THE GENETIC CONSEQUENCES OF CONTRASTING BREEDING SYSTEMS
IN *PLECTRITIS* (VALERIANACEAE)

by

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ABSTRACT

This study describes the consequences of contrasting breeding systems on levels and organization of isozyme variation in two closely related taxa, *Plectritis congesta* (Lindl.) D.C. and *P. brachystemon* F. & M. (Valerianaceae). Both taxa are self-compatible, herbaceous winter annuals which occur along the Pacific coast from British Columbia to California.

Nine enzyme systems were electrophoretically surveyed and fifteen loci identified in *Plectritis*. Genetic models were proposed to explain the observed variation. These models describe the multi-locus organization, subunit structure and allelic variation within each system. The models were tested with crosses and segregation analyses. When progeny classes were sufficiently large, segregations were analyzed using the chi-square statistic. Although *P. congesta* and *P. brachystemon* differed in their allelic complements, the data suggest that the multi-locus organization of the enzyme systems surveyed are identical in both species.

Outcrossing rates were calculated for nine populations of *P. brachystemon* and fifteen populations of *P. congesta*. Their mean outcrossing rates were $2.4 \pm 0.8\%$ and $70.2 \pm 4.8\%$, respectively, and the difference between these means is highly significant ($P \leq 0.01$). A number of genetic parameters were measured within each taxon to assess the effect that breeding system has on population structure. As measured by all parameters, the selfer maintains significantly less variation within populations than the outcrosser although their detected levels of total variation (H_T) are not significantly different.

These two species are ideal for comparison because: (i) they are closely related and have a large proportion of their alleles in common, (ii) the multi-locus organization of the enzyme systems studied is homologous and (iii) they have similar life-cycle strategies and habitat requirements. It is not uncommon, particularly on Vancouver Island, to find the two species growing sympatrically (*i.e.* in mixed populations). When the influence of site variation was controlled by comparing only sympatric populations of the two species, all genetic differences remained significant. The reduced level of variation within populations of the selfer was reflected in the F- statistics and in the analysis of gene diversity. On the average, *P. congesta* populations contained 85% of the variation detected within the species. Only 36% of the variation detected in *P. brachystemon* was maintained within local populations.

In the selfer, the reduced level of genetic variation within populations was accompanied by increased populational differentiation. Preliminary data suggest that differentiation, particularly in the selfer, can occur over short distances. However, whether this represents micro-geographic differentiation or drift in small reproductively isolated populations is problematic. In this taxon, allele frequencies and single locus heterozygosities fluctuated widely and different alleles were often fixed in adjacent populations. In *P. congesta*, allele frequencies and heterozygosities also fluctuated among populations but not with the amplitude observed in *P. brachystemon*. There appears to be no relationship between genetic distance and geographic distance. This implies that gene

flow between populations does not significantly influence allele frequencies. No evidence was found to suggest that the observed isozyme polymorphisms are selectively maintained. However, co-ordinated gene complexes and micro-habitat selection would probably not have been detected. It is concluded that the observed differences in the population structure of *P. brachystemon* and *P. congesta* is primarily the result of their contrasting breeding systems.

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1.0 INTRODUCTION

1.1 *Statement of the problem*

This study describes the consequences of contrasting breeding systems on levels and organization of isozyme variation in two closely related taxa, *Plectritis congesta* (Lindl.) D.C. and *Plectritis brachystemon* F. & M. (Valerianaceae). Both taxa are self-compatible, herbaceous winter annuals which occur along the Pacific coast from British Columbia to California. *P. brachystemon* has a slightly wider distribution, ranging from the Queen Charlotte Islands southward into southern California. These two taxa are morphologically very similar; the only significant differences are in their floral characters.

P. congesta has relatively large flowers. The corollas average 6-10 mm. in length, are bright pink and bear a nectariferous spur. They are strongly fragrant and are actively visited by a number of pollinators. Although self-compatible, *P. congesta* is strongly protandrous. However, since numerous flowers on the inflorescence may be open simultaneously, geitonogamous pollinations are possible.

In contrast, the flowers of *P. brachystemon* are quite small. They are white to pale pink and average only 2-4 mm in length. Corollas may be spurred, but in some populations the spur is reduced to a mere swelling. The flowers are not markedly protandrous. The stigma is in close proximity to the anthers at anthesis. The flowers of *P. brachystemon* have only a very faint fragrance and pollinators were not observed visiting the flowers. This rarity of insect visits has been reported earlier by Dempster (1958), Morey (1962) and Ganders

et al. (1977b). Figure 1 illustrates the size difference between flowers of the two taxa.

The differences in floral characters strongly suggest contrasting breeding strategies. This was investigated and confirmed by Ganders *et al.* (1977a, 1977b) who utilized a seed wing dimorphism, present in both taxa, to estimate outcrossing rates. The inheritance of the seed wing is controlled by a simple one gene, two allele system with the winged allele expressing dominance (Ganders *et al.*, 1977a). Employing the progeny test method of Harding (1970), they found *P. congesta* to be primarily outcrossed; averaging 70% outcrossing over seven populations (Ganders *et al.*, 1977a; Carey and Ganders, in press). In contrast, *P. brachystemon* was found to be almost exclusively self-pollinated, averaging only 2% outcrossing (Ganders *et al.*, 1977b).

These two taxa are closely related; *P. brachystemon* may be a derivative of *P. congesta*. Stebbins (1957) argues that selfers are probably always derived from outcrossers. Many examples of the evolution of autogamous taxa from outcrossers have been demonstrated. Moore and Lewis (1965) presented cytological evidence that normally outcrossing, pink flowered, *Clarkia xantiana* has given rise to two distinct small flowered autogamous populations; one with pink and the other with white flowers. Antonovics (1968) investigated the evolution of self-fertility in heavy metal tolerant populations of *Agrostis tenuis* and *Anthoxanthum odoratum* from normally outcrossing non-tolerant populations. Arroyo (1973) concluded that autogamous *Limnanthes floccosa* has evolved from the outcrosser, *L. alba* (see also Ornduff and Crovello, 1968).

Figure 1. Flowers of (A) *Plectritis congesta* and (B) *P. brachystemon* illustrating differences in size and in the degree of separation of the stigma and anthers in the two species. The illustration shows *P. congesta* after the style has elongated when the stigma is receptive.

P. brachystemon and *P. congesta* have similar geographic ranges, life-cycle strategies and habitat preferences. In fact, sympatric (*i.e.* mixed) populations are not uncommon, at least on southern Vancouver Island. Babbel and Selander (1974) electrophoretically surveyed two pairs of edaphically restricted and widespread plant species and concluded that levels of genetic variability are influenced by ecological amplitude. Recently, Hamrick *et al.* (1979) reviewed all genetically interpretable, higher-plant allozyme literature published prior to June, 1978. Using multivariate techniques, they attempted to correlate twelve life-history characteristics with levels of allozyme variation among 100 plant taxa. The life-history variables which were identified as significantly affecting levels of genetic variability among the studied taxa were: mating system, pollination mechanism, generation length, chromosome number, fecundity, geographic range and successional status. Since *P. congesta* and *P. brachystemon* are closely related (*i.e.* possibly as progenitor and derivative) and have similar life-history characteristics, they offer a unique opportunity to study the genetic consequences of contrasting breeding strategies while controlling for genetic background and other variables which also influence the genetic structure of populations.

1.2 Breeding system and population structure

Populations of a predominantly outcrossed taxon are generally considered to consist of a large number of biotypes, each of which may be represented by only a single individual. The level of variation maintained within the population gene pool depends on the effective population size (a function of the size of the breeding population

and the outcrossing rate), mutation rate, gene flow and the modes and severity of selection.

In contrast, the traditional concept of autogamous populations is one of a number of discrete homozygous biotypes isolated from each other by breeding habit (DuRietz, 1930 and Stebbins, 1950, 1957). Each of these types may occupy a slightly different adaptive peak and be represented by a few or thousands of individuals. Successful biotypes are traditionally considered to maintain themselves as homozygous lines for potentially large numbers of generations. Besides these reproductively isolated sympatric lines, the populations may also consist of numerous hybrids which are the result of occasional crosses between these predominant inbred biotypes. The occurrence of these intermittent cross-pollinations is visualized as the source of great evolutionary activity within the population (Stebbins, 1950, 1957). The proportion of the population composed of these inter-racial hybrids will depend on the level of outcrossing in the population and the modes and severity of selection. The F_1 hybrids will be highly heterozygous. These hybrids may or may not be more vigorous than either of the parental types. If they survive and self, the F_2 generation will show a wide range of segregation. Successive generations will continue to produce new segregants, in diminishing numbers for seven to eight generations. The great majority of these types will be less fit than either of the original parents. But, should a new biotype with superior fitness be synthesized, it can be the progenitor of a new line. As Stebbins visualizes autogamous populations, the predominant biotypes are almost totally homozygous and their inter-racial hybrids are ephemeral. This model predicts that the vast majority of the variation within the population will be distributed between inbred lines

(i.e. families). Consequently, the level of variation maintained within the population will be determined by the number of inbred lines. This will depend on the effective population size, mutation rate, gene flow, habitat diversity and modes and severity of selection. If outcrossing rates are low and hybrids are ephemeral, the two most important factors influencing the levels of variation within populations should be habitat diversity and modes and severity of selection. Baker (1959) predicts that under severe selection only one biotype, or a very few, will exist and autogamous populations will be uniform, if present at all.

1.3 *Morphological variation in selfers*

A number of studies have investigated predominantly self-pollinated taxa to determine if the traditional population model, as proposed by Stebbins and others, agrees with quantitative phenotypic data and observations of morphological gene markers. Jain and Allard (1960) studied variation at nine morphological gene loci within experimental barley populations. They concluded that the variability maintained within these autogamous populations was not associated entirely with sympatric homozygous lines, and that levels of outcrossing lower than those frequently observed in selfers, if accompanied by selection for heterozygotes, could maintain considerable amounts of variability. Similar conclusions were reached when these same barley populations were analyzed for quantitative genetic characters (Allard and Jain, 1962). Allard and Workman (1963) discovered that, in lima beans, wide seasonal fluctuations in fitness were associated with certain morphological markers. However, mean selective values indicated

that heterozygotes left an average of 20% - 30% more progeny than either homozygote. Continuing this work, Harding, Allard and Smeltzer (1966) found that selection for heterozygotes was frequency-dependent. When heterozygotes were numerous, as in early generations following an interracial hybridization, they had little or no selective value. Consequently, they were rapidly reduced in number by inbreeding. This process slowed and an equilibrium was reached when heterozygotes comprised approximately 7% of the experimental population. When their frequency was below this level, their selective values increased and at very low frequencies heterozygotes produced approximately three times as many progeny as homozygotes. When heterozygotes were artificially reduced to a frequency of 2%, the 7% equilibrium value was restored within a single generation and this was maintained in the successive generation. Working with *Avena fatua*, Imam and Allard (1965) discovered that families, derived from single plants collected from natural populations, were highly heterogeneous for most measured phenotypic characters. In addition, families derived from the same site showed a wide range of means for various characters. They concluded that populations of this predominantly self-pollinating taxon consist of large numbers of biotypes and that many if not all individuals in natural populations are heterozygous at numerous loci. Kannenberg and Allard (1967) studied quantitative variation in eleven morphological characters within taxa of the *Festuca microstachys* complex. This complex consists of eight species which frequently occur in mixed populations and are almost exclusively self-pollinating. Since chasmogamous flowers are rare, Kannenberg and Allard estimated that it was unlikely that even one wild plant in 1000 would be an F_1 hybrid and it was probable

that the rate of outcrossing was lower than 1/10,000. However, when the observed variation was partitioned, a large proportion of the total genetic variance in all measured characters was attributed to within-population differences. Species within sites and families within species accounted for 10% and 30%, respectively, of the total phenotypic variance in their experimental plots. Furthermore, when the means of families within sites were compared for each of the eleven measurement characters, it was found that there were few families at any one site which did not differ significantly with respect to at least one measurement character. In other words, any single site contained plants of many different genotypes. They then compared data on three characters to measurements collected for two other species which had contrasting breeding systems; an obligate outcrosser *Lolium multiflorum* and *Avena fatua* in which outcrossing varies between 1% and 10%. Despite the differences in outcrossing rates, there were no consistent differences in the levels of within-population variation among these three taxa.

These studies of morphological variation in selfers are significant because they consistently found that the levels of variation within populations was higher than expected. These results have led several investigators (*e.g.* Jain and Allard, 1960; Allard and Jain, 1962; Imam and Allard, 1965; Kannenberg and Allard, 1967; Solbrig, 1972; Clegg and Allard, 1972; Hamrick and Allard, 1972; Allard *et al.*, 1972; Arroyo, 1975; Solbrig and Rollins, 1977; Levin, 1978; Keeler, 1978 and Hamrick and Holden, 1979) to question whether populations of autogamous taxa are as genetically uniform as traditionally believed. Heterozygote advantage, particularly if it is frequency-dependent,

insures that in highly inbred populations where inter-racial crosses are rare, bursts of segregation may be considerably more persistent than the classical model predicts. Allard and Jain (1962) suggest, given heterosis, that populations in which outcrossing is common (they do not specify how common) might consist largely of heterozygotes associated with numerous hybrid swarms overlapping in time. Such populations would be highly variable and would not differ significantly from populations of predominantly outcrossed taxa.

1.4 Isozyme variation in selfers

More recent studies have utilized gel electrophoresis in order to investigate the distribution of genetic variability within autogamous taxa and to evaluate the effects of breeding system on levels and organization of genetic variability. In *Avena barbata*, Clegg and Allard (1972) found that allele frequencies, at five enzyme loci and two loci governing morphological traits, were distributed in significantly non-random, macro-geographic patterns which were closely associated with the environment. Allard *et al.* (1972) found a large proportion of this variation to be associated with two co-adapted allele complexes. Populations which occupied xeric sites were monomorphic for a specific combination of alleles at five enzyme loci. Populations at mesic sites were monomorphic for an alternate combination of allozymes while populations in intermediate habitats were usually polymorphic at all five loci. Therefore, they concluded that epistatic interactions among alleles were important in the maintenance of genetic variability in *A. barbata*. In addition, Hamrick and Allard (1972) and Hamrick and Holden (1979) have found that these co-adapted allele complexes in

A. barbata are associated with micro-geographical variation within populations. Kahler *et al.* (1980) studied isozyme variation in *A. barbata* in Israel. They found that: each population contained many isozyme phenotypes, populations were differentiated by their phenotypic arrays, isozyme variation was distributed in mosaic patterns, and particular phenotypes were correlated with certain temperature and moisture-related variables. Crawford and Wilson (1977) analyzed isozyme variation at six loci in forty populations of *Chenopodium fremontii*. Most populations were monomorphic and the frequency of observed heterozygotes was extremely low (*i.e.* heterozygosity was detected in only thirteen plants, each one, heterozygous at a single locus). Keeler (1978) studied five enzyme systems in the hexaploid *Veronica peregrina*. No genetic analyses were attempted because of the polyploid inheritance. However, through progeny testing, individual plants were found to be highly variable. It was concluded that hexaploid inheritance allowed the maintenance of individual variation despite almost complete self-pollination. Babbel and Wain (1977) estimated outcrossing rates and analyzed the population structure of the tetraploid weed *Hordeum jubatum*. Four allozyme loci were surveyed and the outcrossing rates within two populations were calculated to be 1% and 3%. The observed heterozygosity at individual loci was low. However, a majority of the families surveyed were fixed heterozygotes at two loci as a result of polyploidy.

1.5 Breeding system and genetic variability

A relatively large number of published studies have examined levels of genetic variation in either selfers or outcrossers. However, only a few investigations have attempted to describe the effects

of different breeding systems on the organization of genetic variation in related species. Solbrig (1972) compared three self-incompatible and four self-compatible species of *Leavenworthia* for levels and organization of enzyme variability. Solbrig did not attempt any inheritance studies with the enzyme systems surveyed. Consequently, observed variation could not be interpreted with respect to allele frequencies and number of loci surveyed. However, based on band frequencies, he concluded that the self-compatible species showed less within-family and within-population variation than did the self-incompatible species. Solbrig and Rollins (1977) studied the evolution of autogamy in *Leavenworthia* (see also Lloyd, 1965). They analyzed both enzyme data (*i.e.* just in terms of band presence or absence and segregating versus non-segregating families) and morphological data on fruit characters. Generally, wider ranges of variation were found in the self-incompatible taxa. However, in one self-compatible species, within-population variability in fruit characters was greater than that found in two of the self-incompatible species. They concluded that the breeding system was the single most important factor determining population structure.

Levin (1977) analyzed allozyme variation at twenty loci in two self-incompatible and one self-compatible species of *Phlox*. The self-compatible species *P. cuspidata* selfs automatically in either the field or greenhouse and sets a full complement of seed. Using the maximum likelihood estimator of Brown and Allard (1970), an outcrossing rate was calculated for a "typical" population of this species and found to be $t = 0.22$. When levels of allozyme variation were compared, *P. cuspidata* had less total and within-population variability

than either of the self-incompatible species. Rick *et al.* (1977) studied genetic variation at 11 enzyme and two morphological loci in *Lycopersicon pimpinellifolium*. This is a self-compatible species in which outcrossing (*i.e.* measured as the proportion of cross-pollination) varied between zero and 40%. In this taxon all measures of population variability (*i.e.* heterozygosity and mean number of alleles per locus) were correlated positively ($P \leq 0.01$) with floral characters (*i.e.* anther length and stigma exsertion) and proportion of cross-pollination (*i.e.* estimated by the proportion of non-parental alleles appearing in progenies). In contrast, Arroyo (1975) detected no clear relationship between enzyme variability and degree of autogamy among populations of the selfer *Limnanthes floccosa*. She also concluded that although the selfer *L. floccosa* has slightly less within-population variation than the outcrosser *L. alba*, the two species have similar genetic structures. However, these conclusions should be accepted cautiously since no inheritance studies were conducted. Consequently, the number of loci surveyed was not determined and enzyme variability could not be interpreted in terms of allelic variation. In addition, Jain (1978) cites unpublished isozyme data which show that the selfer *L. floccosa* is much less variable within populations than the outcrosser *L. alba*.

Investigations of the effects of breeding system on the organization of genetic variability in related plant species has resulted in some conflicting conclusions. Arroyo (1975) detected no clear relationship between outcrossing rate and level of variation in two *Limnanthes* species. Allard *et al.* (1975) reported that *Avena barbata* is genetically less variable than *A. fatua* although their

outcrossing rates are 0.1 - 7.5% and 0.1 - 1.5%, respectively.

Jain (1978) found no relationship between levels of allozyme variation and outcrossing rates among populations of *Limnanthes alba*. In contrast, Levin (1978), Solbrig (1972) and Solbrig and Rollins (1977) found differences in levels of variation within self-compatible versus self-incompatible species of *Phlox* and *Leavenworthia*, respectively. Furthermore, in studies of *Leavenworthia* species (Solbrig and Rollins, 1977) and *Lycopersicon pimpinellifolium* (Rick *et al.*, 1977) it was concluded that the breeding system was the single most important factor influencing population structure.

Jain (1976) emphasizes that comparisons of the genetic structure between related species require quantitative descriptions of the breeding systems in nature and an evaluation of the significance of parameters used to describe the genetic variation. In a comparison of self-compatible and self-incompatible species of *Phlox*, Levin (1978) calculated outcrossing in only one population of the self-compatible species *P. cuspidata*. Although he considered this population to be "typical", it may not describe adequately the breeding system of this species. Outcrossing rates among populations of self-compatible taxa often vary over wide limits; *e.g.* zero to 40% in *Lycopersicon pimpinellifolium* (Rick *et al.*, 1977); 48%-80% in *Plectritis congesta* (Ganders *et al.*, 1977a) and 43%-97% in *Limnanthes alba* (Jain, 1978). Outcrossing frequencies were not calculated for the *Leavenworthia* species (Solbrig, 1972; Solbrig and Rollins, 1977) and the breeding systems in *Limnanthes* (Arroyo, 1975) were "estimated" by their degree of protandry and the ability to set seed in a pollinator-free

environment. In addition, no inheritance studies of enzyme variation were conducted in either *Leavenworthia* or *Limnanthes*. Consequently, variation in enzyme staining activity could not be interpreted with respect to the number of loci scored and allele frequencies within populations. Therefore, most of the studies which have attempted to correlate breeding system with population structure in related taxa have failed to either: (i) quantify the mating system(s), (ii) isolate the effects of the breeding system from other factors which influence population structure (*e.g.* genetic background and life-history characteristics) and/or (iii) describe the organization of variability in genetically significant terms (*e.g.* alleles at loci).

In an effort to clarify some of these issues, the present study analyzes the consequences of contrasting breeding strategies on the levels and organization of isozyme variation in *Plectritis brachystemon* and *P. congesta*. Populations, sampling methods and the electrophoretic techniques which were adapted for *Plectritis* are described in Chapter 2. In Chapter 3, the interpretation of enzyme mobility patterns is discussed. Genetic models which explain the observed variation are proposed and these are tested using the chi-square statistic. Allelic variation at fifteen loci is identified. The geographic distribution of alleles among populations and the relative contributions of various loci to the genetic diversity (Nei, 1975) of each species are described in Chapter 4. In Chapter 5, the analyses of the isozyme data are discussed. Both the outcrossing frequencies, calculated for each population, and the mean outcrossing rates for each taxon are compared. The level of variability maintained within

each population and the hierarchical organization of variation in *P. brachystemon* and *P. congesta* are quantified. Differences between the two species are interpreted with reference to their breeding strategies. In addition to the effects of breeding system on population genetic structure, a number of additional problems are addressed. Comparisons of genetic distance are used to make inferences regarding the levels of gene flow between populations and the taxonomic relationship of *P. congesta* and *P. brachystemon*. Lastly, the factors which influence the maintenance of the observed isozyme polymorphisms are discussed.

2.0 MATERIALS AND METHODS

2.1 *Collection sites and sampling strategy*

Isozyme variation was analyzed in ten collections of *Plectritis brachystemon* and fifteen collection of *P. congesta*.

Within each local population, seeds were collected from individuals in a randomized fashion. Seeds from each plant were kept separate and enzyme variation was scored in the progeny grown from these seeds. An effort was made to collect seeds from an unbiased sample of individuals that represented the genetic variability present in each local population. In the present study, a local population is defined as a group of individuals of the same taxon growing within a specified area: a topodeme in the terminology of Gilmour and Gregor (1939). When *P. brachystemon* and *P. congesta* grow intermixed or in close proximity, this is defined as a sympatric population. The term population does not necessarily imply that these aggregations of individuals are panmictic units (*i.e.* gamodemes).

Table 1 lists collection sites, species present, number of individuals sampled and date of collection. The location of populations is presented in Appendix A. Sites are listed in the order in which they appear in all tables. In order to illustrate possible macro-geographic patterns within each taxon, mainland populations are listed first, then Vancouver Island populations beginning with the Victoria area and moving north. Figure 2 shows the location of all sampled sites.

Table 1. Collection sites.

Sample designation	Locality	Species	Number of plants collected	Date collected
1	Sumas Mountain	<i>P.b.</i>	300	16 June 1977
2(1)	Anacortes, Washington	<i>P.c.</i>	36	17 June 1976
2(2)	Anacortes, Washington	<i>P.c.</i>	81	17 June 1976
13	William Head	<i>P.b.</i>	100	10 June 1977
3	John Dean Park	<i>P.c.</i>	99	20 June 1976
5	Mill Hill Park	<i>P.c.</i>	40	21 June 1976
4	Thetis Lake Park	<i>P.c.</i>	47	21 June 1976
9	Francis Park	<i>P.b.</i>	46	10 June 1977
9	Francis Park	<i>P.c.</i>	40	10 June 1977
10	Prospect Lake Road	<i>P.b.</i>	32	10 June 1977
10	Prospect Lake Road	<i>P.c.</i>	68	10 June 1977
11	Viaduct Avenue	<i>P.c.</i>	100	10 June 1977
7(1)	Malahat Drive	<i>P.b.</i>	55	21 June 1976
7(2)	Malahat Drive	<i>P.c.</i>	50	21 June 1976
7(3)	Malahat Drive	<i>P.b.</i>	100	11 June 1977
8	Crofton Exit	<i>P.c.</i>	60	22 June 1976
14(1)	Jack's Point	<i>P.c.</i>	90	12 June 1977
14(2)	Jack's Point	<i>P.c.</i>	90	12 June 1977
15(1)	Nanoose Hill	<i>P.b.</i>	29	22 June 1976
15(1)	Nanoose Hill	<i>P.c.</i>	22	22 June 1976
15(2)	Nanoose Hill	<i>P.b.</i>	56	12 June 1977
15(2)	Nanoose Hill	<i>P.c.</i>	37	12 June 1977
16	Little Qualicum Falls	<i>P.b.</i>	100	12 June 1977
17	Nile Creek	<i>P.c.</i>	61	24 June 1976
18	Elk Falls Park	<i>P.b.</i>	50	12 June 1977

Figure 2. The location of sampled *Plectritis* populations: ■ designate *P. brachystemon* populations, ● designate *P. congesta* populations and ▲ identify sites where both species were collected.

2.2 *Potential sources of bias in the collections*

2.2.1 *Sample size*

Given random mating, a sample size of thirty individuals per population is sufficient to be 95% confident of detecting alleles within populations which are present at frequencies ≥ 0.05 . It is difficult to determine what constitutes an adequate, and comparable, sample in *Plectritis* because these two taxa have dramatically different outcrossing rates and neither species experiences complete random mating. However, this sample size compares favorably with most studies of isozyme variation in plant populations, regardless of the breeding system. Therefore, an attempt was made to sample at least 30 individuals per population. Although most collections were sufficiently large to achieve this level of sampling, poor germination, combined with technical problems, produced variation in sample sizes among populations and between loci within populations.

2.2.2 *Phenology*

Populations were sampled only once in a given year, consequently, only those plants with mature seed at the time of collection were included in the sample.

2.2.3 *Seed wing*

As seeds mature it is possible that there are differences in the retention of winged versus wingless seed within inflorescences. In populations polymorphic for the seed wing, this would systematically bias frequencies of the winged and wingless alleles in the sample.

Whether this would bias allele frequencies at the enzyme loci surveyed is not known since no linkage analysis was conducted.

2.2.4 Wahlund Effect - an apparent deficiency in heterozygotes as the result of sampling stratified populations

At many sites, plants were distributed over a wide area that encompassed a number of discrete micro-habitats. As a result of autogamy, vicinism and possible micro-site selection, it is possible that topodemes may represent aggregates of sub-populations (*i.e.* neighborhoods), each with unique allele frequencies. Several workers have demonstrated that populations differentiate over small distances (Antonovics, 1968; Antonovics and Bradshaw, 1970; Hamrick and Allard, 1972; Hamrick and Holden, 1979). Bradshaw (1972) states that effective population size should be measured in meters not kilometers. It was assumed, for the purposes of the present study, that all populations of each taxon were homogeneous and were comparable genetic units. However, lumping samples from subdivided populations biases calculated allele frequencies and produces over-estimates of expected heterozygosity (assuming Hardy-Weinberg equilibrium).

2.3 Methods of planting and growth regime

Enzyme variation was surveyed in the progeny grown from the collected seeds. All plants were grown under uniform conditions in order to eliminate possible genotype - environment interactions which might modify isozyme phenotypes.

Plants were grown in a standard potting soil mixture in nursery flats (254 mm. x 508 mm.). All flats were given an initial fertilizing (10 g. Hi-Sol 20-20-20/1800 ml. water), allowed to drain for 24 hrs. and then planted. Seeds were planted 5 mm. to 10 mm. deep, 200 seeds per flat. Wooden semi-micro grid locus markers (*i.e.* toothpicks) were placed next to each seed to identify individuals.

Planted flats were placed in a growth chamber set on a "long day-cool" regime: 16 hrs. of light at 12°C and 8 hrs. of darkness at 7°C with 50% relative humidity. This growth regime was chosen as it promoted germination and prolonged the basal rosette stage of development. Seeds germinated within ten days to two weeks and developed four true leaves three to four weeks after germination. Single basal rosette leaves served as the enzyme source material.

2.4 *Electrophoretic techniques*

Gels were run horizontally and were prepared using Electrostarch (Otto Hiller lot #307) at a 12.5% w/v concentration. Sucrose (10% w/v) was added to the gels to improve the resolution of certain enzymes and to toughen gels for ease of handling. Gels were prepared the afternoon preceding a run. Starch was cooked, degassed and poured into plexiglass gel molds (150 mm. x 200 mm. x 6 mm.). These were allowed to cool to room temperature, wrapped in plastic and stored overnight at 6°C.

Samples were prepared just prior to use as enzyme activity diminished upon freezing and thawing. Leaves were removed from plants and placed on spot plates for manual grinding. These plates were placed on ice to inhibit enzyme activity while samples were prepared for assay. One or two basal leaves were ground with two drops of grinding buffer (0.1 M Tris-HCl, pH 7.5 with 0.5% 2-mercapto-ethanol) and an equal amount of insoluble PVP (*i.e.* polyvinyl-polypyrrolidone). The crude homogenate was then absorbed onto filter paper wicks (4 mm. x 10 mm., Whatman 3 MM paper) and these were inserted into slots cut 20 mm. from one end of the starch gel. Runs were standardized by monitoring the migration of a tracking dye (dilute red food coloring) which was absorbed onto wicks and placed in the outer sample slots of each gel. Genotype standards (*i.e.* identified homozygotes) were run, when needed, to check the mobility of isozyme variants.

Once the samples were loaded, all but 10 mm at either end of the gels were covered with plastic to prevent desiccation during the run. Gels were then placed on the appropriate electrode trays with the origin (*i.e.* samples) at the cathode. Electrode wicks (Handi-wipes) were placed on the unwrapped ends of the gel and current applied until the tracking dye had migrated the appropriate distance. Electrophoresis was conducted inside a large glass front refrigerator that was maintained at 0°-4°C. This prevented overheating which distorts the migrating front and ultimately denatures the proteins.

Two electrophoretic buffer systems were used:

I Discontinuous Li-borate/Tris-citrate buffer, pH 8.0
(adapted from Scandalios, 1969);

II Continuous Tris-citrate buffer, pH 7.0 (Siciliano and Shaw, 1976).

The composition of these buffers is given in Table 2. Electrophoresis was conducted at the following voltages:

I 350 V. ($\sim 60\text{mA}$) until tracking dye migrated 50 mm.,
approximately 4 hrs.;

II 250 V. ($\sim 60\text{mA}$) until tracking dye migrated 110 mm.,
approximately 4.5-5.5 hrs.

Upon completion of the run, gels were removed, sample wicks pulled and the gels trimmed and sliced for staining. Plexiglass strips 1.5 mm. thick were used as slicing guides. Monofilament fishing line (2 lb. test) was used to slice the gel. With a weight on top to prevent buckling, slicing strips were placed on either side of the gel. The fishing line was then held against the strips and drawn through the gel. Once a slice had been made, another pair of strips were placed on either side of the gel and the procedure repeated. It was possible to obtain six slices from a gel. The top slice was discarded. The others, as needed, were placed in the appropriate enzyme staining solutions and incubated at 37°C until the banding patterns could be scored. Table 3 lists the enzymes scored, the number of loci resolved, the appropriate running buffer and the staining reference. The number following the

Table 2 .

Running Buffers

I. DISCONTINUOUS Li-BORATE/TRIS-CITRATE BUFFER pH 8.0

BUFFER A: .03 M LiOH-.19M Boric Acid
 1.20 g Lithium hydroxide (monohydrate)
 11.89 g Boric Acid
 H₂O to 1 liter, adjust pH to 8.0

BUFFER B: .05 M Tris - .008 M Citric Acid
 6.2 g Tris
 1.6 g Citric acid (monohydrate)
 H₂O to 1 liter, adjust pH to 8.0

ELECTRODE: BUFFER A

GEL : 1:9 Mixture of Buffers A & B

REFERENCE: adapted from Scandalios, J.G. (1969) and
 Selander, et al. (1971).

II. CONTINUOUS TRIS-CITRATE BUFFER pH 7.0

ELECTRODE: .13 M Tris - .043 M Citric Acid
 16.35 g Tris
 9.04 g Citric acid (monohydrate)
 H₂O to 1 liter, adjust pH to 7.0

GEL : .013 M Tris - .0043 M Citric Acid
 1:10 dilution of Electrode Buffer (modified from
 reference)

REFERENCE: adapted from Siciliano, M.J. & C.R. Shaw, (1976).

Table 3 . Running Buffer Used, Number of Loci Resolved and Staining References for Enzyme Systems Studied in *P. brachystemon* and *P. congesta*.

ENZYME	# LOCI SCORED	RUNNING BUFFER ¹	STAIN REFERENCE ²
ESTERASE EST E.C. 3.1.1.1	2	I	Gottlieb (1973a)
ISOCITRATE DEHYDROGENASE IDH E.C. 1.1.1.42	1	II	Allendorf, et al. (1977)
LEUCINE AMINO PEPTIDASE LAP E.C. 3.4.11.1	1	I	Roose & Gottlieb (1976)
MALATE DEHYDROGENASE MDH E.C. 1.1.1.37	2	II	Siciliano & Shaw (1976)
MALIC ENZYME ME E.C. 1.1.1.40	1	I	Roose & Gottlieb (1976)
PHOSPHOGLUCOSE ISOMERASE ³ PGI E.C. 5.3.1.9	3	I or II	Gottlieb (1973a)
PHOSPHOGLUCOMUTASE PGM E.C. 2.7.5.1	3	II	Roose & Gottlieb (1976)
6-PHOSPHOGLUCONIC DEHYDROGENASE 6PG E.C. 1.1.1.44	1	I	Allendorf, et al. (1977)
SUPEROXIDE DISMUTASE SOD E.C. 1.15.1.1	1		

¹ Running Buffers: I Discontinuous Li-Borate/Tris-Citrate Buffer pH 8.0
II Continuous Tris-Citrate Buffer pH 7.0

² Most of the staining techniques used are modification of the original reference

³ Due to problems with interpretation, this enzyme was not scored for *P. congesta*.

enzyme abbreviation is its Enzyme Commission code number (Florkin and Stotz, 1973). Table 4 gives the staining procedures and Table 5 provides a list of stock solutions. In addition, Appendix B lists enzyme assays and running buffers which were attempted but proved to be unsuccessful with *Plectritis*.

Gels were scored immediately after staining when the resolution was best. When staining was complete, gels were fixed in either a 1:4:5 acetic acid-methanol-water solution (Allendorf *et al.*, 1977) or a 1:1 glycerine-water solution (Siciliano and Shaw, 1976). The first fixation mixture toughened and preserved gels as semi-permanent records better than the latter. However, the glycerine-water fix better preserved the resolution of certain enzymes (*i.e.* MDH, PGI and PGM) and proved superior for gels that were to be photographed later.

TABLE 4. Summary of Enzyme Staining Procedures.

ENZYME	STAIN BUFFER ¹	NAD	NADP/MgCl ₂ ²	NBT/PMS ³	MTT/PMS ³	OTHER COMPONENTS
1. EST	2					60 mg α-naphthyl acetate 30 mg β-naphthyl acetate 100 mg Fast Blue RR
2. IDH	1		✓	✓		200 mg DL-isocitric acid
3. LAP	3					30 mg l-leucyl-β-naphthylamide (dissolved in 5 ml N, N-dimethyl formamide) 25 mg. Black-K-salt added after 30 min, incubation.
4. MDH	1	25 mg			✓	5 ml 1M Na-L-Malate
5. ME	4		✓	✓		140 mg L-Malic Acid
6. PGI	1		✓		✓	2 ml .018 M Fructose-6- phosphate 20 units G6PDH
7. PGM	1		✓		✓	75 mg Na ₂ α-D-Glucose-1- phosphate 3 ml .00017 M K ₂ α-D-Glucose- 1-phosphate 20 units G6PDH
8. 6PG	1		✓	✓		20 mg 6-Phosphogluconic Acid (Na ₃ Salt)
9. SOD						Develops in light on gels stained for ME, PGI, PGM.

¹ Stain Buffers: 1. .2M Tris-HCl pH 8.0
2. .1M K-PO₄ pH 6.4
3. .1M Tris-Maleate pH 6.5
4. .1M Tris-HCl pH 8.6

² 10 mg NADP + 1 ml 0.5 M MgCl₂

³ 10 mg NBT or, MTT + 5 mg PMS

Table 5.

Stock Solutions

SOLUTION	CONC.	STORAGE	ADDITIONAL
STAINING BUFFERS			
1. Tris-HCl pH 8.0	.2M	R	
2. K-PO ₄ pH 6.4	.1M	R	
3. Tris-Maleate pH 6.5	.1M	R	
4. Tris-HCl pH 8.6	.1M	R	
DYES, COFACTORS, ENZYMES & SUBSTRATES			
Na-L-Malate	1M	R	12.15g Na ₂ CO ₃ H ₂ O in 50 ml. H ₂ O Keep Cool, add 13.4g L-Malic acid H ₂ O to 100 ml.
MgCl ₂	.5M	RT	
G6PDH (Glucose-6-phosphate dehydrogenase)	10 units/ml.	R	Dissolve in .005M citrate pH 7.5
NAD	10 mg/ml	R	Dissolve in .005M citrate pH 7.5
NADP	10 mg/ml	R	Dissolve in .005M citrate pH 7.5
NBT	10 mg/ml	R*	
MTT	10 mg/ml	R*	
PMS	5 mg/ml	R*	

R: refridgerate

RT: room temperature

*: protect from light

3.0 INHERITANCE

3.1 Isozyme nomenclature

Fifteen loci, coding for nine enzymes, were identified in *Plectritus*. These enzymes are listed in Table 3. Interpretations of enzyme mobility patterns on gels are presented and genetic models to explain the observed variation are proposed.

Two terms, isozyme (isoenzyme) and allozyme need to be defined. Isozymes are multiple molecular forms of enzymes. Allozyme designates isozymes which are coded for by alleles at a single locus. The isozyme nomenclature adopted for *Plectritus* was modified from Allendorf and Utter (1979). An enzyme locus is identified by the enzyme's abbreviation, plus a hyphenated numeral if the system is controlled by multiple loci. All isozymes surveyed migrated toward the anode. The locus coding for the most anodally migrating proteins was designated one, the next as two and so on. Allozymes, and their corresponding alleles, are identified by a superscript according to their relative electrophoretic mobility. One allele, generally the most common, was arbitrarily designated 100. Other allozymes were assigned numerical values according to their migration relative to the 100 form. For example, the allele at the most anodal esterase (EST) locus, which codes for the allozyme migrating 14% further than the common form was designated EST-1¹¹⁴. Alleles coding for allozymes with no staining activity (*i.e.* nulls) were designated as 100n (*e.g.* PGI-3¹⁰⁰ⁿ), while allozymes with reduced staining activity are identified by an r following the mobility designation.

As a prelude to the inheritance studies, an initial population survey was conducted to sample the extent of variation present in both species. Whenever variation existed, enzyme systems were analyzed in *P. brachystemon* and *P. congesta* separately, to insure that the mode of inheritance was identical in each species. Heterozygotes were tentatively identified and selfed. Segregation ratios were then compared to the expected 1:2:1 ratio using chi-square analysis whenever progeny classes were sufficiently large to validly apply this statistic. Heterozygotes in *P. brachystemon* were extremely rare and at some loci were never detected. Therefore, to verify allelic variants, crosses between individuals presumed homozygous for alternative alleles were attempted. Because of the floral structure and timing of anthesis in *P. brachystemon*, flowers had to be teased open with forceps and emasculated prior to crossing. *Plectritis* produces achenes and the flowers within an inflorescence mature separately over a period of a few weeks. In consequence, it is not an ideal organism for inheritance studies. Successful pollinations produced a single seed and pollen contamination was often a problem. As a result, progeny classes in the inheritance studies were generally small. It is assumed that the small sample sizes are responsible for the segregation ratios which deviate from the expected. Presumed homozygotes were also selfed and the progeny analyzed to confirm genetic interpretations. Without exception, all such selfs produced progeny expressing the parental genotype.

Each enzyme system surveyed is described with respect to its multilocus organization, sub-unit (*i.e.* quaternary) structure and allelic variation. Polymorphic enzymes are discussed first followed by the monomorphic systems.

3.2 *Esterase* (EST) E.C. 3.1.1.1

Two major zones of activity appeared on gels stained for EST. Various numbers of lightly staining bands were also present but their inheritance could not be determined. The major zones of staining were interpreted to be separate loci. These were designated EST-1 and EST-2. Three alleles were detected at each locus (Figure 3 and Figure 4). The three EST-1 alleles were detected in both species. However, in contrast to the high level of heterozygosity in *P. brachystemon*, variation was rarely detected at this locus in *P. congesta* (Table 17). Three alleles were identified at EST-2 in *P. congesta* while all populations of *P. brachystemon* were monomorphic for EST-2¹⁰⁰ (Table 17).

Heterozygotes at EST-1 and EST-2 were identified by their double banded phenotypes. This was interpreted as evidence that EST in *Plectritis* is a monomeric protein. This observation agrees with the sub-unit structure of EST reported in other taxa:

Cucurbita spp. (Wall and Whitaker, 1971), oats (Clegg and Allard, 1973), barley (Kahler and Allard, 1970), Norway spruce (Bartels, 1971), Scots pine (Rudin and Rasmuson, 1973), *Stephanomeria* (Gottlieb, 1973a), *Gaura* (Gottlieb and Pilz, 1976) and *Drosophila subobscura* (Loukas *et al.*, 1979). Figure 5 is a photograph of two gels stained for EST.

Figure 3. The relative migration of allozymes detected at EST-1.

Figure 4. The relative migration of allozymes detected at EST-2.

Figure 5. Allozyme variation at EST-1; gel 1 - individuals 1-14 are the progeny from a selfed 1^{100} homozygote, individuals 15-29 are the F_1 progeny from the cross of a 1^{114} homozygote X 1^{189} homozygote (single banded 1^{114} individuals are the result of accidental selfs); gel 2- individuals 1-15 demonstrate 1:2:1 segregation in the progeny of a selfed $1^{100}/1^{114}$ heterozygote, individuals 16-29 are the progeny of a selfed 1^{100} homozygote. EST-2 did not photograph well but it is uniformly monomorphic in both gels.

It demonstrates the three alleles detected at EST-1 and illustrates the method used to interpret isozyme patterns. Unfortunately, EST-2 did not photograph well but it is uniformly monomorphic in these gels.

EST-1 was not scored in *P. brachystemon* population 16 (Little Qualicum Falls). Staining activity at this locus was inhibited as a result of spraying the plants with Malathion to combat an aphid infestation. However, the insecticide did not prevent the expression of allozymes at EST-2. This observation suggests that the isozymes coded by these two loci represent different functional classes of EST.

Segregation analyses are presented in Table 6 for EST-1 and Table 7 for EST-2.

3.3 *Leucine aminopeptidase* (LAP) E.C. 3.4.11.1

One locus was resolved on gels stained for LAP. In addition, other lightly staining bands were sometimes observed. This was a highly polymorphic enzyme in both species (Table 17).

Seven alleles were scored in *P. congesta* and all but one of these (LAP-1⁸⁰) were detected in *P. brachystemon* (Figure 6).

Heterozygotes were identified by their two-banded pattern. This agrees with the monomeric structure of LAP in other organisms: corn (Beckman *et al.*, 1964), *Cucurbita* spp. (Wall and Whitaker, 1971), knobcone pine (Conkle, 1971), Norway spruce (Lundkvist, 1974), Scots pine (Rudin, 1977), avocado (Torres *et al.*, 1978a) and *Drosophila subobscura* (Loukas *et al.*, 1979).

Table 6. Analysis of allozyme variation at EST-1

<i>P. brachystemon</i> : Selfed heterozygotes					
Individual	Genotype	Observed allozyme segregation in progeny			χ^2 (2) P
		A_1/A_1	A_1/A_2	A_2/A_2	
77-1-14c	89/114	5	7	3	*
77-1-26c	100/114	0	2	3	*
77-1-73c	100/114	3	11	1	*

<i>P. brachystemon</i> : Crosses					
Individual	Genotype	A_1/A_1	A_1/A_2	A_2/A_2	
77-1-13a x 77-1-1	89/89 x 100/100	0	11	0	
77-18-186 x 77-1-111a	89/89 x 114/114	0	8	0	
77-18-19c x 77-1-110c	89/89 x 114/114	0	10	0	

<i>P. congesta</i> : selfed homozygotes		
Individual	Genotype	Number of progeny**
76-4-5	100/100	7
77-9-8b	100/100	12
77-9-41b	100/100	8
77-9-43b	100/100	8
77-9-53c	100/100	11
77-9-54a	100/100	9
77-9-60c	100/100	14
77-9-64b	100/100	8
77-9-65a	100/100	9
77-9-84a	100/100	13

* sample size insufficient for a valid χ^2 test of significance

** all progeny expressed the maternal genotype

Table 7. Analysis of allozyme variation at EST-2

P. congesta: Selfed heterozygotes

Individual	Genotype	Observed allozyme segregation in progeny			$\chi^2_{(2)}$	P
		A ₁ /A ₁	A ₁ /A ₂	A ₂ /A ₂		
77-9-8b	74/100	0	9	3	*	
77-9-41b	74/100	2	3	3	*	
77-9-64b	74/100	1	4	3	*	
77-9-68b	74/100	0	4	2	*	
77-9-78b	74/100	2	4	1	*	
77-9-81a	74/100	1	3	4	*	
77-9-83a	74/100	7	1	0	*	
77-9-95a	74/100	2	3	3	*	
Σ		15	31	19	0.631	0.50-0.75

P. congesta: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-9-45c	74/74	7
77-9-60c	74/74	14
77-9-45a	100/100	6
77-9-45b	100/100	8
77-9-53c	100/100	11
77-9-64a	100/100	13
77-9-65a	100/100	9
76-4-5	126/126	20

* sample size insufficient for a valid χ^2 test of significance

** all progeny expressed the maternal genotype

Figure 6. The relative migration of allozymes detected at LAP-1.

Figure 7. Allozyme variation at LAP-1 in *P. congesta*: genotypes - 80/95, 95/95, blank, 95/100, blank, 95/100, 85/85, 100/100, 95/100, 80/80, 91/91, 95/95, 100/100, 95/95, 80/80, 95/95, 80/100, blank, 80/91, 91/95, 80/100, 100/100, 80/95, 85/95, 100/100, 100/100.

Figure 7 illustrates a sample of the variation present in *Plectritis*. This gel was typical of the genotype arrays detected in populations of *P. congesta*.

The results of crosses and segregation analyses are presented in Table 8.

3.4 Malate dehydrogenase (MDH) E.C.1.1.1.37

There were three zones of activity on gels stained for MDH. These were arbitrarily designated as zones A, B and C with A being the most anodal zone (Figures 10 and 11). As was found in maize (Longo and Scandalios, 1969), and pitch pine (Guries and Ledig, 1978), these three staining zones may represent isozymes associated with different cellular fractions (*i.e.* cytoplasm and mitochondria).

The zone closest to the origin, C, was a poorly resolved band which may or may not represent activity at a single locus. Zone B was composed of five to seven well defined bands. Most commonly, individuals possessed five bands. The middle band stained darkest, the outer two bands were much lighter and the other bands were intermediate in both position and staining. Variation was observed in the number, the position and the relative staining intensities of bands. The banding patterns suggest that there are at least three loci coding for MDH isozymes which migrate to this zone. The relative staining of this series of bands implies dosage effects caused by the formation of hybrid multimeric isozymes which have overlapping mobilities. MDH has been found to be dimeric in other

Table 8. Analysis of allozyme variation at LAP-1

P. brachystemon: Crosses

Individuals	Genotypes	Observed allozyme segregation in progeny		
		A ₁ /A ₁	A ₁ /A ₂	A ₂ /A ₂
77-1-13a x 77-18-25c	91/91x100/100	0	12	0

P. congesta: Selfed heterozygotes

Individual	Genotype	Observed allozyme segregation in progeny $\chi^2_{(2)}$			P
		A ₁ /A ₁	A ₁ /A ₂	A ₂ /A ₂	
77-9-84a	80/91	1	7	4	*
77-9-64a	80/105	3	6	4	*
77-9-83a	85/100	5	2	0	*
77-9-65a	91/100	0	7	1	*
77-9-78b	91/100	4	0	3	*

P. congesta: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-9-75b	80/80	10
76-4-5	91/91	20
77-9-45a	91/91	6
77-9-60c	91/91	14
77-9-53c	91/91	11
77-9-86	95/95	12
77-9-95a	100/100	13

* sample size insufficient for a valid χ^2 test of significance

** all progeny expressed the maternal genotype

organisms: horseshoe crab (Selander *et al.*, 1970), *Eucalyptis* (Brown *et al.*, 1975), pitch pine (Guries and Ledig, 1978), ponderosa pine (O'Malley *et al.*, 1979), Douglas fir (El-Kassaby, unpublished) and lodgepole pine (Layton and Yeh, unpublished).

Unfortunately, more work is needed to determine the inheritance of variation in zone B, consequently, these loci were not included in the analysis. The variation observed in zone A can be described by the existence of two loci designated MDH-1 and MDH-2. Variation at MDH-1 was common in both species while at MDH-2, no variation was detected in *P. brachystemon* and variation was extremely rare in *P. congesta*. The presence of bands of intermediate mobility between allozymes with different migrations indicate that the active enzymes coded by MDH-1 and MDH-2 are dimers (*i.e.* composed of two sub-units). Isozymes of multimeric enzymes are formed by the random association of protein sub-units. An individual, heterozygous for alleles A and B at a locus which codes for a dimeric enzyme, synthesizes three allozymes AA, AB and BB in a 1:2:1 ratio. Electrophoretically, heterodimers (hybrid dimeric enzymes) can be recognized because they have mobilities intermediate between the homodimeric allozymes and they stain darker, theoretically exhibiting twice the activity of the homodimers. The interpretation of variation at MDH-1 and MDH-2 was complicated. The observed staining patterns were consistent with a model which predicts that isozyme sub-units specified by both loci associate with each other forming both intra- and inter-locus heterodimers. The formation of inter-locus heterodimers of MDH has been observed in ponderosa pine (O'Malley, *et al.*, 1979), lodgepole pine (Layton and Yeh, unpublished) and Douglas fir (El-Kassaby, unpublished).

Figure 8 and Figure 9 depict the relative migration of allozymes scored at MDH-1 and MDH-2 respectively. MDH-1¹⁰⁰ and MDH-1¹¹³ were detected in *P. brachystemon* while MDH-1¹¹³, MDH-1¹²⁵ and MDH-1¹³⁹ were found in *P. congesta*. MDH-1¹⁰⁰ and MDH-2¹⁰⁰ have identical mobilities. The existence of MDH-2¹⁰⁰ was deduced from the presence of inter-locus heterodimers and confirmed with the discovery of the rare MDH-2⁸⁷ variant. Figure 10 and Figure 11 are photographs of gels stained for MDH. The bands visible in zone A of Figure 10 are the inter-locus heterodimers formed between enzyme sub-units coded for by MDH-1 and MDH-2. Heterodimers generally stain twice as darkly as homodimers and often, the MDH-1 homodimeric allozymes were not visible. However, MDH-1 homodimers are visible in Figure 11.

Data from crosses and segregation analyses for MDH-1 are presented in Table 9.

3.5 *Phosphoglucose isomerase* (PGI) E.C.5.3.1.9

There were two zones of activity on gels stained for PGI. These were arbitrarily labeled as zone A and zone B. Variation in the most anodal zone (A) appeared to be controlled by a single locus which was designated PGI-1. A two locus model sufficiently explained the variation observed in zone B and the corresponding loci were designated PGI-2 and PGI-3 (Figure 12). The multilocus organization of PGI in *Plectritis* is identical to that reported by Gottlieb (1977b) for the more advanced section of the genus *Clarkia*.

Figure 8. The relative migration of allozymes detected at MDH-1. The variation in Zone A is determined by two loci, MDH-1 and MDH-2. MDH-1¹⁰⁰ and MDH-2¹⁰⁰ have identical mobilities. The hollow bars represent the position of heterodimers (hybrid dimeric isozymes). In MDH, these bands are interpreted to be inter-locus heterodimers, formed by the random association of protein sub-units coded for by alleles at both MDH-1 and MDH-2. The variation in Zone B is determined by an unknown number of loci and was not interpreted in the present study.

Figure 9. The relative migration of allozymes detected at MDH-2.

Figure 10. Allozyme variation at MDH-1; genotypes - 100/100, 100/100, 100/100, 139/139, 125/125, 125/139, 125/139, 113/113, 125/139, 125/125, 125/139, 125/139, 125/139, 100/100, 125/125, 125/125, 125/139, 125/139, 125/139, 100/100, 100/113, 100/113, 100/100, 100/113, 100/113, 100/100.

Figure 11. Allozyme variation at MDH-1; all individuals are 100/100 homozygotes at MDH-2; note variation in zone B for plants 11 and 28.

Table 9. Analysis of allozyme variation at MDH-1

P. brachystemon: Crosses

Individuals	Genotypes	Observed allozyme segregation in progeny		
		A_1/A_1	A_1/A_2	A_2/A_2
77-1-13a x 77-18-25c	100/100x113/113	0	12	0
77-1-111a x 77-18-18b	100/100x113/113	0	8	0
77-1-110c x 77-18-19c	100/100x113/113	0	10	0

P. brachystemon: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-1-26c	100/100	6
77-1-73a	100/100	14

P. congesta: Selfed heterozygotes

Individual	Genotype	Observed allozyme segregation in progeny $\chi^2_{(2)}$			P	
		A ₁ /A ₁	A ₁ /A ₂	A ₂ /A ₂		
76-4-5	125/139	4	9	6	0.474	0.75-0.90
77-9-95a	125/139	3	4	7	*	
77-9-43b	125/139	2	3	2	*	
77-9-45c	125/139	1	4	0	*	
77-9-60c	125/139	3	9	1	*	
77-9-64a	125/139	4	6	2	*	
77-9-75b	125/139	3	4	3	*	
77-9-78b	125/139	5	2	0	*	
Σ		25	41	21	0.655	0.50-0.75
77-9-84a	113/125	1	1	1	*	

P. congesta: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-9-8b	125/125	12
77-9-45a	125/125	6
77-9-45b	125/125	11
77-9-53c	125/125	11
77-9-54a	125/125	9
77-9-70a	125/125	5
76-4-5	139/139	7

* sample size insufficient for a valid χ^2 test of significance

** all progeny expressed the maternal genotype

In *P. congesta*, an occasional plant appeared to be double banded at PGI-1. This suggests that allozymes at this locus are either functional as monomers or that unlike sub-units do not dimerize. However, this could not be confirmed as mobility differences between variants were small, resolution was not always adequate and the appropriate segregation analyses were not performed.

Variation at PGI-2 and PGI-3 in *P. congesta* produced complex banding patterns which were difficult to interpret. A two locus model, with intra-locus and inter-locus heterodimers, predicted a large percentage of the staining patterns observed in segregation analyses (Figure 13). This model agrees with the dimeric structure of PGI which has been inferred from isozyme studies in other organisms: rabbits (Noltmann, 1964), fish (Avisé and Kitto, 1973), *Gaura* (Gottlieb and Pilz, 1976), *Clarkia* (Gottlieb, 1977b), *Citrus* (Torres *et al.*, 1978b), pitch pine (Guries and Ledig, 1978) and ponderosa pine (Mitten *et al.*, 1979).

As more populations were surveyed, the number of alleles proposed to explain the observed variation became unwieldy. Minor variations in mobility and/or activity, inconsistency in the formation of heterodimers (also reported for AAT in *Citrus* by Torres *et al.*, 1978b) and the presence of nulls at both loci made it impossible to confidently genotype individuals. Figures 14 and 15 illustrate the variation in banding phenotypes observed in *P. congesta*.

Figure 12. Segregation of allozyme variation at PGI-2 and PGI-3 in *P. congesta*: this is the F_1 progeny of a selfed plant, heterozygous at PGI-2 and PGI-3 (individual 12 is *P. brachystemon*).

Figure 13. An allelic interpretation of the allozyme variation pictured in Figure 12 (hollow bars represent the position of heterodimers).

Figure 14. A sample of the allozyme variation detected at PGI-2 and PGI-3 in *P. congesta* (individuals 15, 23, 26 and 27 are *P. brachystemon*).

Figure 15. A sample of the allozyme variation detected at PGI-2 and PGI-3 in *P. congesta* (individual 15 is *P. brachystemon*).

It was determined that more inheritance work was required to consistently identify PGI-2 and PGI-3 allozymes. Consequently, this system was not scored in *P. congesta*. However, *P. brachystemon* proved to be much less polymorphic than *P. congesta* and the proposed inheritance model explained the observed variation. Therefore, PGI was included in the population surveys of this species.

No variation was detected at PGI-1 in *P. brachystemon*. However, the allele fixed in this species was not found in *P. congesta*. This locus was used throughout the study as one of the electrophoretic markers which identify the species. *P. brachystemon* can be differentiated from *P. congesta* in Figures 12, 14, 15, 19 and 20 by the presence of this slowly migrating allozyme. Figures 16 and 17 depict the variation observed at PGI-2 and PGI-3 in *P. brachystemon*. Four alleles were identified at PGI-2 and two alleles were found at PGI-3. PGI-2¹⁰⁰ and PGI-2⁹⁴ are pictured in Figure 18. PGI-2⁹⁴ was extremely rare. It was detected in the heterozygous state in only three individuals, two from population 16 and one from population 9. Since heterozygotes in *P. brachystemon* were rare and these same individuals appeared to be heterozygous at PGM-3, these bands may be artifacts. This variant was tentatively designated as a PGI-2 allozyme because of its staining properties. However, inheritance studies would be needed to confirm this. Figure 19 shows PGI-2⁴² in both the homozygous and heterozygous state. This allozyme is of interest because it was unique to Nanoose Hill and yet, in populations 15(1) and 15(2), its frequency was 0.15.

Figure 16. The relative migration of allozymes detected at PGI-2 in *P. brachystemon*.

Figure 17. The relative migration of allozymes detected at PGI-3 in *P. brachystemon*: the hollow bar represents an inter-locus heterodimer formed between PGI-2¹⁰⁰ and PGI-3^{33r}.

Figure 18. Allozyme variation at PGI-2 in *P. brachystemon*: individuals 8 and 9 are 94/100 heterozygotes, all other plants are homozygous for PGI-2¹⁰⁰; individual 15 is homozygous for PGI-3^{33r} (the low mobility band is a 2¹⁰⁰/3^{33r} inter-locus heterodimer), all other plants are PGI-3¹⁰⁰ⁿ homozygotes.

Figure 19. Allozyme variation at PGI-2 in *P. brachystemon*: individuals 7 and 9-13 are homozygous for PGI-2¹⁰⁰; individuals 14 and 15 are homozygous for PGI-2⁴²; plant 8 is a 100/42 heterozygote; individuals 1, 2, 3, 5 and 6 are *P. congesta* (note the fast PGI-1 allozyme); all *P. brachystemon* plants are homozygous for PGI-3¹⁰⁰ⁿ except individual 4 which has the PGI-3^{33r} allozyme.

Figure 20. A comparison of isozyme variation in PGI between *P. congesta* and *P. brachystemon*: plants 1-19, except 14, are *P. congesta* (note the fast PGI-1 allozyme); plants 20-28 are *P. brachystemon*, the F₁ progeny of a cross between plants homozygous for PGI-3¹⁰⁰ⁿ and PGI-3^{33r}, the progeny are all heterozygotes except for 20, 23 and 27 which are the result of accidental selfs.

PGI-3¹⁰⁰ⁿ was originally assumed to have normal staining properties but a mobility identical to PGI-2¹⁰⁰. This migration distance was designated as relative mobility 100. A slowly migrating and faintly staining allozyme, PGI-3^{33r} was later detected. The relative mobility of the conspicuous band associated with PGI-3^{33r} (*i.e.* intermediate between PGI-3^{33r} and PGI-2¹⁰⁰), combined with the absence of any heterodimer associated with PGI-3¹⁰⁰ⁿ, supported the assumption that PGI-3¹⁰⁰ⁿ and PGI-2¹⁰⁰ had identical mobilities. However, with the detection of PGI-2⁴² homozygotes which show no staining at the PGI-3¹⁰⁰ position (Figure 19), it was possible to identify PGI-3¹⁰⁰ⁿ as a null allele and not a normally staining variant with a mobility identical to PGI-2¹⁰⁰. Null alleles are not uncommon in electrophoretic surveys and their frequencies tend to be underestimated because they are generally not recognized in heterozygotes. However, it is uncommon for the most common allele identified at a locus to be a null.

The results of crosses and segregation analyses for PGI-2 and PGI-3 in *P. brachystemon* are presented in Table 10 and Table 11.

3.6 *Phosphoglucumutase* (PGM) E.C. 2.7.5.1

Gels stained for PGM displayed two zones of activity (A and B). Individuals had either one or two bands in the most anodal zone (A) and two to four bands in the slowly migrating zone (Figure 24). Bands in zone A segregated as alleles at a single locus and was designated PGM-1. Variation in zone B was determined to be controlled by two loci, PGM-2 and PGM-3.

Table 10. Analysis of allozyme variation at PGI-2

P. brachystemon: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-1-13a	100/100	14
77-1-14c	100/100	15
77-1-73a	100/100	14
77-9-11c	100/100	7

** all progeny expressed the maternal genotype

Table 11. Analysis of allozyme variation at PGI-3

P. brachystemon: Crosses

Individuals	Genotypes	Observed allozyme segregation in progeny		
		A ₁ /A ₁	A ₁ /A ₂	A ₂ /A ₂
77-1-13a x 77-18-25c	100n/100n x 33r/33r	0	12	0

P. brachystemon: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-9-11c	33r/33r	7
77-1-13a	100n/100n	14
77-1-14c	100n/100n	15
77-1-73a	100n/100n	14

** all progeny expressed the maternal genotype

Heterozygotes were identified by the presence of two bands which were associated with a single locus. This suggests that PGM in *Plectritris* is a monomeric enzyme which agrees with the sub-unit structure observed for PGM in other organisms: horseshoe crab (Selander *et al.*, 1970), *Citrus* (Torres *et al.*, 1978b), ponderosa pine (Mitten *et al.*, 1979), *Drosophila subobscura* (Loukas *et al.*, 1979) and Douglas fir (El-Kassaby, unpublished).

Four alleles were detected at PGM-1 and PGM-2. Their relative migrations are presented in Figures 21 and 22, respectively. Six alleles were found at PGM-3 (Figure 23). All four PGM-1 alleles were detected in *P. congesta* but only two, PGM-1¹⁰⁶ and PGM-1¹⁰⁰ were found in *P. brachystemon*. At PGM-2, three alleles were found in *P. congesta* while *P. brachystemon* populations were essentially monomorphic for PGM-2¹⁰⁰. The only variant allele detected in *P. brachystemon* was a single individual homozygous for a null (*i.e.* PGM-2¹⁰⁰ⁿ). PGM-3 is of particular interest because the two species were completely differentiated at this locus. Two alleles, PGM-3¹¹⁵ and PGM-3¹⁸² were detected in *P. brachystemon* while four alleles, PGM-3¹⁰⁰, PGM-3¹³⁵, PGM-3⁷⁷ and PGM-3⁴⁹, were observed in *P. congesta*. This was the only locus analyzed in the study in which the two species had no alleles in common. Figures 24 and 25 illustrate the variation observed in PGM.

The results of crosses and segregation analyses in both species are presented for PGM-1 in Table 12, PGM-2 in Table 13 and PGM-3 in Table 14.

Figure 21. The relative migration of allozymes detected at PGM-1.

Figure 22. The relative migration of allozymes detected at PGM-2; (PGM-2¹⁰⁰ⁿ not shown).

Figure 23. The relative migration of allozymes detected at PGM-3.

Figure 24. Allozyme variation in PGM in *P. congesta*.

Figure 25. A comparison of isozyme variation in PGM between *P. congesta* and *P. brachystemon*: plants 1-20, except 15, are *P. congesta*; plants 21-29 are *P. brachystemon*, the F₁ progeny of a cross between plants homozygous for different alleles, all plants are 100/106 heterozygotes at PGM-1 and 115/182 heterozygotes at PGM-3 except plants 21, 24 and 28 which are the result of accidental selfs.

Table 12. Analysis of allozyme variation at PGM-1

P. brachystemon: Crosses

Individuals	Genotypes	Observed segregation of allozymes in progeny		
		A ₁ /A ₁	A ₁ /A ₂	A ₂ /A ₂
77-1-13a x 77-18-25 c	100/100 x 106/106	0	12	0
77-1-111a x 77-18-18b	100/100 x 106/106	0	8	0
77-1-110c x 77-18-19c	100/100 x 106/106	0	10	0

P. brachystemon: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-9-11c	100/100	7
77-1-14c	106/106	15
77-1-26c	106/106	5
77-1-73a	106/106	14

P. congesta: Selfed heterozygotes

Individual	Genotype	Observed segregation of allozymes in progeny			$\chi^2_{(2)}$	P
		A ₁ /A ₁	A ₁ /A ₂	A ₂ /A ₂		
76-4-5	100/106	7	8	5	*	
77-9-45a	100/106	1	3	2	*	
77-9-53c	100/106	1	9	1	*	
77-9-64b	100/106	6	1	1	*	
77-9-68b	100/106	4	1	1	*	
77-9-78b	100/106	3	3	0	*	
77-9-81a	100/106	1	5	2	*	
77-9-83a	100/106	2	3	4	*	
77-9-84a	100/106	3	10	0	*	
Σ		28	43	16	3.322	0.10-0.05

P. congesta: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-9-45b	100/100	11
77-9-60c	100/100	14
77-9-64a	100/100	13
77-9-75b	100/100	10
77-9-65a	106/106	9

* sample size insufficient for a valid χ^2 test of significance

** all progeny expressed the maternal genotype

Table 13. Analysis of allozyme variation at PGM-2

P. brachystemon: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-1-14c	100/100	15
77-1-26c	100/100	6
77-1-73a	100/100	14
77-9-11c	100/100	7

P. congesta: Selfed heterozygotes

Individual	Genotype	Observed segregation of allozymes in progeny $\chi^2_{(2)}$			P
		A ₁ /A ₁	A ₁ /A ₂	A ₂ /A ₂	
77-9-45a	81/100	0	2	4	*
77-9-68b	81/100	1	3	2	*
77-9-78b	81/100	0	1	5	*
77-9-81a	81/100	1	3	4	*
76-4-5	81/118	7	9	4	1.100 0.50-0.75

P. congesta: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-9-64a	81/81	13
77-9-45b	100/100	11
77-9-53c	100/100	11
77-9-60c	100/100	14
77-0-75b	100/100	10
77-0-84a	100/100	13
77-9-95a	100/100	14

* sample size insufficient for a valid χ^2 test of significance

** all progeny expressed the maternal genotype

Table 14. Analysis of allozyme variation at PGM-3

P. brachystemon: Crosses

Individuals	Genotypes	Observed segregation of allozymes in progeny		
		A ₁ /A ₁	A ₁ /A ₂	A ₂ /A ₂
77-1-13a x 77-18-25c	115/115 x 182/182	0	12	0
77-1-111a x 77-18-18b	115/115 x 182/182	0	8	0
77-1-110c x 77-18-19c		0	10	0

P. brachystemon: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-1-14c	115/115	15
77-1-26c	115/115	6
77-1-73a	115/115	14
77-9-11c	115/115	7

P. congesta: Selfed heterozygotes

Individual	Genotype	Observed segregation of allozymes in progeny			$\chi^2_{(2)}$	P
		A ₁ /A ₁	A ₁ /A ₂	A ₂ /A ₂		
77-9-45a	100/135	0	4	2	*	
77-9-45b	100/135	7	4	0	*	
77-9-64b	100/135	5	0	3	*	
77-9-65a	100/135	4	4	1	*	
77-9-75b	100/135	2	7	1	*	
77-9-78b	100/135	1	3	2	*	
77-9-81a	100/135	1	4	3	*	
77-9-83a	100/135	0	4	3	*	
77-9-95a	100/135	4	9	1	*	
Σ		24	39	16	1.633	0.25-0.50

P. congesta: Selfed homozygotes

Individual	Genotype	Number of progeny**
77-9-53c	100/100	11
77-9-60c	100/100	14
77-9-64a	100/100	13
77-9-68b	100/100	6
77-9-84a	135/135	13

* sample size insufficient for a valid χ^2 test of significance

** all progeny expressed the maternal genotype

3.7 Monomorphic enzyme systems

Four enzymes were surveyed and the loci resolved were found to be monomorphic for the same allozyme in both species of *Plectritis*. These enzymes were isocitrate dehydrogenase (IDH: E.C. 1.1.1.42), malic enzyme (ME:E.C. 1.1.1.40), 6-phosphogluconic dehydrogenase (6PG:E.C. 1.1.1.44) and superoxide dismutase (SOD:E.C.1.15.1.1).

Gels stained for IDH displayed two areas of activity, a single monomorphic band and a more slowly migrating, diffusely staining zone. Only the monomorphic band was scored as the slower zone was not resolved. IDH has been shown to be dimeric in horseshoe crabs (Selander *et al.*, 1970), *Drosophila subobscura* (Loukas *et al.*, 1979), ponderosa pine (O'Malley *et al.*, 1979) and Douglas fir (El-Kassaby, unpublished).

Gels stained for ME had a darkly staining monomorphic band plus one or two lightly staining and more slowly migrating bands (Figure 26). Variation was observed in the lightly staining bands but segregations could not be interpreted according to simple Mendelian inheritance. Consequently, only ME-1 was scored. ME appears to be a tetrameric enzyme in *Drosophila subobscura* (Loukas *et al.*, 1979).

A gel stained for 6PG is pictured in Figure 27. A single monomorphic band was observed in all populations screened. 6PG has a dimeric structure in humans (Zouros, 1976), *Drosophila subobscura* (Loukas *et al.*, 1979) and Douglas fir (El-Kassaby, unpublished).

Figure 26. Allozyme activity in ME: note the variation in the number of lightly staining bands.

Figure 27. Allozyme activity in 6PG.

SOD, which is not illustrated, appeared as a single monomorphic white band on gels stained with tetrazolium dye. This enzyme is often reported in the literature as tetrazolium oxidase (TO) because, in vitro, it prevents the oxidation of tetrazolium dyes to blue formazan. Lippitt and Fridovich (1973), demonstrated that TO was actually SOD. Superoxide dismutase (or TO) has been shown to be a dimeric enzyme in the following organisms: humans (Beckman, 1973); dogs (Brewer, 1967); potatoes (Oelschlegal & Stahmann, 1971); two species of *Drosophila* (Richmond and Powell, 1970; Ayala *et al.*, 1972); houseflies (McDonald *et al.*, 1975) and Douglas fir (El-Kassaby, unpublished).

3.8 Summary

Nine enzyme systems were surveyed and fifteen loci identified in *Plectritis*. Genetic models were proposed to explain the observed variation. These models described the multi-locus organization, sub-unit structure and allelic variation within each system. The models were tested with crosses and segregation analyses. When progeny classes were sufficiently large, segregations were analyzed using the chi-square statistic. A model was proposed that explained observed segregations at PGI-2 and PGI-3 in *P. congesta*. However, the level of polymorphism, inconsistency in the formation of heterodimers and the presence of nulls prevented allelic variation at these loci from being confidently scored. Although *P. congesta* and *P. brachystemon*

differed in their allelic complements, the data suggest that the multilocus organization of the systems are identical in both species. Ultimately, fifteen loci were scored in *P. brachystemon* and twelve loci in *P. congesta*. The isozyme variation detected within each population is presented in Appendix C. The level of polymorphism at each locus and the distribution of alleles among populations is described in Chapter 4.

4.0 VARIATION AMONG POPULATIONS

Allele frequencies, expected heterozygosity (h) and observed heterozygosity (h_o) were calculated for each locus and are presented in Table 15 for *P. brachystemon* and Table 16 for *P. congesta*. The expected proportion of heterozygotes at each locus was calculated using the following formula;

$$h = 1 - \sum_i x_i^2 \quad (\text{Nei, 1975})$$

where x_i is the frequency of the i^{th} allele at the locus in question. This is equivalent to the expected frequency of heterozygotes if the population is in Hardy-Weinberg equilibrium.

Calculating expected heterozygosity, using the mean allele frequencies for each species, yielded the following sequence of loci ranked according to their total gene diversity (*i.e.* H_T , Nei, 1973):

P. brachystemon - EST-1 (0.659), MDH-1 (0.484), LAP-1 (0.422), PGM-1 (0.412), PGI-3 (0.226), PGM-3 (0.196), PGI-2 (0.096), EST-2 (0.000), IDH-1 (0.000), MDH-2 (0.000), ME-1 (0.000), PGI-1 (0.000), PGM-2 (0.000), SOD (0.000), 6PG (0.000);

P. congesta - LAP-1 (0.792), MDH-1 (0.509), PGM-3 (0.504), EST-2 (0.502), PGM-2 (0.439), PGM-1 (0.431), EST-1 (0.020), IDH-1 (0.000, MDH-2 (0.000), ME-1 (0.000), SOD (0.000), 6PG (0.000).

In order to emphasize differences in the relative levels of variation at homologous loci and for convenience of discussion, loci surveyed in the two taxa are considered under the following three arbitrary categories:

Table 15. Allele Frequencies and Heterozygosity Values* for Eight Polymorphic Loci in *Plectritus brachystemon*.

Locus	Allele	1	13	9	10	7(1)	7(3)	15(1)	15(2)	16	18
EST-1	114	.45	.00	.00	.00	1.00	.32	.72	1.00		.00
	100	.53	.20	.97	1.00	.00	.12	.22	.00		.00
	89	.02	.80	.03	.00	.00	.56	.06	.00		1.00
	h	.516	.320	.058	.000	.000	.570	.430	.000		.000
	h _o	.046	.029	.023	.000	.000	.000	.111	.000		.000
LAP-1	112	.00	.00	.00	.00	.00	.00	.00	.08	.00	.22
	105	.00	.00	.00	.05	.00	.00	.00	.39	.00	.00
	100	.11	.94	1.00	.95	.95	.40	1.00	.53	1.00	.61
	95	.00	.00	.00	.00	.05	.60	.00	.00	.00	.00
	91	.84	.06	.00	.00	.00	.00	.00	.00	.00	.00
	85	.05	.00	.00	.00	.00	.00	.00	.00	.00	.17
	80	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	h	.280	.113	.000	.095	.095	.480	.000	.561	.000	.551
	h _o	.000	.000	.000	.000	.000	.000	.000	.000	.000	.111
MDH-1	139	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	125	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	113	.00	.00	.00	1.00	.45	.50	.11	.00	1.00	1.00
	100	1.00	1.00	1.00	.00	.55	.50	.89	1.00	.00	.00
	h	.000	.000	.000	.000	.495	.500	.196	.000	.000	.000
	h _o	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000

Table 15. (Continued)

Locus	Allele	1	13	9	10	7(1)	7(3)	15 (1)	15(2)	16	18
PGI-2	100	1.00	1.00	.99	1.00	1.00	.88	.85	.84	.95	1.00
	94	.00	.00	.01	.00	.00	.00	.00	.00	.05	.00
	81	.00	.00	.00	.00	.00	.12	.00	.00	.00	.00
	42	.00	.00	.00	.00	.00	.00	.15	.16	.00	.00
	h	.000	.000	.020	.000	.000	.211	.255	.269	.095	.000
	h _o	.000	.000	.017	.000	.000	.000	.000	.029	.091	.000
PGI-3	100n	1.00	1.00	.81	1.00	1.00	1.00	.38	1.00	1.00	.54
	33	.00	.00	.19	.00	.00	.00	.62	.00	.00	.46
	h	.000	.000	.308	.000	.000	.000	.471	.000	.000	.497
	h _o	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGM-1	117	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	106	1.00	.48	.03	1.00	.00	.16	.23	.00	.00	.00
	100	.00	.52	.97	.00	1.00	.84	.77	1.00	1.00	1.00
	94	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	h	.000	.499	.058	.000	.000	.269	.354	.000	.000	.000
	h _o	.000	.042	.000	.000	.000	.000	.000	.000	.000	.000
PGM-2	118	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	100	1.00	1.00	1.00	1.00	1.00	.96	1.00	1.00	1.00	1.00
	81	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	100n	.00	.00	.00	.00	.00	.04	.00	.00	.00	.00

Table 15. (Continued)

Locus	Allele	1	13	9	10	7(1)	7(3)	15(1)	15(2)	16	18
PGM-2 (con't)	h	.000	.000	.000	.000	.000	.077	.000	.000	.000	.000
	h _o	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
PGM-3	182	.00	.00	.01	.00	.00	.00	.00	.03	.05	1.00
	135	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	115	1.00	1.00	.99	1.00	1.00	1.00	1.00	.97	.95	.00
	100	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	77	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	49	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	h	.000	.000	.020	.000	.000	.000	.000	.058	.095	.000
	h _o	.000	.000	.023	.000	.000	.000	.000	.054	.091	.000

$$* h = 1 - \sum x_i^2$$

h_o = observed heterozygosity

Table 16. Allele Frequencies and Heterozygosity Values* for Eight Polymorphic Loci in *Plectritus congesta*.

		POPULATIONS														
Locus	Allele	2(1)	2(2)	3	5	4	9	10	11	7(2)	8	14(1)	14(2)	15(1)	15(2)	17
EST-1	114	.00	.00	.00	.00	.00	.00	.00	.00	.08	.00	.00	.00	.01	.00	.01
	100	1.00	1.00	.99	1.00	1.00	.98	1.00	1.00	.89	1.00	1.00	1.00	.99	1.00	.99
	89	.00	.00	.01	.00	.00	.02	.00	.00	.03	.00	.00	.00	.00	.00	.00
	h	.000	.000	.020	.000	.000	.039	.000	.000	.201	.000	.000	.000	.020	.000	.020
	h _o	.000	.000	.009	.000	.000	.011	.000	.000	.193	.000	.000	.000	.021	.000	.028
EST-2	126	.00	.00	.04	.00	.00	.00	.00	.00	.08	.09	.07	.00	.06	.00	.02
	100	.94	.93	.69	.19	.63	.68	.51	.55	.16	.86	.78	.89	.13	.17	.94
	74	.06	.07	.27	.81	.37	.32	.49	.45	.76	.05	.15	.11	.81	.83	.04
	h	.113	.130	.450	.308	.466	.435	.500	.495	.390	.250	.364	.196	.323	.282	.114
	h _o	.118	.143	.415	.231	.467	.341	.380	.436	.385	.219	.310	.152	.333	.333	.114
LAP-1	112	.04	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	105	.23	.17	.00	.00	.03	.00	.05	.03	.00	.08	.10	.02	.00	.00	.03
	100	.15	.28	.13	.46	.50	.45	.30	.22	.24	.33	.25	.29	.02	.95	.01
	95	.04	.07	.57	.21	.14	.00	.29	.24	.38	.16	.26	.17	.29	.00	.03
	91	.23	.09	.23	.21	.20	.45	.18	.33	.02	.11	.07	.26	.51	.05	.39
	85	.27	.26	.03	.12	.10	.07	.16	.10	.13	.05	.20	.22	.01	.00	.47
	80	.04	.13	.04	.00	.03	.03	.02	.08	.24	.27	.12	.04	.17	.00	.07
	h	.790	.796	.603	.686	.662	.589	.765	.768	.723	.770	.801	.769	.626	.095	.620
	h _o	.615	.478	.392	.583	.467	.267	.375	.486	.477	.531	.467	.276	.396	.100	.278

* $h = 1 - \sum p_i^2$

h_o = observed heterozygosity

Table 16 . (Continued)

		POPULATIONS														
Locus	Allele	2(1)	2(2)	3	5	4	9	10	11	7(2)	8	14(1)	14(2)	15(1)	15(2)	17
MDH-1	139	.65	.38	.68	.67	.47	.27	.64	.47	.64	.77	.84	.65	.71	.57	.60
	125	.32	.57	.31	.33	.50	.51	.36	.53	.34	.18	.16	.35	.29	.43	.19
	113	.03	.05	.01	.00	.03	.22	.00	.00	.02	.05	.00	.00	.00	.00	.21
	100	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	h	.474	.528	.441	.442	.528	.619	.461	.498	.474	.372	.269	.455	.412	.490	.560
	h _o	.471	.300	.431	.667	.467	.367	.500	.385	.269	.281	.241	.367	.385	.571	.514
MDH-2	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.95	1.00	1.00	1.00	.99	1.00	1.00
	87	.00	.00	.00	.00	.00	.00	.00	.00	.05	.00	.00	.00	.01	.00	.00
	h	.000	.000	.000	.000	.000	.000	.000	.000	.095	.000	.000	.000	.020	.000	.000
	h _o	.000	.000	.000	.000	.000	.000	.000	.000	.075	.000	.000	.000	.026	.000	.000
PGM-1	117	.00	.00	.12	.00	.00	.14	.03	.00	.00	.02	.00	.00	.00	.00	.00
	106	.03	.06	.38	.08	.37	.23	.35	.28	.17	.73	.20	.15	.31	.42	.43
	100	.97	.94	.49	.92	.63	.63	.62	.72	.83	.25	.80	.85	.69	.58	.57
	94	.00	.00	.01	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	h	.058	.113	.601	.147	.466	.531	.492	.403	.282	.404	.320	.255	.428	.487	.490
	h _o	.059	.042	.604	.154	.200	.288	.291	.410	.185	.375	.200	.000	.333	.231	.441

Table 16. (Continued)

POPULATIONS																
Locus	Allele	2(1)	2(2)	3	5	4	9	10	11	7(2)	8	14(1)	14(2)	15(1)	15(2)	17
PGM-2	118	.03	.10	.00	.00	.00	.03	.03	.05	.00	.00	.00	.00	.02	.00	.03
	100	.79	.67	.75	.46	.80	.60	.46	.60	.57	.81	.96	1.00	.62	.71	.51
	81	.18	.23	.25	.54	.20	.37	.51	.35	.43	.19	.04	.00	.36	.29	.46
	100n	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	h	.343	.488	.375	.497	.320	.502	.527	.515	.490	.309	.077	.000	.486	.412	.527
	h _o	.294	.375	.321	.583	.133	.403	.560	.513	.338	.313	.036	.000	.426	.286	.559
PGM-3	182	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	135	.53	.35	.65	.50	.37	.25	.51	.67	.65	.64	.70	.53	.55	.61	.72
	115	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	100	.47	.63	.35	.50	.47	.75	.49	.33	.35	.36	.28	.47	.45	.39	.28
	77	.00	.02	.00	.00	.16	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
	49	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.02	.00	.00	.00	.00
	h	.498	.480	.455	.500	.617	.375	.500	.442	.455	.461	.431	.498	.495	.476	.403
	h _o	.353	.375	.283	.455	.400	.282	.300	.359	.353	.469	.571	.455	.383	.357	.441

$H_T > 0.400$, $0.400 \geq H_T \geq 0.050$ and $H_T < 0.050$.

4.1 Loci polymorphic with $H_T > 0.400$

4.1.1 *P. brachystemon* - Four loci; EST-1 (0.659), MDH-1 (0.484), LAP-1 (0.422) and PGM-1 (0.412) are considered within this category. Allele frequencies at these loci are plotted in Figures 28, 29, 30 and 31. At each of these loci, no single allele is predominant in all populations. However, LAP-1¹⁰⁰ and PGM-1¹⁰⁰ occur with mean frequencies of 0.75 and 0.71 respectively (Table 17). None of the alleles detected at EST-1, LAP-1 and PGM-1 are unique to *P. brachystemon* although EST-1¹¹⁴, EST-1¹⁰⁰, LAP-1¹⁰⁰ and LAP-1⁸⁵ have significantly different frequencies in the two species (Table 17). At these three loci, allele frequencies and heterozygosity values fluctuate over wide ranges with no discernible pattern in the distribution of alleles. On the average, 1.89 EST-1 alleles, 2.00 LAP-1 alleles and 1.40 PGM-1 alleles were detected within each population.

MDH-1 is of particular interest, as this locus proved to be taxonomically diagnostic. Two alleles, MDH-1¹⁰⁰ and MDH-1¹¹³ were detected with mean frequencies of 0.59 and 0.41 respectively (Table 17). MDH-1¹⁰⁰ was unique to *P. brachystemon* while MDH-1¹¹³ was a low frequency allele in *P. congesta*. An average of 1.30 alleles were found within each population. The distribution of these two alleles (Figure 29) somewhat suggests clinal variation. MDH-1¹⁰⁰ was fixed at Sumas Mtn. and two of the three southern Vancouver Island populations. The Malahat Drive and one of the Nanoose Hill populations were polymorphic while

EST-1

Figure 28. The distribution of EST-1 alleles among populations of *P. brachystemon*.

MDH-1

Figure 29. The distribution of MDH-1 alleles among populations of *P. brachystemon*.

LAP-1

Figure 30. The distribution of LAP-1 alleles among populations of *P. brachystemon*.

PGM-1

Figure 31. The distribution of PGM-1 alleles among populations of *P. brachystemon*.

Table 17. Comparison of Mean Allele Frequencies with Heterozygosity Values for Polymorphic Loci in *P. brachystemon* and *P. congesta*.

Locus	Allele	<i>P. brachystemon</i>	<i>P. congesta</i>	t
EST-1	114	.39±.143	.01±.005	2.66*
	100	.34±.134	.99±.007	-4.85***
	89	.27±.133	.00±.002	2.03
	H _T	.659	.020	
EST-2	126	-	.03±.009	-3.33***
	100	1.00±.000	.60±.079	5.06***
	74	-	.37±.079	-4.68***
	H _T	.000	.502	
LAP-1	112	.03±.022	.00±.003	1.36
	105	.04±.039	.05±.018	-.23
	100	.75±.100	.30±.059	3.88***
	95	.06±.060	.19±.041	-1.78
	91	.09±.084	.22±.038	-1.41
	85	.03±.017	.15±.032	-3.33***
	80	-	.09±.022	-4.09***
	H _T	.422	.792	
MDH-1	139	-	.60±.038	-15.79***
	125	-	.36±.033	-10.91***
	113	.41±.142	.04±.019	2.59*
	100	.59±.142	-	4.15***
	H _T	.484	.509	
MDH-2	100	1.00±.000	1.00±.003	.00
	87	-	.00±.003	.00
	H _T	.000	.000	
PGI-2	100	.95±.021	NOT SCORED	
	94	.01±.005		
	81	.01±.012		
	42	.03±.021		
	H _T	.096		

Table 17. (Continued)

Locus	Allele	<i>P. brachystemon</i>	<i>P. congesta</i>	t
PGI-3	100n	.87±.072	NOT SCORED	
	33r	.13±.072		
	H _T	.226		
PGM-1	117	-	.02±.012	-1.67
	106	.29±.128	.28±.047	.07
	100	.71±.128	.70±.050	.07
	94	-	.00±.001	.00
	H _T	.412	.431	
PGM-2	118	-	.02±.007	-2.86**
	100	1.00±.004	.69±.043	7.21***
	81	-	.29±.041	-7.07***
	100n	.00±.004	-	.00
	H _T	.000	.439	
PGM-3	182	.11±.099	-	1.11
	135	-	.55±.036	-15.28***
	115	.89±.099	-	8.99***
	100	-	.44±.033	-13.33***
	77	-	.01±.011	-.91
	49	-	.00±.001	.00
	H _T	.196	.504	
	H _T	.166±.058	.266±.083	

*P < .05

**P < .01

***P < .005

MDH-1¹¹³ replaced MDH-1¹⁰⁰ at Little Qualicum Falls and Campbell River. However, there were notable exceptions to the patterns: population 10 (Prospect Lake) was fixed for MDH-1¹¹³ while 15(2) (Nanoose Hill) was monomorphic for MDH-1¹⁰⁰. In addition, two plants collected at Cape Perpetua, Oregon, were homozygous for MDH-1¹¹³. Unfortunately, based on the present data, it is impossible to determine if there is a selective basis for this pattern or if it is coincidental and the result of stochastic forces.

4.1.2 *P. congesta* - Six loci, LAP-1 (0.792), MDH-1 (0.509), PGM-3 (0.504), EST-2 (0.502), PGM-2 (0.439) and PGM-1 (0.431) fall within this category. Allele frequencies for these loci are plotted in Figures 32 through 37. No single allele was predominant in all populations at any of these loci. However, PGM-2¹⁰⁰ was the most common allele in all but two populations and PGM-1¹⁰⁰ occurred with the highest frequency in fourteen of the fifteen populations (Table 16).

Seven alleles were detected at LAP-1 (Figure 32). LAP-1⁸⁰, with a mean frequency of 0.09, was the only allele not detected in *P. brachystemon* (Table 17). LAP-1¹⁰⁰ and LAP-1⁹¹ were detected in all populations surveyed. On the average, 5.33 alleles were maintained within each population. Three alleles were detected at MDH-1 in *P. congesta* (Figure 33). The two most common alleles, MDH-1¹³⁹ and MDH-1¹²⁵ were not found in *P. brachystemon*. On the average, local populations maintained 2.53 alleles. All populations were polymorphic for MDH-1¹³⁹ and MDH-1¹²⁵. Additionally, MDH-1¹¹³ was detected in eight of the fifteen populations surveyed.

PGM-3 was a taxonomically diagnostic locus (Figure 34). Four alleles were identified at this locus, none of which was detected in *P. brachystemon* (Table 17). Two alleles, PGM-3¹³⁵ and PGM-3¹⁰⁰, were found in all populations while the other two alleles were either absent or occurred at low frequency. An average of 2.20 alleles was detected within each population.

Three alleles were scored at the EST-2 locus (Figure 35). EST-2⁷⁴ and EST-2¹²⁶ were not detected in *P. brachystemon*. The most common allele, EST-2¹⁰⁰, was detected in both taxa but at significantly different frequencies (Table 17). All populations were polymorphic for EST-2¹⁰⁰ and EST-2⁷⁴ while EST-2¹²⁶ was detected in only six populations and always with a frequency of less than 0.10. On the average, 2.40 alleles were maintained within local populations.

Three PGM-2 alleles were detected in *P. congesta* (Figure 36). Two of these alleles were not found in *P. brachystemon*, while the most common allele, PGM-2¹⁰⁰, was detected in both species but at significantly different frequencies (Table 17). All populations except 14(2) (Jack's Point) were polymorphic for PGM-2¹⁰⁰ and PGM-2⁸¹. PGM-2¹¹⁸ was a rare allele. It was detected in only seven populations and with the exception of population 2(2) (Anacortes, WA), it was never present with a frequency greater than 0.05. The average number of alleles detected per population was 2.40.

Four alleles were scored at PGM-1 (Figure 37). Alleles PGM-1¹⁰⁰ and PGM-1¹⁰⁶ were common, occurring in all sampled populations. These alleles were detected also in *P. brachystemon* with almost identical frequencies (Table 17). The two rare alleles, PGM-1¹¹⁷ and PGM-1⁹⁴, were not found in *P. brachystemon*. On the average,

LAP-1

Figure 32. The distribution of LAP-1 alleles among populations of *P. congesta*.

MDH-1

Figure 33. The distribution of MDH-1 alleles among populations of *P. congesta*.

PGM-3

Figure 34. The distribution of PGM-3 alleles among populations of *P. congesa*.

EST-2

Figure 35. The distribution of EST-2 alleles among populations of *P. congesa*.

PGM-2

Figure 36. The distribution of PGM-2 alleles among populations of *P. congesta*.

PGM-1

Figure 37. The distribution of PGM-1 alleles among populations of *P. congesta*.

2.33 alleles were detected within local populations.

4.2 Loci polymorphic with $0.400 \geq H_T \geq 0.050$

4.2.1 *P. brachystemon* - Three loci; PGI-3 (0.226), PGM-3 (0.196) and PGI-2 (0.096) were found to have this level of total gene diversity. At these loci, one allele was generally predominant in all populations.

Allele frequencies for PGI-3 are plotted in Figure 38. PGI-3^{33r} was found in only 3 populations: 9 (Francis Park), 15(1) (NanOOSE Hill) and 18 (Campbell River). However, in those populations it was detected at frequencies of 0.19, 0.62 and 0.46 respectively (Table 15). This locus averaged 1.30 alleles per population.

The two alleles detected at PGM-3 were unique to *P. brachystemon* and were therefore diagnostic of the species. Figure 39 describes the distribution of these alleles. PGM-3¹¹⁵ was either fixed or occurred with a frequency ≥ 0.95 in nine of the ten populations surveyed. Population 18 (Campbell River) was monomorphic for PGM-3¹⁸². Since no other populations from as far north as Campbell River were surveyed, it was impossible to determine if this pattern represents allele replacement along a latitudinal gradient. However, this does not appear to be a simple cline because the two plants collected at Cape Perpetua, Oregon, were also found to be homozygous for PGM-3¹⁸². Unfortunately, without further study, one can only speculate whether there is a selective basis for this polymorphism or if it is merely the result of founder effect or drift in a small population.

Four alleles were scored at PGI-2 (Figure 40). PGI-2¹⁰⁰ was the most common allele in all populations while the other three

Figure 38. The distribution of PGI-3 alleles among populations of *P. brachystemon*.

Figure 39. The distribution of PGM-3 alleles among populations of *P. brachystemon*.

Figure 40. The distribution of PGI-2 alleles among populations of *P. brachystemon*.

Figure 41. The distribution of EST-1 alleles among populations of *P. congesta*.

alleles were rare or locally endemic. On the average, 1.50 alleles were maintained within local populations.

4.2.2. *P. congesta* - None of the loci surveyed had this level of gene diversity.

4.3 *Slightly polymorphic or monomorphic with $H_T < 0.050$*

4.3.1 *P. brachystemon* - Eight loci were monomorphic or nearly so: PGM-2, EST-2, MDH-2, IDH-1, ME-1, PGI-1, SOD and 6PG. No variation was detected at any of these loci except PGM-2. A single plant in population 7(3) (Malahat Dr.) was homozygous for a null allele. Although PGM-2, EST-2 and MDH-2 were not monomorphic in *P. congesta*, the alleles which were fixed in *P. brachystemon* were the most common alleles at the homologous loci in *P. congesta*. All populations surveyed of both species, were fixed for the same alleles at IDH-1, ME-1, SOD and 6PG.

4.3.2 *P. congesta* - Two loci, EST-1 and MDH-2, were slightly polymorphic while four loci, IDH-1, ME-1, SOD and 6PG showed no variation.

Allele frequencies at EST-1 are plotted in Figure 41. This locus was of particular interest because both taxa share the same three alleles. However, EST-1 was the most variable locus in *P. brachystemon* ($H_T = 0.659$) while it was one of the least variable loci in *P. congesta* ($H_T = 0.020$). On the average, populations maintained 1.40 alleles.

At MDH-2, a rare allele, MDH-2⁸⁷ was detected in populations 7(2) (Malahat Drive) and 15(1) (Nanoose Hill) with frequencies of

0.05 and 0.01 respectively (Table 16). MDH-2¹⁰⁰ and the alleles fixed at IDH-1, ME-1, SOD and 6PG were also fixed in *P. brachystemon*.

4.4 Summary

Table 17 compares the mean allele frequencies and total gene diversity values (H_T) for all polymorphic loci surveyed. IDH-1, ME-1, SOD and 6PG were also surveyed but were found to be monomorphic in both species. In addition, no variation was detected at PGI-1 in *P. brachystemon* although it was polymorphic in the other species. Unfortunately, PGI could not be scored consistently in *P. congesta* and was therefore excluded from the study.

In *P. congesta*, thirty-three alleles were detected at the twelve loci surveyed, an average of 2.75 alleles per locus. In *P. brachystemon*, thirty alleles were detected at fifteen loci, an average of 2.00 alleles per locus. When only the twelve loci scored in both species are compared, twenty-three alleles were detected in *P. brachystemon*; an average of 1.92 alleles per locus.

P. congesta had approximately 30% more alleles than *P. brachystemon* at the twelve loci surveyed. Fourteen of its thirty-three alleles, 42%, were not shared with *P. brachystemon*. These unique alleles can be defined as common, rare or endemic. Common alleles were those detected in at least five populations or with a mean frequency ≥ 0.05 . Rare alleles were those which were found in less than five populations and had a mean frequency < 0.05 . Endemics were alleles detected at low frequency in a single population. Nine isozymes

(EST-2¹²⁶, EST-2⁷⁴, LAP-1⁸⁰, MDH-1¹³⁹, MDH-1¹²⁵, PGM-2¹¹⁸, PGM-2⁸¹, PGM-3¹³⁵ and PGM-3¹⁰⁰), 27% of the alleles scored in *P. congesta*, were common but unique to this species. Three alleles (MDH-2⁸⁷, PGM-1¹¹⁷, and PGM-3⁷⁷), 9%, were rare and unique. Two alleles (PGM-1⁹⁴ and PGM-3⁴⁹), 6%, were endemics.

In *P. brachystemon*, four of its twenty-three alleles, 17%, were not found in *P. congesta*. Three of these (MDH-1¹⁰⁰, PGM-3¹⁸² and PGM-3¹¹⁵) were common. The fourth, (PGM-2¹⁰⁰ⁿ), was an endemic detected in only a single plant. Nineteen alleles were present in both species. However, seven of these, 37%, were detected at significantly different frequencies in the two taxa (Table 17).

Populations of the two species were differentiated by alleles and allele frequency differences at three loci (EST-2, MDH-1 and PGM-3).

These loci were considered taxonomically diagnostic. The two species could also be differentiated by their PGI activity patterns. In addition, with the exception of a single plant, PGM-2 was monomorphic in all *P. brachystemon* populations and polymorphic in all but one *P. congesta* populations. None of the PGM-3 alleles were detected in both species and alleles unique to each taxon were present at MDH-1, PGM-2 and EST-2. Generally, the most common allele in *P. congesta* was either fixed or also the most common allele in *P. brachystemon*. However, this was not the case at EST-1, MDH-1 and PGM-3.

In *P. brachystemon*, allele frequencies and single locus heterozygosities fluctuated widely among populations. However, there were no discernable macro-geographic patterns to allele distributions,

except possibly MDH-1 and PGM-3. Populations maintained fewer alleles than in *P. congesta* and different alleles were often fixed in populations. In contrast to *P. brachystemon*, a majority of the alleles detected in *P. congesta* were maintained within each local population. Allele frequencies and single locus heterozygosities fluctuated among populations but not with the amplitude found in *P. brachystemon*. These observations suggest that in *P. brachystemon*, the levels of gene flow among populations and/or recombination within populations are lower than that which occurs in *P. congesta*.

5.0 ANALYSES AND DISCUSSION

This chapter consists of five sections. Within each section, analyses and their rationale are explained, data presented and the results discussed. Section 5.1 describes the analysis of the breeding system and the calculation of outcrossing rates in *P. brachystemon* and *P. congesta*. Section 5.2 discusses levels of genetic variability within populations, contrasting the two species. Section 5.3 analyzes the hierarchical distribution of variation within each taxon. Section 5.4 compares intra-specific and inter-specific estimates of genetic identity and genetic distance. This section has four sub-sections which utilize these indices to make inferences relevant to various taxonomic and evolutionary questions. Lastly, section 5.5 summarizes the data presented in the previous four sections, emphasizing differences between the two species and describing the genetic consequences of their contrasting breeding strategies.

5.1 Analysis of the breeding system

The breeding system of any taxon can be estimated using Wright's fixation index, F (Wright, 1965). The fixation index measures the extent to which observed genotypic distributions deviate from Hardy-Weinberg proportions (*i.e.* p^2 , $2pq$, q^2).

$$F = 1 - \frac{H}{2pqn}$$

where H is the observed number of heterozygotes and $2pqn$ is the expected number of heterozygotes assuming Hardy-Weinberg equilibrium.

Values of F range from -1 to $+1$. Negative values indicate a percentage of heterozygotes in excess of expectations and positive values represent a deficiency of heterozygotes. Wright's equilibrium law describes observed genotype distributions in terms of allele frequencies and the fixation index F :

genotype -	AA	Aa	aa
frequency -	$p^2 + pqF$	$2pq(1-F)$	$q^2 + pqF$

When $F=0$, the genotype distribution is described by a Hardy-Weinberg equilibrium. Deviations from Hardy-Weinberg proportions (F) are the result of the joint effect of all factors acting on alleles and genotypes at a given locus: non-random mating, mutation, selection, gene flow and genetic drift. When inbreeding is the only factor causing deviations from expectations, F is identical to the classical inbreeding coefficient. F analysis measures the probability that uniting gametes, taken at random from a population, will be related by descent. This value is defined as the correlation between uniting gametes.

Fixation indexes were calculated for each polymorphic locus within each population. These values are presented in Table 18 for *P. brachystemon* and in Table 19 for *P. congesta*. In addition, the statistic F_{is} was calculated for each locus. This value is defined as the average correlation between uniting gametes relative to their own local population. Large values of F_{is} (*i.e.* few heterozygotes) indicate that within local populations, a large percentage of the uniting gametes are related

TABLE 18. Wrights Fixation Index (F)¹ for Seven Polymorphic Loci in
P. brachystemon.

POPULATION	Locus						
	EST-1	LAP-1	MDH-1	PGI-2	PGI-3	PGM-1	PGM-2
1	.911	1.000	-	-	-	-	-
13	.911	1.000	-	-	-	.917	-
9	.599	-	-	-	1.000	1.000	-
10	-	1.000	-	-	-	-	-
7(1)	-	1.000	1.000	-	-	-	-
7(3)	1.000	1.000	1.000	1.000	-	1.000	1.000
15(1)	.742	-	1.000	1.000	1.000	1.000	-
15(2)	-	1.000	-	.894	-	-	-
16	not scored	-	-	-	-	-	-
18	-	.798	-	-	1.000	-	-
$F_{is}^2 =$	.865	.988	1.000	.949	1.000	.968	1.000

¹ $F = 1 - \frac{H}{2pqn}$: H = observed number of heterozygotes; 2pqn = expected number.

² F_{is} = weighted mean of F for all populations

TABLE 19. Wright's Fixation Index (F)¹ for Eight Polymorphic Loci
in *P. congesta*.

POPULATION	EST-1	EST-2	LAP-1	MDH-1	MDH-2	PGM-1	PGM-2	PGM-3
2(1)	-	-.041	.221	.007	-	.042	.143	.291
2(2)	-	-.099	.399	.432	-	.631	.232	.219
3	.057	.078	.350	.022	-	-.005	.145	.378
5	-	.251	.150	-.508	-	-.047	-.174	.091
4	-	-.001	.295	.116	-	.571	.583	.352
9	.709	.215	.547	.408	-	.458	.198	.249
10	-	.240	.510	-.085	-	.409	-.063	.400
11	-	.119	.367	.228	-	-.018	.004	.188
7(2)	.040	.014	.340	.433	.215	.345	.310	.224
8	-	.125	.310	.244	-	.072	-.011	-.017
14(1)	-	.147	.417	.103	-	.375	.072	-.326
14(2)	-	.227	.641	.194	-	1.000	-	.083
15(1)	-.064	-.032	.368	.067	-.282	.221	.124	.226
15(2)	-	-.182	-.053	-.166	-	.526	.307	.250
17	-.389	-.003	.552	.082	-	.100	-.060	-.095
F _{is} ² =	.180	.096	.398	.155	.032	.300	.128	.188

¹ $F = 1 - \frac{H}{2pqn}$: H = observed number of heterozygotes; 2 pqn = expected number.

² F_{is} = weighted mean of F for all populations

by descent. The differences in F_{is} values for the two species are striking. At all polymorphic loci surveyed, except MDH-2 where variation was rare, local populations of *P. congesta* show an average deficiency of heterozygotes between 10% (EST-2) and 40% (LAP-1). In contrast, local populations of *P. brachystemon* had an average deficiency of heterozygotes between 86% (EST-1) and 100% (MDH-1, PGI-3 and PGM-2). This difference in the observed levels of heterozygosity within populations of the two species suggests that in *P. brachystemon*, a much larger percentage of the uniting gametes are related.

When populations are at equilibrium and non-random mating is the only factor affecting the distribution of genotypes at a locus, the fixation index (F) is related to the outcrossing frequency (t) according to the relationship

$$F = \frac{1-t}{1+t} \quad (\text{Nei and Syakudo, 1958}).$$

Outcrossing rates can be calculated from F by transforming the above equation to

$$t = \frac{1-F}{1+F} \quad (\text{Nei and Syakudo, 1958}).$$

This method assumes that populations are at equilibrium and therefore is not a rigorous estimator of t (Jain, 1979). Since t is a function of F , single locus estimates of t are sensitive to evolutionary forces, besides breeding system, which affect genotype frequencies within populations (Workman, 1969). However, breeding system should be the only factor which affects all loci within a population equally. Consequently, when F is averaged over

a number of loci and this $\bar{F}$ is used to calculate outcrossing, the resulting t , if not an absolute estimator, is certainly a relative estimator of outcrossing among populations. The average net deviation from Hardy-Weinberg proportions ($\bar{F}$) and the outcrossing rate (t) were calculated for each population and are presented in Table 20 for *P. brachystemon* and Table 21 for *P. congesta*.

Wright's fixation index can be calculated only for polymorphic loci. Consequently, the number of loci contributing to the outcrossing estimates varies among populations. An outcrossing frequency was not listed for *P. brachystemon* population 16 (Little Qualicum Falls) because the only variation scored in the population was detected in two plants, heterozygous at two loci (PGI-2 and PGM-3). This apparent anomaly produced an outcrossing rate which was unrealistically high for this species ($t=0.988$).

The estimates of outcrossing for *P. brachystemon* were between 0 and 7.4% (Francis Park). The average ($\pm$ standard error) for the nine populations was $2.4 \pm 0.8\%$ outcrossing. In *P. congesta* the estimates ranged from a low of 42% (Francis Park and Jack's Point -2,) to a high of 100% (Mill Hill Park). The average outcrossing frequency for the fifteen populations was $70.2 \pm 4.8\%$. These estimates are in excellent agreement with those of Ganders *et al.* (1977a, 1977b) and Carey and Ganders (in press), who utilized a seed wing dimorphism of known inheritance to estimate outcrossing in both taxa. Using this polymorphism and the progeny test method of Harding (1970), they found outcrossing

TABLE 20. Estimates of Outcrossing Frequency for Ten Populations of *P. brachystemon*.

POPULATION	$\bar{F}^1$	t^2
1	.922	.041
13	.924	.040
9	.863	.074
10	1.000	.000
7(1)	1.000	.000
7(3)	1.000	.000
15(1)	.959	.021
15(2)	.948	.027
16	-	-
18	.978	.011
AVERAGE		.024±.008

¹ $\bar{F}$ = weighted mean of F for all polymorphic loci

² $t = \frac{1-\bar{F}}{1+\bar{F}}$

TABLE 21. Estimates of Outcrossing Frequency for Fifteen Populations of *P. congesta*.

POPULATION	$\bar{F}^1$	t^2
2(1)	.105	.801
2(2)	.307	.530
3	.146	.745
5	-.037	1.077
4	.319	.516
9	.407	.421
10	.252	.597
11	.146	.745
7(2)	.244	.608
8	.121	.784
14(1)	.137	.759
14(2)	.406	.422
15(1)	.090	.835
15(2)	.151	.738
17	.028	.946
AVERAGE:		.702±.048

¹ $\bar{F}$ = weighted mean of F for all polymorphic loci

² $t = \frac{1-\bar{F}}{1+\bar{F}}$

in *P. brachystemon* to average 2% and outcrossing in *P. congesta* to range from 48% to 80% with a mean of 70%.

The difference between the mean outcrossing rates for these two closely related species was highly significant ($P \leq 0.01$) but not surprising. The observed divergence in breeding strategies had been anticipated on the basis of floral morphology and field observations and confirmed the earlier outcrossing estimates in these taxa.

5.2 Genetic variability within populations

In order to evaluate the consequences of contrasting breeding strategies on the population structure of the two taxa, five genetic parameters were measured within each population. The statistics used to describe levels of genetic variability within local populations are the percentage of loci that are polymorphic, average number of alleles per locus, average number of alleles per polymorphic locus, expected percentage of heterozygous loci per individual and the observed percentage of heterozygous loci per individual. These values were calculated separately for each population then averaged over all populations ($\pm$ standard error) for *P. brachystemon* and *P. congesta* in Tables 22 and 23 respectively.

The proportion of loci that are polymorphic per population varied in *P. brachystemon* between 6.67% (Prospect Lake) and 40% (Malahat Drive-2) with an average of $20.76 \pm 3.49\%$. The range in *P. congesta* was not as great. Population 14(2) (Jack's Point)

Table 22. Summary of Various Genetic Parameters for Ten Populations of *Plectritus brachystemon*.

Population	Percentage Loci Polymorphic ¹	Average Number Alleles/Locus	Average Number Alleles/Polymorphic Locus	Expected Percentage of Heterozygous Loci/Individual ² (H)	Observed Percentage of Heterozygous Loci/Individual (H _{obs.})
1	13.33	1.27±.18	3.00±.00	5.31±3.79	.31±.31
13	20.00	1.20±.11	2.00±.00	6.21±3.82	.47±.33
9	33.33	1.33±.22	2.00±.00	3.09±2.05	.42±.23
10	6.67	1.07±.07	2.00±.00	0.63±.63	.00±.00
7(1)	13.33	1.13±.09	2.00±.00	3.93±3.32	.00±.00
7(3)	40.00	1.47±.17	2.17±.17	14.05±5.49	.00±.00
15(1)	33.33	1.40±.16	2.20±.20	11.37±4.58	.74±.74
15(2)	20.00	1.27±.15	2.33±.33	5.92±4.01	.55±.40
16 ³	14.28	1.14±.10	2.00±.00	1.36±.92	1.30±.88
18	13.33	1.20±.14	2.50±.17	6.99±4.77	.74±.74
AVERAGE	20.76±3.49	1.25±.04	2.22±.10	5.89±1.33	.45±.13

¹ The frequency of the most common allele is $\leq .99$.

² $(H = \sum_{k=1}^r \frac{h}{r})$ for r loci; $h = 1 - \sum_{i=1}^2 x_i^2$ expressed as a percentage.

³ All estimates with this population based on a sample of 14 loci (EST-1 Excluded)

Table 23. Summary of Various Genetic Parameters for Fifteen Populations of *Plectritis congesta*.

Population	Percentage Loci Polymorphic ¹	Average Number Alleles/Locus	Average Number Alleles/Polymorphic Locus	Expected Percentage of Heterozygous Loci/Individual ² (H)	Observed Percentage of Heterozygous Loci/Individual (H _{obs.})
2(1)	50.00	2.08±.50	3.17±.79	18.97±7.78	15.92±6.30
2(2)	50.00	2.08±.43	3.17±.60	21.13±8.14	14.28±5.35
3	58.33	2.17±.39	3.00±.44	24.54±7.52	20.46±6.50
5	50.00	1.67±.26	2.33±.33	21.50±7.41	22.28±7.85
4	50.00	2.00±.43	3.00±.63	26.49±8.05	17.78±6.11
9	58.33	2.00±.30	2.71±.29	25.75±7.78	16.33±4.98
10	50.00	2.00±.43	3.00±.63	27.04±8.44	20.05±6.40
11	50.00	1.92±.42	2.83±.65	26.01±8.23	21.58±6.61
7(2)	66.67	2.17±.34	2.75±.37	25.92±7.12	18.96±5.02
8	50.00	2.08±.43	3.17±.60	21.38±7.37	18.23±5.96
14(1)	50.00	2.00±.43	3.00±.63	18.85±7.36	15.21±5.92
14(2)	41.67	1.75±.41	2.80±.80	18.11±7.57	10.42±4.85
15(1)	66.67	2.08±.34	2.63±.38	23.41±7.14	19.19±5.60
15(2)	50.00	1.50±.15	2.00±.00	18.68±6.42	15.65±5.61
17	58.33	2.17±.42	3.00±.53	22.78±7.63	19.79±6.67
AVERAGE	53.33±1.78	1.98±.05	2.84±.08	22.64±.79	17.74±.81

¹The frequency of the most common allele is $\leq .99$.

²($H = \sum_{k=1}^r \frac{h}{r}$ for loci; $h = 1 - x_i^2$) expressed as a percentage.

with 41.67%, had the lowest percentage of loci that were polymorphic. Populations 7(2) (Malahat Drive) and 15(1) (Nanoose Hill) with 66.67%, had the highest percentage of loci that were polymorphic. The average for the fifteen populations was $53.33 \pm 1.78\%$. The difference between the means for the two species was highly significant ($P \leq 0.01$).

The average number of alleles detected per locus in *P. brachystemon* ranged between 1.07 (Prospect Lake) and 1.47 (Malahat Drive-3) with a mean of 1.25 ± 0.04 . The values in *P. congesta* were consistently higher with a low of 1.67 (Mill Hill) and a high of 2.17 (John Dean Park, Malahat Drive and Nile Creek). The mean for *P. congesta* was 1.98 ± 0.05 and this was significantly larger than the mean observed in *P. brachystemon* ($P \leq 0.01$).

When average number of alleles per polymorphic locus was calculated, *P. congesta* was again consistently more variable than *P. brachystemon*. The range in the selfer was between 2.00, found in five populations, and 2.33 (Nanoose Hill-2). *P. brachystemon* averaged 2.22 ± 0.10 alleles per polymorphic locus. The corresponding values in the outcrosser were from 2.00 (Nanoose Hill-2) to 3.17 (Anacortes-1, -2 and Crofton exit). The mean for *P. congesta* was 2.84 ± 0.08 , and again the difference between the two taxa was highly significant ($P \leq 0.01$).

The expected percentage of heterozygous loci per individual (H) was calculated from the observed allele frequencies within each population. This is the best single statistic for describing levels of genetic variability within populations

(Nei and Roychoudhury, 1974) and is defined in the next section. In *P. brachystemon*, H ranged between 0.63% (Prospect Lake) and 14.05% (Malahat Drive-3) with an average value of $5.89 \pm 1.33\%$. In *P. congesta*, expected heterozygosity varied between 18.11% (Jack's Point-2) and 27.04% (Prospect Lake) for an average of $22.64 \pm 0.79\%$. This value was significantly higher than the mean calculated for the selfer ($P \leq 0.01$).

Although the absolute value of heterozygosity was lower in *P. brachystemon*, the relative range of values was much larger than that observed in *P. congesta*. The magnitude of the fluctuations among populations of the selfer suggests heightened population differentiation. This was particularly striking when *P. brachystemon* collections from sympatric sites 7(1):7(3) (Malahat Drive) and 15(1):15(2) (Nanoose Hill) were compared. These sympatric pairs had large differences in their levels of detected variation (H) and yet they were separated by distances of only thirty meters at Malahat Drive and no greater than one hundred meters at Nanoose Hill (the exact distance is unknown). The magnitude of these observed differences is interpreted to be a result of the breeding system. Autogamy restricts recombination and gene flow via pollen vectors and therefore enhances the relative contributions of founder effect and drift in determining population structure.

The observed percentage of heterozygous loci per individual ($H_{obs.}$) was calculated by direct count and the differences between the two species was highly significant

($P \leq 0.01$). The range in *P. brachystemon* was from zero (Prospect Lake, Malahat Drive-1 and -3) to 1.30% (Little Qualicum Falls) with a mean of only $0.45 \pm 0.13\%$. In *P. congesta*, $H_{obs.}$ varied between 10.42% (Jack's Point-2) and 22.28% (Mill Hill) with a mean of $17.74 \pm 0.81\%$.

Outcrossing rates and levels of expected heterozygosity (H) were compared among populations within each species. This is plotted in Figure 42 for *P. brachystemon* and Figure 43 for *P. congesta*. However, as was found in *Limnanthes alba* (Jain, 1978), and *Pinus contorta* (Yeh and Layton, 1979), there was no clear relationship between estimated outcrossing rates and genetic variability among populations of the same taxon. Intuitively, one might predict a positive relationship between these two parameters and there could be several reasons for the lack of correlation. Sample sizes within populations may have been too small to detect differences. Jain (1978) genotyped 20-30 families per population, Yeh and Layton (1979) genotyped 15 families per population and from 15 to 60 plants were genotyped per population of *Plectritis*. Secondly, the range of outcrossing rates among populations of the same species may be too small to be accurately measured with the estimators used. Although the technique employed by Jain (1978) does not assume that the populations are in Hardy-Weinberg equilibrium, the method used to calculate outcrossing in lodgepole pine (Yeh and Layton, 1979) and *Plectritis* makes that assumption. Any deviations between the observed and expected frequencies of heterozygotes are attributed solely to the rate of outcrossing.

Figure 42. The relationship between the outcrossing rate and the expected heterozygosity among populations of *P. brachystemon*.

Figure 43. The relationship between the outcrossing rate and the expected heterozygosity among populations of *P. congesta*.

This assumption allows the combined influence of other factors such as micro-habitat selection, heterosis, Wahlund effect and stochastic forces to minimize breeding system differences. Lastly, the indicator used to measure levels of variation among populations presents problems because estimates of heterozygosity have a large standard error. This occurs because each locus does not contribute equally to a species' heterozygosity. Some loci are monomorphic while others are highly polymorphic. Consequently, without a very large sample of loci, the S.E. associated with the estimate obliterates subtle differences in the levels of variability between populations of the same taxon (Figures 42 and 43).

Although no relationship was detected between outcrossing rates and levels of heterozygosity among populations of the same species, the data suggest that the breeding system is a major determinant of population structure. The contrast in the outcrossing rates of these two taxa is paralleled by observed differences in levels of variation maintained within populations. As measured by all parameters, populations of *P. congesta* maintain significantly higher levels of genetic variability than do populations of its autogamous relative, *P. brachystemon*. When site variation was controlled by comparing only those populations of both taxa growing sympatrically, all detected differences remained significant.

5.3 *Analysis of gene diversity*

The level of genic variability and its hierarchical organization can be quantified with the analysis of gene diversity (Nei, 1973, 1975). This analysis permits genetic variation to be partitioned between different hierarchical levels of population structure (*i.e.* within versus between local or sub-populations). This technique is a modification of Wright's F-statistics expanded to multiple alleles. However, unlike F-analysis, it is not dependent on the detection of genotype frequencies because it does not measure deviations between observed and expected levels of heterozygosity.

The basis of the analysis is the calculation of expected, or theoretical, heterozygosity per locus (h):

$$h = 1 - \sum x_i^2,$$

where x_i is the frequency of the i^{th} allele at the locus in question. This is designed to measure the amount of genetic variation at a locus within a population. In this use, the terms heterozygosity and gene diversity are synonymous. The concepts of heterozygosity and homozygosity ($j = \sum x_i^2$) in populations were originally developed with respect to random mating populations. If a population is in Hardy-Weinberg equilibrium, the expected heterozygosity equals the observed heterozygosity ($h = h_0$). When equilibrium conditions are not met, as when mating is non-random, h does not equal the frequency of heterozygotes in the population. In order to avoid confusion when studying populations with non-random mating systems, Nei (1973) proposed

the term "gene diversity" for the heterozygosity statistic (H) and "gene identity" for the homozygosity statistic (J).

The expected heterozygosity of a population is estimated by

$$H = \frac{r}{\sum_{k=1}^r} h_k / r$$

which is the average of h over the r loci sampled. This can be interpreted as the average proportion of heterozygotes per locus or the proportion of loci at which an individual can be expected to be heterozygous.

The organization of gene diversity within a taxon can be described by the relationship

$$H_T = H_S + D_{ST}.$$

The total gene diversity (H_T) can be partitioned into the gene diversity within local populations (H_S) and that between local populations (D_{ST}). These values are calculated for each locus over all populations sampled, then averaged over all loci to get a grand estimate for the taxon studied.

H_T estimates the total genic variation sampled over all populations as it is a function of mean allele frequencies;

$$H_T = 1 - \sum \bar{x}_i^2.$$

$\bar{x}_i$ is the mean frequency of the i^{th} allele over all sampled populations. This statistic was calculated from the data in Table 17.

H_S is an estimate of the average amount of genetic variation maintained within local populations. If n populations are surveyed, the average gene diversity within populations at a

particular locus is

$$H_S = \sum_{k=1}^n h_k / n$$

where h_k is the expected heterozygosity in the k^{th} local population.

If all populations are members of a single large panmictic unit and there is no selection for local adaptation, then all alleles will be equally distributed over the entire range of the taxon and H_T will equal H_S . However, in nature this does not occur. Natural populations tend to differentiate over time into sub-populations as a result of mutation, selection, random drift and restricted gene flow. Therefore, H_S is a subset of H_T . The level of differentiation between populations is measured by D_{ST} , the gene diversity between populations, and is given by

$$D_{ST} = H_T - H_S.$$

The greater the level of sub-division within the taxon, the larger the value of D_{ST} .

The relative amount of genetic differentiation between local populations (G_{ST}) can be estimated by

$$G_{ST} = D_{ST} / H_T.$$

This value varies from zero to one and is termed the coefficient of gene differentiation. The sampling variance of G_{ST} can be calculated (Chakraborty, 1974), a standard error obtained and the significance of the genetic differentiation assessed.

The analyses of gene diversity in *P. brachystemon* and *P. congesta* are presented in Tables 24 and 25 respectively. There is a large amount of inter-locus variation in heterozygosity in both species which is reflected by the magnitude of the standard errors of the means. Similar results have been obtained in most other organisms that have been extensively surveyed for electrophoretic variability. The standard errors are often one-fifth to two-fifths as large as the mean heterozygosities (Lewontin, 1974). Since all loci do not contribute equally to a species average gene diversity, it is important to survey as large a number of loci as possible in order to realistically estimate this parameter (Nei and Roychoudhury, 1974).

The values of H_T for *P. brachystemon* were between zero and 0.659 with a mean over all loci of 0.166 ± 0.058 . In *P. congesta*, the values ranged between zero and 0.792 with a mean of 0.266 ± 0.083 . Although *P. brachystemon* generally has less total gene diversity at each locus than *P. congesta*, the pattern of heterozygosity among loci of each species is similar. However, the EST loci are notable exceptions. Both species share the same three alleles at EST-1 yet this locus had the largest H_T value of all loci surveyed in *P. brachystemon* and one of the lowest values observed in *P. congesta*. In contrast, EST-2 had three alleles and was extremely heterozygous in *P. congesta* while it was monomorphic in *P. brachystemon*. Esterases are a large and heterogeneous class of enzymes which are identified electrophoretically solely by their ability to react

Table 24. Analysis of Gene Diversity and Degree of Differentiation for 15 Loci in *Plectritis brachystemon*.

Locus	Total Gene Diversity (H_T)	Gene Diversity Within Populations (H_S)	Proportion of Interpopulation Gene Differentiation (G_{ST})
EST-1	.659	.210	.681
EST-2	.000	.000	.000
IDH-1	.000	.000	.000
LAP-1	.422	.218	.483
MDH-1	.484	.119	.754
MDH-2	.000	.000	.000
ME -1	.000	.000	.000
PGI-1	.000	.000	.000
PGI-2	.096	.085	.115
PGI-3	.226	.128	.434
PGM-1	.412	.118	.714
PGM-2	.000	.008	.000
PGM-3	.196	.017	.913
SOD	.000	.000	.000
6PG	.000	.000	.000
Combining All Loci:			
	.166±.058	.060±.021	.639±.053

Table.25. Analysis of Gene Diversity and Degree of Differentiation for 12 Loci in *Plectritis congesta*.

Locus	Total Gene Diversity (H_T)	Gene Diversity Within Populations (H_S)	Proportion of Interpopulation Gene Differentiation (G_{ST})
EST-1	.020	.020	.000
EST-2	.502	.321	.361
IDH-1	.000	.000	.000
LAP-1	.792	.671	.153
MDH-1	.509	.468	.081
MDH-2	.000	.008	.000
ME -1	.000	.000	.000
PGM-1	.431	.365	.153
PGM-2	.439	.391	.109
PGM-3	.504	.472	.063
SOD	.000	.000	.000
6PG	.000	.000	.000
Combining All Loci:			
	.266±.083	.226±.071	.150±.039

with a class of synthetic substrates. The differential loss of allozyme activity at EST-1 following the application of Malathion suggests that allozymes at EST-1 and EST-2 may represent different functional classes of EST. If these polymorphisms are adaptive (Johnson, 1976), this contrasting organization of diversity in EST may represent an evolutionary divergence of adaptive strategies in the two species. However, whether this observed difference is of physiological significance or the result of genetic drift is purely speculative.

The difference between the mean H_T values of the two species was compared using a t-test with 25 d.f. and found to be non-significant ($P \leq 0.40$). The non-significance of the difference between H_T averaged over all loci may be attributed partially to the large standard error of the means. However, this comparison demonstrates that despite its high level of inbreeding, *P. brachystemon* maintains a level of total variation which is somewhat reduced, but comparable to the predominantly outcrossed *P. congesta*.

It is the gene diversity within populations, H_S , which reflects the contrasting breeding systems. Inter-locus variation in H_S was again large. Values ranged from zero to 0.218 in *P. brachystemon* and zero to 0.671 in *P. congesta*. When averaged over all loci, the mean genetic diversity within local populations was 0.060 ± 0.021 and 0.226 ± 0.071 for *P. brachystemon* and *P. congesta* respectively. The mean H_S is equal to the mean expected heterozygosity (H) averaged over all populations. Consequently, H_S can be interpreted as the

average proportion of heterozygotes per locus or the proportion of loci at which an individual can be expected to be heterozygous. On the average, *P. congesta* was heterozygous at 23% of its loci while *P. brachystemon* was heterozygous at only 6%. Despite the inter-locus variation, the difference between the means was significant ($P \leq 0.05$). This illustrates that the level of population sub-division in the selfer is much larger than the level in the outcrosser. As the gene diversity within populations (H_S) decreases, the gene diversity between (*i.e.* differentiating) populations (D_{ST}) increases. Accordingly, the relative magnitude of gene differentiation between populations (G_{ST}) also increases because it is described by the relationship

$$G_{ST} = D_{ST}/H_T$$

When G_{ST} was averaged over all loci, the values for *P. brachystemon* and *P. congesta* were 0.639 ± 0.053 and 0.150 ± 0.039 respectively. The difference between the means was highly significant ($P \leq 0.01$). In *P. brachystemon*, 64% of the total gene diversity could be attributed to inter-population differences. This compared to only 15% in *P. congesta*. In other words, populations of *P. congesta* maintain, on the average, 85% of the variation detected over the sampled range of the species. In contrast, single populations of *P. brachystemon* maintain an average of only 36% of the selfer's array of detected variation.

Although G_{ST} is a good measure of the relative amount of gene differentiation between populations, its value is highly dependent on H_T (Nei, 1975). When H_T is low, G_{ST} may overestimate the magnitude of interpopulation gene differentiation. Although

H_T was not found to be significantly different between the two taxa, another measure of population differentiation was calculated. Standard genetic distance, D (Nei, 1972) estimates the number of net codon differences per locus between populations. It is independent of the gene diversity within populations and therefore can be used for comparing the degree of gene differentiation in different organisms. The average genetic distance ($\bar{D} \pm$ inter-locus S.E.) among populations of *P. brachystemon* and *P. congesta* was 0.137 ± 0.083 and 0.052 ± 0.034 respectively. In other words, for every 100 loci, there were approximately 5.2 codon differences between populations of *P. congesta* and 13.7 codon differences between populations of *P. brachystemon*. Although the $\bar{D}$ between populations of the selfer was more than two and a half times greater than the corresponding value in the outcrosser, the difference was non-significant ($P \leq 0.40$). However, this lack of significance can be attributed to the inter-locus variation in heterozygosity.

5.4 *Comparisons of genetic identity and genetic distance*

Nei's (1972, 1975) index of genetic identity (I) and standard genetic distance (D) were calculated for all intra-specific and inter-specific pairwise combinations of populations. There were four objectives in this analysis:

- i. to assess the relative levels of inter-population differentiation within each species and correlate this with the breeding system;

- ii. to determine if genetic distance reflects geographic distance between populations;
 - iii. to calculate the inter-specific similarity of the two taxa in order to evaluate their taxonomic relationship and
 - iv. to determine if sympatric populations are genetically more similar than other inter-specific combinations.
- The purpose of this comparison was to test for the possible selective maintenance of the observed isozyme polymorphisms.

Nei's genetic identity statistic compares populations for both the presence/absence of identical alleles and their occurrence at similar frequencies. This measure is calculated for each locus:

$$I = \frac{\sum (P_{jx} P_{jy})}{\sqrt{(\sum (P_{jx})^2) (\sum (P_{jy})^2)}}$$

where P_{jx} and P_{jy} are the frequencies of the j^{th} allele in populations X and Y respectively. The numerator is calculated for all alleles at the locus and then divided by the geometric mean of identical homozygote frequencies. Two populations which have identical allele frequencies at a locus, have a genetic identity of one. Their genetic identity is zero if they have no alleles in common. When all gene loci are considered, the genetic identity between two populations is calculated by

$$I = \frac{I_s}{\sqrt{(\sum_j p_{ijx}^2)(\sum_j p_{ijy}^2)}L^2}$$

The numerator, I_s , is an index of genetic similarity (Sokal and Sneath, 1963):

$$I_s = \frac{1}{L} \sum_{ij}^{LK} p_{ijx} \cdot p_{ijy}$$

In both the numerator and denominator, p_{ijx} and p_{ijy} are the corresponding frequencies of the j^{th} allele at the i^{th} locus in populations X and Y. L is the number of loci surveyed. Spiess (1977) presents a more detailed discussion of the theory and calculation of this statistic.

The standard genetic distance between populations X and Y is defined as $D = -\log_e I$. This is approximately equal to $1-I$. This value estimates the accumulated number of gene substitutions per locus, between populations, assuming the rate of gene substitutions is identical for all loci.

The genetic identities and standard genetic distances between populations of *P. brachystemon* and *P. congesta* are presented in Tables 26 and 27 respectively. All calculations were based on allele frequencies from fifteen loci in *P. brachystemon* and twelve loci in *P. congesta*. Population 16 (Little Qualicum Falls) of *P. brachystemon* was excluded from all genetic distance calculations because allele frequencies for EST-1 were not available.

Table 26. Genetic identity (I) and standard genetic distance (D)
between populations of *P. brachystemon*

	1	13	9	10	7(1)	7(3)	15(1)	15(2)	18
1		0.9030	0.8665	0.8687	0.8468	0.8709	0.8690	0.8708	0.6856
13	0.1020		0.9422	0.8687	0.9113	0.9451	0.9291	0.9112	0.8174
9	0.1433	0.0595		0.8657	0.9167	0.9136	0.9466	0.9154	0.7830
10	0.1408	0.1408	0.1442		0.8433	0.8671	0.8366	0.7818	0.7721
7(1)	0.1663	0.0929	0.0868	0.1704		0.9492	0.9577	0.9731	0.8174
7(3)	0.1382	0.0565	0.0904	0.1426	0.0521		0.9180	0.9317	0.8617
15(1)	0.1404	0.0735	0.0549	0.1784	0.0432	0.0856		0.9523	0.8031
15(2)	0.1384	0.0930	0.0884	0.2461	0.0273	0.0707	0.0489		0.7675
18	0.3775	0.2016	0.2446	0.2586	0.2016	0.1488	0.2193	0.2646	
$\bar{I}$ pop.	0.8451	0.9026	0.8923	0.8372	0.9002	0.9066	0.8999	0.8850	0.7869
$\bar{D}$ pop.	0.1683	0.1025	0.1140	0.1777	0.1051	0.0981	0.1055	0.1222	0.2396
Estimates of I above the diagonal					$\bar{I} = 0.8720$				
Estimates of D above the diagonal					$\bar{D} = 0.1370 \pm 0.0825$				

Table 27. Genetic identity (I) and standard genetic distance (D) between populations of *P. congesta*.

	2(1)	2(2)	3	5	4	9	10	11	7(2)	8	14(1)	14(2)	15(1)	15(2)	17
2(1)		.9930	.9576	.9269	.9705	.9534	.9555	.9673	.9203	.9396	.9859	.9956	.9133	.8877	.9677
2(2)	.0070		.9427	.9256	.9777	.9723	.9549	.9647	.9154	.9247	.9593	.9820	.8976	.8940	.9479
3	.0433	.0590		.9372	.9750	.9403	.9792	.9819	.9510	.9721	.9779	.9701	.9537	.9178	.9578
5	.0759	.0773	.0649		.9623	.9464	.9859	.9748	.9917	.8831	.9261	.9232	.9802	.9732	.9019
4	.0299	.0226	.0253	.0384		.9857	.9849	.9892	.9528	.9623	.9698	.9831	.9493	.9686	.9462
9	.0477	.0281	.0616	.0551	.0144		.9667	.9684	.9185	.9218	.9220	.9541	.9293	.9317	.9359
10	.0455	.0461	.0210	.0142	.0152	.0339		.9922	.9777	.9488	.9559	.9534	.9730	.9579	.9566
11	.0332	.0359	.0183	.0255	.0109	.0321	.0078		.9741	.9454	.9648	.9687	.9737	.9513	.9606
7(2)	.0831	.0884	.0502	.0083	.0484	.0850	.0226	.0262		.8995	.9389	.9219	.9803	.9569	.9031
8	.0623	.0783	.0283	.1243	.0384	.0814	.0526	.0561	.1059		.9662	.9541	.9030	.9067	.9586
14(1)	.0142	.0416	.0223	.0768	.0307	.0812	.0451	.0358	.0630	.0344		.9924	.9255	.9116	.9557
14(2)	.0044	.0182	.0304	.0799	.0170	.0470	.0477	.0318	.0813	.0470	.0076		.9188	.9076	.9550
15(1)	.0907	.1080	.0474	.0200	.0520	.0733	.0274	.0267	.0199	.1020	.0774	.0847		.9393	.9097
15(2)	.1191	.1121	.0858	.0272	.0319	.0707	.0430	.0499	.0441	.0979	.0925	.0970	.0626		.8678
17	.0328	.0535	.0431	.1033	.0553	.0663	.0444	.0402	.1019	.0423	.0453	.0460	.0946	.1418	
$\bar{I}$ pop.	.9520	.9461	.9580	.9451	.9697	.9460	.9672	.9698	.9425	.9344	.9534	.9553	.9387	.9261	.9370
$\bar{D}$ pop.	.0492	.0554	.0429	.0565	.0308	.0555	.0333	.0307	.0592	.0679	.0477	.0457	.0633	.0767	.0651

Estimates of I above the diagonal

$\bar{I} = 0.9493$

Estimates of D above the diagonal

$\bar{D} = 0.0520 \pm 0.0344$

5.4.1 Levels of inter-population differentiation

Levels of inter-population differentiation within taxa can be compared by calculating mean genetic identities ($\bar{I}$) from all pairwise combinations of populations. On the average, populations of *P. brachystemon* were genetically more distinct ($\bar{I} = 0.8720$) than populations of *P. congesta* ($\bar{I} = 0.9493$). Based on a sample of twenty-eight species, Gottlieb (1977a) estimated the mean identities of intra-specific plant populations to be $0.95 \pm .02$. He therefore concluded that in general, a sample of one or a few populations constitute an adequate sample of a species variability. While the value of $\bar{I}$ for *P. congesta* is in excellent agreement with Gottlieb's estimate, the calculated value for *P. brachystemon* is much lower. In fact, it is lower than estimates of $\bar{I}$ between five congeneric species pairs (Gottlieb, 1977a): *Clarkia biloba* and *C. lingulata* ($\bar{I}=0.88$), *Gaura longiflora* and *G. demareei* ($\bar{I} = 0.99$), *Hymenopappus scabrosaeus* and *H. artemisiaefolius* ($\bar{I} = 0.90$), *Oenothera strigosa* and *O. biennis* ($\bar{I} = 0.97$), and *Stephanomeria exigua* subsp. *coronaria* and *S. malheurensis* ($\bar{I} = 0.94$).

The observed differences in the mean genetic identities in *Plectritis* reflects the relative degree of partitioning in the gene pool of each species. In order for I to be large, local populations must maintain a large proportion of the taxon's array of variation. On the average, 85% of the variation in *P. congesta* was detected within each population. In contrast, *P. brachystemon* populations contained only 34% of the variation of the species. Large fluctuations in allele frequencies between populations were much more prevalent in the selfer than the outcrosser (Figures 27-40). The

distribution of many alleles in *P. brachystemon* might best be described as a "random walk." This pattern of allele distribution is explained most easily to be a consequence of the breeding system. Autogamy restricts gene flow and lowers the effective population size. This enhances the importance of stochastic forces in determining allele frequencies within populations. This is not to say that founder effect and genetic drift are not important within predominantly outcrossing taxa, only that these forces become relatively more important when gene flow is reduced.

5.4.2 Comparisons between genetic distance and geographic distance

Genetic distance estimates are summarized in Table 28 for *P. brachystemon* and in Table 29 for *P. congesta*. In addition, dendrographs (McCammon, 1968) were generated using the estimates of genetic identity and these are presented in Figures 44 and 45 for *P. brachystemon* and *P. congesta* respectively. Although certain comparisons suggest a relationship between geographic distance and genetic distance, overall there appears to be no clear relationship between these two parameters. These observations are in agreement with those of Kahler *et al.* (1980), who found that isozyme variability in *Avena barbata* was distributed in mosaic patterns which were not related to geographic distance.

In *P. brachystemon*, population 1 (Sumas Mountain) and population 18 (Campbell River) are separated by the greatest geographic distance and also had the largest genetic distance ($\bar{D} = 0.3775$). However, population 18 was the most genetically dissimilar of all *P. brachystemon* populations ($\bar{D} = 0.2396$). This large $\bar{D}$ is comparable to some inter-specific comparisons within other genera. It reflects

Table 28. Summary of Genetic Distances in *P. brachystemon*

Population	$\bar{D}$	Most similar to		Most distant from	
		Population	D	Population	D
1	0.1683	13	0.1020	18	0.3775
13	0.1025	7(3)	0.0565	18	0.2016
9	0.1140	15(1)	0.0549	18	0.2446
10	0.1777	1 and 13	0.1408	18	0.2586
7(1)	0.1051	15(2)	0.0273	18	0.2016
7(3)	0.0981	7(1)	0.0521	18	0.1488
15(1)	0.1055	7(1)	0.0432	18	0.2193
15(2)	0.1222	7(1)	0.0273	18	0.2646
18	0.2396	7(3)	0.1488	1	0.3775

Table 29. Summary of genetic distances in *P. congesta*

Population	$\bar{D}$	Most similar to		Most distant from	
		Population	D	Population	D
2(1)	0.0492	14(2)	0.0044	15(2)	0.1191
2(2)	0.0554	2(1)	0.0070	15(2)	0.1121
3	0.0429	10	0.0210	15(2)	0.0858
5	0.0565	7(2)	0.0083	8	0.1243
4	0.0308	11	0.0109	17	0.0553
9	0.0555	4	0.0144	7(2)	0.0850
10	0.0333	11	0.0078	8	0.0526
11	0.0307	10	0.0078	8	0.0561
7(2)	0.0592	5	0.0083	8	0.1059
8	0.0679	3	0.0283	5	0.1243
14(1)	0.0477	14(2)	0.0076	15(2)	0.0925
14(2)	0.0457	2(1)	0.0044	15(2)	0.0970
15(1)	0.0633	7(2)	0.0199	2(2)	0.1080
15(2)	0.0768	5	0.0272	17	0.1418
17	0.0651	2(1)	0.0328	15(2)	0.1418

Figure 44. A dendrogram depicting the genetic relationships among the sampled *P. brachystemon* populations (population 16 excluded): correlations are based on Nei's Index of Genetic Identity calculated from the allele frequencies at fifteen loci.

Figure 45. A dendrogram depicting the genetic relationships among the sampled *P. congesta* populations: correlations are based on Nei's Index of Genetic Identity calculated from the allele frequencies at twelve loci.

the observation that this population was monomorphic for or maintained at relatively high frequency, alleles which- were generally either rare or absent in other *P. brachystemon* populations (*i.e.* EST-1⁸⁹, MDH-1¹¹³, PGI-3^{33r} and PGM-3¹¹⁵). Sympatric populations 7(1):7(3) (Malahat Drive) and 15(1):15(3) (Nanoose Hill) had relatively low genetic distances: 0.0521 and 0.0489 respectively. However, populations 15(1) and 15(2) were more similar to population 7(1), and 7(1) was more similar to 15(2), than they were to their respective sympatric populations. Populations 7(1) and 7(3) were collected approximately 30 m. apart (although in successive years). Their allele frequencies were compared using a t-test (Spiess, 1977). Although these two populations were separated by such a small distance, they maintained the following alleles at significantly different frequencies: EST-1⁸⁹, EST-1¹¹⁴, LAP-1⁹⁵, LAP-1¹⁰⁰, PGM-1¹⁰⁰ and PGM-1¹⁰⁶ ($P \leq 0.01$); and EST-1¹⁰⁰, PGI-2⁸¹ and PGI-2¹⁰⁰ ($P \leq 0.05$). In addition, these populations had significantly different heterozygosity values ($P \leq 0.01$) at EST-1, LAP-1, PGI-2 and PGM-1.

P. brachystemon populations 15(1) and 15(2) were separated by a distance of not more than 100 m. (the exact distance is unknown). Within these two populations, the following alleles were observed at significantly different frequencies ($P \leq 0.01$); EST-1¹⁰⁰, EST-1¹¹⁴, LAP-1¹⁰⁰, LAP-1¹⁰⁵, PGI-3^{33r}, PGI-3¹⁰⁰ⁿ, PGM-1¹⁰⁰ and PGM-1¹⁰⁶. These populations also had significantly different heterozygosity values at LAP-1, PGI-3 and PGM-1 ($P \leq 0.01$); and EST-1 ($P \leq 0.05$). In contrast, *P. congesta* populations 15(1) and 15(2) were compared

and significantly different frequencies ($P \leq 0.01$) were detected for only LAP-1⁹¹, LAP-1⁹⁵ and LAP-1¹⁰⁰. These data suggest that the level of genetic differentiation between populations of *P. brachystemon* is not necessarily associated with the geographic distance which separates them.

When populations of *P. congesta* were compared, the correspondence between geographic distance and genetic distance appeared better than that found in *P. brachystemon*. However, there were still notable exceptions to any pattern. Figure 45 depicts a major dichotomy within *P. congesta*. Populations segregated into two unequal sub-groups with populations 15(1) and 15(2) (Nanoose Hill); 7(2) (Malahat Drive) and 5 (Mill Hill Park) differentiated from the other eleven populations. This dichotomy was generated by the relative frequency of allele EST-2⁷⁴ and there was no discernible pattern to its distribution (Figure 35). In populations 15(1), 15(2), 7(2) and 5, it was detected at frequencies >0.75 while in the other eleven populations it was never found with a frequency >0.50 . Within the small sub-group, populations 15(1) and 15(2) were much more similar to populations 7(2) and 5 respectively, than they were to each other. Populations 2(1) and 2(2) (Anacortes, WA) occur on a serpentine outcrop (Kruckeberg, 1969). Although these populations were quite similar ($D = 0.0070$), population 2(1) was more similar to population 14(2) (Jack's Point: $D = 0.0044$) than it was to population 2(2). Population 10 (Prospect Lake) and 11 (Viaduct Ave) were collected approximately one km apart.

and they were genetically more similar to each other ($D = 0.0078$) than they were to any other populations. However, population 17 (Nile Creek) was most similar to population 2(1) (Anacortes, WA) although these populations are separated by the greatest distance.

The general lack of correspondence between geographic distance and genetic distance suggests that gene flow is not a major factor influencing the macro-geographic distribution of alleles in *Plectritis*. Regardless of the geographic distances involved, *P. congesta* populations were, in general, genetically more similar to each other than were *P. brachystemon* populations; $\bar{D} = 0.0520$ and $\bar{D} = 0.1370$ respectively. This indicates either that gene flow between populations is more prevalent in the outcrosser and/or that as a result of recombination within populations, the loss of variability due to genetic drift is less of a factor than it is in the selfer. Since seeds of both species should be equally dispersible and there is no evidence to suggest that pollen vectors in *P. congesta* are effective over large distances, stochastic forces are probably more important than gene flow in determining allele distributions and the relative levels of genetic differentiation observed in these taxa. However, Lewontin (1974) cautions that even small migration rates (*i.e.* one individual in a thousand per generation) are sufficient to prevent differentiation between populations of moderate size.

5.4.3 Taxonomic and evolutionary relationships

Although Morey (1959, 1962) did not give *P. brachystemon* specific rank, recognizing it as *P. congesta* ssp. *brachystemon* (F. & M.) Morey, the isozyme data support the recognition of two distinct species. Standard genetic distance was averaged over all inter-specific pairwise combinations of populations (Table 30) so that the genetic divergence between these two taxa could be compared to other species pairs of known taxonomic rank. These data were used also to construct a dendrograph (McCammon, 1968) which is presented in Figure 46. Gottlieb (1977a) surveyed isozyme data on twenty-eight plant species in order to evaluate the applications of electrophoretic data in plant systematics. Reviewing available data for thirteen pairs of related species, he calculated an average genetic identity for congeneric species, $\bar{I} = 0.67 \pm 0.07$ (equivalent to $\bar{D} = 0.40$). This demonstrates that in general, congeneric species are significantly more differentiated than intra-specific populations ($\bar{I} = 0.95 \pm 0.02$). Based on a sample of twelve loci, the genetic identity of *P. brachystemon* and *P. congesta* was $I = 0.7311$ ($\bar{D} = 0.3132$). This level of differentiation is in excellent agreement with Gottlieb's value for congeneric species.

However, the evolutionary relationship of the two species is not so clear. Gottlieb had data on three species pairs which were known to be related as progenitor and derivative with the derivative being of relatively recent origin: *Stephanomeria exiqua* subsp. *coronaria* and *S. malheurensis* ($\bar{I} = 0.94$); *Clarkia biloba* and *C. lingulata*

Figure 46. A dendrograph depicting the genetic relationships among the sampled *Plectritis* populations: correlations are based on Nei's Index of Genetic Identity calculated from the allele frequencies at twelve loci.

($\bar{I} = 0.88$), and *Guara longiflora* and *G. demareei* ($\bar{I} = 0.99$).

This high average genetic identity was interpreted as evidence that shortly after their origin, derivative species are limited genetic versions of the progenitor. In other words, reproductive isolation had evolved without a major genetic reorganization.

P. brachystemon has been considered to be a derivative of *P. congesta*. Yet, their genetic identity was much lower than those calculated for the three species pairs known to be related as progenitor and derivative. When the gene pools of each species were compared (section 4.4), 42% (14/33) of the alleles detected in *P. congesta* were not scored in *P. brachystemon*. Only 17% (4/23) of the selfer's alleles were not also found in the outcrosser. Nineteen alleles were present in both species but seven of these were detected with significantly different frequencies (Table 17). Based on their level of allozyme differentiation, it is highly unlikely that *P. brachystemon* is a recent derivative of *P. congesta*. The inability to hybridize *P. congesta* and *P. brachystemon* (Carey and Ganders, personal communication) also indicates considerable divergence. However, the majority of the selfer's allozymes (83%) was a sub-set of the variation detected in the outcrosser. The average genetic distance ($\bar{D}=0.3132$) between the two species was smaller than the intra-specific genetic distance ($D=0.3775$) between *P. brachystemon* populations-1 (Sumas Mountain) and 18 (Campbell River). Therefore, it is possible that the selfer is a derivative of the outcrosser but that enough time has elapsed since divergence for a considerable

amount of differentiation to have occurred. These observations are based, however, on the study of a region where both species have invaded since the retreat of Pleistocene glaciers . If

P. brachystemon evolved from *P. congesta* , it probably did so south of the glacial boundary. The genotypes that moved north may not represent a random sample of the genetic variation of the two species. Consequently, it is possible that the two species are genetically more similar further south: however, this may not necessarily be the situation. In conclusion, the isozyme data confirmed the recognition of *P. brachystemon* as a distinct species. However, it is impossible to make a definitive statement on the evolutionary relationship of these two taxa without sampling populations in California and also studying other species in the genus.

5.4.4 *Maintenance of observed isozyme polymorphisms*

A controversy has developed within population genetics because of efforts to explain the maintenance of observed enzyme polymorphisms. The neutralists (neo-classicists) believe that most of the electrophoretic variation, which is observed, is selectively neutral and maintained by a balance between mutation rate and genetic drift. In contrast, the selectionists (neo-Darwinians) believe that observed protein polymorphisms are selectively maintained. A thorough discussion of these issues can be found in Ayala (1974), Lewontin (1974), Johnson (1973, 1975, 1976) and Powell (1975).

Genetic distances were compared between *P. brachystemon* and *P. congesta* at sympatric and non-sympatric sites to determine if sympatric populations were genetically more similar than other inter-specific comparisons. The purpose of this analysis was to test for the possible selective maintenance of isozyme polymorphisms. The rationale for this approach was that if both taxa share the same alleles and the observed allozyme polymorphisms are influenced by selection, similar environmental regimes at the sympatric sites should favor the same alleles in both species. This would be reflected by their greater genetic similarity. Genetic distances between sympatric populations are listed in italics in Table 30. The mean standard genetic distance for sympatric comparisons was $\bar{D} = 0.3393$ while it was $\bar{D} = 0.3120$ for non-sympatric comparisons. Therefore, these data do not support the assumption that the observed isozyme polymorphisms are selectively maintained. It should be noted, however, that *P. congesta* and *P. brachystemon* may have differentiated sufficiently that this comparison is inappropriate. The existence of multi-locus allele complexes have been demonstrated in slender wild oats (*e.g.* Clegg and Allard, 1972; Hamrick and Allard, 1972; Allard *et al.*, 1972; Hamrick and Holden, 1979; Kahler *et al.*, 1980) and barley (Clegg *et al.*, 1972). These authors present evidence and argue that selection has structured the genetic resources of these species into "highly interacting, co-adapted gene complexes." If fitness is determined by the coordinated interaction of large numbers of loci, then alleles which are selective at a few marker loci in one species of *Plectritis* may not necessarily be equally advantageous in the other.

Table 30. Inter-specific comparisons of standard genetic distance (D)
in *Plectritris*

		<i>P. brachystemon</i> populations								
		1	13	9	10	7(1)	7(3)	15(1)	15(2)	18
<i>P. congesta</i> populations	2(1)	0.3242	0.3060	0.1960	0.2991	0.2882	0.2417	0.2693	0.2854	0.2868
	2(2)	0.3393	0.2960	0.1868	0.2817	0.2791	0.2378	0.2587	0.2881	0.2836
	3	0.2858	0.3249	0.2552	0.2662	0.3585	0.2524	0.3161	0.3700	0.3669
	5	0.4524	0.3983	0.2758	0.3843	0.3836	0.3461	0.3590	0.4121	0.4057
	4	0.2735	0.2595	0.1824	0.2098	0.2827	0.2408	0.2417	0.3111	0.3029
	9	0.2896	0.2943	0.2121	0.2335	0.3017	0.2664	0.2730	0.3402	0.3027
	10	0.3386	0.3452	0.2588	0.2921	0.3694	0.2998	0.3277	0.3862	0.3834
	11	0.3140	0.3330	0.2409	0.2944	0.3473	0.2801	0.3118	0.3594	0.3584
	7(2)	0.4412	0.4022	0.3003	0.3853	0.3840	0.3192	0.3636	0.4020	0.4023
	8	0.2406	0.2846	0.2483	0.1873	0.3536	0.2816	0.2912	0.3718	0.3663
	14(1)	0.3200	0.2975	0.2046	0.2724	0.2997	0.2388	0.2703	0.3089	0.3089
	14(2)	0.2903	0.2763	0.1825	0.2608	0.2748	0.2251	0.2478	0.2892	0.2845
	15(1)	0.3677	0.4379	0.3427	0.3915	0.4562	0.3676	0.4197	0.4588	0.4618
	15(2)	0.3990	0.3232	0.2452	0.2678	0.3540	0.3560	0.3041	0.4087	0.3998
	17	0.2695	0.3407	0.2699	0.2586	0.3625	0.2878	0.3281	0.3740	0.3385

Comparisons between sympatric populations are in italics

$\bar{D}$ (*P.b.:P.c.*) = 0.3132

$\bar{D}$ (sympatric) = 0.3393

$\bar{I}$ (*P.b.:P.c.*) = 0.7311

$\bar{I}$ (sympatric) = 0.7123

A second approach to this question was to compare the observed variance of inter-locus heterozygosity (h) to a theoretical variance which assumes that neutral mutations and genetic random drift are balanced (Nei and Roychoudhury, 1974). The theoretical variance is given by

$$\text{Var } (h) = \frac{2\theta}{(1+\theta)^2(2+\theta)(3+\theta)}$$

where $\theta = 4N\mu$; N is the effective population size and μ is the mutation rate per locus per generation. The value of θ may be estimated by $H/(1-H)$; in which H is the estimate of average heterozygosity, as the expectation of H is $\theta/(1+\theta)$. The estimates of θ , the observed variance of heterozygosity and the theoretical variance of heterozygosity are presented for *P. brachystemon* and *P. congesta* in Tables 31 and 32 respectively. The expected and observed values are generally in excellent agreement for *P. brachystemon* and reasonable agreement for *P. congesta*.

Unfortunately, it is not possible to assess the significance of departures from the theoretical value. In addition, it cannot be determined whether the difference in the level of agreement between variances in *P. brachystemon* and *P. congesta* has any implication in regard to the relative roles of selection and drift in maintaining observed polymorphisms within the two species. However, in general it would appear that the levels of isozyme variation, particularly in *P. brachystemon*, are in agreement with the expectations of a stochastic model. This does not eliminate the possibility of selection because certain types of selection and

Table 31. A comparison between the observed and the theoretical inter-locus variances of heterozygosity (h) in populations of *P. brachystemon*

Population	θ^1	Observed variance ²	Theoretical variance ³
1	0.056	0.022	0.016
13	0.066	0.022	0.018
9	0.032	0.006	0.010
10	0.006	0.001	0.002
7(1)	0.041	0.017	0.012
7(3)	0.164	0.045	0.035
15(1)	0.128	0.033	0.030
15(2)	0.063	0.024	0.018
16	0.014	0.001	0.004
18	0.075	0.034	0.020

¹ $\theta = 4N\mu$, where N = effective population size and μ = mutation rate per locus per generation, θ estimated by $H/(1-H)$ since the expectation of $H = (\theta/(1+\theta))$

² Observed inter-locus variance of heterozygosity: $V(h) = \frac{r}{\sum_{k=1}^r} (h_k - H)^2 / (r-1)$
 where h_k = estimate of heterozygosity at the k^{th} locus and r = the number of loci scored

³ Theoretical inter-locus variance of heterozygosity:

$$\text{Var}(H) = \frac{2\theta}{(1+\theta)^2(2+\theta)(3+\theta)}$$

Table 32. A comparison between the observed and the theoretical inter-locus variance of heterozygosity (h) in populations of *P. congesta*

Population	θ	Observed variance	Theoretical variance
2(1)	0.234	0.073	0.043
2(2)	0.268	0.080	0.045
3	0.325	0.068	0.048
5	0.274	0.066	0.045
4	0.342	0.078	0.049
9	0.347	0.073	0.049
10	0.371	0.086	0.049
11	0.352	0.081	0.049
7(2)	0.350	0.061	0.049
8	0.272	0.065	0.045
14(1)	0.232	0.065	0.042
14(2)	0.221	0.069	0.042
15(1)	0.306	0.061	0.047
15(2)	0.230	0.049	0.042
17	0.295	0.070	0.047

varying mutation rates may produce the same patterns (Li, 1978).

In addition, there are a number of plant studies which have demonstrated micro-habitat selection (Antonovics, 1968; Hamrick and Allard, 1972; Mitten *et al.*, 1977; Hamrick and Holden, 1979; Kahler *et al.*, 1980) and the sampling strategies used in the present study would not resolve variation patterns at this level. Considering the quantity of variation maintained within local populations and the vulnerability of individuals, particularly seedlings, selection may be most important operating at the level of micro-site differences.

5.5 Summary: the genetic consequences of contrasting breeding systems

Outcrossing rates were calculated for nine populations of *P. brachystemon* and fifteen populations of *P. congesta*. The difference between the mean outcrossing rates in the two species was highly significant ($P \leq 0.01$). These two species are ideal for comparison because: (i) they are closely related and have a large proportion of their alleles in common, (ii) the multi-locus organization of the enzyme systems studied is homologous and (iii) they have similar life-cycle strategies and habitat requirements. It is not uncommon, particularly on southern Vancouver Island, to find the two species growing sympatrically. A number of genetic parameters were measured within each taxon to assess the effect that breeding system has on population structure. The results are summarized in Table 33. As measured by all parameters, the selfer maintained significantly less variation within local populations than the outcrosser although their detected levels of total variation

Table 33. Summary of genetic differences between *P. brachystemon* and *P. congesta*

Parameter	<i>P. brachystemon</i>	<i>P. congesta</i>	t
Outcrossing frequency	0.024±0.008	0.702±0.048	13.84**
Percentage of loci polymorphic per population	20.76±3.49	53.33±1.78	8.31**
Average number of alleles/locus per population	1.25±0.04	1.98±0.05	11.41**
Average number of alleles/polymorphic locus per population	2.22±0.10	2.84±0.08	4.84**
Expected % of heterozygous loci/individual	5.89±1.33	22.64±0.79	10.83**
Observed % of heterozygous loci/individual	0.45±0.13	17.74±0.81	21.09**
Total gene diversity (H_T)	0.166±0.021	0.266±0.083	0.99
Gene diversity within populations (H_S)	0.060±0.021	0.266±0.071	2.24*
Proportion of inter-population gene differentiation (G_{ST})	0.639±0.053	0.150±0.039	7.41**
Mean standard genetic distance between population ($\bar{D}$)	0.137±0.083	0.052±0.034	0.94
Mean genetic identity between populations ($\bar{I}$)	0.872	0.949	

* (P 0.05)

** (P 0.01)

(H_T) were not significantly different. When the influence of site variation was controlled by comparing only sympatric populations of the two species, all genetic differences remained significant. The reduced level of variation within populations of the selfer was reflected in the F-statistics and in the analysis of gene diversity. On the average, *P. congesta* populations contained 85% of the species' variation while only 36% of the variation detected in *P. brachystemon* was maintained within local populations.

In the selfer, the reduced level of genetic variation within populations was accompanied by increased populational differentiation. This is graphically depicted in Figure 46 which shows the reduced genetic identity among populations of *P. brachystemon*. Preliminary data suggest that differentiation, particularly in the selfer, can occur over short distances. However, whether this represents micro-geographic differentiation or drift in small reproductivity isolated populations is problematic. In this taxon, allele frequencies and single locus heterozygosities fluctuated widely and different alleles were often fixed in adjacent populations. In *P. congesta*, allele frequencies and heterozygosities also fluctuated among populations but not with the amplitude observed in *P. brachystemon*. There appears to be no relationship between genetic distance and geographic distance. This implies that gene flow between populations does not significantly influence allele frequencies. No evidence was found to suggest that the observed isozyme polymorphisms are selectively maintained. However, co-ordinated gene complexes and micro-habitat selection would probably not have

been detected.

It is concluded that the observed differences in the population structure of *P. brachystemon* and *P. congesta* is primarily the result of their contrasting breeding systems. Autogamy restricts gene flow between populations and recombination within populations. Inbreeding strongly influences the levels of variation maintained within populations by lowering the effective population size. This increases the potential that stochastic forces will significantly affect allele frequencies within populations. Although small migration rates are sufficient to prevent differentiation between populations (Lewontin, 1974), there is no evidence that pollen flow in *P. congesta* is effective over large distances. Therefore, the relative amounts of recombination and drift within local populations are probably the most important factors influencing the contrasting levels and organization of genetic variation in *P. brachystemon* and *P. congesta*.

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APPENDIX A. POPULATION LOCATIONS

Sample designation	Location	Location and comments
1	Sumas Mountain	large, open south-facing slope of Sumas Mtn. (east of Abbotsford, B.C), visible from Trans-Canada Hwy.
2(1)	Anacortes, WA	open slope overlooking Puget Sound, Washington Park (Anacortes City Park): Fidalgo Head, Fidalgo Island; on ultramafic soils derived from periodotite (Kruckeberg, 1969).
2(2)	Anacortes, WA	large open meadow, Washington Park: ultramafic soils
13	William Head	large population growing among Garry oak and arbutus, Metchosin Rd. .6 km SW of Pearson College Dr. Metchosin, B.C.
3	John Dean Park	plants were collected on a small ledge and along a 15 m transect at the base of a clearing located below a DND radar installation, Central Saanich, B.C.
5	Mill Hill Park	open south-facing slope; plants at this site bloom 2-4 wks. earlier than other collected populations, near Victoria, B.C.
4	Thetis Lake Park	open meadow with scattered Garry oak and arbutus, near Victoria, B.C.
9	Francis Park	sympatric population, <i>P.c.</i> is more abundant than <i>P.b.</i> ; Douglas-fir woodland, a much shadier site than most; Saanich, B.C.
10	Prospect Lake Rd.	sympatric population, <i>P.c.</i> is more widely distributed than <i>P.b.</i> ; an open and somewhat disturbed site, evidence of a burn; <i>Gaultheria shallon</i> and <i>Cytisus scoparius</i> are abundant; Prospect Lake Rd. .5 km N of Prospect Lake Golf Club. Saanich, B.C.
11	Viaduct Avenue	small population in a depression between two rock outcrops; plants were robust and density was high, intersection of Prospect Lake Rd. and Viaduct Ave., Saanich, B.C.

APPENDIX A. POPULATION LOCATIONS

Sample designation	Location	Location and comments
7(1)	Malahat Drive	sympatric population, an exposed, SE-facing rocky knoll beneath three Hydro transmission towers overlooking Saanich Inlet; pulloff located 4 km N of "entering Malahat Dr." sign on Trans-Canada Hwy. 7(1) - <i>P.b.</i> collected along a 15 m transect running down the slope beginning approx. 10 m N of transmission tower #1.
7(2)	Malahat Drive	<i>P.c.</i> - located beneath transmission tower #2
7(3)	Malahat Drive	<i>P.b.</i> - NE slope of knoll, top of knoll with a few <i>P.c.</i>
8	Crofton Exit	SE-facing ledge approx. 5 m above Hwy. site was quit damp at time of collection and herb cover was lush; plants were collected along a 30 m transect running NW from the ledge to the back of the clearing; Trans-Canada Hw. 1.7 km south of Crofton Exit (Saltspring Island ferry).
14(1)	Jack's Point	open site bisected by a gravel road, <i>P.c.</i> very dense; site has been destroyed by construction of Duke Point Industrial Complex, Nanaimo, B.C.
14(2)	Jack's Point	open site along gravel road .3 km from A(1); site also destroyed by construction.
15(1), 15(2)	Nanoose Hill	sympatric site with <i>P.b.</i> being the most abundant; large S-facing slope with Garry oak and arbutus, both collections from semi-shaded sites beneath <i>Quercus garryana</i> . site is visible from Trans-Canada Hw. at Nanoose Bay, north of Nanaimo.
16	Little Qualicum Falls	plants were located on a small open ledge overlooking the lower falls; Little Qualicum Falls Prov. Park.

APPENDIX A. POPULATION LOCATIONS

Sample designation	Location	Location and comments
17	Nile Creek	plants collected approx. 50 m from beach in a grassy area of stabilized sand among scattered <i>Pseudotsuga menziesii</i> . Access to site is a gravel road off the Trans-Canada Hwy. along the north bank of Nile Creek, just south of Bowser, B.C.
18	Elk Falls Park	plants were collected on an exposed grassy ledge below the dam; Elk Falls Prov. Park, Campbell River, B.C..

APPENDIX B. ENZYME SYSTEMS WHICH WERE NOT ADEQUATELY RESOLVED WITH AVAILABLE TECHNIQUES

ENZYME	RUNNING BUFFER	STAIN REFERENCE	COMMENTS
1. Acid phosphatase APH E.C. 3.1.3.2	I-VI	Roose & Gottlieb (1976)	Adequate staining, but resolution was poor.
2. Alcohol dehydrogenase ADH E.C. 1.1.1.1	I,II,IV,V	Scandalios (1969) Gottlieb (1973b)	No Activity No Activity
3. Alkaline phosphatase ALPH E.C. 3.1.3.1	I,II	Scandalios (1969)	No Activity
4. Aspartate aminotransferase ATT (=GOT) E.C. 2.6.1.1	I-VII	Delorenzo & Ruddle (1970)	With buffer VI, and to a lesser degree I, resolution was sometimes very good but staining was unreliable. AAT appears to be a 2 or 3 locus system with the slowest locus showing dimeric inheritance.
5. Catalase CAT E.C. 1.11.1.6	I,II,IV,VI	Shaw & Prasad (1970) Siciliano & Shaw (1976)	Little or No Activity No Activity
6. Fructose-1, 6-diphosphatase FDP E.C. 3.1.3.11	I,II,IV-IX	Allendorf, et al. (1977)	Very light staining with buffers, II, VI & VII if 10 mg. NADP added to gel, however staining is unreliable.
7. Glutamate dehydrogenase GDH E.C. 1.4.1.3	I,II,IV,V	Roose & Gottlieb (1976) Allendorf, et al. (1977)	Staining unreliable, sometimes resolved a single monomorphic band with buffer I. There is some question as to whether this is GDH as a single band was sometimes resolved on other gels without the addition of substrate.

APPENDIX B. (Continued)

ENZYME	RUNNING BUFFER	STAIN REFERENCE	COMMENTS
8. Glucose-6-phosphate dehydrogenase G6P E.C. 1.1.1.49	I,II IV-IX	Roose & Gottlieb (1976) Allendorf, et al. (1977)	Staining very diffuse, was unable to resolve.
9. Peroxidase PER E.C. 1.11.1.7	I,II,IV,V,VI	Brown & Allard (1969) Gottlieb (1973a) Siciliano & Shaw (1976)	Had success with Gottlieb's stain and running buffers I & VI, however, staining was inconsistent so system was not used. PER appears to be a multilocus system with both anodal & cathodal variants.
10. Sorbitol dehydrogenase SDH E.C. 1.1.1.14	I,II V-IX	Allendorf, et al. (1977)	Single monomorphic band sometimes resolved with Systems I & II. However this band stains without the addition of substrate and is therefore of unknown function.
11. Succinate dehydrogenase SUCDH E.C. 1.3.99.1	I,II V-IX	Allendorf, et al. (1977)	Results identical to SDH.
12. Xanthine dehydrogenase XDH E.C. 1.2.1.37	I,II V-IX	Allendorf, et al. (1977)	Results identical to SDH & SUCDH.

RUNNING BUFFERS

- I Discontinuous Li-Borate/Tris-Citrate pH 8.0
- II Continuous Tris-Citrate pH 7.0
- III 0.3 M Borate pH 8.0
- IV Poulik
- V Histidine-Citrate
- VI Tris-Versene-Borate pH 8.0
- VII Tris-Maleate pH 7.4
- VIII Continuous Tris-Citrate I pH 6.3
- IX Continuous Tris-Citrate II pH 8.0

REFERENCE

- Scandalios (1969), Selander, et al. (1971)
- Siciliano & Shaw (1976)
- Shaw & Prasad (1970)
- Selander, et al. (1971)
- Gottlieb (1973a)
- Siciliano & Shaw (1976)
- Selander, et al. (1971)
- Selander, et al. (1971)
- Selander, et al. (1971)

APPENDIX C. GENE AND GENOTYPE FREQUENCIES WITHIN POPULATIONS

H = expected percentage of heterozygous loci/individual (see Tables 22 and 23)

$H_{\text{obs.}}$ = observed percentage of heterozygous loci/individual (see Tables 22 and 23)

n = number of individuals genotyped

(F) = number of families represented by genotyped individuals.

APPENDIX C.1. *Plectritis brachystemon* populations

Population #77-1

Sumas Mtn.

H=.0531

H_{obs.} =.0031

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	89	.02	130	(130)	89/89	2
	100	.53			100/100	66
	114	.45			114/114	56
	h	.516			100/114	6
	h _o	.046				
EST-2	100	1.00	19	(19)		
LAP-1	85	.05	19	(19)	85/85	1
	91	.84			91/91	16
	100	.11			100/100	2
	h	.280				
	h _o	.000				
MDH-1	100	1.00	19	(19)		
MDH-2	100	1.00	19	(19)		
PGI-1	100	1.00	131	(131)		
PGI-2	100	1.00	131	(131)		
PGI-3	100n	1.00	131	(131)		
PGM-1	106	1.00	112	(112)		
PGM-2	100	1.00	131	(131)		
PGM-3	115	1.00	131	(131)		
IDH-1	100	1.00	19	(19)		
ME-1	100	1.00	19	(19)		
6PG	100	1.00	19	(19)		
SOD	100	1.00	19	(19)		

Population 77-13

William Head

H=.0621

H_{obs.} = .0047

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	89	.80	70	(70)	89/89	55
	100	.20			100/100	13
	h	.320			89/100	2
	h _o	.029				
EST-2	100	1.00	30	(30)		
LAP-1	91	.06	16	(16)	91/91	1
	100	.94			100/100	15
	h	.113				
	h _o	.000				
MDH-1	100	1.00	16	(16)		
MDH-2	100	1.00	16	(16)		
PGI-1	100	1.00	73	(73)		
PGI-2	100	1.00	73	(73)		
PGI-3	100n	1.00	73	(73)		
PGM-1	100	.52	48	(48)	100/100	24
	106	.48			106/106	22
	h	.499			100/106	2
	h _o	.042				
PGM-2	100	1.00	73	(73)		
PGM-3	115	1.00	73	(73)		
IDH-1	100	1.00	30	(30)		
ME-1	100	1.00	30	(30)		
6PG-1	100	1.00	30	(30)		
SOD	100	1.00	30	(30)		

Population 77-9

Francis Park

H=.0309

H_{obs} = .0042

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	89	.03	43	(43)	89/89	1
	100	.97			100/100	41
	h	.058			89/100	1
	h _o	.023				
EST-2	100	1.00	14	(11)		
LAP-1	100	1.00	14	(11)		
MDH-1	100	1.00	14	(11)		
MDH-2	100	1.00	14	(11)		
PGI-1	100	1.00	56	(43)		
PGI-2	94	.01	56	(43)	100/100	55
	100	.99			94/100	1
	h	.020				
	h _o	.017				
PGI-3	33	.19	43	(43)	33/33	8
	100n	.81			100n/100n	35
	h	.308				
	h _o	.000				
PGM-1	100	.97	40	(40)	100/100	39
	106	.03			106/106	1
	h	.058				
	h _o	.000				
PGM-2	100	1.00	43	(43)		
PGM-3	115	.99	43	(43)	115/115	42
	182	.01			115/182	1
	h	.020				
	h _o	.023				
IDH-1	100	1.00	14	(11)		
6PG	100	1.00	14	(11)		
ME-1	100	1.00	14	(11)		
SOD	100	1.00	14	(11)		

Population 77-10

Prospect Lake

H=.0063

H_{obs}=.0000

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	1.00	8	(8)		
EST-2	100	1.00	21	(19)		
LAP-1	100	.95	22	(19)	100/100	21
	105	.05			105/105	1
	h	.095				
	h _o	.000				
MDH-1	113	1.00	22	(19)		
MDH-2	100	1.00	22	(19)		
PGI-1	100	1.00	22	(19)		
PGI-2	100	1.00	22	(19)		
PGI-3	100n	1.00	22	(19)		
PGM-1	106	1.00	22	(19)		
PGM-2	100	1.00	22	(19)		
PGM-3	115	1.00	22	(19)		
IDH-1	100	1.00	22	(19)		
ME-1	100	1.00	22	(19)		
6PG	100	1.00	22	(19)		
SOD	100	1.00	22	(19)		

Population 76-7(1)

Malahat Dr.

 $H = .0393$ $H_{obs} = .0000$

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	114	1.00	20	(20)		
EST-2	100	1.00	20	(20)		
LAP-1	95	.05	20	(20)	95/95	1
	100	.95			100/100	19
	h	.095				
	h_o	.000				
MDH-1	100	.55	20	(20)	100/100	11
	113	.45			113/113	9
	h	.495				
	h_o	.000				
MDH-2	100	1.00	20	(20)		
PGI-1	100	1.00	20	(20)		
PGI-2	100	1.00	20	(20)		
PGI-3	100n	1.00	20	(20)		
PGM-1	100	1.00	20	(20)		
PGM-2	100	1.00	20	(20)		
PGM-3	115	1.00	20	(20)		
IDH-1	100	1.00	20	(20)		
ME-1	100	1.00	20	(20)		
6PG	100	1.00	20	(20)		
SOD	100	1.00	20	(20)		

Population 77-7(3)

Malahat Dr.

H=.1405 H_{obs}=.0000

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	89	.56	25	(24)	89/89 100/100 114/114	14
	100	.12				3
	114	.32				8
	h	.570				
	h _o	.000				
EST-2	100	1.00	25	(24)		
LAP-1	95	.60	25	(24)	95/95 100/100	15
	100	.40				10
	h	.480				
	h _o	.000				
MDH-1	100	.50	24	(23)	100/100 113/113	12
	113	.50				12
	h	.500				
	h _o	.000				
MDH-2	100	1.00	24	(23)		
PGI-1	100	1.00	25	(24)		
PGI-2	81	.12	25	(24)	81/81 100/100	3
	100	.88				22
	h	.211				
	h _o	.000				
PGI-3	100n	1.00	25	(24)		
PGM-1	100	.84	25	(24)	100/100 106/106	21
	106	.16				4
	h	.269				
	h _o	.000				
PGM-2	100	.96	25	(24)	100/100 100n/100n	24
	100n	.04				1
	h	.077				
	h _o	.000				
PGM-3	115	1.00	25	(24)		
IDH-1	100	1.00	25	(24)		
ME-1	100	1.00	25	(24)		
6PG	100	1.00	25	(24)		
SOD	100	1.00	25	(24)		

Population 76-15(1)

Nanoose Hill

H=.1137 H_{obs}=.0074

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	89	.06	9	(9)	100/100	2
	100	.22			114/114	6
	114	.72			89/114	1
	h	.430				
	h _o	.111				
EST-2	100	1.00	13	(13)		
LAP-1	100	1.00	13	(13)		
MDH-1	100	.89	9	(9)	100/100	8
	113	.11			113/113	1
	h	.196				
	h _o	.000				
MDH-2	100	1.00	9	(9)		
PGI-1	100	1.00	13	(13)		
PGI-2	42	.15	13	(13)	42/42	2
	100	.85			100/100	11
	h	.255				
	h _o	.000				
PGI-3	33	.62	13	(13)	33/33	8
	100n	.38			100n/100n	5
	h	.471				
	h _o	.000				
PGM-1	100	.77	13	(13)	100/100	10
	106	.23			106/106	3
	h	.354				
	h _o	.000				
PGM-2	100	1.00	13	(13)		
PGM-3	115	1.00	13	(13)		
IDH-1	100	1.00	13	(13)		
ME-1	100	1.00	13	(13)		
6PG	100	1.00	13	(13)		
SOD	100	1.00	13	(13)		

Population 77-15(2)

Nanoose Hill

 $H = .0592$ $H_{obs} = .0055$

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	114	1.00	7	(2)		
EST-2	100	1.00	7	(2)		
LAP-1	100	.53	36	(32)	100/100	19
	105	.39			105/105	14
	112	.08			112/112	3
	h	.561				
	h _o	.000				
MDH-1	100	1.00	37	(32)		
MDH-2	100	1.00	37	(32)		
PGI-1	100	1.00	37	(32)		
PGI-2	42	.16	35	(30)	42/42	5
	100	.84			100/100	29
	h	.269			42/100	1
	h _o	.029				
PGI-3	100n	1.00	37	(32)		
PGM-1	100	1.00	37	(32)		
PGM-2	100	1.00	37	(32)		
PGM-3	115	.97	37	(32)	115/115	35
	182	.03			115/182	2
	h	.058				
	h _o	.054				
IDH-1	100	1.00	37	(32)		
ME-1	100	1.00	37	(32)		
6PG	100	1.00	37	(32)		
SOD	100	1.00	37	(32)		

Population 77-16

Little Qualicum Falls

 $H = .0136$ $H_{obs} = .0130$

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	Not Scored					
EST-2	100	1.00	33	(33)		
LAP-1	100	1.00	33	(33)		
MDH-1	113	1.00	33	(33)		
MDH-2	100	1.00	33	(33)		
PGI-1	100	1.00	33	(33)		
PGI-2	94	.05	33	(33)	100/100	30
	100	.95			94/100	3
	h	.095				
	h_o	.091				
PGI-3	100n	1.00	33	(33)		
PGM-1	100	1.00	33	(33)		
PGM-2	100	1.00	33	(33)		
PGM-3	115	.95	33	(33)	115/115	30
	182	.05			115/182	3
	h	.095				
	h_o	.091				
IDH-1	100	1.00	33	(33)		
ME-1	100	1.00	33	(33)		
6PG	100	1.00	33	(33)		
SOD	100	1.00	33	(33)		

Population 77-18

Elk Falls

H=.0699 H_{obs}=.0074

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	89	1.00	74	(41)		
EST-2	100	1.00	12	(6)		
LAP-1	85	.17	9	(6)	85/85	1
	100	.61			100/100	5
	112	.22			112/112	2
	h	.551			85/100	1
	h _o	.111				
MDH-1	113	1.00	9	(6)		
MDH-2	100	1.00	9	(6)		
PGI-1	100	1.00	74	(41)		
PGI-2	100	1.00	74	(41)		
PGI-3	33	.46	74	(41)	33/33	34
	100n	.54			100n/100n	40
	h	.497				
	h _o	.000				
PGM-1	100	1.00	74	(41)		
PGM-2	100	1.00	74	(41)		
PGM-3	182	1.00	74	(41)		
IDH-1	100	1.00	14	(6)		
ME-1	100	1.00	14	(6)		
6PG	100	1.00	14	(6)		
SOD	100	1.00	14	(6)		

APPENDIX C.2. *Plectritis congesta* populations

Population 76-2(1)

Anacortes

H=.1897 H_{obs}=.1592

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	1.00	12	(12)		
EST-2	74	.06	17	(17)	100/100	15
	100	.94			74/100	2
	h	.113				
	h _o	.118				
LAP-1	80	.04	13	(13)	85/85	1
	85	.27			91/91	1
	91	.23			100/100	1
	95	.04			105/105	2
	100	.15			80/105	1
	105	.23			85/91	3
	112	.04			85/95	1
	h	.790			85/100	1
	h _o	.615			91/100	1
					105/112	1
MDH-1	113	.03	17	(17)	125/125	2
	125	.32			139/139	7
	139	.65			113/139	1
	h	.474			125/139	7
	h _o	.471				
MDH-2	100	1.00	17	(17)		
PGM-1	100	.97	18	(18)	100/100	17
	106	.03			100/106	1
	h	.058				
	h _o	.059				
PGM-2	81	.18	17	(17)	81/81	1
	100	.79			100/100	11
	118	.03			81/100	4
	h	.343			100/118	1
	h _o	.294				
PGM-3	100	.47	17	(17)	100/100	5
	135	.53			135/135	6
	h	.498			100/135	6
	h _o	.353				
IDH-1	100	1.00	18	(18)		
ME-1	100	1.00	18	(18)		
6PG	100	1.00	18	(18)		
SOD	100	1.00	18	(18)		

Population 76-2(2)

Anacortes

H=.2113

H_{obs}=.1428

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	1.00	21	(20)		
EST-2	74	.07	21	(20)	100/100	18
	100	.93			74/100	3
	h	.130				
	h _o	.143				
LAP-1	80	.13	23	(22)	80/80	2
	85	.26			85/85	3
	91	.09			91/91	1
	95	.07			95/95	1
	100	.28			100/100	3
	105	.17			105/105	2
	h	.796			80/100	2
	h _o	.478			85/91	1
					85/100	3
					85/105	2
					91/100	1
					95/105	1
					100/105	1
MDH-1	113	.05	20	(19)	125/125	9
	125	.57			139/139	5
	139	.38			113/125	1
	h	.528			113/139	1
	h _o	.300			125/139	4
MDH-2	100	1.00	20	(19)		
PGM-1	100	.94	24	(23)	100/100	22
	106	.06			106/106	1
	h	.113			100/106	1
	h _o	.042				
PGM-2	81	.23	24	(23)	81/81	2
	100	.67			100/100	12
	118	.10			118/118	1
	h	.488			81/100	6
	h _o	.375			81/118	1
					100/118	2
PGM-3	77	.02	24	(23)	100/100	11
	100	.63			135/135	4
	135	.35			77/100	1
	h	.480			100/135	8
	h _o	.375				
IDH-1	100	1.00	24	(23)		
ME-1	100	1.00	24	(23)		
6PG	100	1.00	24	(23)		
SOD	100	1.00	24	(23)		

Population 76-3

John Dean Park H=.2454

 $H_{obs.} = .2046$

Locus	Allele	Frequency	n	(F)	Genotype	# Individuals
EST-1	89	.01	53	(53)	100/100	52
	100	.99			100/89	1
	h	.020				
	h _o	.009				
EST-2	74	.27	53	(53)	74/74	5
	100	.69			100/100	26
	126	.04			74/100	18
	h	.450			74/126	1
	h _o	.415			100/126	3
LAP-1	80	.04	51	(51)	91/91	9
	85	.03			95/95	20
	91	.23			100/100	2
	95	.57			80/95	4
	100	.13			85/91	1
	h	.603			95/95	2
	h _o	.392			91/95	4
					91/100	1
					95/100	8
MDH-1	113	.01	51	(51)	125/125	5
	125	.31			139/139	24
	139	.68			113/125	1
	h	.441			125/139	21
	h _o	.431				
MDH-2	100	1.00	51	(51)		
PGM-1	94	.01	53	(53)	100/100	14
	100	.49			106/106	7
	106	.38			94/106	1
	117	.12			100/106	18
	h	.601			100/117	6
	h _o	.604			106/117	7
PGM-2	81	.25	53	(53)	81/81	5
	100	.75			100/100	31
	h	.375			81/100	17
	h _o	.321				
PGM-3	100	.35	53	(53)	100/100	11
	135	.65			135/135	27
	h	.455			100/135	15
	h _o	.283				
IDH-1	100	1.00	53	(53)		
ME-1	100	1.00	53	(53)		
6PG	100	1.00	53	(53)		
SOD	100	1.00	53	(53)		

Population 76-5

Mill Hill Park

 $H = .2150$ $H_{obs} = .2228$

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	1.00	12	(3)		
EST-2	74	.81	13	(3)	74/74	9
	100	.19			100/100	1
	h	.308			74/100	3
	h _o	.231				
LAP-1	85	.12	12	(3)	91/91	1
	91	.21			95/95	2
	95	.21			100/100	2
	100	.46			85/100	3
	h	.686			91/100	3
	h _o	.583			95/100	1
MDH-1	125	.33	12	(3)	139/139	4
	139	.67			125/139	8
	h	.442				
	h _o	.667				
MDH-2	100	1.00	12	(3)		
PGM-1	100	.92	13	(3)	100/100	11
	106	.08			100/106	2
	h	.147				
	h _o	.154				
PGM-2	81	.54	12	(3)	81/81	3
	100	.46			100/100	2
	h	.497			81/100	7
	h _o	.683				
PGM-3	100	.50	11	(3)	100/100	3
	135	.50			135/135	3
	h	.500			100/135	5
	h _o	.455				
IDH-1	100	1.00	13	(3)		
ME-1	100	1.00	13	(3)		
6PG	100	1.00	13	(3)		
SOD	100	1.00	13	(3)		

Population 76-4

Thetis Lake Park

H=.2549 H_{obs}=.1778

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	1.00	15	(10)		
EST-2	74	.37	15	(10)	74/74	2
	100	.63			100/100	6
	h	.466			74/100	7
	h _o	.467				
LAP-1	80	.03	15	(10)	91/91	1
	85	.10			95/95	1
	91	.20			100/100	6
	95	.14			80/91	1
	100	.50			85/91	1
	105	.03			85/95	1
	h	.662			85/100	1
	h _o	.467			91/100	2
					95/105	1
MDH-1	113	.03	15	(10)	125/125	4
	125	.50			139/139	4
	139	.47			113/125	1
	h	.528			125/139	6
	h _o	.467				
MDH-2	100	1.00	15	(10)		
PGM-1	100	.63	15	(10)	100/100	8
	106	.37			106/106	4
	h	.466			100/106	3
	h _o	.200				
PGM-2	81	.20	15	(10)	81/81	2
	100	.80			100/100	11
	h	.320			81/100	2
	h _o	.133				
PGM-3	77	.16	15	(10)	77/77	1
	100	.47			100/100	4
	135	.37			135/135	4
	h	.617			77/100	3
	h _o	.400			100/135	3
IDH-1	100	1.00	15	(10)		
ME-1	100	1.00	15	(10)		
6PG	100	1.00	15	(10)		
SOD	100	1.00	15	(10)		

Population 77-9

Francis Park

 $H = .2575$ $H_{obs} = .1634$

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	89	.02	88	(29)	89/89	1
	100	.98			100/100	86
	h	.039			89/100	1
	h _o	.011				
EST-2	74	.32	41	(12)	74/74	6
	100	.68			100/100	21
	h	.435			74/100	14
	h _o	.341				
LAP-1	80	.03	30	(12)	85/85	1
	85	.07			91/91	10
	91	.45			100/100	11
	100	.45			80/85	1
	h	.589			80/91	1
	h _o	.267			85/91	1
					91/100	5
MDH-1	113	.22	30	(12)	113/113	6
	125	.51			125/125	10
	139	.27			139/139	3
	h	.619			113/125	1
	h _o	.367			125/139	10
MDH-2	100	1.00	30	(12)		
PGM-1	100	.63	66	(25)	100/100	34
	106	.23			106/106	6
	117	.14			117/117	7
	h	.531			100/106	15
	h _o	.288			106/117	4
PGM-2	81	.37	72	(25)	81/81	14
	100	.60			100/100	29
	118	.03			81/100	25
	h	.502			100/118	4
	h _o	.403				
PGM-3	100	.75	71	(25)	100/100	43
	135	.25			135/135	8
	h	.375			100/135	20
	h _o	.282				
IDH-1	100	1.00	44	(12)		
ME-1	100	1.00	44	(12)		
6PG	100	1.00	44	(12)		
SOD	100	1.00	44	(12)		

Population 77-10

Prospect Lake

H=.2704 H_{obs}=.2005

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	1.00	42	(13)		
EST-2	74	.49	50	(20)	74/74	15
	100	.51			100/100	16
	h	.500			74/100	19
	h _o	.380				
LAP-1	80	.02	48	(18)	85/85	4
	85	.16			91/91	7
	91	.18			95/95	8
	95	.29			100/100	11
	100	.30			80/91	2
	105	.05			85/95	4
	h	.765			85/100	3
	h _o	.375			91/105	1
					95/100	4
					95/105	4
MDH-1	125	.36	36	(11)	125/125	4
	139	.64			139/139	14
	h	.461			125/139	18
	h _o	.500				
MDH-2	100	1.00	36	(11)		
PGM-1	100	.62	55	(20)	100/100	26
	106	.35			106/106	13
	117	.03			100/106	13
	h	.492			100/117	3
	h _o	.291				
PGM-2	81	.51	50	(19)	81/81	12
	100	.46			100/100	10
	118	.03			81/100	25
	h	.527			81/118	2
	h _o	.560			100/118	1
PGM-3	100	.49	50	(19)	100/100	17
	135	.51			135/135	18
	h	.500			100/135	15
	h _o	.300				
IDH-1	100	1.00	50			
ME-1	100	1.00	50			
6PG	100	1.00	50			
SOD	100	1.00	50			

Population 77-11

Viaduct Ave.

 $H = .2601$ $H_{obs} = .2158$

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	1.00	39	(20)		
EST-2	74	.45	39	(20)	74/74	9
	100	.55			100/100	13
	h	.495			74/100	17
	h _o	.436				
LAP-1	80	.08	37	(18)	85/85	1
	85	.10			91/91	6
	91	.33			95/95	7
	95	.24			100/100	4
	100	.22			105/105	1
	105	.03			80/91	4
	h	.768			80/95	1
	h _o	.486			80/100	1
					85/91	3
					85/100	2
					91/95	2
					91/100	4
					95/100	1
MDH-1	125	.53	39	(20)	125/125	13
	139	.47			139/139	11
	h	.498			125/139	15
	h _o	.385				
MDH-2	100	1.00	39	(20)		
PGM-1	100	.72	39	(20)	100/100	20
	106	.28			106/106	3
	h	.403			100/106	16
	h _o	.410				
PGM-2	81	.35	39	(20)	81/81	5
	100	.60			100/100	14
	118	.05			81/100	16
	h	.515			81/118	1
	h _o	.513			100/118	3
PGM-3	100	.33	39	(20)	100/100	6
	135	.67			135/135	19
	h	.442			100/135	14
	h _o	.359				
IDH-1	100	1.00	39	(20)		
ME-1	100	1.00	39	(20)		
6PG	100	1.00	39	(20)		
SOD	100	1.00	39	(20)		

Population 76-7(2)

Malahat Dr.

H=.2592 H_{obs}=.1896

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	89	.03	57	(19)	100/100	45
	100	.89			114/114	1
	114	.08			89/100	4
	h	.201			100/114	7
	h _o	.193				
EST-2	74	.76	65	(19)	74/74	38
	100	.16			126/126	2
	126	.08			74/100	18
	h	.390			74/126	5
	h _o	.385			100/126	2
LAP-1	80	.24	65	(19)	80/80	11
	85	.13			85/85	4
	91	.02			95/95	12
	95	.38			100/100	7
	100	.24			80/95	6
	h	.723			80/100	3
	h _o	.477			85/95	6
					85/100	3
					91/95	2
MDH-1	113	.02	67	(19)	113/113	1
	125	.34			125/125	14
	139	.64			139/139	34
	h	.474			125/139	18
	h _o	.269				
MDH-2	87	.05	67	(19)	87/87	1
	100	.95			100/100	61
	h	.095			87/100	5
	h _o	.075				
PGM-1	100	.83	65	(19)	100/100	48
	106	.17			106/106	5
	h	.282			100/106	12
	h _o	.185				
PGM-2	81	.43	68	(19)	81/81	18
	100	.57			100/100	27
	h	.490			81/100	23
	h _o	.338				
PGM-3	100	.35	68	(19)	100/100	12
	135	.65			135/135	32
	h	.455			100/135	24
	h _o	.353				
IDH-1	100	1.00	68	(19)		
ME-1	100	1.00	68	(19)		
6PG	100	1.00	68	(19)		
SOD	100	1.00	68	(19)		

Population 76-8

Crofton Exit

H=.2138 H_{obs}=.1823

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	1.00	32	(17)		
EST-2	74	.05	32	(17)	100/100	24
	100	.86			126/126	1
	126	.09			74/100	3
	h	.250			100/126	4
	h _o	.219				
LAP-1	80	.27	32	(17)	80/80	2
	85	.05			85/85	1
	91	.11			91/91	2
	95	.16			95/95	3
	100	.33			100/100	5
	105	.08			105/105	2
	h	.770			80/91	2
	h _o	.531			80/95	2
					80/100	9
					85/95	1
					91/95	1
					95/100	1
					100/105	1
MDH-1	113	.05	32	(17)	125/125	2
	125	.18			139/139	21
	139	.77			113/125	1
	h	.372			113/139	2
	h _o	.281			125/139	6
MDH-2	100	1.00	32	(17)		
PGM-1	100	.25	32	(17)	100/100	2
	106	.73			106/106	18
	117	.02			100/106	11
	h	.404			100/117	1
	h _o	.375				
PGM-2	81	.19	32	(17)	81/81	1
	100	.81			100/100	21
	h	.309			81/100	10
	h _o	.313				
PGM-3	100	.36	32	(17)	100/100	4
	135	.64			135/135	13
	h	.461			100/135	15
	h _o	.469				
IDH-1	100	1.00	32	(17)		
ME-1	100	1.00	32	(17)		
6PG	100	1.00	32	(17)		
SOD	100	1.00	32	(17)		

Population 14(1)			Jack's Point		H = .1885	H _{obs.} = .1521
Locus	Allele	Frequency	n	(F)	Genotype	# Individuals
EST-1	100	1.00	30	(30)		
EST-2	74	.15	29	(29)	74/74	1
	100	.78			100/100	18
	126	.07			126/126	1
	h	.364			74/100	7
	h _o	.310			100/126	2
LAP-1	80	.12	30	(30)	80/80	2
	85	.20			85/85	2
	91	.07			91/91	1
	95	.26			95/95	6
	100	.25			100/100	3
	105	.10			105/105	2
	h	.801			80/100	3
	h _o	.467			85/91	1
					85/95	1
					85/100	4
					85/105	2
					91/95	1
					95/100	2
MDH-1	125	.16	29	(29)	125/125	1
	139	.84			139/139	21
	h	.269			125/139	7
	h _o	.241				
MDH-2	100	1.00	29	(29)		
PGM-1	100	.80	30	(30)	100/100	21
	106	.20			106/106	3
	h	.320			100/106	6
	h _o	.200				
PGM-2	81	.04	28	(28)	100/100	26
	100	.96			81/100	2
	h	.077				
	h _o	.036				
PGM-3	49	.02	28	(28)	135/135	12
	100	.28			49/100	1
	135	.70			100/135	15
	h	.431				
	h _o	.571				
IDH-1	100	1.00	30	(30)		
ME-1	100	1.00	30	(30)		
6PG	100	1.00	30	(30)		
SOD	100	1.00	30	(30)		

Population 14 (2)			Jack's Point		H = .1811	H _{obs.} = .1042
Locus	Allele	Frequency	n	(F)	Genotype	# Individuals
EST-1	100	1.00	27	(27)		
EST-2	74	.11	33	(33)	74/74	1
	100	.89			100/100	27
	h	.196			74/100	5
	h _o	.152				
LAP-1	80	.04	29	(29)	80/80	1
	85	.22			85/85	3
	91	.26			91/91	7
	95	.17			95/95	5
	100	.29			100/100	5
	105	.02			85/91	1
	h	.769			85/100	6
	h _o	.276			100/105	1
MDH-1	125	.35	30	(30)	125/125	5
	139	.65			139/139	14
	h	.455			125/139	11
	h _o	.367				
MDH-2	100	1.00	30	(30)		
PGM-1	100	.85	27	(27)	100/100	23
	106	.15			106/106	4
	h	.255				
	h _o	.000				
PGM-2	100	1.00	33	(33)		
PGM-3	100	.47	33	(33)	100/100	8
	135	.53			135/135	10
	h	.498			100/135	15
	h _o	.455				
IDH-1	100	1.00	33	(33)		
ME-1	100	1.00	33	(33)		
6PG	100	1.00	33	(33)		
SOD	100	1.00	33	(33)		

Population 76-15(1)

Nanoose Hill

H=.2342 H_{obs}=.1919

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	.99	47	(19)	100/100	46
	114	.01			100/114	1
	h	.020				
	h _o	.021				
EST-2	74	.81	42	(18)	74/74	27
	100	.13			100/100	1
	126	.06			74/100	9
	h	.323			74/126	5
	h _o	.333				
LAP-1	80	.17	48	(19)	80/80	1
	85	.01			91/91	18
	91	.51			95/95	10
	95	.29			80/91	8
	100	.02			80/95	6
	h	.626			85/91	1
	h _o	.396			91/95	2
					91/100	2
MDH-1	125	.29	39	(16)	125/125	4
	139	.71			139/139	20
	h	.412			125/139	15
	h _o	.385				
MDH-2	87	.01	39	(16)	100/100	38
	100	.99			87/100	1
	h	.020				
	h _o	.026				
PGM-1	100	.69	48	(19)	100/100	25
	106	.31			106/106	6
	h	.428			100/106	16
	h _o	.333				
PGM-2	81	.36	47	(19)	81/81	8
	100	.62			100/100	19
	118	.02			81/100	18
	h	.486			100/118	2
	h _o	.426				
PGM-3	100	.45	47	(19)	100/100	12
	135	.55			135/135	17
	h	.495			100/135	18
	h _o	.383				
IDH-1	100	1.00	48	(19)		
ME-1	100	1.00	48	(19)		
6PG	100	1.00	48	(19)		
SOD	100	1.00	48	(19)		

Population 77-15(2)

Nanoose Hill

H=.1868

H_{obs}=.1565

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	1.00	7	(3)		
EST-2	74	.83	6	(3)	74/74	4
	100	.17			74/100	2
	h	.282				
	h _o	.333				
LAP-1	91	.05	10	(8)	100/100	9
	100	.95			91/100	1
	h	.095				
	h _o	.100				
MDH-1	125	.43	14	(10)	125/125	2
	139	.57			139/139	4
	h	.490			125/139	8
	h _o	.571				
MDH-2	100	1.00	14	(10)		
PGM-1	100	.58	13	(9)	100/100	6
	106	.42			106/106	4
	h	.487			100/106	3
	h _o	.231				
PGM-2	81	.29	14	(10)	81/81	2
	100	.71			100/100	8
	h	.412			81/100	4
	h _o	.286				
PGM-3	100	.39	14	(10)	100/100	3
	135	.61			135/135	6
	h	.476			100/135	5
	h _o	.357				
IDH-1	100	1.00	14	(10)		
ME-1	100	1.00	14	(10)		
6PG	100	1.00	14	(10)		
SOD	100	1.00	14	(10)		

Population 76-17

Nile Creek

H=.2278

H_{obs} = .1979

Locus	Allele	Frequency	n	(F)	Genotype	#Individuals
EST-1	100	.99	36	(24)	100/100	35
	114	.01			100/114	1
	h	.020				
	h _o	.028				
EST-2	74	.04	35	(23)	100/100	31
	100	.94			74/100	3
	126	.02			100/126	1
	h	.114				
	h _o	.114				
LAP-1	80	.07	36	(24)	80/80	1
	85	.47			85/85	13
	91	.39			91/91	10
	95	.03			95/95	1
	100	.01			105/105	1
	105	.03			80/85	1
	h	.620			80/91	2
	h _o	.278			85/91	6
					85/100	1
MDH-1	113	.21	35	(23)	113/113	3
	125	.19			125/125	1
	139	.60			139/139	13
	h	.560			113/125	2
	h _o	.514			113/139	7
					125/139	9
MDH-2	100	1.00	35	(23)		
PGM-1	100	.57	34	(22)	100/100	12
	106	.43			106/106	7
	h	.490			100/106	15
	h _o	.441				
PGM-2	81	.46	34	(22)	81/81	7
	100	.51			100/100	8
	118	.03			81/100	17
	h	.527			100/118	2
	h _o	.559				
PGM-3	100	.28	34	(22)	100/100	2
	135	.72			135/135	17
	h	.403			100/135	15
	h _o	.441				
IDH-1	100	1.00	36	(24)		
ME-1	100	1.00	36	(24)		
6PG	100	1.00	36	(24)		
SOD	100	1.00	36	(24)		