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# Reproductive System and Pattern of Genetic Variation in Two *Limnanthes* Species

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**Summary.** Several populations of two species of the genus *Limnanthes (L. alba*, an outbreeder and *L. floccosa*, an inbreeder) were examined with respect to variability of fifteen quantitative characters, allozyme variation at 11 loci, and response to different pollination conditions and moisture stress. Nearly equal amounts of phenotypic variability were found in the two species. *L. alba* had higher within-family variability than *L. floccosa*, but this result was highly heterogeneous among characters. A study of between-and within-population variance estimates did not reject the null hypothesis that *L. alba* and *L. floccosa* are similar with regard to the partitioning and amount of variability for quantitative characters. However, allozyme variation at 11 loci in a large number of populations showed *L. alba* to be highly polymorphic in contrast to the virtual monomorphism within *L. floccosa* populations. The average number of alleles per locus in *L. alba* and *L. floccosa* was 1.97 and 1.02, respectively, and on an average, *L. alba* and *L. floccosa* populations had 63% and 3% loci with polymorphism, respectively. Three groups of allozyme allelic combinations emerged which correlated well with the taxonomic delineation of allogamous *L. alba*, three semi-autogamous *L. floccosa* forms and two autogamous *L. floccosa* forms.

All taxa showed a significant reduction in the seed output per plant due to moisture stress. *L. alba* suffered a further loss of fecundity under the paucity of pollinators, *L. floccosa* ssp. *floccosa* showed no significant effect from this factor, whereas *L. floccosa* ssp. *grandiflora* exhibited a curvilinear response which peaked at 'partial pollination' and decreased to a lower level at 'full pollination.'

The geographic distribution of the two species with regard to the temperature and rainfall distribution did not suggest *L. floccosa* to be living in drier marginal areas. Patterns of variation in flowering time showed *L. alba* to be less variable than *L. floccosa*. Overall, there seemed to be little direct support for the thesis that inbreeding species originated from outcrossing taxa in marginal environments as a direct adaptation to a shortened growing season of xeric environments and to the lack of pollinators. Alternative hypotheses suggest that autogamy in *L. floccosa* might have evolved as a reproductive isolating barrier acting through either cleistogamy or divergence in flowering times.

**Key words:** Evolution of inbreeding – Electrophoretic variation – Phenotypic plasticity – Variation patterns

## Introduction

The genus *Limnanthes* has drawn considerable attention as a potential new oil crop to serve as a substitute for the sperm whale oil in many industrial uses. Agronomic and genetic researches on its domestication and the development of genetic resources are underway in California, Oregon and Maryland. For experimental ecology and evolution, *Limnanthes* offers such advantages as the relative ease of seed collection and cultivation, feasibility of demographic work, a great diversity in morphology and distribution patterns among species and most important of all, the population structure features of highly isolated and well defined populations in vernal pools and streams. A vernal pool represents one distinct population that may have more than one effective neightborhood. Plant populations in vernal pools have been treated with the biogeographic theory of islands (Holland and Jain, in press) and their structures would allow the effects of sub-division, migration, selection and random drift to be sorted out in a study of genetic variation patterns of related species.

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Naturalists have long had an interest in the adaptive aspects of reproductive processes in plants. A particular focus of this interest has been on the comparative effects of cross- and self-fertilization on the genetic structure of populations and the biosystematic relationships. *Limnanthes alba* Bentham is an outcrossing species in which Mason (1952) described two varieties, namely, *L. a.* 'alba' and *L. a.* 'versicolor'. *L. floccosa* Howell is an inbreeding complex of five subspecies as described by Arroyo (1973b). All these taxa are self-compatible but represent at least three levels of outbreeding (Table 1). *L. alba* achieves allogamy primarily through protandry. Two of the subspecies of *L. floccosa* (viz. *L. f. floccosa, L. f. bellingeriana*) are classified as fully autogamous and produce cleistogamous flowers. The remaining three subspecies (*L. f. pumila, L. f. grandiflora, L. f. californica*) are labeled as semi-autogamous owing to their relatively more chasmogamous flowers and the presence of a small degree of protandry. The potential difference in the rates of outcrossing between the latter two groups has not been studied but may be reflected by a slightly higher percentage of polymorphic loci and higher average number of alleles per locus in the semi-autogamous populations assayed.

Arroyo (1973a, 1975) postulated with respect to the phylogenetic relationships that the *floccosa* complex was derived from an outcrossing ancestor (similar to the present-day *L. alba*) as a result of selection for a short-lived and self-pollinated type. 'Fluctuations in pollinator availability and seasonal variations in soils drying in marginal populations of *L. alba* indicate that autogamy in *L. floccosa* has arisen in relation to its prefertilization effect of securing seed set under circumstances in which insect-mediated cross-pollination is unreliable' (Arroyo 1973a). Darwin (1876) recognized that selection could act to alter mating systems in plants. Stebbins (1950) suggested that genetic uniformity and insured seed production, resulting from increased autogamy, would be advantageous in marginal environments. Cleistogamy in the two subspecies of *L. floccosa*, associated with smaller flowers, a smaller quantity of pollen, and seed production in the absence of pollinating insects might have evolved as an adaptation for economizing resources such as seed development time and reproductive effort. Kalin's above mentioned hypothesis of economy of life-cycle duration and plant size was proposed earlier for *Leavenworthia* by Lloyd (1965) and was more recently discussed in detail by Solbrig and Rollins (1977).

A recent review of literature on the evolution of inbreeding in plant populations (Jain 1976) showed the need for a critical evaluation of various alternative hypotheses with the help of genetic tools in the estimation of levels of outbreeding, extensive work on the mapping of

**Table 1.** Summary of descriptions of intraspecific taxa of *L. alba* and *L. floccosa* (After Arroyo 1973b and Mason 1952)

| Taxa | Mating system | Important characters | Geographical distribution |
|---|---|---|---|
| *L. alba* var 'alba' | outcrossing | Pubescent herbage and sepals, tuberculate nutlets, showy flowers | Central Valley, California |
| *L. alba* var 'versicolor' | outcrossing | Glabrous herbage and calyx, smooth to wrinkled nutlets, showy flowers | Sierra foothills and mountains, California |
| *L. floccosa* ssp. *floccosa* | autogamous | Glabrous herbage and calyx. Tuberculate nutlets, inconspicuous flowers | Shasta County, California Jackson County, Oregon |
| *L. floccosa* ssp. *bellingeriana* | autogamous | Glabrous herbage and calyx, tuberculate nutlets, inconspicuous flowers | Eastern margin of *L. floccosa* ssp. *floccosa* distribution in Jackson Co., Oregon |
| *L. floccosa* ssp. *californica* | semi-autogamous | Pubescent herbage and calyx, tuberculate nutlets, semi-inconspicuous flowers | Central Valley, limited to Butte County sites |
| *L. floccosa* ssp. *grandiflora* | semi-autogamous | Pubescent herbage and calyx, minor tuberculation on nutlets, semi-inconspicuous flowers | Medford plains, Jackson County, Oregon |
| *L. floccosa* ssp. *pumila* | semi-autogamous | Glabrous herbage and calyx, sharply tuberculated nutlets, semi-inconspicuous flowers | Table Rocks, Jackson County, Oregon |

species distributions and assays of genetic variation. Further, to test whether inbreeding favors the colonization of marginal habitats would require several experimental approaches. First, we must define criteria that are to be used to designate *L. floccosa* as a colonizer of marginal habitats. Can a series of artificial colonies be utilized to measure the relative colonizing success? Is a comparison of the genetic and phenotypic variability of an inbreeding species and its related outcrossing ancestor relevant to a discussion of the genetic consequences of inbreeding in evolution? Gottlieb (1977) elegantly reviewed the role of electrophoretic evidence in such comparisons. Finally, would the reproductive success of such related species under various stress environments test experimentally the Darwinian hypothesis on the role of inbreeding in assuring reproduction? This study explores the contemporary patterns of variability and adaptation of two *Limnanthes* species in relation to these questions.

## Materials and Methods

### *A. Quantitative Variation*

For the purpose of studying the relative variability in quantitative traits, individual plant families from four populations of *L. alba* and three populations of *L. floccosa* were grown in 1975. Table 2 lists the sites utilized and specifies the sample sizes involved. Seedlots were planted in 3.8 X 3.8 cm peat pots and placed on benches outdoors to allow the germination to occur. The soil mixture consisted of Yolo clay loam, sphagnum peat and sand in the proportions 2:1:1. Most seedlings had emerged three weeks after sowing. The plants were transplanted into 10.2 X 10.2 cm plastic pots containing the same soil mixture and raised to maturity. A series of 15 quantitative traits were measured on individual plants. A hierarchical analysis of variance was carried out for a completely randomized design.

### *B. Allozyme Variation*

Individual plants and bulk seed were collected from 8 *L. alba* populations and 18 *L. floccosa* populations (Fig. 1) These were assayed for allozyme variation using starch gel electrophoresis following the standard procedures described by Shaw and Prasad (1970). Genotypic frequencies for a total of 11 loci were scored for each population, based on sample sizes in the range of 15 to 40 plants. The sites utilized were chosen on the basis of available seed, with the objective of sampling each species as completely as possible with respect to its infra-specific taxonomic groupings and geographical distribution. Nutlets were germinated in petri dishes placed in a growth chamber at 10 C. Seedlings were analyzed approximately nine days after germination.

### *C. Moisture and Pollination Stress*

One population each of three different taxa (namely, *L. alba, L. floccosa* ssp. *grandiflora* and *L. floccosa* ssp. *floccosa*) representing

**Table 2.** Populations and sample sizes utilized in the study of quantitative variation

| Site code | Taxon | Site description | Number of | |
|---|---|---|---|---|
| | | | families | plants |
| 219 | *Limnanthes floccosa* ssp. *floccosa* | Field north of Tresham Lane, Jackson County, Oregon | 11 | 217 |
| 224 | *Limnanthes floccosa* ssp. *grandiflora* | Pasture west of Table Rock Rd., 0.2 mi, south of Rogue River; Jackson County, Oregon | 27 | 215 |
| 227 | *Limnanthes floccosa* ssp. *pumila* | Summit, Lower Table Rock; Jackson County, Oregon | 30 | 215 |
| 232 | *Limnanthes alba* | Streambed 2.2 mi. east of Hwy. 299/Interstate 5 intersection; Shasta County, California | 12 | 154 |
| 233 | *Limnanthes alba* var. 'versicolor' | Pasture 4.3 mi. west of Ingot on Hwy. 299; Shasta County, California | 29 | 305 |
| 237 | *Limnanthes alba* var. 'alba' | Pasture, 1.1 mi. north of junction of Hwy. 99 and Hwy. 162; Butte County, California | 18 | 299 |
| 238 | *Limnanthes alba* | Pasture on Excelsior Rd., 2.1 mi. south of Hwy. 16; Sacramento County, California | 19 | 264 |

allogamous, semi-autogamous and fully autogamous groups, respectively) was chosen for this study. The objective was to measure the effects of two environmental factors on fecundity. Seeds from each population were sown in plastic pots containing a Yolo clay loam, sphagnum peat and sand mixture in proportions of 2:1:1. Two or three seeds were sown in each pot and seedlings were thinned down to one plant per pot.

A split split-plot experimental design was used with four blocks, each block having three pollination treatments as main plots and each pollination plot further divided into two water regimes. Each water regime contained from five to ten pots of each of the three taxa.

The main plots were allowed three levels of pollinator availability, as follows: A small hive of honey bees (*Apis mellifera* L.) was placed at one end of the greenhouse. The main plots were covered by wire cages with removable sides. Access of bees to the main plots was controlled by opening or closing the open ends of cages with nylon window screening. The 'full' main plot treatment was accessible to pollinators during the entire course of the experiment; the 'partial' treatment was carried out by closing off the ends at an arbitrary midpoint in the flowering period simulating shortened pollinator life-cycle and flight time; for 'no pollinator' treatment, bees were excluded for the entire duration of the experiment. The 'high' water treatment consisted of placing the pots in trays filled with water whereas the 'low' water treatment involved watering of the pots once every three days during which time the soil dried considerably and loss of leaf turgor occurred intermittently. An analysis of variance was performed using unweighted plot and subplot means.

## Results

### 1 *Quantitative Variation*

To determine whether *L. alba* and *L. floccosa* differ in their patterns of variability, the following parameters are discussed: (a) phenotypic variation in natural populations; (b) population means; (c) population variances, $s^2_{pop}$, and (d) within-and between-family variance components estimated from data on individual plant families.

#### (a) Phenotypic Variability of Natural Populations

A total of 35 populations of *L. floccosa* and *L. alba* were sampled in order to compare the variability of phenotypic expression of two characters, number of flowers per plant and number of basal branches per plant. These characters were chosen because they were easily scored and are closely allied with reproductive effort. A nonparametric test, the Mann-Whitney U-test, for unpaired observations was used for the comparison of coefficients of variability (CV). The test failed to reject the null hypothesis that the relative ranks of the CVs of *L. alba* are different from those of *L. floccosa* for the number of flowers/plant. For number of basal branches/plant *L. alba* populations ranked higher than *L. floccosa* ($0.02 < P < 0.05$).

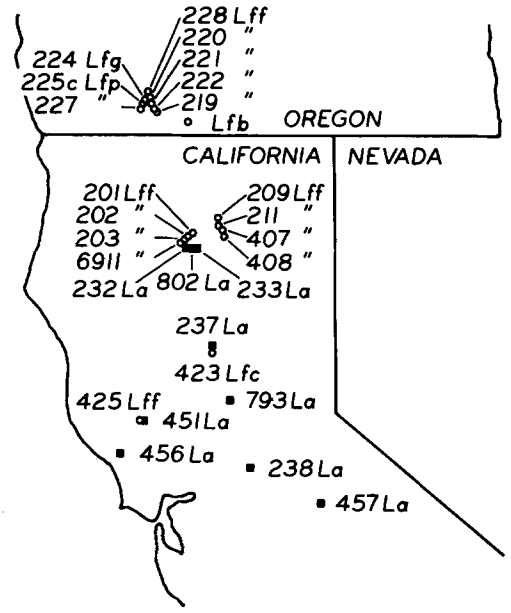


Fig. 1. Geographical locations of populations utilized in allozyme and quantitative variation studies o Lf, ■ La

#### (b) Population Means

Species and population means for the 15 characters measured on individual plant families are given in Table 3. The Student-Newman-Keuls (SNK) test for multiple comparisons between means was utilized to test the significance of differences among means. The results of the tests are denoted by lower case letters following each mean. Means that share the same letter are not significantly different while those that do not share the same letter are significantly different at the 5% level (Sokal and Rohlf 1969). Characters related to the size of floral parts (e.g. corolla diameter, sepal length, petal length, pistil length and stamen length) show population means which are often significantly different from one another. Both *L. alba* and *L. floccosa* show this interpopulation diversity. An important contrast between the two species can be noted for the characters pistil-stamen index and pistil-stamen ratio. The population means of *L. floccosa* are not significantly different from each other ($P > 0.05$) but those of *L. alba* are significantly different ($P < 0.05$).

Populations 232 and 233 of variety 'versicolor' and populations 237 and 238 representing variety 'alba' are separable by several characters, e.g. sepal length, pistil length, fertility. Other characters, however, do not support this varietal subdivision.

#### (c) Intrapopulation Variance

The estimates of $s^2_{pop}$ for all seven populations are given in Table 4. When populations of both species are

rank ordered, again *L. alba* shows a generally higher ranking for $s^2_{pop}$ than *L. floccosa*; when each population is given a ranking (1 to 7) for each character, the generally higher ranking of *L. alba* is statistically significant ($X^2_{(6)} = 14.35$, $0.05 > P > 0.025$).

(d) Between and Within Family Variance Components

To examine the variation patterns of *L. alba* and *L. floccosa* in genetic terms, an analysis of variance was performed on each population to obtain the estimates of

**Table 3.** Population means for all characters derived from populations used in quantitative variation study

| | *L. floccosa* | | | | *L. alba* | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chacacter | 219 | 224 | 227 | Species mean | 232 | 233 | 237 | 238 | Species mean |
| 1 DF | 112.3a | 122.1b | 115.4d | 116.6 | 120.2c | 123.5e | 123.6e | 125.4f | 123.5 |
| 2 CD | 4.9a | 8.8b | 9.0b | 8.0 | 13.6c | 14.4d | 19.4e | 21.4f | 17.3 |
| 3 SL | 6.6a | 9.6d | 8.1c | 8.3 | 7.0b | 7.1b | 8.3c | 9.2d | 8.0 |
| 4 PL | 7.5a | 9.2c | 8.5b | 8.5 | 10.8d | 10.6d | 13.0e | 14.1f | 12.1 |
| 5 PSL | 2.8a | 4.3d | 3.7c | 3.8 | 3.1b | 2.8a | 4.3d | 4.6d | 3.7 |
| 6 STL | 2.9a | 4.5c | 4.0b | 3.9 | 4.6cd | 4.7d | 7.0e | 7.8f | 6.1 |
| 7 PH | 12.7d | 7.4a | 9.4b | 9.9 | 12.0c | 14.6e | 15.4f | 18.0g | 15.4 |
| 8 BB | 6.5a | 5.4d | 6.6f | 6.2 | 4.9c | 5.1cd | 2.2b | 1.8a | 3.3 |
| 9 NF | 26.4d | 11.9a | 14.2b | 17.7 | 21.9c | 27.7e | 12.8ab | 11.8a | 18.2 |
| 10 FER | 1.2a | 3.1e | 2.9d | 2.4c | 1.8b | 1.9b | 2.6c | 3.2e | 2.5 |
| 11 FEC | 32.2a | 36.9abc | 44.14c | 36.7 | 40.3a | 53.5d | 33.5ab | 37.9bc | 41.5 |
| 12 PI | 0.90c | −0.41*a | 0.32b | 0.18 | 3.80e | 3.43d | 4.72f | 4.91f | 4.19 |
| 13 PSI | 0.12a | 0.17a | 0.25a | 0.19 | 1.53b | 1.83c | 2.71d | 3.24e | 2.38 |
| 14 PR | 0.89c | 1.05e | 0.96d | 0.98 | 0.65ab | 0.69b | 0.64a | 0.66ab | 0.66 |
| 15 PSR | 1.50a | 1.05a | 1.09a | 1.07 | 1.55c | 1.68de | 1.67d | 1.76e | 1.69 |

* Negative value indicates that sepal axis exceeds petal axis

DF = days to flowering from the date of planting, CD = corolla diameter (mm), SL = sepal length (mm), PL = petal length, PSL = pistil length (mm), STL = stamen length (mm), PH = petal height, BB = no. of basal branches, NF = no. of flowers, FER = seed set per flower (= fertility), FEC = fecundity (no. of seeds/plant), PI = pistil index, PSI = pistil-stamen index, PR = perianth ratio, and PSR = pistil-stamen ratio

**Table 4.** Intrapopulation variance ($s^2_{pop}$)

| | *L. floccosa* | | | | *L. alba* | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Character[a] | 219 | 224 | 227 | Species mean | 232 | 233 | 237 | 238 | Species mean |
| 1 DF | 13.3 | 25.2 | 51.9 | 30.1 | 9.5 | 11.8 | 6.0 | 15.5 | 10.7 |
| 2 CD | 2.1 | 4.1 | 4.6 | 3.6 | 5.1 | 4.9 | 8.5 | 6.7 | 6.3 |
| 3 SL | 0.29 | 1.0 | 0.53 | 0.61 | 0.84 | 1.0 | 1.6 | 2.5 | 1.5 |
| 4 PL | 0.66 | 0.81 | 0.56 | 0.68 | 1.1 | 1.8 | 2.4 | 3.3 | 2.15 |
| 5 PSL | 0.09 | 0.43 | 0.37 | 0.30 | 0.55 | 0.27 | 0.66 | 0.80 | 0.57 |
| 6 STL | 0.07 | 0.24 | 0.21 | 0.17 | 0.276 | 0.35 | 0.65 | 0.64 | 0.48 |
| 7 PH | 5.2 | 3.8 | 4.8 | 4.6 | 3.1 | 8.4 | 3.1 | 7.4 | 5.5 |
| 8 BB | 6.7 | 5,6 | 4.2 | 5,5 | 2.4 | 4.6 | 1.0 | 1.0 | 2.2 |
| 9 NF | 54 | 27 | 29 | 70 | 47 | 149 | 12 | 19 | 56.8 |
| 10 FER | 0.20 | 1.4 | 1.1 | 0.90 | 0.46 | 0.68 | 0.78 | 1.4 | 0.83 |
| 11 FEC | 338 | 396 | 460 | 398 | 388 | 1065 | 189 | 384 | 504 |
| 12 PI | 0.50 | 0.44 | 0.48 | 0.47 | 1.2 | 1.2 | 1.6 | 2.8 | 1.7 |
| 13 PSI | 0.06 | 0.23 | 0.30 | 0.20 | 0.40 | 0.34 | 0.74 | 0.87 | 0.59 |
| 14 PR | 0.028 | 0.005 | 0.006 | 0.013 | 0.007 | 0.007 | 0.007 | 0.014 | 0.009 |
| 15 PSR | 0.011 | 0.018 | 0.035 | 0.021 | 0.072 | 0.082 | 0.081 | 0.088 | 0.081 |

[a] Refer to Table 3 for description of character symbols

variation within families, $s_w^2$, and variation between families, $s_b^2$. It is possible to make twelve *alba* vs. *floccosa* pairwise comparisons of three populations of *floccosa* with four populations of *alba* for each character. No significant difference between *L. alba* and *L. floccosa* was noted for $s_b^2$, although different characters gave a notable heterogeneity in this pattern. For example, several characters like 'days to anthesis' and 'number of basal branches' deviated strongly from the equality of $s_b^2$ values of the two species. The chi square distribution based on the non-parametric sign test may be used to test whether $s_b^2/s_w^2$ (alba) = $s_b^2/s_w^2$ (floccosa). The $s_w^2$ estimates in *L. alba* were on an average greater than *L. floccosa* ($X^2_{(1)} = 3.14$, $P < 0.05$). Table 5 presents the F-ratios ($s_b^2/s_w^2$) for each character and population. Based on the fact that *L. alba* is an outbreeder and *L. floccosa* an inbreeder, one would expect in theory this ratio to be higher for *L. floccosa* than in *L. alba*, since inbreeding populations are assumed to be highly homozygous with $s_b^2$ rather low. A two-way ANOVA, performed on these F-values (Table 5) failed to show a significant difference between populations but emphasized the heterogeneity of values for different characters. These results point to the problem in pooling characters for such comparative surveys of variation. Further, they show that the prediction of smaller within-family variance for inbreeding populations was not realized.

Finally, a hierarchical ANOVA of all three components of variation (namely, within-family, between-family and between-population) showed that for nearly all quantitative characters, the amounts of variability among populations within species were higher than the mean species differences. In other words, a larger component of variability was found between populations than between species although *L. alba* and *L. floccosa* are easily distinguished by the use of several qualitative floral characteristics.

### 2 *Allozyme Variation*

The allozyme data presented in this section are based on six enzyme systems, namely, esterase (2 loci), phosphoglucomutase (2 loci), acid phosphatase (1 locus), leucine aminopeptidase (2 loci), and glutamate oxaloacetate transaminase (4 loci).

The genetics of enzyme loci was based on a Mendelian analysis of several segregating progenies in *L. alba* and inferred for highly monomorphic *L. floccosa* by assuming homology with the mobility classes in *L. alba*. A USDA Plant Introduction accession # 283721 (*L. floccosa* ssp. *pumila*) was used as a control, allowing migration distances to be standardized. Three parameters were estimated from the data on allelic frequencies in 8 *L. alba* populations and 18 *L. floccosa* populations: Jaccard's Similarity Index, number of alleles per locus, and the percentage of polymorphic loci per population.

Jaccard's Similarity Index, based on the proportion of alleles common between taxa in pairs, was used to categorize the similarity between groups of populations. A summary of results in terms of the frequency diagrams is shown in Fig. 2 for six sets of similarity indices. One can test if the allelic composition of a population of *L. alba* is more similar to another *L. alba* population or to a *L.*

**Table 5.** F-ratios (between SS/within SS) ANOVA for each population

| | *L. floccosa* | | | | *L. alba* | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Character[a] | 219 | 224 | 227 | Species mean | 232 | 233 | 237 | 238 | Species mean |
| 1 DF | 8.61 *** | 2.12 *** | 7.45 *** | 6.06 | 3.85 *** | 6.59 *** | 6.59 *** | 12.56 *** | 5.53 |
| 2 CD | 13.84 *** | 2.08 *** | 4.65 *** | 6.86 | 0.98 N.S. | 1.27 N.S. | 2.12 *** | 2.42 *** | 1.70 |
| 3 SL | 0.96 N.S. | 1.33 ± | 2.56 *** | 1.62 | 0.92 N.S. | 1.51 ** | 2.73 *** | 4.56 *** | 2.43 |
| 4 PL | 3.89 *** | 1.41 * | 0.97 N.S. | 2.09 | 1.97 ** | 1.83 *** | 2.87 *** | 1.41 ± | 2.02 |
| 5 PSL | 2.17 ** | 1.49 * | 2.55 *** | 2.24 | 1.88 ** | 1.10 N.S. | 0.91 N.S. | 2.55 *** | 1.61 |
| 6 STL | 2.56 *** | 1.16 ± | 1.08 N.S. | 1.60 | 2.28 *** | 0.58 N.S. | 1.55 * | 2.70 *** | 1.78 |
| 7 PH | 5.42 *** | 10.96 *** | 5.08 *** | 7.02 | 5.54 *** | 8.59 *** | 5.25 *** | 5.67 *** | 6.26 |
| 8 BB | 8.50 *** | 9.07 *** | 3.84 *** | 7.14 | 5.42 *** | 6.70 *** | 2.50 *** | 8.70 *** | 5.83 |
| 9 NF | 3.83 *** | 12.94 *** | 5.91 *** | 7.56 | 7.16 *** | 13.32 *** | 2.20 *** | 12.56 *** | 8.81 |
| 10 FER | 1.04 *** | 2.78 *** | 1.93 *** | 1.92 | 1.70 * | 1.73 *** | 2.25 *** | 2.67 *** | 2.09 |
| 11 FEC | 1.54 N.S. | 7.17 *** | 3.46 *** | 4.06 | 3.90 *** | 5.29 *** | 1.57 ± | 6.41 *** | 4.29 |
| 12 PI | 3.89 *** | 11.85 * | 2.70 *** | 2.81 | 1.11 N.S. | 3.28 *** | 2.76 *** | 2.92 *** | 3.36 |
| 13 PSI | 1.11 N.S. | 1.19 N.S. | 1.93 ** | 1.41 | 0.92 N.S. | 0.86 N.S. | 0.98 N.S. | 1.87 * | 1.16 |
| 14 PR | 1.55 N.S. | 1.83 * | 2.61 *** | 2.00 | 0.78 N.S. | 3.30 *** | 2.47 *** | 3.34 *** | 2.47 |
| 15 PSR | 0.97 N.S. | 1.12 N.S. | 1.93 * | 1.34 | 0.75 N.S. | 0.93 N.S. | 0.92 N.S. | 2.40 *** | 1.25 |

[a] Refer to Table 3 for description of character trait symbols

± $0.10 > P > 0.05$, * $0.05 > P > 0.01$, ** $0.01 > P$ 0.001, *** $P > 0.001$, N.S. = not significant

*floccosa* population. For example, histograms A, B and C show the distributions of similarity indices from the following intergroup comparisons: A, allogamous populations versus autogamous populations; B, allogamous populations versus semi-autogamous populations; and C, semi-autogamous populations versus autogamous populations. Histograms in Fig. 2D give the distribution of similarity indices for *L. alba*, of *floccosa* ssp. *floccosa* and *floccosa* ssp. *bellingeriana* populations in Fig. 2E, and of similarity indices for *floccosa* ssp. *pumila*, ssp. *grandiflora* and ssp. *californica* in Fig. 2F.

The means and standard errors of each of these distributions are as follows: A, 0.235 ± 0.005; B, 0.207 ± 0.010; C, 0.460 ± 0.010; D, 0.605 ± 0.017; E, 0.716 ± 0.013; and F, 0.789 ± 0.061. Thus, larger similarity within rather than among groups (viz. D, E, F vs. A, B, C) supports the scheme of genetic differentiation of these taxa into three groups, earlier derived by Mason (1952) and Arroyo (1973b), based on corolla morphology and breeding system. However, note that in certain cases two populations of *L. floccosa* might be less similar genetically than the species pair *L. alba* and *L. floccosa* as a whole.

The next question is: how do *L. alba* and *L. floccosa* compare in two parameters of genetic variation, namely, the average number of alleles per locus and percentage loci polymorphic per population. Table 6 lists the estimates of

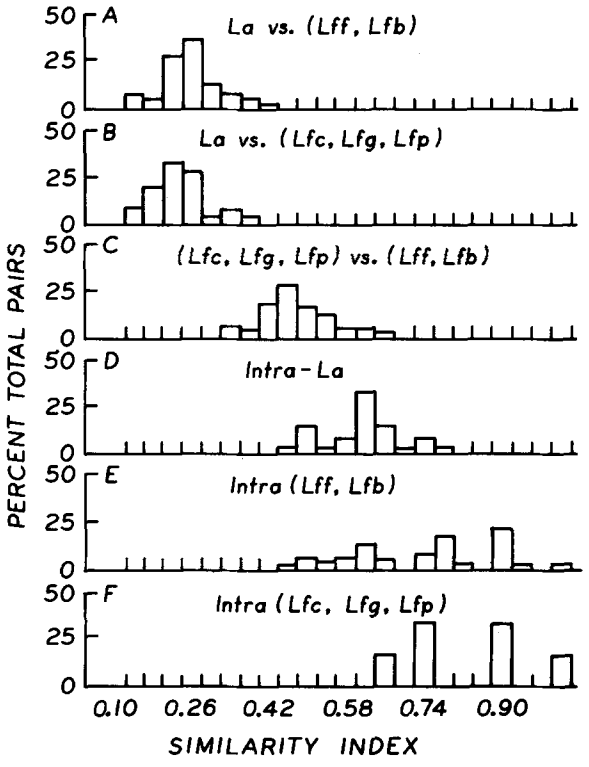


Fig. 2. Frequency distributions of Jaccard's index for various groupings of taxa

Table 6. Two parameters of allozyme diversity over all populations

| *L. alba* | Average number of alleles/locus | Percentage loci polymorphic |
|---|---|---|
| 793 | 2.07 | 79 |
| 802 | 1.57 | 43 |
| 233 | 2.14 | 79 |
| 238 | 1.86 | 57 |
| 237 | 1.86 | 57 |
| 457 | 1.71 | 43 |
| 456 | 2.50 | 79 |
| 451 | 2.08 | 67 |
| Species mean | 1.97 | 63 |
| *L. floccosa* | | |
| 423 | 1.29 | 21 |
| 224 | 1.00 | 0 |
| 225c | 1.00 | 0 |
| 227 | 1.14 | 14 |
| 6911 | 1.07 | 7 |
| 407 | 1.07 | 7 |
| 208 | 1.07 | 7 |
| 209 | 1.00 | 0 |
| 201 | 1.00 | 0 |
| 202 | 1.00 | 0 |
| 211 | 1.00 | 0 |
| 203 | 1.00 | 0 |
| 218 | 1.00 | 0 |
| 220 | 1.00 | 0 |
| 221 | 1.00 | 0 |
| 222 | 1.00 | 0 |
| Lfb | 1.00 | 0 |
| 425 | 1.00 | 0 |
| Species mean | 1.02 | 3 |

Table 7. Analysis of variances for split split plot design of moisture and pollination stress experiment

| Source | df | Fecundity (seeds/plant) MS | F |
|---|---|---|---|
| Block (B) | 3 | 0.2534 | |
| Pollination (P) | 2 | 4.5577 | 3.10 N.S. |
| BxP (error a) | 6 | 1.4711 | |
| Water (W) | 1 | 4.0705 | 12.51 ** |
| WxP | 2 | 0.3386 | |
| BxW + BxPxW (error b) | 9 | 0.3254 | |
| Taxa (T) | 2 | 5.1801 | 10.95 *** |
| PxT | 4 | 2.1636 | 4.58 ** |
| WxT | 2 | 0.3575 | |
| PxWxT | 4 | 0.1057 | |
| BxT + BxPxT + BxWxT + BxPxWxT (error c) | 36 | 0.4729 | |

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

these parameters. It is evident that *L. alba* has a significantly greater diversity of alleles and a higher proportion of polymorphic loci than *L. floccosa* which is almost totally monomorphic. This result is strikingly in disagreement with the observations and conclusions of Arroyo (1975) about the high levels of electrophoretic variation in *L. floccosa*. Furthermore, the two groups of 'subspecies' of *L. floccosa* (Arroyo 1973b) are fixed for all different alleles for the loci for glutamate oxalvacetate transaminase-I, acid phosphatase-I, and acid phosphatase-II.

### 3 *Moisture and Pollination Stress Experiment*

Individual plants were scored for fecundity (number of seeds per plant) in order to measure the effects of stress in moisture availability and visitation by pollinating insects. Here, one is testing whether the differences in phenology associated with the different mating systems represented by *L. alba* and two sets of *L. floccosa* subspecies truly result in different abilities to produce seed under such stress environments. Results of an analysis of variance for fecundity are given in Table 7. The analysis was performed on the fecundity data expressed as the proportion of seed output under 'full' pollination and 'high' watering level.

The pollination treatment gave no significant differences over the whole experiment ($P > 0.05$) but the water levels did make a significant contribution to the variance, as did, also the variation due to taxa. The pollination-level by taxon interaction is significant. Specifically, there was a significantly lower fecundity of *L. alba* under the 'no pollination' regime. *L. floccosa* ssp. *floccosa* showed no response to the variation in pollinator availability whereas the response of *L. floccosa* ssp. *grandiflora* was curvilinear with the highest seed output under 'medium' pollination. A reduction at the 'full' level of pollination might have resulted from interovular competition for photosynthate but whether this is related to the partial autogamy is not clear from these data.

### 4 *Phenological Consideration*

Arroyo (1973a, b; 1975) had postulated the derivation of *L. floccosa* from an ancestral *L. alba* in relation to the role of inbreeding in xeric and marginal environments.

Certain changes in phenology associated with autogamy might have been selected for in northeastern California and Southern Oregon under a more variable climatic regime. Specifically, autogamous types would flower earlier and finish the reproductive phase (i.e., anthesis and seed maturation) within a shorter period of time. Figure 3 shows the relative distribution of flowering times scored on families sampled from a series of populations. The position of the vertical lines on the horizontal axis denotes the mean flowering time of that progeny and the vertical dimension of lines represent the standard deviations of respective mean values. Not all the subspecies of *L. floccosa* are earlier than the populations of *L. alba*; moreover, the standard deviation of flowering time within family is not smaller in *L. floccosa* than *L. alba*.

Field observations were made in a number of localities where *L. alba* and *L. floccosa* ssp. *floccosa* existed in neighboring sympatry. In April and May of 1975 it was noted that ssp. *floccosa* populations which cohabited sites with *L. alba* were approximately a month earlier in flow-

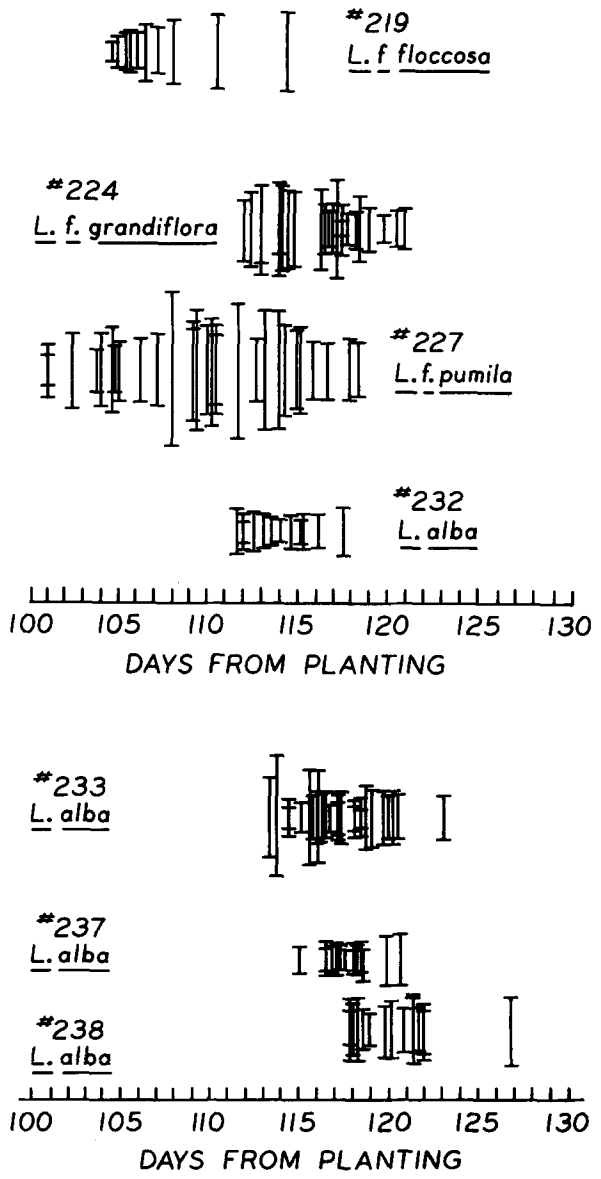


Fig. 3. Mean and standard deniation of progenies for the character days to anthesis

ering time. In contrast the ssp. *floccosa* populations not sharing a habitat with *L. alba* flowered nearly simultaneously with *L. alba* populations of the same geographical region. During a May 1974 collection trip in Oregon, it was noted that individuals of ssp. *floccosa* growing intermixed with the ssp. *grandiflora* population were actually later in flowering time.

Another prediction from Arroyo's hypothesis is that the present day distribution of *L. alba* and *L. floccosa* sites might reflect an increased colonizing ability of the latter in marginal areas. Burney, California is geographically an outpost for *L. floccosa* and has an intermediate rainfall and low May temperatures. These *floccosa* sites near Burney are at a relatively high elevation (over 900 m). Moreover, the microgeographic patterns of sympatry between *L. alba* and *L. floccosa* did not often show that *L. floccosa* grows in more xeric patches. At several locations, e.g. populations # 231 and # 451, *L. floccosa* was in fact observed to be growing in a closer proximity to a pool or stream-bed than much of the *L. alba* stand. These observations do not support the premise that *L. floccosa* is more successful in colonizing more xeric habitats than *L. alba*.

## Discussion

The results of this study suggest that the comparative patterns of variability in *L. alba* and *L. floccosa* are complex. Although an overall pattern of greater genetic variability in *L. alba* was observed, there are some important exceptions for characters, such as the number of days to anthesis, number of basal branches, number of flowers per plant, and fecundity. Hillel et al. (1973) discussed the theoretical circumstances which would allow the population variance, $s^2_{pop}$, and within-family variance, $s^2_w$, of a selfing population to exceed that of an outcrossing population. Considering one-locus model, population variance of an inbreeder may exceed that of the outcrosser due to strong selection favoring heterozygotes, or if inbreeding somehow lowers homeostasis. Brown (in press) discussed many theoretical aspects of the 'paradoxical observation' that many outbreeders have lower levels of heterozygosity than expected from random mating while inbreeding populations may often carry an excess of heterozygotes. Of course, one sees clearly the importance of better quantitative estimates of outbreeding rates and of more integrated studies on variation of different characters. Higher phenotypic plasticity seemed to characterize *L. floccosa* variability which in the absence of genetic polymorphism may be an alternative adaptive strategy. The notion of genetic strategies, first discussed by Levins (1967), was further explored by Jain (1978). Inbreeders, including both colonizing and noncolonizers, may often rely on plasticity. Results of quantitative analyses of variation showed neither inter-family nor inter-population differences to be more pronounced in the inbreeder *L. floccosa* than the outbreeder *L. alba*. However, the results of allozyme assays of variation strongly suggest a difference in their genetic structures. Earlier, Arroyo (1973b) reported allozyme data at several acid phosphatase and peroxidase loci which suggested very high levels of heterozygosity in *L. floccosa*. In fact, she had concluded that the two species have a similar population structure, and extrapolating from electrophoretic data, similar allelic composition of gene pools. In contrast, a dissimilarity of gene pools (Fig. 2, histograms A and B) strongly suggests genetic divergence between the two mating system groups.

It is evident that quantitative and allozyme variants gave different variability patterns in the two species. It is especially interesting to note that flowering time, fertility and fecundity are characters in which *L. floccosa* showed larger values of $s^2_{pop}$ and $s^2_b$ than *L. alba*. In contrast *L. alba* showed larger values of these parameters for corolla diameter, sepal axis, petal axis, pistil length and stamen length. One interpretation of larger interpopulation variance for flowering time in autogamous *L. floccosa* might be that it need not be tightly aligned with the emergence and flight period of pollinators and could therefore accumulate more genetic variability than *L. alba*. Thus, the argument of selection for autogamy as a means of achieving earlier flowering with shorter duration becomes less tenable. Many oligolectic native solitary bees are known to show hatching times which coincide remarkably well with the flowering time of the species of host plant they visit (Thorp 1969). The oligolectic pollinators of *L. alba* have been reported by Kalin (1971). Such a co-evolved system leaves little opportunity for deviation of an entomophilous species such as *L. alba* except in conjunction with an adaptation insuring fertility (e.g. autogamy). Moreover, the differences noted between *L. alba* and the two subspecies of *L. floccosa* in the moisture and pollination stress study, with regard to fecundity under different pollination intensities, point out the vulnerability of *L. alba* to the vagaries of pollinator availability. Flowering time may be a highly regulated feature of the phenology of this species.

In order to understand the relative roles of various factors of evolution, estimates of certain genetic parameters would be essential. Foremost among these would be the outcrossing rates and selection coefficients for selected morphological and allozyme loci. In addition we need estimates of certain demographic parameters (e.g. total population size, effective population size, density and seed carry-over in the soil), and life-history characteristics (e.g. rates of development, flowering times and reproductive versus vegetative allocation of energy). In this regard, the definition of habitat tolerance in terms of in-

undation and saturation of the soil during the rainy season, inter- and intraspecific competition and the limitations imposed by the physical process of salt accumulation in the habitat are all aspects that may lead to a more comprehensive understanding of variation in these species. The principal thought here is that predictions of genetic variability must incorporate many factors ranging from description of various aspects of population ecology in the field to certain specific parameters of genetic loci, measured with suitable experimental tools and population genetic models.

To recapitulate our observations on the breeding systems, as noted above, there exist several levels of autogamy within *Limnanthes* depending on such factors as protandry, flower size, availability of pollinators and degree of cleisotogamy. Arroyo's (1975) observations on the similar genetic structure of *alba* and *floccosa* are not supported by our data. Likewise, Arroyo's (1975) observations and hypotheses as to the role of xericness of environment is not fully supported by our own field studies. For example, the microhabitat separation of *L. alba* and *L. floccosa* in populations where they are sympatric and neighboring, or, of *L. floccosa* var. '*bellingeriana*' and *L. floccosa* var. '*floccosa*' did not appear to be correlated with microgeographic moisture clines. *L. floccosa* var. '*floccosa*' distribution is northerly but not necessarily peripheral geographically. In relation to marginal stress environments we tested the role of pollinator availability and moisture stress on fecundity and overall reproductive rates. Inbreeding in *L. floccosa* is not correlated with an increased reproductive rate, dispersal, etc. that are commonly discussed as the features of colonizing species. The often suggested role of inbreeding in the colonization of new habitats is tenuous in this case since many outbreeding populations are known throughout the range of distribution of the genus. With genetic and demographic data in natural and artificially founded colonies, we hope to test various alternative hypotheses on the origin of autogamy and on its adaptive significance (Jain 1976, for a review).

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