unintrogressed plants of *P. davidsonii* and *P. rupicola*. Open bars indicate *P. davidsonii*-typical bands; hatched bars indicate *P. rupicola*-typical bands. Error bars are one standard deviation.
Figure 3.5. Mean frequency of species-typical bands in unintrogessed populations surveyed. A. P. davidsonii populations; B. P. rupicola populations. Open bars indicate P. davidsonii-typical bands; hatched bars indicate P. rupicola-typical bands. Error bars are standard deviation.
Figure 3.6. Proportions of species-typical marker band frequencies in each HI class. Values represent means from all individuals in all three hybrid subpopulations surveyed. Open bars indicate *P. davidsonii*-typical bands; hatched bars indicate *P. rupicola*-typical bands. Error bars are standard deviation.
CHAPTER 4

POLLINATOR VISITATION AND SEED SIRING SUCCESS IN *PENSTEMON DAVIDSONII* AND *P. RUPICOLA*

4.1 INTRODUCTION

Models of hybrid zone structure vary in the relative importance of genetic and ecological factors in maintaining hybrid zones (reviewed in Arnold 1997). Models focusing on ecological factors stress the importance of selection against hybrids and dispersal across the hybrid zone (Barton and Hewitt 1985). Such models typically make the assumption that hybrids are maladaptive. In contrast, some models of hybrid zone structure assume that hybrids have higher fitness relative to parental species in hybrid zones (Anderson 1948; Endler 1985). A few examples of hybridization occurring in intermediate or disturbed habitats include *Silene* (Runyon-Lager and Prentice 2000), *Iris* (Anderson 1948; Arnold and Bennett 1993), *Helianthus* (Carney et al. 2000), and *Cercocarpus* (Rieseberg et al. 1989). The factors influencing hybridization and hybrid zone structure are likely to include aspects of reproductive biology, including pollinator preferences (Hodges and Arnold 1994) and flight pattern (Leebens-Mack and Milligan 1998), pollen tube competition (Carney and Arnold 1997; Carney et al. 1996; Daehler 76
and Strong 1997), seed-siring success (Carney et al. 1994), and genotype-by-environment interactions of hybrids in hybrid zones (Johnston et al. 2001a; Johnston et al. 2001b).

Selection in hybrid zones may result in either symmetrical or asymmetrical patterns of gene flow. Studies of a hybrid zone between *Aquilegia formosa* and *A. pubescens* have demonstrated the importance of ethological isolation on maintenance of species boundaries, although symmetrical introgression of nuclear markers was observed across the hybrid zone (Chase and Raven 1975; Grant 1952; Hodges and Arnold 1994). In *Aesculus* (dePamphilis and Wyatt 1989; dePamphilis and Wyatt 1990) and *Penstemon* (Wolfe and Elisens 1993; Wolfe and Elisens 1994; Wolfe and Elisens 1995), asymmetries in observed patterns of gene flow have been attributed to unidirectional pollen movement resulting from hummingbird migration patterns. In these scenarios, ethological barriers appear to play a causal role in hybrid zone structure through limitation of interspecific pollen movement. Extensive studies of Louisiana irises have revealed asymmetries in hybrid zone structure that have been attributed to a combination of ethological barriers, pollen competition, and differential seed siring abilities (Arnold and Bennett 1993; Carney and Arnold 1997; Carney et al. 1994; Carney et al. 1996; Cruzan and Arnold 1993). In these studies, pollen competition was found to be a key determinant in the frequency of F1 hybrid formation. In the formation of F1 seeds in an *Iris hexagona* X *fulva* hybrid zone, *I. hexagona* always served as the seed parent, and this difference was attributed to the relative abundance of *I. fulva* flowers relative to *I. hexagona* flowers (Arnold and Bennett 1993). Conspecific pollen grew faster than heterospecific pollen, and would therefore reach the ovules first unless heterospecific pollen was given a sufficient headstart (Carney et al. 1996). In studies of the *Piriqueta*
caroliniana complex (Martin and Cruzan 1999), chromosomal races of *Caledia captiva* (Shaw et al. 1990), and *Notropis cornutus* and *N. chrysoscepalus* (Dowling and Hoeh 1991), apparent asymmetries across broad hybrid zones were attributed to recent migration of the hybrid zone, resulting in a genetic "trail" following behind the leading edge of the hybrid zone (Arnold 1997). In these cases, selection for changing climatic conditions were used to explain the apparent asymmetry in hybrid zone structure by recent migration of species ranges.

In *Penstemon* subgenus *Dasanthera*, hybridization has been well-documented between many different species pairs (Every 1977). Furthermore, studies of hybrid zone structure on Wizard Island in Crater Lake National Park have demonstrated an asymmetry in the genetic makeup of *P. davidsonii*-like hybrids, where *P. davidsonii*-like hybrids have a greater than expected proportion of *P. rupicola*-typical genetic markers (see Chapter 3). In contrast, *P. rupicola*-like hybrids show *P. davidsonii*-typical bands at frequencies similar to unintrogressed individuals of *P. rupicola*. One possible explanation for the observed asymmetry in gene flow might be related to differing pollination syndromes.

*Penstemon davidsonii* and *P. rupicola* also exhibit differences in floral morphology that have been attributed to differences in the primary pollinators of these species (Every 1977). *Penstemon davidsonii* has smaller, purple flowers, with anthers that are inserted within the corolla tube. The two sets of anthers face one another and are held together by lanate hairs forming a dense mat between the anther sacs and the anther sacs dehise inward toward the mat of hairs. In addition, the corolla tube is relatively narrow, limiting the entry of very large bees. In contrast, *P. rupicola* has pink to magenta
flowers with anthers exerted from the corolla tube. Flowers are larger than *P. davidsonii* flowers, and have a much wider corolla tube. The anthers in *P. rupicola* are oriented downward toward the palate such that the four anther sacs are oriented on a plane. Although lanate hairs occur on the surface of the anthers, the hairs do not serve to hold the anther sacs together into a unit as in most other species in the subgenus. Because of these differences in floral morphology, *P. davidsonii* is thought to be pollinated primarily by small bees, whereas *P. rupicola* is hypothesized to be pollinated primarily by hummingbirds (Every 1977; Thompson et al. 2000).

Several potential scenarios may be acting separately or in concert to maintain the present structure of the *Penstemon* hybrid zone on Wizard Island. First, differences in pollinator visitation to pure species and to hybrids may result in differential pollen transfer between species. Furthermore, differential pollen transfer may influence hybrid zone structure. Additionally, the difference in floral size between *P. davidsonii* and *P. rupicola* may render *P. rupicola* a better pollen donor relative to *P. davidsonii*. Because *P. rupicola* has larger flowers than *P. davidsonii*, *P. davidsonii* may serve as a more efficient maternal donor relative to *P. rupicola* because the pollen tubes are adapted to growing down longer styles than pollen tubes of *P. davidsonii*. The pollen tubes of *P. rupicola* may be better adapted to growing down the short style of *P. davidsonii* than the pollen tubes of *P. davidsonii* growing down the longer style of *P. rupicola*. It has long been noted that heterospecific pollination is often more efficient if there is a difference in floral size (Blakeslee 1945). Finally, selection for *P. rupicola*-like genetic elements may also play a role in the observed asymmetry in the hybrid zone. Exogenous selection in
the form of greater fitness of *P. rupicola* genotypes might explain the observed asymmetry across the hybrid zone.

In this study, the contributions of endogenous selection pressures were assessed in the maintenance of hybrid zone structure in *Penstemon* by examining ethological isolation between *P. davidsonii* and *P. rupicola*, and post-pollination and post-fertilization factors in terms of differential seed siring abilities and germination rates for conspecific and heterospecific pollinations on *P. davidsonii* and *P. rupicola*.

4.2 METHODS

4.2.1 Pollinator observations.— Pollinator visitation to *P. davidsonii* and *P. rupicola* was examined during peak flowering in early- to mid-July in 1999 and 2000 in Crater Lake National Park. All observations for *P. davidsonii* were conducted on Wizard Island along the summit trail. Observations for *P. rupicola* were conducted on Wizard Island along the Fumarole Bay trail and at the Cleetwood Cove trail on the rim of the lake. Observations were made in 1 X 2 m$^2$ plots. For each plot, the density of flowering stems was recorded. Visitation was recorded in 10-minute observation intervals. In each interval, all of the visitors that landed on flowers were recorded. A visit was defined as a potential pollinator landing on a flower and either probing for nectar or removing pollen from the anthers. For each visitor, the number of flowers visited and behavior during floral visits was recorded during the visitation interval. A visitation interval was defined as all of the consecutive floral visits by a single visitor in the absence of any other behavior, such as grooming. When a pollinator was observed to engage in another
behavior, a new visitation interval was started. For each visitation interval, the number of flowers visited and the behavior of the visitor was recorded (collecting nectar, collecting pollen, or both). For each visitor, the family (for insects), number of flowers visited, and behavior was recorded.

For both *P. davidsonii* and *P. rupicola*, the proportion of visits by each potential pollinator was calculated. However, sampling is probably biased against hummingbird visitation because of the proximity of observers to the plot during observations. Differences in the types of potential pollinators visiting each species were examined using the G-test of independence (Sokal and Rohlf 1995), with species and visitor as the two factors. Furthermore, differences in pollinator behavior during floral visits (nectar probe vs. pollen collection) were tested using the G-test of independence (Sokal and Rohlf 1995).

4.2.2 Cross-pollinations.— To test for differences in seed-siring ability and seed viability, conspecific and heterospecific pollinations were conducted for both *P. davidsonii* and *P. rupicola*. In July 2000, 40 plants of *P. davidsonii* and *P. rupicola* were marked for study and numbered sequentially in Crater Lake National Park, Oregon. All pollinations were done on plants on Wizard Island. On each plant, four flowers were marked, three of which were emasculated and bagged and the fourth flower was marked and left unmanipulated for open pollination. Each of the emasculated flowers on marked plants received a different treatment: one received conspecific pollen, one received heterospecific pollen, and the third was bagged but not pollinated to test for spontaneous seed production. Of the 40 bagged flowers that were emasculated and left unpollinated, only two *P. davidsonii* flowers produced fruits and six *P. rupicola* flowers produced
fruits. In all of these fruits, seed set and seed germination rates were low. Therefore, these data were not included in further analyses.

During the study period, all flowers were checked daily for stigma receptivity. Stigmas were considered receptive when the style turned downward and secretions were evident on the stigmatic surface. On the second day of stigma receptivity, flowers were hand-pollinated by rubbing a recently-opened anther of the appropriate species across the stigma. Fresh anthers were collected daily from outside the study areas to avoid inadvertent geitonogamous pollinations. Hand-pollinated flowers were then left bagged until fruit collection (approx. four weeks). All fruits that were retained on plants were collected and placed in coin envelopes for temporary storage until seeds were counted. Upon return to the lab, each fruit was scored as empty or filled based on the presence of seeds in the fruit. For all fruits that had seeds, the number of filled seeds was counted and all seeds were placed on moist filter paper and cold stratified for five months at 4°C.

Following cold stratification, seeds were removed from the cold and the number of germinated seeds was recorded. In addition, up to twenty seeds per fruit were planted in 420-cell flats, with one seed per cell. Flats were kept on mist benches for two weeks and were then moved to open benches and watered daily. Seed germination was recorded daily for three weeks, after which no seeds had germinated for over one week. Seeds were considered germinated when the seed coat fell off and cotyledons opened. Percent seed germination was calculated by dividing the total number of seeds germinated (including those emerging during cold stratification) by the total number of seeds considered. For example, if 30 seeds had germinated during cold stratification and 10
germinated and 10 ungerminated seeds were planted, only five of which germinated after this point, total seed germination would be calculated as 30+5/40, or 0.875.

4.2.3 Data analysis.— Fruit set was analyzed to test for independence of species and cross type, and presence/absence of a fruit using a three-way contingency table approach (Zar 1996). Tests of partial independence of species and cross type were also performed using a G-test of partial independence (Zar 1996).

Seed set and percent germination were analyzed using a two-way ANOVA with species and cross type as fixed factors and plants as random factors. Percent germination was arcsine square root transformed to normalize variance prior to analysis. Analyses were performed using the general linear model function of SPSS version 10.0.

Mean separation was achieved by calculating least significant difference (LSD) for planned comparisons of (1) conspecific vs. open pollination for each species, (2) conspecific vs. heterospecific pollination for each species, and (3) conspecific pollination between species.

4.3 RESULTS

4.3.1 Pollinator visitation.— For each species, a total of 64, 10-minute intervals were observed, for a total of over 20 hours of observations. The number of visits per interval was about equal for P. davidsonii and P. tropicola with 5.6 and 5.4 visits per ten minute interval, respectively. Although similar suites of pollinators visited both species, the proportion of visits by each potential pollinator type varied by species (G<sub>.05</sub>=217.6; Fig. 4.1). Whereas the primary visitors to P. davidsonii were bees in the
Megachilidae (76.5%), the primary visitors to *P. rupicola* were members of the Halictidae (46.4%). Although *P. rupicola* has been hypothesized to have a hummingbird pollination syndrome, only 2% of visitors were hummingbirds. Hummingbirds were observed to visit nearby plants at moderate frequency in populations of both *P. rupicola* and *P. davidsonii*.

In addition to differences in visitation rate between species, potential pollinators showed significant differences in feeding behavior during floral visits ($G_{10, <0.001}=312.6$; Fig. 4.5). Whereas megachilid bees and hummingbirds were primarily observed collecting nectar during floral visits, halictid bees and syrphid flies were observed collecting pollen more frequently than probing for nectar. Minor pollinators, such as bumblebees, collected both nectar and pollen, and hummingbirds visited flowers exclusively for nectar (Fig. 4.2).

4.3.2 Cross-pollinations.— Of 160 marked flowers for each species, 108 fruits (68%) were recovered from *P. davidsonii* and 97 fruits (60%) were recovered from *P. rupicola*. Although the ultimate fate of unrecovered fruits is unknown, many of these are presumably the result of aborted fruits and, in some cases, herbivory by small mammals (personal observation). Percent fruit set was high (>80%) for both conspecific and open pollinated treatments for both species (Fig. 4.3). For heterospecific pollination, fruit set was 95% for *P. rupicola*, but only 50% for *P. davidsonii* (Fig. 4.3). Fruit set differed significantly among treatments and species ($G_{10, <0.001}=70.8$). Tests of partial independence demonstrate that fruit set is dependent on both species ($G_{9, 0.05}=18.0$) and cross type ($G_{7, <0.001}=63.9$).
Although mean seed set for all treatments of *P. davidsonii* was lower than for all treatments of *P. rupicola*, there was no significant species effect on seed set (Table 4.1, Fig. 4.4). There is a significant cross type effect on seed set, suggesting that both species demonstrate similar patterns with respect to cross type (Table 4.1). Least significant difference detected a significant difference in seed set between conspecific treatments for *P. davidsonii* and *P. rupicola*. Although heterospecific crosses with *P. davidsonii* as the maternal parent resulted in about half as many seeds per fruit relative to conspecific and open pollinated flowers (Fig. 4.4), this difference was not significant. The power to detect a difference was reduced substantially as a result of low fruit set for *P. davidsonii* heterospecific crosses. Open-pollinated fruits of *P. rupicola* had significantly more seeds per fruit relative to conspecific crosses (Fig. 4.4). However, there was no significant difference in mean seeds per fruit for *P. rupicola* conspecific vs. heterospecific crosses.

Seed germination was significantly higher for conspecific and heterospecific crosses of *P. davidsonii* relative to open pollinated flowers and all treatments for *P. rupicola* (Fig. 4.5). For *P. davidsonii*, open pollinated fruits showed significantly lower germination rates relative to manipulated treatments. There was no significant difference among any of the *P. rupicola* treatments (Fig. 4.5). Percent germination showed a significant species X cross interaction (Table 4.2; Fig. 4.5), suggesting that *P. davidsonii* serves as a better maternal donor than does *P. rupicola* with regard to seed germination.
Hybrid zone structure is a complex dynamic balanced by differences in pollen and seed dispersal, gamete competition and survivorship, and fitness of hybrids in hybrid zones. The results presented in this study suggest that pollinator visitation patterns, seed-siring ability and selection for fitness characteristics are important in maintaining hybrid zone structure in Penstemon.

4.4.1 Pollinator dynamics.— Pollinator visitation to *P. davidsonii* and *P. rupicola* demonstrates that there is a great deal of overlap in the potential pollinators that visit both species. Sweat bees (Halictidae) and leaf-cutter bees (Megachilidae) accounted for the majority of floral visits. Although *P. rupicola* has been considered to demonstrate a hummingbird pollination syndrome, the incidence of hummingbird visitation was very low. The actual visitation by hummingbirds is probably greater than observed in this study because hummingbirds tended not to visit plants in which humans were in close proximity. Hummingbirds were observed visiting flowers of both *P. davidsonii* and *P. rupicola* at moderate frequencies outside the observation plots. Therefore, the relative importance of hummingbird pollination may be greater than is suggested by these data.

Differences in the behavior of floral visitors are likely to influence the effectiveness of pollination. For example, both syrphid flies and halictid bees visit flowers primarily for pollen and rarely come into contact with the stigmatic surface (pers. obs.). Therefore, these visitors are unlikely to serve as effective pollinators. Although few coflowering species were observed in the study area, both halictid bees and syrphid flies tend to be generalists and are likely to visit other species that are flowering.
concurrently, potentially resulting in heterospecific pollen transfer from unrelated species. In contrast, megachilid bees, bumblebees and hummingbirds visit flowers most frequently for nectar, and in probing for nectar contacted both the anthers and the stigma. Hummingbirds and bumblebees visited *Penstemon* flowers at relatively low frequency on Wizard Island. Both of these pollinator types are also generalists, but may serve as effective pollinators when *Penstemon* flowers are in bloom because of the abundance of *Penstemon* flowers and their visitation behavior. In contrast to other pollinator types, megachilid bees tend to be loyal in visitation to *Penstemon* flowers and their visitation behavior suggests that these bees effectively contact both the anthers and the stigma, making megachilid bees a likely candidate for the primary pollinators of *Penstemon* species on Wizard Island.

Pollinator flight distances were not recorded in this study, but the observed flight distances varied for the visitors observed. Although hummingbirds are capable of long flight distances, the birds observed on Wizard Island were territorial, and visited a large number of flowers on the same plant. Thus they are likely to result in a high frequency of geitonogamous pollinations. Bumblebees were observed to visit many flowers in a given visit interval, but flew long distances in between visits, making the potential for long-distance pollen transfer likely. Megachilid bees were observed to visit just a few flowers per visit interval, but appeared to be faithful to *Penstemon* flowers and returned frequently for floral visits.

The primary visitors to *P. rupicola* flowers were by pollen-collecting insects. There are several possible explanations for this. First, *P. rupicola* may produce more pollen than *P. davidsonii*, thereby making *P. rupicola* an abundant food source for pollen
collectors. Second, \textit{P. rupicola} might provide easier access to pollen than \textit{P. davidsonii} because of the difference in position and orientation of the anthers between these two species. Finally, there may be a difference in the nutritional value of the pollen of these species. In addition to pollen rewards, differences in nectar volume and concentration may influence pollinator visitation. Flower color, flower size, diameter of the corolla tube, and position and orientation of the anthers may also influence pollinator visitation and/or the efficiency of pollen transfer.

Although there is overlap in the primary pollinators to \textit{P. davidsonii} and \textit{P. rupicola}, pollinator movements between species, and floral cues and rewards influencing visitation are important aspects in understanding pollinator dynamics. Several studies have found floral constancy of visitors, even in hybrid zones. A few systems that show pollinator preferences in mixed populations include a \textit{Baptisia} hybrid zone (Leebens-Mack and Milligan 1998), mixed populations of \textit{Cercidium} (Jones 1978), floral color morphs of \textit{Raphanus sativus} (Stanton 1987), and different species of \textit{Mimulus} (Schemske and Bradshaw 1999; Sutherland and Vickery 1993). Furthermore, worker bumblebees also display individual preferences with regard to floral visitation (Heinrich 1979). Contrary to these findings, both hummingbirds and bumblebees visited some hybrid genotypes at higher frequency than either of the parental species in an \textit{Iris} hybrid zone (Wesselingh and Arnold 2000).

\textit{Penstemon} is quite diverse in floral morphology with pollinators including a diverse array of \textit{Hymenoptera}, \textit{Lepidoptera}, \textit{Diptera}, and hummingbirds (Kampny 1995; Pennell 1935; Straw 1956; Thompson et al. 2000). Hummingbird pollination has arisen from bee-pollination in many different \textit{Penstemon} lineages, including subgenus
Dasanthera (Wolfe et al., unpubl.). Furthermore, visitation by bumblebees and other small bees, such as Megachilidae, has been reported in hummingbird-pollinated species of Penstemon. However, in P. barbatus and P. pinifolius, visitation by small bees does not effect pollination (Lange et al. 2000). Megachilid bees, especially Osmia spp. have been hypothesized to be primary pollinators to many Penstemon species, including P. gracilis (Crosswhite and Crosswhite 1966), P. pallidus (Crosswhite and Crosswhite 1966), and P. penlandii (Tepedino et al. 1999). Penstemon newberryi, which is closely-related to P. rupicola and has similar floral morphology, is visited by hummingbirds, bumblebees, and Osmia (Thompson et al. 2000). Furthermore, in a comparison of bee-pollinated P. cyananthus and hummingbird-pollinated P. eatonii (Bateman 1980), P. cyananthus had more insect visits from more insect families than did P. eatonii. However, P. eatonii had higher fruit set and seed set, suggesting that hummingbirds serve as more effective pollinators in this system. My results are similar in finding higher seed set for the hummingbird-pollinated species, but differ in the diversity of pollinators visiting these species. Penstemon rupicola was visited by the same diversity of floral visitors as P. davidsonii. Visitation by a variety of insects could suggest that P. rupicola has maintained floral scents, or ultraviolet nectar guides, attracting insect visitors in addition to hummingbirds.

4.4.2. Pollination effectiveness.— Although fruit set is about equal for P. davidsonii and P. rupicola for both conspecific and open pollinated treatments, P. davidsonii has much lower fruit set for heterospecific crosses relative to P. rupicola. These results are contrary to the hypothesis that P. davidsonii serves as a better maternal parent than does P. rupicola. The lower than expected fruit production appears to be the
result of fruit abortion. However, it is not clear whether pollen tubes were formed and reached the ovules from this study. Studies of pollen tube growth rate would be necessary to determine if, or where pollen tube growth arrested.

Seed set data demonstrate that *P. davidsonii* produces significantly fewer seeds than *P. rupicola*. Additionally, *P. davidsonii* as the maternal donor shows a greater reduction in fruit set and overall seed set in heterospecific crosses relative to conspecific than does *P. rupicola*, although this difference was not significant for *P. davidsonii*. However, this is a conservative estimate of seed set because only the fruits that set seed were considered and a large proportion of heterospecific fruits in *P. davidsonii* were aborted.

The difference in number of seeds produced in *P. davidsonii* vs. *P. rupicola* might be related to differences in life history strategy. Although *P. rupicola* produces more seeds per fruit than does *P. davidsonii*, these seeds tend to be smaller. Furthermore, variance in germination rate is higher for *P. rupicola* (Figs. 4 & 5). Fruits for *P. rupicola* tended to have a bimodal distribution of seed germination, where seed germination was very high for some fruits and very low for other fruits. *Penstemon davidsonii* had much more consistent germination rates. Smaller seeds of *P. rupicola* might be an adaptation to cliff-dwelling habit. Smaller, lighter seeds may more easily transported by wind to suitable habitat than *P. davidsonii* seeds, which tend to be larger and could be weighted down and drop to the base of cliffs.

The two species differ significantly by cross interaction for percent germination, and *P. davidsonii* shows higher germination rates than does *P. rupicola* for all cross types, suggesting greater fitness of *F*₁ hybrids with *P. davidsonii* as a maternal donor.
However, this advantage in seed germination may not present a numerical advantage for *P. davidsonii* because of the greater overall seed set for *P. rupicola* fruits. Furthermore, this initial fitness measure might not translate into a fitness advantage in other important characters, such as early life history survivorship, reproductive fitness, and fitness in different habitat types. Therefore, it will be important to consider exogenous selection pressures such as seedling establishment, fitness of seedlings, and fertility of hybrids in assessing hybrid zone structure. Both species exhibited a high germination rate for heterospecific crosses, suggesting that hybrids are fit and may display some hybrid vigor.

Hybrid zone structure on Wizard Island might also be maintained in part by the large proportion of *P. rupicola*-like hybrids in the hybrid zone despite the greater overall proportion of *P. davidsonii* plants on the island. However, the large proportion of *P. rupicola*-like hybrids might reflect a fitness advantage of *P. rupicola* in the hybrid zone. If *P. rupicola*-like genetic elements were to confer a fitness advantage on *P. davidsonii*-like hybrids, this could explain the observed asymmetry in the hybrid zone. Although few studies have addressed fitness advantages of different genotypes in hybrid zones, there is evidence for selection for chromosomal blocks in hybrid species of *Helianthus* that may confer a selective advantage in hybrids (Rieseberg et al. 1998; Rieseberg and Linder 1999; Rieseberg et al. 1995; Rieseberg et al. 1996b). Further studies of the fitness advantages of introgressed characters and genetic elements may serve to test such a hypothesis.

The data presented here suggest that the factors maintaining hybrid zone structure on Wizard Island include both endogenous selection pressures such as pollinator visitation and behavior, differential seed-siring ability, and exogenous selection pressures...
such as differential fitness of hybrids. These factors may be acting in concert to maintain hybrid zone structure on Wizard Island. Future studies detailing pollinator visitation behavior and floral cues, pollen tube growth rates in different maternal backgrounds, and differences in fitness of $F_1$ and backcross hybrids, may help tease apart the contribution of factors to maintaining hybrid zone structure in *Penstemon* subg. *Dasanthera*.
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Table 4.1. ANOVA for seed set per fruit.
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Table 4.2. ANOVA for percent germination.
Figure 4.1. Percentage of floral visits by each of the major pollinator types to *Penstemon* flowers. A. *P. davidsonii*. B. *P. rupicola*
Figure 4.2. Number of visits by each of the major pollinators to *Penstemon* flowers broken down by pollinator behavior during visitation.
Figure 4.3. Percent fruit set for conspecific, heterospecific, and open pollinated treatments of *P. davidsonii* and *P. rupicola*. 
Figure 4.4. Mean number of filled seeds for conspecific, heterospecific, and open pollinated fruits of *P. davidsonii* and *P. rupicola*. Error bars show one standard deviation.
Figure 4.5. Percent seed germination in conspecific, heterospecific and open pollinations for *P. davidsonii* and *P. rupicola*. Error bars are one standard deviation.
BIBLIOGRAPHY


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103
dePamphilis, C. W., R. Wyatt. 1989. Hybridization and introgression in buckeyes
(Aesculus: Hippocastanaceae): a review of the evidence and a hypothesis to

dePamphilis, C. W., R. Wyatt. 1990. Electrophoretic confirmation of interspecific
hybridization in Aesculus (Hippocastanaceae) and the genetic structure of a

Dice, L. R. 1945. Measures of the amount of ecologic association between species.

Dowling, T. E., W. R. Hoeh. 1991. The extent of introgression outside the contact zone
between Notropis cornutus and Notropis chryscephalus (Teleostei: Cyprinidae).
Evolution 45: 944-956.


Emms, S. K., M. L. Arnold. 1997. The effect of habitat on parental and hybrid fitness:

Press.

diversity in the rare Calamagrostis porteri ssp. insperata (Poaceae): Comparative
results for allozymes and random amplified polymorphic DNA (RAPD) and

granule-bound starch synthase (GBSSI) gene in the Rosaceae: Multiple loci and

153 p.

within the genus Citrus (Rutaceae) and related genera as revealed by RFLP and


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