

Breeding systems and population structure in *Limnanthes*

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Summary. The breeding systems of seven *Limnanthes* (*Limnathaceae*) populations, including one “inbreeding” and three “outbreeding” taxa, were quantified using a multilocus outcrossing rate estimator (t_m) and autofertility estimates. Along with the assays of heterozygosity levels, these data were used to separate components of “effective” outcrossing in terms of Wright’s equilibrium inbreeding coefficient (F_e) and adult (F_A) and zygotic (F_Z) fixation indices. The patchy distribution of alleles as a potential source of “substructure inbreeding” was tested from the allelic frequencies mapped along a linear transect. Evidence for consanguineous matings in restricted neighborhoods and for selection at two different life cycle stages, and the efficiency of the protandrous breeding system were noted and discussed. Multilocus estimates of outcrossing are useful for their greater precision and unbiased nature while single locus estimates can help in detecting the effects of selection and population substructure. The data generally support the “heterozygosity paradox” noted by Brown (1979) but further suggest that the paradox may often result from a lack of precision of outcrossing estimates and from overlooking the stages of the life cycle being sampled.

Key words: Breeding system – Population subdivision – Multilocus estimator – Inbreeding – Selection

Introduction

Breeding systems have predictable and large effects on the amount and distribution of genetic variability in

populations (Jain 1975; Brown 1979) and the genome organization within individuals (Lerner 1954; Allard 1975; Hedrick et al. 1978). Hence, quantitative information on the levels and variability in outcrossing is of critical importance.

Numerous codominant genetic markers detected by protein electrophoresis coupled with statistical models for outcrossing rate estimation now offer tools for this quantification (Clegg 1980). Additionally, the earlier single locus outcrossing rate estimators have evolved to much more consistent, unbiased and efficient multilocus estimators that also estimate the maternal genotype arrays and the pollen pool allele frequencies (Shaw et al. 1981b; Ritland and Jain 1981). With these data, the “effective” (Allard and Workman 1963) or “apparent” (Ellstrand et al. 1978) outcrossing rate which measures the combined effects of self-fertilization, inbreeding from consanguineous matings, Wahlund effects and selection against heterozygotes, can now be partitioned into these different components (Brown 1979; Shaw and Allard 1981a; Ritland 1983). This allows a more critical analysis of true outcrossing rates both for their genetic basis and their interaction with the population genetic factors of population size, density and substructure, seed and pollen dispersal, and the stages and intensity of heterotic selection (Fig. 1).

The genus *Limnanthes* (*Limnathaceae*) contains species with a range of sexual breeding system, from cleistogamy to apparently outcrossed chasmogamy (Mason 1952; Ornduff and Crovello 1968; McNeill and Jain 1983; Kesseli and Jain 1984b). All species are hermaphroditic and self-compatible, but protandry is a developmental mechanism which presumably enhances outcrossing in many taxa. Allozyme variation of open pollinated single plant progeny arrays (families) and bulks, in seven collections representing four taxa, along with autofertility studies, provided data for partitioning the “effective” outcrossing rates. Fixation indices (Wright 1965) for adults (F_A) and zygotes (F_Z) and inbreeding coefficients (F_e) calculated from multilocus outcrossing rates (Ritland and Jain 1981) measure

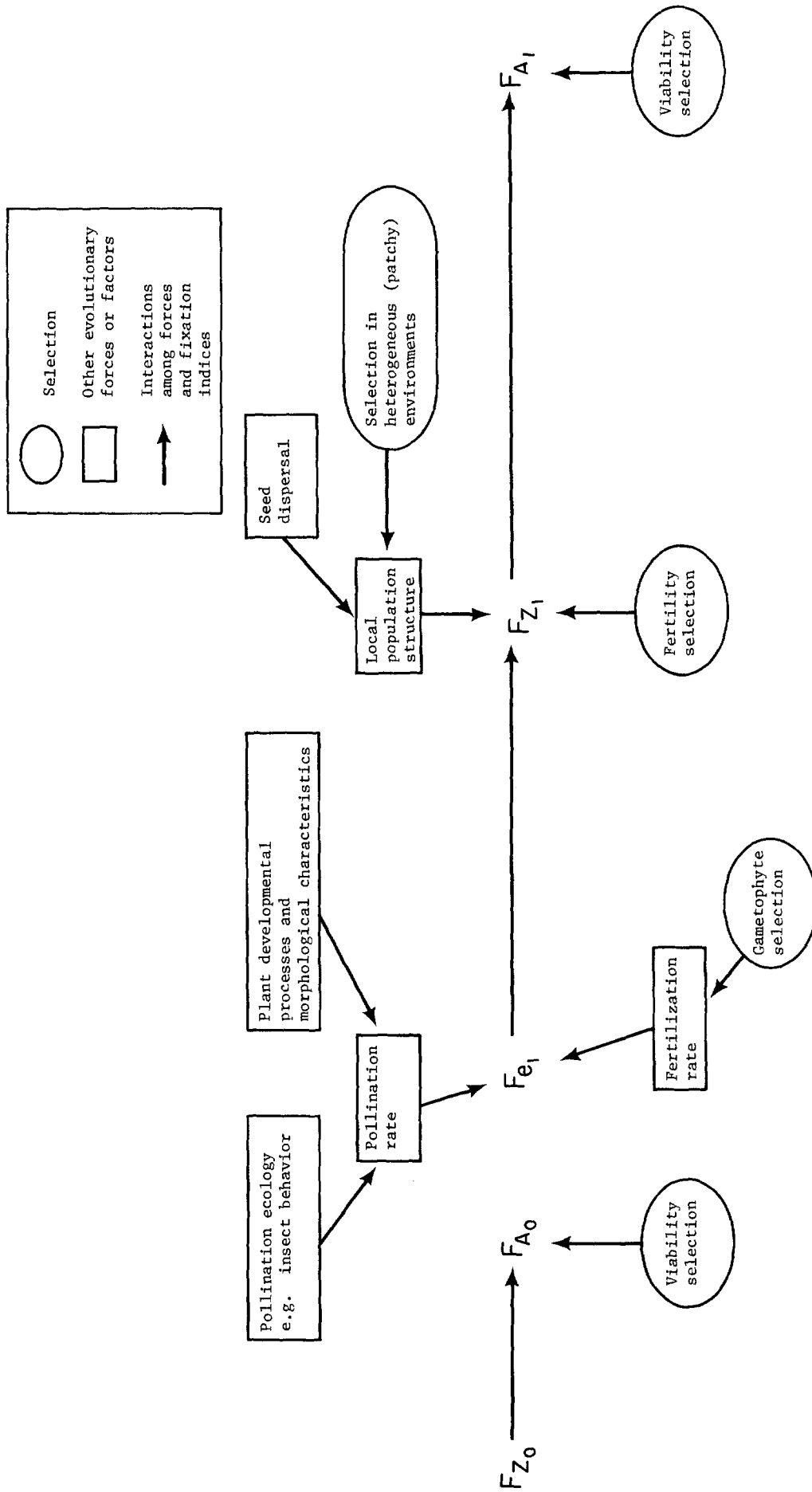


Fig. 1. A life cycle diagram indicating components of selection and other factors which affect fixation indices in a population. Fz is the fixation index of the zygotes, F_A is the fixation index of the adults and F_e is the expected inbreeding coefficient predicted from the multifocus estimator

changes in the allozyme heterozygosity in populations at two different life-cycle stages.

Specifically, data on autofertility estimate the degree of autogamy (self-pollination within a flower) whereas any disparity of autogamy (autofertility studies) and self-pollination rates determined from reliable multilocus outcrossing estimator would show insect-mediated geitonogamy. These unbiased multilocus outcrossing rate estimators, contrasted to the heterogeneous single locus estimators could show differential spatial patchiness of alleles which would bias downward the single locus outcrossing rate of that locus. Heterozygote deficiencies at zygote stages (high F_Z) relative to that predicted from the breeding system (F_e) would show inbreeding from consanguineous matings to be prevalent in specific populations and this may be a function of seed flow, past bottlenecks or patch dynamics. Heterozygote excess at zygote stages (low F_Z relative to F_e) or adult stages (low F_A relative to F_Z) would indicate that differential reproduction and heterosis during the seedling to adult stages, respectively, were possible selective forces. (Fig. 1).

Materials and methods

Seven population samples comprised of four *Limnanthes* taxa from different habitats were used in this study. Three of these taxa [*L. douglasii* var. 'rosea' (UCL accession nos. 511, 528, and 529 scored in years 1978 and 1980), *L. douglasii* var. 'nivea' (UCL 435) and *L. d.* var. 'douglasii' (UCL 438)] are considered outbreeders on the basis of floral morphology (Mason 1952; Ornduff and Crovello 1968; Kesseli and Jain 1984a). The other taxon *L. bakeri* (UCL 144) is considered a predominant selfer.

In order to estimate seed set in the absence of insect pollinators 10–20 plants in three populations (*L. d.* var. 'rosea', 511 and 529 and *L. bakeri*, 144) were grown on outdoor benches but under separate insect proof cages. The plants were spaced out but no attempt was made to prevent flower contact within a plant. Additionally, in the same three populations 20–30 randomly chosen plants from nature were scored for the average number of nutlets per flower. The first five flowers were scored on each plant.

Twenty to fifty open pollinated single plant progeny arrays (families) and random bulks were collected from each of the seven populations. An average of 10–14 seeds per family (except 80–529 with only five seeds per family) and a minimum of 44 bulk seeds per population were assayed for allozyme marker loci using electrophoretic techniques (McNeill and Jain 1983; Kesseli and Jain 1984a). The examined isozymes were: phosphoglucosmutase (PGM), glutamate oxalacetic transaminase (GOT), phosphoglucosomerase (PGI), malate dehydrogenase (MDH), shikimate dehydrogenase (SKD), peroxidase (PRX), acid phosphatase (ACP). A multilocus outcrossing rate estimator, t_m , (Ritland and Jain 1981) was used to predict the maternal genotypic and pollen pool allele frequencies as well as measure the outcrossing rates in the families with data from four to 11 polymorphic enzyme

loci (19 loci were scored but many had low levels of variability which were insufficient for calculating outcrossing rates cf. Kesseli and Jain 1984a). Single locus outcrossing estimates (t_i) were calculated and heterogeneity of the single locus estimates was tested by chi-square.

A systematic sampling of families every 10 cm along a transect in one population (529–78) allowed for a test of the predicted effects of spatial heterogeneity on single locus estimates. Presence or absence of the rare alleles in the progeny of individuals was scored (the common allele for each locus was generally present in all samples). At three loci, *Mdh-2*, *Skd-2* and *Prx-1*, in which two alleles are common, the allele frequencies of the rarer allele were subdivided into classes of 0, 0.001–0.25, 0.251–0.50, > 0.50. A run test (Pielou 1974) was used to detect spatial patchiness of allelic distribution for the 11 loci. Any spatial patchiness for a particular locus is expected to bias its single locus outcrossing rate estimate downward. Thus, with these data, we tested the hypothesis that heterogeneity of single locus outcrossing rate estimates is caused by variation in the levels of patchiness of different loci.

Wright's (1965) fixation index [$F = 1 - H_{obs}/(2pq)$], where H_{obs} is the observed frequency of heterozygotes and p and q are allele frequencies, was calculated at each polymorphic locus for the bulk seed (zygote) collections (F_Z) and the adult population predicted from the family arrays (F_A). The values of F for each locus in a population were weighted by the inverse of their variance and averaged in order to obtain a mean F value for each population (Brown 1979). Differences between F_A and F_Z represent the effects of different forces acting between the adult to zygote or zygote to adult life cycle stages (Fig. 1).

The expected value of the equilibrium coefficient of inbreeding due to selfing alone was calculated from the multilocus outcrossing rates [$F_e = (1 - t_m)/(1 + t_m)$]. The quantity ($F_Z - F_e$; Brown 1979) represents deviation of the realized fixation index from the expected value which assumed selfing to be the only source of departure from the Hardy-Weinberg equilibrium; and therefore, ($F_Z - F_e \neq 0$) is an indicator of other sources of inbreeding or selective forces involving underdominance or overdominance. These relationships between the different "F" coefficients and certain population features are sketched in a life-cycle diagram (Fig. 1).

Results

Autofertility and seed set

In *L. bakeri* (UCL 144), reduced protandry is associated with a high autofertility level. The average number of nutlets per flower was 2.11 out of a possible five (Table 1). In two *L. douglasii* populations (UCL 511, UCL 529), autofertility was significantly lower (0.056 and 0.048 nutlets per flower, respectively). Most likely, seed set results from geitonogamy with younger flowers at pollen shedding stages contacting the older flowers. The average seed set of *L. bakeri* in nature was not significantly different from that of the naturally open-pollinated *L. douglasii* populations; all values were between 3.0 and 3.5 of the five nutlets per flower. Comparing mean autofertility and mean seed set, we estimated that less than 2% (0.056/3.30) of the seeds

Table 1. Estimates of autofertile components of self-fertilization

Component		Taxon and population		
		<i>L. douglasii</i> 511	<i>L. douglasii</i> 529	<i>L. bakeri</i> 144
Autofertility (nutlets/flower with pollinators excluded)	Mean	0.056	0.048	2.11
	SE	0.023	0.019	0.32
	No. of plants	20	20	10
Total seed set (nutlets/flower in nature)	Mean	3.48	3.13	3.29
	SE	0.31	0.16	0.20
	No. of plants	20	30	20
Approximate frequency of autofertile seed		0.016	0.015	0.64
Selfing rate (1-t _m) (see Table 2)		0.05	0.25	0.79

Table 2. Estimates of single locus outcrossing rates

	Population						
	511	528	529-1978	529-1980	438	435	144
	<i>L.d.</i> 'rosea'				<i>L.d.</i> 'douglassii'		<i>L.d.</i> 'nivea'
No. families	34	31	30	50	22	21	43
No. individuals	392	322	418	252	300	220	457
Locus	Single locus estimates (t _i):						
<i>Pgm-2</i>	0.96±0.06	0.27±0.07	0.49±0.10	0.55±0.14	–	1.00±0.12	–
<i>Got-1</i>	–	0.59±0.11	0.82±0.18	0.63±0.19	1.00±0.14	0.69±0.16	–
<i>Got-2</i>	0.90±0.23	0.37±0.14	0.70±0.25	0.71±0.26	0.93±0.12	0.80±0.41	0.21±0.10
<i>Got-3</i>	1.00±0.07	1.00±0.07	0.86±0.12	0.18±0.17	0.99±0.08	0.78±0.12	–
<i>Pgi-1</i>	0.82±0.10	0.78±0.33	0.87±0.13	0.35±0.11	–	–	0.14±0.03
<i>Pgi-2</i>	0.93±0.09	0.49±0.07	0.88±0.13	0.36±0.26	0.90±0.26	0.80±0.41	–
<i>Mdh-2</i>	1.00±0.06	0.74±0.08	0.79±0.11	0.54±0.10	1.00±0.13	–	–
<i>Skd-1</i>	0.94±0.06	1.00±0.10	0.66±0.07	0.52±0.06	–	–	–
<i>Prx-1</i>	1.00±0.07	0.87±0.26	0.55±0.06	0.30±0.05	0.90±0.20	0.41±0.12	0.27±0.05
<i>Prx-2</i>	0.90±0.30	–	0.69±0.23	0.56±0.30	1.00±0.09	–	0.22±0.04
<i>Prx-3</i>	–	–	0.70±0.20	–	–	–	–
<i>Acp-2</i>	0.99±0.07	–	–	–	0.29±0.13	0.80±0.31	–
<i>Acp-3</i>	–	–	–	–	0.77±0.12	–	–
χ ² -test for the heterogeneity of t _i estimates	NS	P < 0.001	P < 0.05	P < 0.05	P < 0.01	P < 0.10	P < 0.10

produced in *L. douglasii* were of autofertile origin compared with over 60% (2.11/3.29) in *L. bakeri*.

Variability in outcrossing rates

In all natural populations the multilocus outcrossing rate estimates (t_m) were less than 1.0 (0.21–0.95, Table 3) with the more autofertile, smaller-flowered

taxon *L. bakeri* having the lowest outcrossing rate. While the range of outcrossing rates (0.75–0.95) among the *L. d.* var. 'rosea' populations was wide, populations 528 and 529 which are genetically very similar (Nei's distance D=0.01, see Kesseli and Jain 1984a) and geographically close (200 m apart), had nearly the same outcrossing rate. In addition, the two samples of population 529 collected in 1978 and 1980, gave the same t_m value (Table 3). These data suggest for *Limnanthes* taxa

Table 3. Estimates of multilocus outcrossing rates and F-statistics

	Population						
	511	528	529-1978	529-1980	438	435	144
	<i>L.d. 'rosea'</i>				<i>L.d. 'douglassii'</i>	<i>L.d. 'nivea'</i>	<i>L. bakeri</i>
t_m	0.95 ± 0.03	0.77 ± 0.4	0.75 ± 0.03	0.75 ± 0.04	0.87 ± 0.04	0.82 ± 0.04	0.21 ± 0.02
F_e	0.03	0.13	0.14	0.14	0.07	0.10	0.65
F_z	0.23 ± 0.05	0.20 ± 0.05	0.15 ± 0.06	0.27 ± 0.05	0.15 ± 0.11	0.30 ± 0.07	0.54 ± 0.06
<i>t</i> -test for $F_z = F_e$	$P < 0.01$	NS	NS	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$
F_A	-0.19 ± 0.06	-0.011 ± 0.07	-0.004 ± 0.07	-0.04 ± 0.05	+0.05 ± 0.10	+0.012 ± 0.13	+0.25 ± 0.08
<i>t</i> -test for $F_z = F_A$	$P < 0.01$	$P < 0.01$	$P < 0.10$	$P < 0.01$	NS	$P < 0.05$	$P < 0.05$

NS = not significantly at $P < 0.05$

t_m = multilocus outcrossing rate estimates

F_e = Wright's inbreeding coefficient, $1 - t_m / 1 + t_m$

F_z = the fixation index at the zygotic life cycles stage as calculated from bulk seed collections and equals one minus the ratio of the observed over the expected frequency of heterozygotes assuming Hardy-Weinberg equilibrium

F_A = the fixation index at the adult life cycle stage

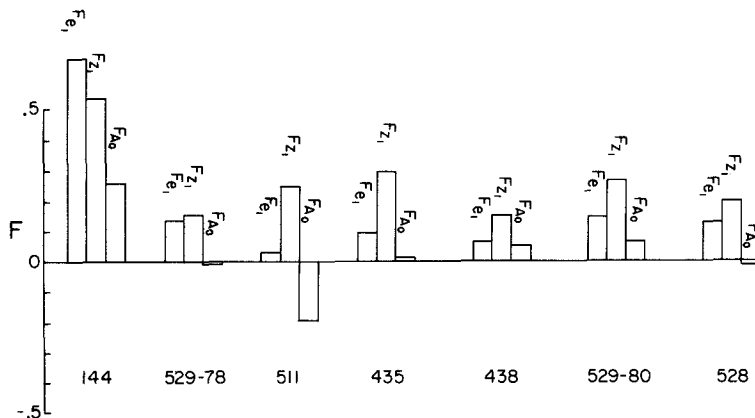


Fig. 2. Changes in the fixation index values through the life cycle stages of the seven populations. Symbols and life cycle stage are described in Fig. 1

that outcrossing rates as measured by the multilocus estimator may be relatively invariant in successive years and within small regions.

Heterogeneity of single locus estimates

There was a significant heterogeneity among the single locus outcrossing rate estimates. A chi-square homogeneity test showed four populations with significant ($P < 0.05$) and two with nonsignificant but notable ($P < 0.10$) heterogeneity between loci. However, no locus had either a consistently high or low value across populations; thus, for these marker loci there was no evidence of a "global" heterozygote advantage or of linkage to an "outcrossing locus." These factors would have biased upwardly the single locus estimate in all populations.

Single locus estimates could also be negatively biased by the Wahlund effect of nonhomogeneous gene dispersion in space. The transect in population 529-78 showed significant patchiness of alleles at the *Pgm-2* and *Pgi-1* loci ($P < 0.05$) and marginally significant patchiness at *Got-3* and *Prx-1* ($P < 0.07$) loci. Whereas two of these loci displayed the lowest single locus outcrossing rate estimates (*Pgm-2* and *Prx-1*, Table 2) and thus support the hypothesis of Wahlund effect, the two other loci with patchy distributions had high outcrossing rate estimates.

Fixation indices and the predicted effects of selfing: adult to zygote transition

The values of weighted fixation index, F_z , of the bulk seed collections were significantly greater than zero in

six of seven populations ($P < 0.05$, see Table 3 and Fig. 2), indicating departure from the Hardy-Weinberg equilibrium. The predicted equilibrium inbreeding coefficient, F_e , which assumes that self-fertilization is the only violation of Hardy-Weinberg equilibrium conditions, fully accounted for this deficit in only one population (529–578). In three populations, $F_Z - F_e > 0$ was significant ($P < 0.05$) indicating a substantial heterozygote deficit larger than that predicted by the outcrossing parameter alone. In one population, *L. bakeri* the inbreeder, $F_Z - F_e < 0$ was significant ($P < 0.05$) indicating an excess of heterozygotes.

Fixation indices and genotypic changes: zygote to adult transition

As judged from the lack of consistently high (single locus) outcrossing rate estimates across populations for any locus, there is no evidence for heterotic selection at these marker loci during the mating stage. However, in all populations the fixation index of the initial adult population, F_A , was less than that of the seed population, F_Z ($P < 0.05$ in six of seven populations, Table 3 and Fig. 2). This suggests heterotic selection between the zygote or seedling to the adult life cycle stages.

Genotype-outcrossing rate correlations

The range of outcrossing rates among individuals in some populations was limited (i.e. population 511) while others were considerably larger (i.e., populations 144 and 528, Fig. 3). The genotype of each individual was compared to the outcrossing rate of that individual to test for any association with their individual outcrossing rates. No significant correlations of outcrossing

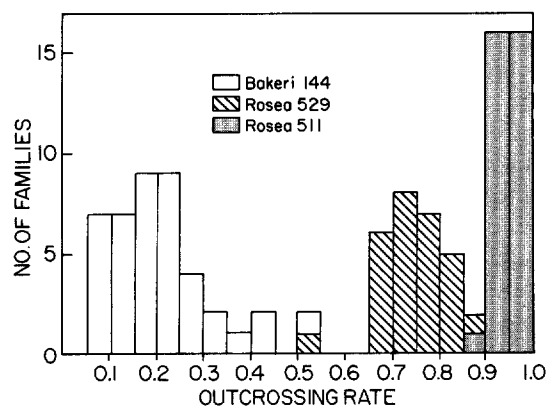


Fig. 3. Outcrossing rate variation among the individuals of three different populations, *L. bakeri* (UCL 144) and *L. d. 'rosea'* (UCL 529 and 511)

rate with any allele or genotype were found in any of the populations. A positive non-significant correlation was detected between the number of heterozygous loci per individual and outcrossing rate. Since the maximum number of loci per population was only 11, probably larger data sets would be needed to accurately test the significance of this correlation.

Discussion

Components of self-fertilization

Inbreeding as an evolutionary force affecting population genetic structure is pervasive in many populations (Wright 1965; Brown 1979) and has at least three components: autogamy, vector-mediated geitonogamy, and consanguineous matings in populations with sub-structure or limited dispersal. Autogamy in *L. douglasii* is inhibited by protandry and therefore extremely limited or non-existent. However, vector mediated geitonogamy was present in all populations since 5% to 25% of the seed set in these populations does result from self-fertilization. With an average seed set in nature of between 3.0 and 3.5 nutlets per flower and autofertility of approximately 0.05 nutlets per flower in the absence of pollinators, it appears that only about 1.5% of the total seed set results from wind-mediated (flower collisions) geitonogamous, self-pollination (Table 1). Thus, much of self-fertilization requires pollen transmission by insects. In early or late season when pollinators may be rare no pattern of an increased autofertility has been observed. But this needs rigorous testing. Since other studies with artificially inbred populations have demonstrated significant levels of inbreeding depression in *L. douglasii* populations (Kesseli and Jain 1984 b), the observed levels of inbreeding, largely resulting from geitonogamy and consanguineous matings, may be unavoidable by-products of the mode of plant development and the population structure.

In *L. bakeri* the situation is clearly different. Autogamy is possible and much higher levels of autofertility are found. Because of the lack of protandry, autogamy probably produces most of the total autofertile seed set in nature (64% of total). Of the remaining seed set approximately 15% is geitonogamous selfed seed and 21% is outcrossed seed. Thus the majority of the insect-mediated pollinations in *L. bakeri* result in outcrossed progeny.

The self-compatible, protandrous outcrossing mechanism of these taxa possesses some interesting properties. It is surprising that this system is so efficient at reducing self-fertilization in some *L. douglasii* populations given a profuse flowering pattern (numer-

ous flowers on different branches simultaneously open) coupled with short pollinator movements (Kesseli et al., unpublished data). It appears that the system not only prevents autogamy but also largely inhibits geitonogamy. Insect-mediated pollen transfer probably assures all pollen loads to be comprised of a mixture of pollen types and selection at the gametophytic stage may favor foreign pollen over selfed pollen which leads to the observed high outcrossing rates. It appears that gametophytic competition, which along with pollination efficiency are components of outcrossing rates, could have a stabilizing effect on outcrossing rates in a background of environmental unpredictability. Interestingly, gametophytic selection was found to be a key factor in a reexamination of some other outcrossing systems (e.g. gametophytic self-incompatibility; Mulcahy and Mulcahy 1983).

Variation in "true" outcrossing rates and the effects of the environment

Often studies have reported variation in the outcrossing rates among populations, sometimes associated with environmental parameters (Phillips and Brown 1977; Brown et al. 1978; Moran and Brown 1980). We have also found significant differences among populations. Thus, breeding systems appear to be highly variable within taxa and greatly influenced by environmental variables (Hamrick 1982). However, we find that: 1) little is known about the genetic control of outcrossing rates since frequently heritability has not been estimated and 2) as Ellstrand et al. (1977) and Ennos and Clegg (1982) have noted, much of the variation recorded is variation in "apparent" outcrossing rates, which are by definition strongly influenced by the mating among relatives, Wahlund effects and selection. Recent reports of multilocus outcrossing rate estimates show them to be highly invariant in some taxa. In 26 populations of *Hordeum spontaneum*, Brown et al. (1978) found that the rates generally range from 0 to 0.049 (one unusual population with a value of 0.096 was found). There were some large scale regional differences, but local populations had similar values. In eight stands of Douglas Fir, representing a variety of age classes densities and environmental conditions, estimates range from 0.86 to 0.96 (Shaw and Allard 1981a). These findings of low interpopulation variation in t_m , at least within local areas, are clearly in contrast to the single locus estimates in numerous other studies (Harding et al. 1974; Rick et al. 1977; Allard et al. 1977; Sanders and Hamrick 1980). More detailed genetic and ecological studies are needed before making any general statements about the breeding system variability or flexibility even in a closely related group of taxa.

Variation in "effective" outcrossing rates and the effects of the environment

Brown (1979) noted that populations of outcrossing taxa often show a deficiency of heterozygotes and populations of self-fertilizing taxa show an excess of heterozygotes when compared to the expected equilibrium inbreeding coefficients. He discussed the

contradictory nature and possible explanations of this observation, termed the "heterozygosity paradox". Clegg (1980) states that heterozygote excess is widespread even in outcrossing populations. Our data support Brown's paradox, as five of six populations of outcrossers possess, at the seedling stage of the life cycle, a marked deficiency of heterozygotes greater than the levels predicted by the t_m estimates, i.e. ($F_z - F_e > 0$), thus suggesting other forms of inbreeding, substructuring or selection against heterozygotes in these populations. Population 511 ($F_z - F_e = 0.20$) is a discretely subdivided population occurring in several closely neighboring vernal pools. Population 435 ($F_z - F_e = 0.20$) lacks these subdivisions, but inhabits a relatively dry meadow containing a high density of grasses. A posteriori it appears likely that both the populations with large heterozygote deficits have restricted seed flow and consequently a higher frequency of sib-matings because water dispersal is confined or limited.

Evidence for patchiness, even when water flow did not appear limited, came from population 529. The two loci with the lowest t_i values did show the expected large run lengths, although several transects placed perpendicular to the original one, are needed to map accurately all patches. In addition, patchiness of genotypic distribution appeared greater in 1980 than in 1978. This was suggested by a higher F_z (0.27) and consistently lower t_i values in 1980. We do not know whether the patchiness is stable and whether the sampled areas of 1978 and 1980 were simply from different founded subpopulations.

Brown (1979) was able to detect the additional consanguineous inbreeding in outcrossers because of his reliance on self-incompatible taxa (with outcrossing rates of 1.0 by definition) which preclude the need to estimate the rates. Detecting this in self-compatible taxa would be difficult since single locus estimates, which would incorporate this additional inbreeding into the estimate, have generally been used in outcrossing studies. We expected to find similar effects of population structure in the selfing *L. bakeri* population, but a surprising excess of heterozygotes ($F_z - F_e = -0.11$) indicated that selection between the adult and seedling stage may counterbalance any inbreeding due to population subdivision and consanguinity. A higher contribution to the bulk seed pool by the heterozygous or outcrossed individuals may be this component of selection.

While our data support Brown's (1979) observation of heterozygote deficiency in outcrossers among the zygotes detected by early seedling census, they also support Clegg's (1980) statement that generalized heterosis is prevalent in outcrossers and selfers alike. This arises from heterotic selection during the post-emergence stages of the life cycle. In all populations, the maternal adult genotypic frequencies

showed less of a heterozygote deficiency or even an excess when compared to the seedling stage. This shift is drastic in some populations and common to both the selfer and outcrosser taxa (Fig. 2). For example, F decreased from 0.23 to -0.19 in population UCL 511 and from 0.50 to 0.25 in population UCL 144 (Fig. 2). While the number of years and populations observed is much too small to invoke any broad conclusions about selection, it is interesting to speculate that the heterosis detected between the adult and seedling stages in the inbreeder (*L. bakeri*) may simply be an earlier manifestation of the general heterosis detected in all populations between the seedling and adult stages. Why inbreeders would register heterotic effects at the earlier stages is not clear, but selection at different life cycle stages has been reported (Allard et al. 1977; Phillips and Brown 1977).

This study showed that comparisons of single versus multilocus outcrossing rate estimates allow us to detect natural selection and the role of spatial substructure in genetic variation patterns. This is especially useful when populations are systematically sampled at different life cycle stages. In conjunction with the knowledge of the genetic controls of mating system per se and the role of gametic selection, life cycle analyses as shown here will also help in the discussions of the evolution sex and of breeding systems (Jain 1976; Antonovics and Ellstrand 1984).

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