GENETIC DIVERSITY AND ORIGIN OF
TWO QUEEN CHARLOTTE ISLANDS PLANTS:
SENECIO NEWCOMBEI AND SAXIFRAGA TAYLORI

by

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We accept this thesis as conforming
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Questions concerning the history and origins of two vascular plants endemic to the Queen Charlotte Islands, *Senecio newcombei* and *Saxifraga taylori*, are examined from a biosystematic and population genetics standpoint.

Both species maintain levels of genetic variation lower than would be expected from their breeding system and geographic range. Historical factors such as population bottlenecks and founder events may have contributed to the genetic profiles of these species, a suggestion consistent with their hypothesized survival in Pleistocene glacial refugia.

The allozyme variability of a third species, *Saxifraga vespertina*, was assessed to compare its genetic complement to *S. taylori*. Although complicated by an overall lack of variation, a low estimation (0.224) of Nei's genetic identity does not support the hypothesis of a historically recent divergence of the two species.
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for my Mom and Dad.
Chapter 1

Introduction

From the lone shieling of the misty island
Mountains divide us and the waste of seas
John Galt

1.1 The Queen Charlotte Islands

The islands of the world have provided generously for the advance of evolutionary theory. Their isolation, practicable size, and often discernible age make them nearly ideal natural laboratories, and they have fostered many significant innovations in thought (Carlquist, 1974). The modern concept of evolution, it may be argued, crystallized in the mind of Charles Darwin during his exploration of the Galápagos Islands (Darwin, 1839). Islands, both oceanic and continental, remain fertile ground to modern investigations of the evolutionary process (Crawford et al., 1987; Ganders, 1989; Inoue & Kawahara, 1990; Wendel & Percy, 1990).

The Queen Charlotte Islands form a crescent-shaped archipelago of 10,000 km² that lies between latitude 52 and 54°N, 100 km off the north-central coast of British Columbia, Canada (Fig. 1.1). The original inhabitants captured something of the islands' rugged and unique beauty in the name they gave their home, Haida Gwaii.

The islands' luxuriant forests and nearly pristine alpine habitats support a surprising array of endemic and disjunct organisms. Early explorations by Europeans revealed plants and animals unfamiliar to them (Greene, 1897; Kindberg, 1899; Osgood, 1901),
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Figure 1.1: The Queen Charlotte Islands
and as the potential for novelty in the flora and fauna became apparent, major surveys were launched to describe and explain the occurrence of this unexpected biodiversity.

Zoologists identified several endemic forms of insects, fish, birds and mammals (McCabe & Cowan, 1945; Lindroth, 1961; Foster, 1965). Extensive collection and analysis of the bryoflora on the islands has led to the description of several endemics and spectacular species disjunctions (Persson, 1958; Schofield, 1965, 1968, 1969). Botanical surveys of the islands between 1957 and 1964 provided the material for the landmark *Flora of the Queen Charlotte Islands* (Calder & Taylor, 1968). This publication treated a flora four times larger than was known preceding it, including fourteen endemic (Table 1.1) and many disjunct taxa.

Recent investigations of the Queen Charlotte Islands continue to furnish discoveries of biogeographical significance (Roemer & Ogilvie, 1983; Ogilvie, 1994; Roemer in Douglas, 1995; W. Schofield, pers. comm.).

1.2 Pleistocene Glacial Refugia

Hypotheses that attempt to explain the existence of endemic and disjunct organisms at high latitudes must consider the role of glaciation. At regular intervals during the Pleistocene epoch (ca. $1.6 \times 10^6 - 10^4$ ybp) massive glaciers flowed from the polar areas and mountains of the northern hemisphere to envelop intervening land forms in upwards of 2000 m of ice (Flint, 1971). Organisms that survived the extreme rigour of these "ice-age" conditions would necessarily have done so in ice-free areas called refugia. Biogeographers have long considered these refugia in their attempt to explain the occurrence of endemic and disjunct taxa in areas with evidence of glaciation. Fernald (1925) developed a theory which suggested that 'nunataks', mountain peaks untouched by the glaciers that surrounded them, were the refuge and subsequent dispersal centre of plant
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Table 1.1: The vascular plant endemics of the Queen Charlotte Islands as described by Calder and Taylor (1965). Taxa marked with a star were subsequently found on the Brooks Peninsula, Vancouver Island.

endemics. Hultén (1937) emphasized the importance of a larger refugium in Alaska to the present-day distribution of the arctic and boreal flora.

A similar interpretation of the origins of the endemic flora and fauna in the Queen Charlotte Islands exposes conflict between the geological and biological evidence for refugia.

Of the several glacial episodes experienced by the Pacific coast of North America during the Pleistocene, extensive evidence is available only for the most recent (Clague, 1989a). During this event, the Late Wisconsinan or Fraser glaciation, the Cordilleran Ice Sheet covered the entire coast north of the conterminous United States and extended well beyond the present coastline (Flint, 1971; Clague, 1989b). Continental islands, including the Queen Charlottes, were understood to have been heavily glaciated.
Early geological reports claimed to be quite definitive about the extent of glaciation in the Queen Charlottes, and declared the islands to have been completely covered in ice at the height of the Fraser glaciation (Dawson, 1880 cited in Schofield, 1989; Sutherland Brown & Nasmith, 1962). Recent analyses of the geological record on the islands and nearby coast have led to similar conclusions about their recent and widespread glaciation (Dyke & Prest, 1987; Blaise et al., 1990).

Schofield (1989) points out that despite the unequivocal remarks of Sutherland Brown & Nasmith (1962), they actually conclude that a small percentage of land was unglaciated. Sutherland Brown (1968) repeats this apparent contradiction, which may simply reflect those authors’ opinion that areas of potential refugia are “insignificant” when compared to the extensive glaciation of the islands (Sutherland Brown & Yorath, 1989).

Some evolutionary studies have accepted the geological evidence and attributed biological distinctiveness in the Queen Charlotte Islands to rapid, post-glacial divergence (Moodie & Reimchen, 1976). But even the earliest researchers contended that the amount of post-glacial time available to evolutionary processes is inadequate to account for the observed divergence in plants and animals, suggesting the possibility of refugia on the islands (McCabe & Cowan, 1945).

Biologists studying the endemic vascular plants, bryophytes, insects, birds and mammals have all invoked refugia of some kind as an explanation for the large degrees of divergence from the mainland relatives of these groups (Foster, 1965; Schofield, 1969; Kavanaugh, 1980). Similar theories have been advanced to explain the presence of the widely disjunct species in these groups (Miller & Hubbs, 1969; Ogilvie & Ceska, 1984; Schofield, 1989).

Randhawa and Beamish (1972) include their own survey of ploidy levels in Saxifraga ferruginea with the other evidence of northwest coast refugia that they review. S. ferruginea is polyploid through most of its range except for apparently relictual populations
in the Queen Charlottes, Kodiak Island, and southeastern Alberta. They hypothesize that diploid populations of *S. ferruginea* represent a once widespread species, eradicated by glacial advance but for refugial populations. The present distribution of polyploid *S. ferruginea* indicates the ability of these individuals to rapidly colonize newly opened terrain in the slipstream of retreating glaciers.

A survey of mitochondrial DNA restriction site variation in the song sparrow *Melospiza melodia* over its entire continental range identified basal haplotypes from the Queen Charlotte Islands and Newfoundland (Zink & Ditmann, 1993). This implies that the mitochondrial genomes of individuals found throughout North America today are directly descended from populations that survived the most recent glaciation in the ice-free refugia of those islands.

In contrast, the recent construction of mitochondrial DNA phylogenies for the mammals of the Queen Charlottes does not support a distinct island lineage for the endemic black bear or pine marten. Coastal populations in Alaska and British Columbia, however, appear to be derived from a mtDNA lineage different from interior or eastern populations, data consistent with other hypotheses of coastal refugia. Morphological divergence in at least these two groups appears to be recent in origin and likely the result of post-glacial colonization and subsequent adaptation to a maritime selective regime (T. Reimchen, pers. comm.).

The strongest support for the existence of refugia is found in the paleobotanical data, which clearly demonstrates a lack of glaciation in certain areas at specific times.

Heusser’s (1955) analysis of post-glacial pollen profiles from several locations in the Queen Charlotte Islands reveals a diverse and well-developed flora unlikely to have resulted from recent migration. Heusser (1955, 1989) develops the ideas of Dahl (1946) to support the possibility of refugia in which such a flora may have persisted. While recognizing that even the ocean-buffered Queen Charlottes would have developed their
own cirque and valley glaciers in the Pleistocene climate, Heusser believes the islands escaped burial by the Cordilleran Ice Sheet (Heusser, 1989). In addition, the islands' outer coast may have presented considerable obstacles to the formation of large glaciers. There, Pacific Plate subduction has worn away much of the continental shelf (Sutherland Brown & Yorath, 1989) and the water plunges to depths exceeding 2500 m over a steep, 10–15% grade. The mountains along the coast of the Charlottes reach heights of nearly 1000 m, continuing the sharp incline observed in the submarine profile (Heusser, 1955). This descent from high mountains into very deep water immediately offshore would not have supported a significant accumulation of ice. Active ice sheets would continually calve into the sea, rather than backing up and engulfing nearby valleys (Heusser, 1955). Large areas near the coast, on and around various mountain peaks, could have been refugia to plants and animals during the Wisconsinan glaciation.

Subsequent paleobotanical investigations supported Heusser's findings, and extend the duration of known ice-free conditions on the Queen Charlotte Islands. Plant fossils from the Cape Ball area are dated to 16,000 ± 570 ybp (Warner et al., 1982), indicating ice-free conditions on part of Graham Island during the maximum Wisconsinan glaciation of the mainland. These data, combined with a well-documented eustatic drop in sea levels, suggest that life may also have persisted on a now-submerged platform in the Hecate Strait (Warner et al., 1982).

Although this evidence is strongly suggestive, it must be pointed out that an undisputed, continuous pollen core or sediment sample is not available to provide direct evidence of a continuous refugium in the Queen Charlotte Islands. Until such time as one is found, the question of refugia is likely to remain open.
1.3 Historical Biogeography of the Endemic Vascular Flora

Calder and Taylor (1968) embraced the idea of refugia in the Queen Charlotte Islands. Their chapter on phytogeography cites the work of Heusser (1960) and Foster (1965) to support their theory of a progressive, post-glacial migration of plants from refugial areas on the islands to the rest of the Queen Charlottes and nearby mainland (Calder & Taylor, 1968).

There is some indication that Calder and Taylor considered glacial refugia to be significant to the evolutionary history of some of the endemic species. In their discussion of the generic affinities of *Senecio newcombei*, they include that species, with *Saxifraga taylori*, *Ligusticum calderi*, and *Isopyrum savilei*, in a group of endemics whose closest relatives are found south of the Wisconsinan glacial boundary. By describing a pattern of relationships in this way, they appear to invoke a Pleistocene-refugia isolating mechanism in the origin of the vascular plant endemics.

The possibility of the endemic species’ survival or genesis in glacial refugia suggested several interesting and intractable problems to Calder and Taylor. The pre-glacial distribution of the various endemic taxa, and the evolutionary events which produced them were considered likely to remain unknown.

Botanical exploration of Vancouver Island in the early 1980’s opened a new chapter in the history of the Queen Charlotte Islands’ endemics. The Brooks Peninsula, (Fig. 1.1) a mountainous and remote area on the northwest coast, proved to contain several of the vascular plants previously known only from the Queen Charlottes (Pojar, 1980; Ogilvie & Ceska, 1984). Although the discovery was initially restricted to two species, the list has expanded to include nine of the original fourteen species considered endemic by Calder and Taylor (Table 1.1) (Ogilvie, 1989). The presence of these endemic species, other alpine disjuncts (Ogilvie & Ceska, 1984), and abundant fossil pollen of *Ligusticum*
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*calderi* dating from 11,000 ybp (Hebda, in press), provides evidence for a glacial refugium in this area. It seems unlikely that parallel colonization events could explain the similarity in the endemic flora of the two areas. Rather the presence of the endemics in the Brooks Peninsula argues strongly that they possessed a wider, coastal distribution in the past, and were restricted by glaciation to the two refugia where they survived independently (Ogilvie & Roemer, 1984).

1.4 Thesis Objectives

Despite the interesting evolutionary questions associated with the Queen Charlotte Islands/Brooks Peninsula endemics and disjuncts, none of the vascular plant species has been investigated genetically or biosystematically. The historical setting revealed by the recent discoveries on Vancouver Island suggests some particular lines of inquiry.

1.4.1 Genetic Variation

The amount of genetic variation within a species, and its distribution among populations has several important implications. Genetic variability may reflect a species' evolutionary past, as well as its evolutionary potential (Soltis & Soltis, 1991). Broad surveys of genetic variation in plants have documented its correlation with several life-history characters (Hamrick, *et al.*, 1979; Karron, 1987; Hamrick & Godt, 1989). In particular, geographic range and breeding system are strongly associated with gene diversity (Hamrick & Godt, 1989). Historical contingencies may account for the considerable portion of variability in gene diversity not attributed to ecological or life-history traits (Karron, 1991, Lewis & Crawford, 1995). For example, a presently widespread species may have experienced a population bottleneck through which its genetic variation was forced to flow.

It has been hypothesized that such an event did occur in the recent history of the
Queen Charlotte Islands’ endemics, where a prolonged survival in glacial refugia would have lowered population size for many generations (Ogilvie & Roemer, 1984; Schofield, 1989; Taylor, 1989).

Population genetic theory predicts that genetic variability is reduced in such circumstances (Wright, 1938; Kimura & Crow, 1964). The loss of variation associated with random genetic drift increases dramatically with the cumulative sampling effects in an extended population bottleneck (Nei, 1975; Barrett & Kohn, 1991). The probability of inbreeding is also greater in small populations and invariably leads to a reduction of genetic variation (Hamrick & Loveless, 1989; Soltis & Soltis, 1991). A less commonly considered, but important factor in a protracted range reduction, is the operation of strong natural selection for a limited variety of habitat types, promoting genetic uniformity (Babbel & Selander, 1974; Karron, 1991). These predictions have been tested by estimates of the genetic variation in plant species with either restricted distributions and population sizes, or the possibility of such a bottleneck in their recent history. Several of these studies have confirmed the predictions of theory (Levin et al., 1979; Schwartz, 1985; Waller et al., 1987 Pleasants & Wendel, 1989; Soltis et al., 1992; Qiu & Parks, 1994; Purdy & Bayer, 1995).

One objective of the present thesis will be to examine the levels of genetic variation in the Queen Charlotte Islands’ endemics Senecio newcombei and Saxifraga taylori, and determine whether these levels are consistent with the hypothesized population bottleneck encountered in Pleistocene refugia.

Enzyme electrophoresis has become the standard technique for investigating population genetic variation in plants (Kephart, 1990), and is used in this study. Measuring allozyme variability is efficient, cost-effective and provides a reliable estimate of genetic variation in natural populations (Soltis & Soltis, 1991).

Breeding system was found to be one of the most significant correlates of gene diversity
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in the survey of Hamrick and Godt (1989). A breeding system analysis provides important controls for interpreting the significance of genetic variation. For example, predominantly self pollinated populations are already characterized by low gene diversity, which would make bottlenecks difficult to identify. An objective of this thesis will be to examine the mating systems of the study species and determine the extent of inbreeding. Crossing experiments are particularly informative in this regard, as is an estimate of deviation in allele frequencies from values expected under Hardy-Weinberg equilibrium (Wright, 1965).

Useful comparisons can be made between the estimates of genetic variation in this study and those of wide-scale surveys in seed plants (e.g., Hamrick & Godt, 1989). Another helpful comparison could be made to variation in a closely related species, effectively removing the “background noise” generated by diverging evolutionary pathways (Layton & Ganders, 1984). In this study, genetic variation in the endemic *Saxifraga taylori* is compared to that in its presumed close relative *S. vespertina*.

1.4.2 Phylogenetic Affinities of the Endemic Vascular Plants

As noted above, the presence of the Queen Charlotte Islands' endemics on the Brooks Peninsula suggests that they were more widely distributed along the Pacific coast prior to Wisconsinan glaciation. The possibility of sympatry with their mainland congeners at some time in the past implies a different relationship among these species than that of recent progenitor-derivatives.

The information obtained by enzyme electrophoresis also provides a means to examine the genetic similarity of the study species. Allele frequency is the basis for the calculation of Nei's genetic identity (Nei, 1972).

Isozyme differentiation between sister species should be correlated with the time since
separation. If divergence were recent, possibly initiated by isolation in refugia, then genetic identities would be fairly high (Soltis, 1985; Witter & Carr, 1988). If the separation were a much older event, even pre-Pleistocene, lower identities could be expected (Elisens & Crawford, 1988).

An objective of this thesis is to compare the genetic identity of the two *Saxifraga* species with values known for other congeneric species pairs. Do they, for example, exhibit a genetic identity characteristic of sister species only recently diverged, or that of more distantly related congeners?

Again, it is important to have some knowledge of the mating system of the species being compared when interpreting their genetic similarity. The populations of autogamous species, for example, may be further diverged from one another or from populations of related species, providing a spurious indication of greater phylogenetic distance (Weller et al., 1996).

### 1.5 Study Species

**Senecio newcombei** Greene

First collected by Dr. C. F. Newcombe in 1897 on the West Coast of Moresby Island (Douglas, 1982), *Senecio newcombei* remains one of the true endemics of the Queen Charlotte Islands (Taylor, 1989). *S. newcombei* is a distinctive perennial with showy yellow ray flowers bordering its solitary head (Figs. 1.2, 1.3). Although not found on Vancouver Island or the nearby mainland, *S. newcombei* enjoys a wide distribution throughout the montane regions of the Islands, growing particularly well on open, rocky, or boggy slopes (Calder & Taylor, 1968).

It was initially intended that *Senecio porteri*, a rare high alpine endemic of Colorado and Oregon, would be included in this study. *Senecio porteri*, known from disjunct
Figure 1.2: *Senecio newcombei*, a true Queen Charlotte Islands endemic. Modified from Calder & Taylor, 1968.
Figure 1.3: *Senecio newcombei*. Photo courtesy of H. Roemer.
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populations in the Rocky Mountains of Colorado and Wallowa Mountains of Oregon (Peck, 1941; Weber, 1987) was considered to be closely related to Senecio newcombei (Calder & Taylor, 1968). Both species are included in section Aurei (Barkley, 1962), a result of similarities in leaf morphology and solitary flower heads (Calder & Taylor, 1968).

Although duplicates of Cusick's 1899 collection of S. porteri from northeastern Oregon are widely distributed in herbaria, the species has not been found there again and is now considered officially extirpated from that state (J. Kagan, pers. comm.). To collect living material from the several high-alpine areas of Colorado where it is available (T. Ranker, pers. comm.), was deemed prohibitively expensive. More importantly, its taxonomic affinity with S. newcombei has been brought into question by recent micromorphological studies (T. Barkley, pers. comm.).

Saxifraga taylori Calder & Savile

An alpine species widely distributed in rocky habitats of the Queen Charlottes, Saxifraga taylori has also been found on the Brooks Peninsula (Fig 1.4) (Ogilvie & Ceska, 1984). It forms extensive colonies on talus slopes, where it is often a pioneer colonizer (Calder & Savile, 1959). Saxifraga taylori is a striking endemic with relatively wide-open flowers bearing unspotted, white petals (Fig 1.5). Although diverging in several respects from other members of the section Trachyphyllum, an overall similarity and occurrence near the distribution centre of the section support its inclusion (Calder & Savile, 1959).

Saxifraga vespertiha (Small) Fedde

Saxifraga vespertina was considered by Calder and Savile (1959) to be a possible close relative of S. taylori (Fig 1.6). This species has a disjunct distribution among the Olympic Peninsula, southern Cascades of Washington, and the Columbia River Gorge (Fig 1.4).
Figure 1.4: Geographic distribution of *Saxifraga taylori* (triangles) and *S. vespertina* (circles). The star indicates the location of the Columbia River Gorge populations of *S. vespertina* cited in this thesis. Expanded from Ogilvie (1989) and Calder & Savile (1959).
Figure 1.5: *Saxifraga taylori*. Photo courtesy of H. Roemer.
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The fragmentary specimen of *Saxifraga taylori* that initiated the modern botanical investigation of the Queen Charlotte Islands was originally identified as *S. vespertina*. Describing *S. taylori* in their treatment of the section *Trachyphyllum* in North America, Calder and Savile (1959) detail similarities in the two species that may have contributed to the misinterpretation. In particular, the three-lobed leaves and soft texture placed the species together (Calder & Savile, 1959). *Saxifraga vespertina* is sometimes designated a variety of the *S. bronchialis* complex (Hitchcock et al., 1964).

1.6 Summary

*Senecio newcombei, Saxifraga taylori, and S. vespertina* were studied with several objectives in mind.

The first goal of this thesis was to make estimates of the genetic variation in the above species from electrophoretically detected variation at glycolytic enzyme loci. The levels in these species are compared to each other and to average values for plants with similar characteristics. Determining the mating system of the study species strengthens these comparisons by providing an additional framework in which to interpret the observed levels of variation.

The second major objective was to compare the genetic complement of the Queen Charlotte Islands/Brooks Peninsula endemic *Saxifraga taylori* to its presumed near relative *S. vespertina*, by calculating Nei's genetic identity. This ratio is compared to values for species pairs believed to represent various degrees of relatedness. Knowledge of the mating systems of these species is also important when interpreting this estimation of their phylogenetic affinity.
Figure 1.6: *Saxifraga vespertina*. Photo courtesy of C. Shaughnessy.
Chapter 2

Materials and Methods

There are some enterprises in which a careful disorderliness is the true method.
Herman Melville, *Moby Dick*

2.1 Collection Localities

2.1.1 *Senecio newcombei* and *Saxifraga taylori*

Living plants and herbarium voucher specimens (deposited at UBC) were collected from the Queen Charlotte Islands in October, 1993 and June, 1994. Locations (Fig. 2.7), population codes, and species collected, including a brief description of habitat and associated vegetation, are described below.

Tasu Mountain (TS). West coast, Moresby Island, south shore of Tasu Sound, alpine area 600–800 m above and to the south of the Tasu mine. Approximately thirty specimens each of *Senecio newcombei*, *Saxifraga taylori*, and *Cassiope lycopodiodes* ssp. *cristapilosa* were collected (October, 1993).

Tana Bay (TN). West coast, Graham Island. Rhyolite outcrops, 300–600 m above and due east of Tana Bay. Approximately thirty individuals of *Senecio newcombei* were collected around the nutrient rich seeps and small ponds, where they were frequently associated with *Senecio moresbiensis*. *Cassiope lycopodiodes* ssp. *cristapilosa*, *Ligusticum calderi*, and *Lloydia serotina* ssp. *flava* were also present and collected in small numbers (June, 1994).
Chapter 2. Materials and Methods

Chaatl Island (CH). Western mouth of Skidegate Channel. Approximately twenty specimens each of *Senecio newcombei* and *Cassiope lycopodiodes* ssp. *cristapilosa* were collected on the boggy, terraced slopes 300–500 m above a waterfall on the north-central part of the island. *Ligusticum calderi* was also present and a few specimens were collected. Near the peak of the island's highest point, at the first signs of exposed rock, seven specimens of *Saxifraga taylori* were collected (June, 1994).

Sleeping Beauty (SB). Northwest of Queen Charlotte City, Graham Island. Alpine area around Mt. Genevieve, at end of hiking trail above Forest Service Rd. 10. Halfway up to peak, 450–500 m, ten specimens of *Senecio newcombei* (these with large, glossy leaves and broad petiolar bases clasping the stem) were collected in two subalpine areas nicely defined by dense carpets of the moss *Rhytidiopsis robusta*, and *Fauria crista-galli*. At the base of the cirque on the eastern side of a ridge, in a boggy area around a pond, *Senecio newcombei* grew in abundance with *Dodecatheon jeffreyi*, *Viola langsdorfii*, and *Geum calthifolium*, among thick mats of *Cassiope mertensiana*. Approximately forty individuals of *S. newcombei* were collected here and further up the slope. At the 900 m peak, and down the west face on dangerously steep slopes, *Saxifraga taylori* was found with *Lloydia serotina* ssp. *flava* and *Pedicularis parviflora* ssp. *parviflora*. In wetter areas around peak, *S. taylori* grew with *Ranunculus cooleyae*, *Romanzoffia sitchensis*, and *Douglasia laevigata* ssp. *ciliolata*. Approximately forty individuals of *S. taylori* were collected here (June, 1994).

2.1.2 *Saxifraga vespertina*

In April 1994 a total of forty-five living plants of *Saxifraga vespertina* were collected from three populations in Multnomah County, Oregon (Fig 1.4). The specimens were found 100–300 m above the Scenic Highway off Interstate–84, on the south side of the Columbia river. Population codes and a brief description of locality are provided below.
Figure 2.7: Collection localities in the Queen Charlotte Islands
SVI. On trail east of Horsetail Falls, approaching upper falls. Dry rock face on nearly vertical cliffs, bound into crevices with various bryophytes, approximately 5 m above path.

SVII. 10 m above trail west of Horsetail Falls. In dry spots on vertical rock wall, avoiding wet seeps where \textit{Saxifraga occidentalis} flourishes.

SVX. Oneonta Gorge Trail #400, west of Oneonta Gorge, where trail begins to descend to Scenic Highway. Specimens were taken from a point 25 m above path, although the bulk of the population was out of reach beyond this point.

2.2 Electrophoretic Procedures

Leaf tissue was removed from the field collected plants growing in growth chambers at UBC. Although adult plants were used almost exclusively, seedlings of \textit{Senecio new-combei} were also sampled. Approximately 0.75 cm$^2$ sized samples from individual plants were placed into the wells of a chilled ceramic dish, softened with 3–4 drops of 0.1 M Tris-HCl (pH 7.5) extraction buffer (after Soltis \textit{et al.}, 1983) and ground into liquid consistency with a frozen pestle. Two to three, 3 x 10 mm wicks cut from Whatman 3 mm chromatography paper were used to absorb the extract from each well. Wicks were blotted with paper towel, and speed-loaded into a pre-cut slot in a chilled, 12.5% starch gel, along with an additional wick saturated with a marker dye. Additional wicks were preserved in a $-90^\circ$C freezer for future use.

Electrophoresis was performed with the continuous buffer systems of Soltis \textit{et al.}\,(1983), and a slight modification of their lithium-borate buffer described in Ashton (1990). Table 2.2 provides a summary of buffer systems employed, enzymes resolved, and typical run conditions.

Gels were electrophoresed at 4$^\circ$C in a deli-style refrigerator and then visualized using
<table>
<thead>
<tr>
<th>Buffer System</th>
<th>Enzymes Stained</th>
<th>Typical Run Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>LAP, ME</td>
<td>10 h @ 70 mA</td>
</tr>
<tr>
<td>8</td>
<td>PGI, TPI</td>
<td>10 h @ 70 mA</td>
</tr>
<tr>
<td>9</td>
<td>SkDH, 6PGD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PGM, IDH, GDH</td>
<td>6 h @ 45 mA</td>
</tr>
<tr>
<td>AA</td>
<td>LAP, PGI</td>
<td>9 h @ 55 mA</td>
</tr>
</tbody>
</table>

Table 2.2: A summary of buffer systems used in enzyme electrophoresis. Lithium-borate buffers (7 & 8) and Histidine-citrate buffer (9) after Soltis et al., 1983. Modified lithium-borate buffer (AA) from Ashton, 1990.
the staining protocols of Soltis et al. (1983), and Wendel and Weeden (1989). For each enzyme system, the fastest or most anodal isozyme was scored as locus ‘1’. Within each isozyme locus, the most anodal allozyme was scored as allele ‘a’.

2.3 Estimates of Genetic Variation

To quantify genetic variation in the study species, and provide a means of comparison to other taxa, several standard statistics were derived from allele frequencies. These values are normally calculated for individual populations, and mean values of these population data applied to the species as a whole (Soltis & Soltis, 1991). All statistics were generated by GeneStat-PC v.3.3 (Lewis, 1993).

Levels of genetic variation were estimated with: $P$, the percentage of polymorphic loci; $A$, the mean number of alleles per locus; $A_p$, the mean number of alleles per polymorphic locus; $H_{obs}$, the observed heterozygosity, and $H$, the average expected heterozygosity or gene diversity. The heterozygosity of a locus is defined as

$$h = 1 - \sum_{i=1}^{m} x_i^2,$$

where $m$ equals the number of alleles, and $x_i$ is the frequency of the $i$th allele (Nei, 1987). The average of this value over all loci is $H$.

Nei’s genetic diversity statistics (Nei, 1973) may be used to partition the genetic variation within and among populations (Hamrick, 1989). The gene diversity in all populations ($H_T$) has been defined by Nei as 1 minus the gene identity ($1 - J_T$) where

$$J_T = \sum_{i=1}^{m} x_i^2.$$

The derivation of within-population variation ($H_S$) is identical to that of $H$ above, and since total variation is equal to the sum of within and among-population variation,
Chapter 2. Materials and Methods

the value for diversity among populations \( (D_{ST}) \) is easily obtained from

\[
H_T = H_S + D_{ST}.
\]

To facilitate comparison between species, it is useful to calculate the relative magnitude of gene differentiation among populations. The proportion of the total variation distributed among populations is represented by

\[
G_{ST} = \frac{D_{ST}}{H_T}
\]

which is also referred to as the coefficient of gene differentiation.

GeneStat-PC provides estimates of \( D_{ST} \) and \( G_{ST} \) unbiased for sample size, after Nei and Chesser (1983).

2.4 Calculation of Genetic Identity

A useful estimate of the closeness of the relationship between two species is provided by Nei’s genetic identity (Nei, 1972). The calculation of identity, \( I \), utilizes allele frequencies and is based on the probability of any two randomly chosen, normally segregating genes at a particular locus being identical. In population \( X \) this probability is \( j_X = \sum x_i^2 \), while it is \( j_Y = \sum y_i^2 \) in population \( Y \) (where \( x_i \) and \( y_i \) represent frequencies of the \( i \)th allele). The probability of a similar identity between alleles in the populations \( X \) and \( Y \) is \( j_{XY} = \sum x_i y_i \).

For all loci, between the two populations, identity is defined as

\[
I = \frac{J_{XY}}{\sqrt{J_X J_Y}}
\]

where \( J_X, J_Y, \) and \( J_{XY} \) are the arithmetic means of \( j_X, j_Y, \) and \( j_{XY} \), respectively, over all loci (Nei, 1972). This ratio of probabilities is equal to one if two populations have an identical allelic complement, and is zero if they have no alleles in common.
2.5 Analysis of Breeding Systems

2.5.1 Senecio newcombei

Crossing experiments were performed on plants housed in growth chambers maintained by the Department of Botany at UBC. Plants were placed on a 16:8 h light:dark regimen, and subject to a temperature that fluctuated between 14 and 7°C over the same period of time.

Pollen transfer was accomplished in two ways. Pollen was collected on a white index card from one flower head and redistributed with a fine paint brush to others. For more precise transfers, cat whiskers were employed. These delicate fibers have a semi-glutinous surface which picks up and releases pollen beautifully.

To examine the extent of self-compatibility in Senecio newcombei, approximately thirty flowering individuals were either manually self-pollinated or allowed to complete a normal flower maturation sequence. This developmental process can include mechanisms for self-pollination (Douglas, 1982). In the latter procedure, flowering individuals were not disturbed, separated somewhat from others in flower and allowed to form a mature seed head. Flower heads of other individuals were covered with small cheesecloth bags and similarly isolated. For manual selfs, pollen was collected at anthesis and brushed onto the ring of flowers exhibiting the most receptive stigmas. More precise transfers onto the female, ray flowers were performed with a cat's whisker.

The heaviest pollen output of one plant frequently corresponded to the apparent maximum pollen receptivity of another, a condition that was inferred from an individual plant's presentation of a large number of flowers with open styles. This facilitated a series of outcrossing experiments in which pollen was collected from flowers presenting pollen, but whose style branches had not yet separated to expose the stigmatic surface.

\footnote{No cats were harmed in this study.}
This pollen was transferred with a brush onto the flower heads of other plants, and with a cat’s whisker to individual receptive stigmas on disk or ray flowers.

Mature achenes from selfs and outcrosses were harvested prior to their falling from the inflorescence, sterilized in a 3% sodium hypochlorite solution (after Schultz, 1993), and placed on filter paper in flat, plastic containers. Seeds were kept moist, and the percentage that germinated was recorded.

2.5.2 *Saxifraga taylori* and *S. vespertina*

Individuals of *Saxifraga taylori* were selfed in a fashion similar to that detailed above. The flowers are protandrous, and the anthers remain distant from the pistil until later in the flower’s development when they begin to curl inward and reach a point above the stigmatic surface. Typically the stigmas do not appear receptive until the second set (inner whorl) of five stamens has matured. Plants were artificially selfed by the manual transfer of pollen to the receptive or near-receptive stigmas.

Interspecific crosses between plants of *Saxifraga taylori* that were collected while in flower, or which flowered shortly after collection, and *Saxifraga vespertina* were attempted in the growth chambers. Pollen was transferred from the most mature anthers of one species onto the receptive stigmas of an emasculated flower. Both species were used as pollen donors and pollen recipients, and plants were sampled from all populations equally, with the exception of the Tasu (TS) population, which was not in flower at the same time as the others.
Chapter 3

Results

_Every form of refuge has its price._
D. H. Henley

3.1 Electrophoretic Patterns

The interpretation of the gel banding patterns is based on the known quaternary structure of the enzyme and its subcellular compartmentalization (Wendel & Weeden, 1989; Weeden & Wendel, 1989; Kephart, 1990).

3.1.1 _Senecio newcombei_

Of the twelve enzyme systems for which significant attempts at staining were made, eight resolved well and provided fourteen loci that could be interpreted genetically. Three additional loci resolved sufficiently but interpretation was prevented by intractable banding patterns. Inconsistent or lack of staining precluded the remaining systems from this analysis.

Three loci were polymorphic, _Pgi-2, Pgi-3_, and _Pgm-2_ (Table 3.3). The remaining eleven loci were invariable, displaying banding patterns similar to that of _Me-1_ shown in Fig. 3.8.

Below is a description of the genetic interpretation of the banding patterns recorded for polymorphic loci, and other observations of potential significance.
Table 3.3: Loci resolved and allele frequencies in *Senecio newcombei*. The three Queen Charlotte Islands’ populations are from Chaatl Island (CH), Sleeping Beauty (SB), and Tana Bay (TN).
Phosphoglucoisomerase (PGI)

Expression of PGI in diploid plants is usually controlled by two isozymes, a plastid and cytosolic form (Kephart, 1990). Three were scored in all populations examined, suggesting a duplication of one locus consistent with the claim that *S. newcombei* is polyploid (Taylor & Mulligan, 1968).

Although the locus *Pgi-1* was fixed for one allele, *Pgi-2* and *Pgi-3* were each interpreted as having two alleles. The most common banding pattern, a 9:6:1 (from the bottom band up) intensity ratio, is expected from the interaction of a heterozygote and homozygote at a duplicated locus (Fig 3.9). The predominance of the allele designated *Pgi-3b*, and consideration of the lack of variability at other enzyme loci, led to a conservative interpretation of the following banding patterns. A 1:2:1 ratio was observed for at least some plants in all populations. This could be the result of interaction between two heterozygotes or two loci homozygous for alternate alleles, since the darker, interlocus heterodimer would be observed in either case. Because of the rarity of the *Pgi-3a* allele inferred from the large number of 'unbalanced heterozygotes', the *Pgi-3* locus was scored as homozygous for *Pgi-3b*. This interpretation requires a *Pgi-2* locus homozygous for *Pgi-2a* to produce the observed 1:2:1 banding pattern.

Although nearly fixed for the *Pgi-3b* allele, all three populations had at least one individual with the typical 9:6:1 pattern reversed, indicating maintenance of a *Pgi-3a* allele at the second locus. Interpreting the fairly frequent 1:2:1 banding pattern as an
Figure 3.9: Diagrammatic representation of the assignment of alleles and sample banding phenotypes for the dimeric enzyme PGI in *Senecio newcombei*. The genotypes are recorded as *Pgi-2/Pgi-3*. The 3-banded, 9:6:1 staining intensity ratio seen in lanes 1 and 2 is characteristic of an interaction between a heterozygote and homozygote at loci *Pgi-2* and *Pgi-3*. The conservative interpretation of two homozygous loci for the 1:2:1 ratio is shown in lane 3. The middle band in lanes 1–3 is an interlocus heterodimer.

Interaction between two heterozygotes would make the *Pgi-3a* allele more common, and less unusual an occurrence in all three populations of *S. newcombei*.

**Phosphoglucomutase (PGM)**

Poor resolution and inconsistent scoring prevented the inclusion of the *Pgm-1* locus (likely the plastid form) in these results. The complex, albeit unresolved, multi-banded patterns may indicate a duplication of the *Pgm-1* locus.

The cathodal staining patterns were interpreted as the product of two loci, one fixed for the allele *Pgm-3a*, and the other maintaining three alleles bracketing the first. The fastest-migrating allele of the *Pgm-2* locus was detected only in the large Sleeping Beauty population. Since diploid plants typically possess two loci of this monomeric enzyme (Weeden & Wendel, 1989), the occurrence of three loci for PGM in *S. newcombei* may represent a duplication event.
6-Phosphogluconate dehydrogenase (6PGD)

A three-banded pattern in the anodal staining region was recorded for all individuals. The absence of any variation in this pattern suggests a phenomenon known as fixed heterozygosity. Two loci of a dimeric enzyme, both fixed for a single allele, will form homodimers and an intergenic heterodimer, producing the typical 1:2:1 ratio.

All individuals in all populations expressed a single allele at 6Pgd-3.

3.1.2 Saxifraga taylori and S. vespertina

In Saxifraga taylori, successful staining of seven enzyme systems provided eleven loci for interpretation. Three of these loci were polymorphic, Pgm-2, 6Pgd-1, and Skdh-1.

In Saxifraga vespertina, banding patterns suggested ten loci, five of which were variable, Idh-2, Lap-1, Pgm-2, 6Pgd-1, and Skdh-2. The remaining loci observed in both species were fixed for a single allele. The loci analysed in S. taylori and S. vespertina and their allele frequencies are displayed in Table 3.4.

A small degree of inter-population difference in allelic complements was observed in both Saxifraga species. In two instances, the small Chaatl Island population of S. taylori lacked several alleles present in the larger populations surveyed. For the isozyme 6Pgd-1, both the CH and TN populations lacked a rare allele found in the SB population. The Idh-2b allele, found in all other populations of both species, was absent in the SVX population of S. vespertina.

The genetic interpretation of the banding patterns is described below, with significant observations made concerning particular loci.
<table>
<thead>
<tr>
<th>Locus (N) allele</th>
<th>Population</th>
<th>Saxifraga taylori</th>
<th>Saxifraga vespertina</th>
</tr>
</thead>
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<tr>
<td></td>
<td>TS</td>
<td>CH</td>
<td>SB</td>
</tr>
<tr>
<td>Idh-1</td>
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<td>0.00</td>
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<td>0.00</td>
</tr>
<tr>
<td>b</td>
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<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>c</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Idh-3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>a</td>
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<td>1.00</td>
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<td>Lap-1</td>
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</tr>
<tr>
<td>b</td>
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<td>Pgi-1</td>
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<td>Pgm-2</td>
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<tr>
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<td>1.00</td>
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<td>Skdh-1</td>
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</tr>
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<td>0.60</td>
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<td>0.59</td>
</tr>
<tr>
<td>Skdh-2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
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<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>b</td>
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<tr>
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</tr>
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<td>Tpi-1</td>
<td></td>
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</tr>
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<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>b</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 3.4: Loci resolved and allele frequencies in Saxifraga taylori and S. vespertina.
Isocitrate dehydrogenase (IDH)

At the *Idh-1* locus, *Saxifraga taylort* and *S. vespertina* were fixed for alternate alleles, similar to the situation observed at *Tpi-1*.

At the *Idh-2* locus, *S. taylort* was homozygous for the *Idh-2b* allele, also present in two of the *S. vespertina* populations although at a lower frequency. Two additional alleles, one migrating faster and one slower than *Idh-2b* were present in all *S. vespertina* populations. The *Idh-2* locus in *S. taylort* formed an intergenic heterodimer with the locus designated *Idh-3*, forming a 1:2:1 banding pattern in all individuals, typical of fixed heterozygosity.

Leucine aminopeptidase (LAP)

*Saxifraga taylort* exhibited a single allele, *Lap-la*, for this monomeric enzyme in all populations examined. *S. vespertina* populations, although often homozygous for *Lap-lb*, contained heterozygous plants expressing *Lap-lc*. Although no individuals homozygous for *Lap-lc* were observed, this does not represent a statistically significant deviation from the genotype frequencies expected under Hardy-Weinberg equilibrium.

Phosphoglucoisomerase (PGI)

Both species of *Saxifraga* were fixed for the same allele at *Pgi-1*, a pattern observed at only one other locus, *6pgd-2*.

Although *Pgi-2* stained well and banding patterns were recorded for most individuals, there were inconsistencies in its interpretation, and it could not be included in these results.
Chapter 3. Results

Figure 3.10: Scanned image of a starch gel stained for the isozyme 6Pgd-1 in Saxifraga taylori. Arrows indicate heterozygous individuals with the rare 6Pgd-1a allele.

Phosphoglcomutase (PGM)

The two largest populations of Saxifraga taylori possess some variability at the Pgm-2 locus, maintaining two or three alleles. Only one allele, Pgm-2d, was detected in the seven individuals collected from the Chaatl Island population. Saxifraga vespertina maintains a similar pattern of variability at Pgm-2, i.e. two allozymes in addition to Pgm-2c, one migrating slower and one faster than this most commonly expressed allele.

Saxifraga taylori and S. vespertina share only one allele for Pgm-2. Pgm-2e, apparently rare in S. taylori, has an average frequency of 0.36 in the Columbia River Gorge populations of S. vespertina.

6-Phosphogluconate dehydrogenase (6-PGD)

Two loci of 6PGD were scored consistently in both species. Two populations of S. taylori, CH and TS, were isomorphic for 6Pgd-1b. The population SB, however, contained a faster allele, 6Pgd-1a, present in four individuals. The presence of this allele is easily determined from the three-banded staining pattern produced by plants heterozygous for this dimeric enzyme (Fig. 3.10). Saxifraga vespertina has two slower alleles at 6pgd-1, which are present in all three populations.
Figure 3.11: Diagrammatic representation of the assignment of loci and alleles to the enzyme SkDH in *Saxifraga taylori* (lanes 1-3) and *S. vespertina* (lanes 4-5). *Saxifraga taylori* is fixed for the allele Skdh-2a, while *S. vespertina* is fixed for Skdh-1b.

**Shikimate dehydrogenase (SkDH)**

The behaviour of SkDH in both *Saxifraga* species is controlled by two genes. The assignment of alleles to loci of SkDH in both species is described in Fig. 3.11. *Saxifraga taylori* was polymorphic at Skdh-1, while *S. vespertina* expressed only the allele Skdh-1b. *Saxifraga vespertina* was, however, polymorphic at the Skdh-2 locus, while *S. taylori* was fixed for the allele Skdh-2a. The monomorphic nature of this locus in *S. taylori* can be seen in Fig. 3.12. Interestingly, the fairly infrequent allele, Skdh-1a was detected in both the Tasu and Sleeping Beauty populations of *S. taylori*. The two populations are fairly distant, in high alpine situations approximately 50 km apart, and on different islands.

### 3.2 Estimates of Genetic Variation

#### 3.2.1 Species Level Genetic Diversity Parameters

Values for several standard estimates of genetic variation are found in Table 3.5. The percentage of polymorphic loci was calculated by dividing the number of loci that are
Chapter 3. Results

Figure 3.12: Isolation of the Skdh-2 locus in ten individuals of Saxifraga taylori.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>L</th>
<th>A</th>
<th>$A_p$</th>
<th>$P$</th>
<th>$H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senecio newcombei</td>
<td>74</td>
<td>14</td>
<td>1.29</td>
<td>2.30</td>
<td>0.21</td>
<td>0.078</td>
</tr>
<tr>
<td>Saxifraga taylori</td>
<td>74</td>
<td>11</td>
<td>1.45</td>
<td>2.67</td>
<td>0.27</td>
<td>0.064</td>
</tr>
<tr>
<td>Saxifraga vespertina</td>
<td>45</td>
<td>10</td>
<td>1.70</td>
<td>2.40</td>
<td>0.50</td>
<td>0.206</td>
</tr>
</tbody>
</table>

Table 3.5: A summary of allozyme diversity in the study species. N = the # of individuals, L = the # of loci. Included are: $A$, the mean number of alleles per locus; $A_p$, the mean number of alleles per polymorphic locus; $P$, the percentage of polymorphic loci; and $H$, the average expected heterozygosity or gene diversity.

The observed levels of heterozygosity in populations of each species were compared to those values expected under Hardy-Weinberg equilibrium. There are no statistically significant differences between the observed and expected genotype frequencies in any of the populations examined for the three study species.

3.2.2 Nei’s Statistics of Genetic Diversity

Nei’s genetic diversity statistics were calculated for each species as presented in Table 3.6.
Chapter 3. Results

<table>
<thead>
<tr>
<th>Species</th>
<th>pops.</th>
<th>$H_T$</th>
<th>$H_S$</th>
<th>$D_{ST}$</th>
<th>$G_{ST}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senecio newcombei</td>
<td>3</td>
<td>0.0812</td>
<td>0.0808</td>
<td>0.0004</td>
<td>0.0049</td>
</tr>
<tr>
<td>Saxifraga taylori</td>
<td>3</td>
<td>0.0671</td>
<td>0.0650</td>
<td>0.0021</td>
<td>0.0313</td>
</tr>
<tr>
<td>Saxifraga vespertina</td>
<td>3</td>
<td>0.1942</td>
<td>0.1925</td>
<td>0.0017</td>
<td>0.0088</td>
</tr>
</tbody>
</table>

Table 3.6: Nei’s genetic diversity statistics unbiased for sample size. $H_T$ = Total gene diversity; $H_S$ = Gene diversity within populations; $D_{ST}$ = Gene diversity among populations; $G_{ST}$ = Coefficient of gene differentiation.

3.3 Genetic Identity

Nei’s genetic identity for *Saxifraga taylori* and *S. vespertina* is estimated as 0.224.

3.4 Breeding System Experiments

3.4.1 *Senecio newcombei*

The flower heads of *Senecio newcombei* that were outcrossed appeared to mature much faster than those that were self-pollinated. Their ligules wilted and fell from the inflorescence in less time, and the formation of a mature seed head was faster than in flowers either self-pollinated or covered with cheesecloth and left to self.

None of the ‘achenes’ (essentially dead flowers) collected from the selfed or isolated plants germinated. In contrast, a high percentage, approximately 70–80%, of the plump, dark achenes collected from outcrossed plants germinated within a week. This demonstrates the self-incompatibility of *S. newcombei*, at least in the individuals tested.

3.4.2 *Saxifraga taylori* and *S. vespertina*

No seed was produced from any of the self pollinations or crosses within each species, or from the attempted hybrid crosses.
Chapter 4

Discussion

*The race is not always to the swift, nor the battle to the strong,*

...*but time and chance happeneth to them all.*

*Ecclesiastes 9:11*

4.1 Genetic Variation

The significance and interpretation of genetic variability in natural populations remains one of the central problems of evolutionary biology. The attempts to characterize the configuration, maintenance, and evolutionary consequence of this variation has essentially defined the discipline of population genetics.

Despite the rigorous mathematical foundation the field had established, population genetic theory did not predict and could not explain the massive levels of variation detected with the advent of protein electrophoresis (Mani, 1984; Gillespie, 1991). Claims of widespread polymorphism and high heterozygosity (*e.g.*, Lewontin & Hubby, 1966) rendered untenable the "classical" theory of the early geneticists, Muller and Fisher (Minkoff, 1983). Differences of opinion over the mechanisms which alter the genetic structure of populations were polarized in a fierce debate, at the heart of which lay the neutralist-selectionist controversy (Lewontin, 1974; Selander, 1976). Although the more general problem of the relative importance of the forces effecting evolutionary change is unresolved, several attempts have been made to understand these phenomena in relation to protein diversity.
Chapter 4. Discussion

The neutral models of Kimura, Ohta, and Nei have been tested empirically with isozyme data (Nei, 1975; Soulé, 1976). The biochemical and physiological significance of allozymes have been investigated (Johnson, 1979), and connections sought between genetic and environmental factors (Bryant, 1974; Hamrick et al., 1979; Nevo et al., 1984). The latter method has demonstrated that levels of genetic variation vary nonrandomly among species and populations, establishing strong correlations with ecological, demographic and life-history characteristics (Nevo et al., 1984; Hamrick & Godt, 1989). The broad sampling of taxa and loci found in these studies provides a strong framework in which to place individual data sets for interpretation.

Two recent summaries of the plant electrophoretic literature provide especially useful points of comparison for the results in this thesis (Table 4.7). The comprehensive review of Hamrick and Godt (1989) separated the genetic diversity estimates from 653 studies into 32 categories of eight ecological and reproductive traits. The summary of DeJoode and Wendel (1992) reported average values of allozyme variability for 62 island endemic plant species.

Compared to the “average” plant species, *Senecio newcombei* and *Saxifraga taylori* maintain relatively low levels of genetic variation (Table 4.7). Although the populations of *Saxifraga vespertina* from the Columbia River Gorge have a slightly lower than average number of alleles per locus, the proportion of polymorphic loci is nearly identical to the mean value for other plant species. The average heterozygosity, although high, falls within the same range due to standard error.

A more informative comparison may be made between the study species and those plants with which they share certain geographic and life-history traits.
Table 4.7: A comparison of species level genetic diversity parameters between the Queen Charlotte Islands’ endemics and selected categories of seed plants. $A$, the mean number of alleles per locus; $P$, the percentage of polymorphic loci; and $H$, average expected heterozygosity or gene diversity. Standard errors are in parentheses.

<table>
<thead>
<tr>
<th>Species or Category</th>
<th>$A$</th>
<th>$P$</th>
<th>$H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senecio newcombei</td>
<td>1.29 (0.02)</td>
<td>21.4 (0.0)</td>
<td>0.078 (0.045)</td>
</tr>
<tr>
<td>Saxifraga taylori</td>
<td>1.45 (0.04)</td>
<td>27.3 (5.3)</td>
<td>0.064 (0.044)</td>
</tr>
<tr>
<td>Saxifraga vespertina</td>
<td>1.70 (0.06)</td>
<td>50.0 (3.0)</td>
<td>0.206 (0.069)</td>
</tr>
<tr>
<td>Mean for 449 plant species $^a$</td>
<td>1.96 (0.05)</td>
<td>50.5 (1.4)</td>
<td>0.149 (0.006)</td>
</tr>
<tr>
<td>Dicots</td>
<td>1.79 (0.04)</td>
<td>44.8 (1.5)</td>
<td>0.136 (0.007)</td>
</tr>
<tr>
<td>Short-lived, herbaceous perennial</td>
<td>1.70 (0.06)</td>
<td>41.3 (2.2)</td>
<td>0.116 (0.009)</td>
</tr>
<tr>
<td>Temperate region</td>
<td>1.91 (0.05)</td>
<td>48.5 (1.5)</td>
<td>0.146 (0.006)</td>
</tr>
<tr>
<td>Endemic distribution</td>
<td>1.80 (0.08)</td>
<td>40.0 (3.2)</td>
<td>0.096 (0.010)</td>
</tr>
<tr>
<td>Narrow distribution</td>
<td>1.83 (0.08)</td>
<td>45.1 (2.8)</td>
<td>0.137 (0.011)</td>
</tr>
<tr>
<td>Sexual reproduction</td>
<td>2.00 (0.05)</td>
<td>51.6 (1.5)</td>
<td>0.151 (0.006)</td>
</tr>
<tr>
<td>Sexual and asexual</td>
<td>1.69 (0.08)</td>
<td>43.8 (3.7)</td>
<td>0.138 (0.016)</td>
</tr>
<tr>
<td>Mixed mating system</td>
<td>1.68 (0.08)</td>
<td>40.0 (3.5)</td>
<td>0.120 (0.015)</td>
</tr>
<tr>
<td>Selfing</td>
<td>1.69 (0.09)</td>
<td>41.8 (2.9)</td>
<td>0.124 (0.011)</td>
</tr>
<tr>
<td>Outcrossing (animal pollinated)</td>
<td>1.99 (0.07)</td>
<td>50.1 (2.0)</td>
<td>0.167 (0.010)</td>
</tr>
<tr>
<td>Gravity dispersed seed</td>
<td>1.81 (0.05)</td>
<td>45.7 (1.9)</td>
<td>0.136 (0.008)</td>
</tr>
<tr>
<td>Wind dispersed seed</td>
<td>2.10 (0.09)</td>
<td>55.4 (3.0)</td>
<td>0.144 (0.010)</td>
</tr>
<tr>
<td>Island endemics $^b$</td>
<td>1.32</td>
<td>25.0</td>
<td>0.064</td>
</tr>
</tbody>
</table>

$^a$ from Hamrick & Godt (1989)

$^b$ from DeJoode & Wendel (1992)
4.1.1 Life-history Characteristics of the Study Species

All three study species are herbaceous, perennial dicots of temperate climate (Greene, 1897; Calder & Savile, 1959; Calder & Taylor, 1968). *Senecio newcombei* remains strictly endemic to the Queen Charlotte Islands, despite numerous attempts to find it on the Brooks Peninsula and nearby mainland (B. Ogilvie, pers. comm.). *Saxifraga taylori* may also be considered to have an endemic distribution, although small, disjunct populations are known from Vancouver Island. *Saxifraga vespertina* has several populations in the Olympic Mountains, the southern Cascades of Washington, and the Columbia River Gorge. Encompassing an area of less than 25,000 km², its range is best defined as narrow.

Reproduction in *Senecio* species may incorporate both sexual and asexual processes. Agamospermy has not been reported in the genus (R. Kowal, pers. comm.), but other means of vegetative propagation (apomixis) have been described (e.g., Fernald, 1950). While typical *Senecio newcombei* individuals possess a stem arising singly from a fairly short caudex (pers. obs.), Barkley's (1978) description includes the possibility that several individuals may arise from a branched rhizome.

In general, flower development in the Asteraceae effects a mixed mating system. The protandrous flowers initially encourage outcrossing but eventually allow self-pollination to occur as the style branches separate (J. Pojar in Douglas, 1982). In *Senecio*, conspicuous female ray flowers mature earlier than the disk flowers on the same flower head. This attracts insect pollinators, and enhances outcrossing (Marshall & Abbott, 1984). The relatively unspecialized flowers may attract hundreds of different visitors (Harper & Wood, 1957; Pojar, 1974). Tiny flies have been observed on *Senecio newcombei*, covered in pollen and moving between flowers (pers. obs.).

Many plant species have self-incompatibility systems and thus avoid the deleterious
effects of inbreeding depression in addition to promoting outcrossing (Charlesworth & Charlesworth, 1987). The inability of selfed Senecio newcombei flowers to produce viable seed (reported in Section 3.4), is consistent with the presence of a self-incompatibility system. There is conspicuous uniformity in the self-incompatibility system operating in a given family (Hiscock et al., 1995). Homomorphic, sporophytic self-incompatibility is characteristic of self-incompatible species in the Asteraceae (Bateman, 1952), and has been demonstrated in Senecio (Abbott & Forbes, 1993). It seems probable that the highly effective self-incompatibility system apparently possessed by Senecio newcombei is of the homomorphic sporophytic type.

The barbellate pappus attached to the achene in Senecio newcombei represents an adaptation to wind dispersal typical of the Asteraceae (J. Pojar in Douglas, 1982).

Nearly all Saxifraga species reproduce by seed (Spongberg, 1972), however, vegetative reproduction by aerial bulbils or offsets is common in many species and the exclusive means of reproduction for a few (Webb & Gornall, 1989). Saxifraga taylori will set fertile seed and spreads across rocky slopes with vigorous vegetative offsets (Calder & Savile, 1959). Saxifraga vespertina appears to grow in more discrete units, likely establishing itself in crevices via dispersed seed (Jolley, 1988).

Saxifraga flowers are protandrous, at least with respect to the first (outer) whorl of mature stamens, which increases the probability of outcrossing, compared to a lack of dichogamy. Extended flowering periods, however, may result in pollen transfer between flowers on the same individual, which is the equivalent of selfing (Webb & Gornall, 1989). Self-incompatibility has been documented in two species of Saxifraga (Elvander, 1984), but most are believed to be at least partly self-compatible.

Saxifraga flowers are adapted to entice insect pollinators, by secreting nectar from a disc that surrounds the ovary or styles (Spongberg, 1972). Beetles, flies, and less
commonly, bees, are typical visitors to the flowers (Webb & Gornall, 1989). Two interesting features of *Saxifraga taylori* suggest an enhanced ability to attract insects: the nectar-secreting disc is strongly developed, considerably more so than other species in the section; and the petals are widely divergent, making the flower saucer-shaped and more conspicuous than the cup flowers of its close relatives (Calder & Savile, 1959).

Although the genus *Saxifraga* has no specialized anatomy for seed dispersal, the dehiscent capsules are often held high on stems which oscillate in the wind and spread the seed a short distance (Savile, 1975). Water from melting snow may also play a significant role in seed dispersal for some alpine species (Webb & Gornall, 1989).

### 4.1.2 Comparison of Genetic Diversity

Hamrick and Godt's (1989) analysis revealed that geographic range and breeding system were the best indicators of species-level genetic diversity. Based on those characters and the other generalizations in Table 4.7, the Queen Charlotte Islands' species should maintain levels of variation lower than the average values for plants. Despite having wide-spread, large populations, both species are endemics, a factor to which gene diversity seems especially sensitive. The possibility of vegetative reproduction may also suggest slightly lower values for these estimates of variation, similar to the effect that a mixed-mating system in *Saxifraga taylori* may produce. In *Senecio newcombei* however, obligate outcrossing and wind-dispersed seed are more consistent with average to slightly higher levels of variation, and the effect of these characters may counteract its limited distribution.

The geographic range and reproductive strategy of *Saxifraga vespertina* may contribute to moderate levels of genetic diversity in that species. A 'narrow' distribution apparently depresses variation a small amount, but the absence of clonal growth and seed dispersal aided by a nearly vertical habitat may well compensate for limited range
and the level of selfing that occurs within a mixed-mating system. 

*Saxifraga vespertina* does indeed meet the expectations of the above hypothesis, displaying a perfectly average value of $P$ (percent polymorphic loci), and estimated $A$ (average number of alleles per locus) that corresponds closely to that parameter in plants with similar characteristics. Although the average value for expected heterozygosity ($H$) appears high, the associated error of this statistic increases its range to near the mean for narrowly distributed species ($0.206 - 0.069 = 0.137$), and well within the margin of sexual reproducers. This high estimate, however, coupled with the observed lack of deviation in genotype frequencies from Hardy-Weinberg equilibrium values, may suggest the relative unimportance of selfing to *S. vespertina*, despite its assumed capacity for mixed-mating.

The generalizations of Hamrick and Godt (1989) are borne out to some extent in the Queen Charlotte Islands' endemics. Estimates of gene diversity in *Senecio newcombei* are lower than those in similar outcrossers, but correspond well with values for other endemic species. The 'endemic distribution' character accounts for low levels of $H$ in *Saxifraga taylori* equally well.

In contrast, the small number of alleles and low percentages of polymorphic loci in the endemic species belie both reproductive characters and distribution. The estimates of $A$ and $P$ are lower than those expected for endemics, or any other category of life-history trait included in Table 4.7.

The lack of predictive power in the generalizations of Hamrick and Godt results in part from the kind of species included in their 'endemics' category. The papers from which their data were culled had examined only a few insular species. And while the "smaller, ecologically limited species" that they considered as endemics may have experienced reduced population levels during their evolutionary history, they are not as likely to have experienced the extremes of colonization events or population bottlenecks typical of oceanic island endemics (Carlquist, 1974). The apparent dearth of insular taxa in the
review of Hamrick and Godt prompted a reply from DeJoode and Wendel (1992) who reported averages from 62 oceanic island endemics (included in Table 4.7).

The Queen Charlotte Islands, which lie some 100 km off the coast of British Columbia are continental, not oceanic islands. Nonetheless, the genetic diversity parameters for Senecio newcombei and Saxifraga taylori bracket near perfectly those means from DeJoode and Wendel. Perhaps these Queen Charlotte Islands’ endemics have experienced genetic restructuring similar to that of the oceanic islands’ species. An extended survival in glacial refugia, the associated reduction in population numbers, inbreeding, and subsequent founding of post-glacial populations, may have an effect on genetic variation analogous to the long-distance dispersal related bottlenecks experienced by island endemics.

The possibility of unknown fluctuations in population size or mating system in a species’ evolutionary past undoubtedly represents a fundamental source of error to predictions of present levels of genetic variation. The eight traits used to separate the taxa analysed by Hamrick and Godt (1989) accounted for only 24% of the total genetic variation at the species level. Despite the strong associations found for range and breeding system, the greater part of variation in gene diversity may be a response to historical contingencies (Karron, 1991).

Hypotheses invoking historical factors such as population bottlenecks or founder events are frequently employed when reproductive or life-history characters cannot be used to explain genetic profiles (Qui & Parks, 1994). An especially pertinent class of examples, endemic species which maintain less genetic variation than would be expected from their simply having a restricted distribution, is discussed in Lewis and Crawford (1995). Past population restructuring was offered as explanation for unexpectedly low levels of variation in all of these cases (e.g., Kesseli & Jain, 1984; Soltis et al., 1991).
It seems reasonable to speculate that unknown historical events may be responsible for the lack of congruence between the observed levels of genetic variation in the Queen Charlotte Islands' endemics and the levels predicted by the suite of traits listed in Table 4.7.

The survival of *Senecio newcombei* and *Saxifraga taylori* populations in small refugia at the height of Pleistocene glaciation would provide a scenario consistent with the observed loss of gene diversity in those species. The distribution and population sizes of both species were certainly reduced, and the effect of genetic drift on their respective gene pools may have been amplified over the duration of the population bottleneck (Barrett & Kohn, 1991). According to Grant (1985), the long term action of genetic drift results in fixation at polymorphic loci, or the extinction of alleles. This outcome has apparently been realized in all populations of both endemic species.

These small and isolated populations would probably experience the effects of inbreeding. Even in the obligately outcrossing *Senecio newcombei*, consanguineous mating would be highly likely and ultimately increase homozygosity (Qiu & Parks, 1994). Long-term survival of harsh conditions in a restricted environment implies strong selection for the limited habitat possibilities and may encourage genetic uniformity (Babbel & Selander, 1974).

In post-glacially established populations, founder effects are crucial determinants of genetic architecture. The populations sampled in this study may well be secondarily established from genetically depauperate progenitor populations within refugial areas. For example, some specimens of *Saxifraga taylori* from Mt. Tasu were collected from the inner face of a large and very obvious glacial cirque.
4.1.3 Population Differentiation

The intra-species compartmentalization of variation may provide additional support for the refugial hypothesis.

Low $G_{ST}$ values in Table 3.6 indicate that very little of the observed variation is related to differences between populations. In *Saxifraga vespertina*, population-level variability is typical of a genetically vagile species, with low $G_{ST}$ resulting from high gene flow among the relatively close populations. This explanation is less likely to be applicable to the Queen Charlotte Islands' endemics, whose populations are remote from one another. It is more probable that multiple founder events from a source population with limited diversity is responsible for the similarity in allele frequencies. Even small amounts of migration provides an effective counterpoise to drift-related genetic divergence of isolated populations (Cabe & Alstad, 1994).

Alternatively, the similarity of fixed alleles in distant populations may represent the independent operation of genetic drift, pushing allele frequencies in small populations to the same end, *i.e.*, fixation of common, and extinction of rare alleles.

4.2 Genetic Identity

Recent compilations of genetic identity between congeneric species have strongly supported the findings of Gottlieb (1977), who reported a mean for interspecific genetic identity of 0.67 (Crawford, 1989). The accumulation of data has increased the range of values reported, however, from 0.25 to complete identity of 1.0 (Crawford, 1989). The characteristics of the species found at the extremes of this range may help to interpret the genetic identity of 0.224 computed for *Saxifraga taylori* and *S. vespertina*.

Recently diverged, progenitor-derivative species pairs consistently display high genetic identities (Crawford, 1990). A classic study in the genus *Stephanomeria* revealed almost
no divergence ($I = 0.94$) between an hypothesized progenitor-derivative pair (Gottlieb, 1973, cited in Crawford, 1990). Similarly, rapid radiation of island (Lowrey & Crawford, 1985) or continental (Soltis, 1985) taxa may result in high interspecific genetic identities. Hawaiian *Bidens* exhibit a range of 0.886–0.995 for interspecific identity (Helenurm & Ganders, 1985).

Species that have diverged gradually over a longer period of time may accumulate sufficient allelic novelty to effect greater genetic distance (Witter & Carr, 1988). Average genetic identity among three groups of *Layia* (which virtually defined the geographical model of speciation) is estimated at 0.598 in Warwick and Gottlieb (1985).

### 4.2.1 Genetic Similarity of *Saxifraga taylori* and *S. vespertina*

The incredibly low genetic identity between *Saxifraga taylori* and *S. vespertina* would seem to extinguish the possibility of a recent, refugial-driven divergence. In fact, a value of $I = 0.224$ requires explanation beyond long-term separation of the two taxa.

Estimations of $I$ are particularly affected by differences between individual loci. The number of loci which are completely different or exactly the same accounts for the greater part of interspecific distance (Ayala, 1975). It is clear from Table 3.4 that genetic differentiation of the two species of *Saxifraga* is more likely to be a function of fixed allele differences than divergence in allele frequencies at polymorphic loci.

Variability in breeding system may contribute to unexpectedly high levels of genetic divergence among related species (Weller et al., 1996). Increased inbreeding will result in the loss of genetic variation, higher homozygosity, and greater divergence between individual loci. While self-fertilization alone will produce this effect, consanguineous mating may be equally important in smaller populations (Qui & Parks, 1994). The existence of such conditions in refugial populations of *Saxifraga taylori* would be consistent with an exaggerated genetic distance between it and *S. vespertina*. 
Chapter 4. Discussion

Genetic drift is another mechanism that would operate on refugial populations. Population bottlenecks during the necessary restriction of population size, or due to post-glacial founder events, may also result in the loss of rare alleles and fixation of others. Although such historical events are not subject to disproof, they have been invoked to explain low genetic identities between related species (Sytsma & Schaal, 1985).

Limited sample size has a stochastic effect on the calculation of Nei’s identity not unlike genetic drift. Alleles shared by both species may not have been detected in this analysis. These populations sampled may maintain only a subset of their respective species’ actual diversity, or happen to possess genetic complements least similar to each other.

4.2.2 Phylogenetic Affinities of *Saxifraga taylori*

The unusually low genetic identity between *Saxifraga taylori* and *S. vespertina* may also reflect a true phylogenetic distance. The original discussion of the relationship between the two species offers little concrete evidence of a closer affinity (Calder & Savile, 1959). Calder and Savile specifically mention the three-lobed leaves of *S. taylori*, which “possibly indicate some relationship to *S. vespertina* and *S. tricuspidata*”. Yet *Saxifraga vespertina*, according to their description, displays small teeth (“sometimes little more than large cilia”) only some of the time on its otherwise entire lamina. The specimens of *S. vespertina* collected from the Columbia River Gorge did not display this tricuspidate morphology. Calder and Savile (1959) also cite the soft feel (despite a cartilaginous margin) of both species as a character separating them from *S. bronchialis* and *S. tricuspidata*.

The same treatment, however, describes the clawed petals of *Saxifraga taylori*, which dry a clear yellow, two characters it shares with *S. bronchialis* ssp. *funstonii*. This apparently innocuous suggestion of an alternative relationship for *Saxifraga taylori* has since
been indirectly endorsed by some other biosystematic research on the Queen Charlotte Islands’ endemics.

A review of the cytology and micromorphological characters of *Senecio newcombei* has strongly suggested its transfer to *Sinosenecio* B. Nordenstam, a segregate genus endemic to southeast Asia (T. Barkley, pers. comm.). It may not be unreasonable to look for affinities of *Saxifraga taylori* within the Asian branches of the *S. bronchialis* complex, or elsewhere in the section *Trachyphyllum*.

Such a reinterpretation would correspond well with Schofield’s (1969) suggestion that the species-level Queen Charlotte Islands’ endemics represent remnants of a much older flora, possibly Tertiary in origin.

### 4.3 Conclusions

The endemic plants display lower levels of genetic variation than average plants. Their low levels can be explained in some part by their distribution, but historical factors have to be considered to explain a further drop in variability.

Such historical events, namely population bottlenecks and founder events, are expected in a long-term survival in glacial refugia.

While several factors may explain the low genetic identity between the two species of *Saxifraga*, the ultimate explanation may simply be that they are less closely related than previously believed.


