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AN ECOLOGICAL GENETIC STUDY OF GYNODIOECY IN LIMNANTHES DOUGLASII (LIMNANTHACEAE)[1]

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ABSTRACT

Gynodioecy in two populations of *Limnanthes douglasii* var. *rosea* was studied for its maintenance requirements, role in population structure and its influence on the levels of outbreeding. The mode of inheritance of male sterility appeared to be nucleo-cytoplasmic, although the precise number of nuclear genes and dominance relationships could not be ascertained. Measurements on the plants sampled from natural stands and controlled experiments showed that male-steriles and their progeny had greater biomass and more flowers per plant than hermaphrodites, though these results varied with the environmental conditions. The hermaphrodites of gynodioecious populations had higher rates of selfing than the hermaphrodite individuals in populations lacking male sterility. Estimates of the inbreeding depression and homozygosity levels were also higher in gynodioecious populations. Variation in these parameters of breeding system and relative heterozygosis among populations may explain why male sterility has a restricted distribution, as theoretical models also predict rather specific conditions for this stable polymorphism. These data suggest that the advantage of male sterility is associated with lowered inbreeding depression. However, the potential ecological resource reallocation to the female function needs to be investigated. The fitness differences observed in this study between male-steriles and hermaphrodites appear inadequate to maintain the nucleo-cytoplasmic male sterility but could account for the observed frequencies (10–20%) of male-steriles in nature if the genetic system has evolved from a cytoplasmic system.

NATURAL gynodioecious populations, comprised of hermaphrodites and male-steriles, have been recognized since the time of Darwin (1877) and the practical uses of male sterility have been known and extensively exploited in the agricultural populations (Frankel and Galun, 1977). However, evolutionary interests in natural gynodioecious populations are just unfolding along with the research on the mode of inheritance, mechanism of gene action, and on the maintenance of male sterility using population genetic models.

In natural populations carrying male-steriles, often complex inheritance models are invoked (Godley, 1963; Aalders and Hall, 1963; Storey, 1953). In some gynodioecious populations contradictory results have been reported on the mechanisms of inheritance. In particular, it appears as though some earlier work indicating nuclear control of male sterility (Lewis and Crowe, 1956) has been superseded by the recent work showing cytoplasmic modes with nuclear fertility restorer loci (Kheyr-Pour, 1981). Other nuclear control models (Ross, 1969; Connor, 1973) are also suspect, since analyses of specific between-family crosses are required to establish the nucleo-cytoplasmic control mechanisms (Ganders, 1978; Kheyr-Pour, 1980). Cytoplasmic control of male sterility has now been postulated for over 100 agricultural species in 26 genera and 16 families. In nearly all of the cases with adequate genetic analyses, cytoplasmic male sterility seemed to originate from hybridization between different taxa (Edwardson, 1970).

Theoretical models of the evolution and maintenance of male sterility have been intensively studied with over 30 articles published which have shown male sterility to persist only when there is selection favoring the male-steriles or disfavoring the hermaphrodites at some stage in the life cycle. This is necessary in order to compensate for the lack of pollen contribution to the next generation by the male-steriles. The magnitude of fitness differences depends on the model of inheritance chosen, as they range from only a slight female fertility advantage in a cytoplasmic model (Lloyd, 1974; Charlesworth and Ganders, 1979), to a two-fold or more advantage in the nuclear models (Lewis and Crowe, 1956; Ross and Shaw, 1971;

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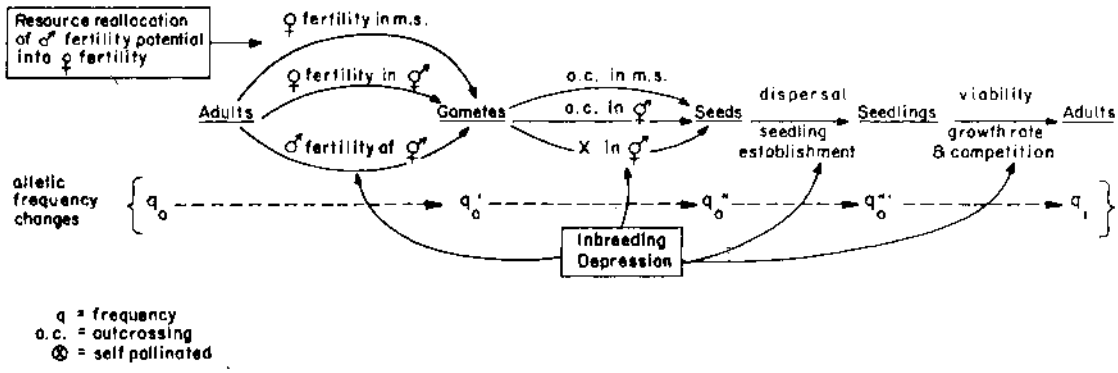

Fig. 1. Diagram showing life cycle stages which can be scored for changes in the frequency of male-steriles and in genotypic frequencies. Transitional frequency estimates in turn provide the estimates of various parameters of selective forces.

Lloyd 1974; Charlesworth and Charlesworth, 1978). Large selection pressures must be involved in gynodioecious systems at equilibrium which therefore offer an interesting area for ecological genetic research. Numerous workers have suggested different modes of selection (cf. Charlesworth and Ganders, 1979; Gregorius, Ross and Gillet, 1982); a generalized life cycle diagram (Fig. 1) is used here to illustrate the stages present and the possible evolutionary forces contributing to the maintenance of male sterility. In contrast to this extensive theoretical work, rather few studies (e.g., Lewis and Crowe, 1956; Assoud et al., 1978; Horovitz and Beille, 1980; Uno, 1982; Van Damme, 1982) have reported any experimental tests of these models or even estimates of many of the parameters from the natural populations.

Baker (1966) first discovered a few isolated gynodioecious populations of *Limnanthes douglasii* var. *rosea*. Jain, Hauptli and Boussy (1978) reported male sterility in a U.S. Department of Agriculture collection of *L. douglasii*. In addition, extensive information on the distribution patterns and the genetic and morphological variability in this largely endemic California genus is now at hand (Kesseli and Jain, in review; McNeill and Jain, 1983).

The aims of this study were the following: 1) describe male sterility and its associated morphological features; 2) evaluate segregation ratios in the progenies of certain hybrid combinations for determining the mode of inheritance; and 3) estimate some of the ecological parameters of fitness and spatial distribution, viz. a) female fertility, b) outcrossing rates, c) the extent of inbreeding depression upon selfing, and d) relative seedling survival of male-steriles and hermaphrodites.

MATERIALS AND METHODS—The variety *L. d.* var. *rosea* is confined to the vernal pools of the Central Valley of California. Population densities can range up to 300 plants/m^2, depending on the location and year (unpub. data). The plants are self-compatible, protandrous, pollinated primarily by *Apis mellifera* and native *Andrena* spp. The flowers are 5-merous with ten stamens and five ovules and produce a maximum of five nutlets per flower. The nutlets readily abscise and fall at maturity; thus, precise estimates of seed set are difficult to obtain.

Two natural gynodioecious populations of *L. d.* var *rosea*, (collection nos. UCL 528 and UCL 529), designated as "Pasture" and "Lakeshore" populations respectively, were sampled in the vernal pools located 16 km south of Dixon, Solano County. Morphological characters were scored from the field-collected male-sterile and hermaphroditic individuals. Relative frequency of male-steriles in a stand and relative plant density were estimated with two sampling methods: 1) by scoring the nearest plant at 15-cm intervals along a 30-m to 50-m-long line transects; and 2) by scoring all plants within ¼ m^2-quadrats placed at 4.5-m intervals along several transects.

Seeds from male-steriles and hermaphrodites were grown in a greenhouse. Controlled cross- and self-pollinations were performed with an appropriate genetic design over three generations in order to deduce the inheritance mechanism of male sterility. Fertility components were estimated for the field-collected plants from the quadrats. Fertility and other fitness components were also estimated from the greenhouse experiments in which male-sterile and hermaphrodite individuals were grown in 10 × 10-cm^2 pots using a random-

TABLE 1. *Summary of morphological comparisons between the male steriles (♀) and hermaphrodites (♂) of Lakeshore population, scored in spring 1978*

Plant type	Statistic and sample size (*N*)	Pollen fertility (%)	Flower color	Petal length mm	Sepal length mm	Stamen length mm	Style length mm	Plant diam[a] cm	Plant height Sample 1 cm	Plant height Sample 2 cm	Relative frequency among plants flowering on: March 12	Relative frequency among plants flowering on: March 20
♀	Mean	0	White	8.7	5.5	3.1	7.7	12.1	7.5	17.4	0.005	0.103
	S.E.			0.33	0.15	0.31	0.37	1.97	0.84	1.42		
	N			11	11	11	11	11	11	12	200	107
♂	Mean	96 to 100	Pink	12.8	6.8	7.6	7.6	15.3	8.4	20.0	0.995	0.897
	S.E.			0.33	0.27	0.25	0.24	1.42	0.53	2.07		
	N			11	11	11	11	11	11	6	200	107
	t-test			***[b]	***	***	NS[b]	NS	NS	NS		

[a] Length of longest precumbent branch.
[b] *** *t*-test for means, $P < 0.001$. NS not significant at $P = 0.05$.

ized block design. All plants were simultaneously scored and harvested at the peak flowering of each experiment and with the fitness of male-steriles set at 1.0, the relative fitness of hermaphrodites was calculated.

Open-pollinated single plant progenies (30 in the Lakeshore site and 31 in the Pasture site) were collected from the populations at 1.5-m intervals along transects, in the spring of 1978. In addition, 34 families in a population with no male-steriles (UCL 511), approximately 65 km north of Dixon, were sampled. Ten to fourteen seeds from each of these families were grown and the seedlings were scored for 11 electrophoretic allozyme loci (procedures reported by McNeill and Jain, 1983; Kesseli and Jain, in review). Outcrossing rates and their standard errors were estimated from allozyme data using a multilocus estimator (Ritland and Jain, 1981). At the Pasture site (population UCL 528), the effect of male-steriles on enhanced outcrossing rates was assessed from the heterozygosity levels at allozyme loci.

Inbreeding depression was estimated in four populations, namely, Pasture (gynodioecious), Maughn, Shippee and Vina (the latter three are totally hermaphroditic accessions, nos. UCL 511, 512 and 516, respectively). Twenty to twenty-five individual plants grown from their bulk seed collections were self-pollinated in a greenhouse. Selfed progenies (S_1) were grown in a randomized block design, replicated four times. Also included as controls were an equivalent number of plants grown from the parental bulk seed (S_0) as well as a few artificially produced hybrids (F_1) between different plants of a population. Fitness components were estimated from the data on: 1) relative seedling survival, flower number and biomass output per plant as measures of reproductive success and total growth, and 2) frequency of phenodeviants (cf. Lerner, 1954, for definition) in the selfs vs. bulks or crosses within each population. The following abnormalities were classified as phenodeviants: compound flowers, polypetalous or apetalous flowers, petaloid stamens, contorted stems, and dwarfs. Analysis of variance BMDP program P2V (Dixon, 1981) was used to analyze the original and log transformed data.

Single cotyledons were removed from the mature plants grown for the above inbreeding depression study, and assayed for 13 allozyme loci. Observed heterozygosity was scored and compared with the expected heterzygosity, assuming Hardy-Weinberg equilibrium conditions and using Wright's (1965) fixation index for the selfed versus open-pollinated progenies.

RESULTS—*Morphological description and frequency*—Several different types of male-steriles are found within the Lakeshore and Pasture populations. The most common form has aborted, whitish, small anthers with short stamen filaments. Others have yellowish, red or white anthers with varying anther sizes. Cytological examinations showed the former type to have a complete lack of pollen development past the stage of pollen mother cell differentiation or first meiotic division whereas the others apparently had pollen development aborted at some stage between tetrad formation and anthesis. Rare intermediate plants with low amounts of pollen were also observed. Only complete male-steriles were used in the genetic and fitness estimation studies.

Male-sterile individuals are characterized by several floral and vegetative traits (Table 1). The gynoecia of the male steriles appear to be the only parts of the flower which are not sig-

TABLE 2. *Frequency of male-sterility, scored in spring 1978*

Population	Sampling procedure	No. of plants in sample	Relative frequency of male-steriles
Lakeshore	Transect	50	0.14
	Transect	57	0.07
	Transect	50	0.24
Pasture	Transect	41	0.10
	Transect	54	0.04
	Quadrats	418	0.17 ± (S.E. = 0.08)

nificantly reduced in comparison to those of hermaphrodites. Petals, sepals and stamens are all significantly smaller ($P < 0.001$) in male-steriles. Flowers of male-steriles are distinctly white in color, instead of being typically pink. Male-sterile plants appear slightly shorter in stature and more branched, although the two separate samples showed no statistically significant differences for these traits. Male-sterile individuals flowered on an average two to three days later than hermaphrodites.

Frequencies of male-steriles vary among and within transects (0.04 to 0.24, Table 2). The samples from quadrats showed patchiness in the spatial distribution of male-steriles, as frequencies ranged from 0 to 0.63 among these quadrats.

Genetics of male sterility—Crosses among several male-sterile and hermaphrodite plants were scored in an attempt to determine the genetic basis of male sterility. Eight models were analyzed in an order of increasing complexity but only the diagnostic crosses useful in testing a particular genetic model are presented (Table 3). The first step was to determine whether nuclear control models could sufficiently account for our results, or whether a cytoplasmic component must be invoked. Of the five nuclear models, numbers 1–4 could be eliminated quite readily. In each case, a cross between male-sterile and hermaphrodite would either yield: 1) all hermaphroditic progeny, or 2) segregate for male-steriles and hermaphrodites, male steriles not exceeding 50% of the progeny; yet we found several crosses (four examples (1–4) are shown under the crossed parents (Gen. 1) column in Table 3) which yielded only male-sterile progeny. A nuclear model (no. 5, Table 3) was proposed in *Origanum vulgare* (Lewis and Crowe, 1956) and *Cortaderia* (Connor, 1973); one cross in this two-locus epistatic model can produce all male-sterile progeny. However, if a male-sterile offspring from this cross was to be crossed to any hermaphrodite, a minimum of 50% hermaphrodites would be expected in the resulting progeny. Complete male-sterile progenies were detected for both generations in several cases (e.g., Generation 2 of Cross 4). No single one- or two-locus, two-allele model with any other combination of genotypes or level of dominance appears to be compatible with our data without invoking gametophytic selection. Other nuclear models could be devised, but none was as parsimonious as the nucleo-cytoplasmic models. Strict cytoplasmic inheritance (no fertility restorers) has also been eliminated since some male-steriles did segregate (see Model 6 and Crosses 5–7, Table 3).

Likewise, we were able to eliminate two of the nucleo-cytoplasmic models as explanations. In both models (single locus with a dominant fertility restorer in the presence of "sterile" cytoplasm or with a recessive fertility restorer in the presence of sterile cytoplasm) any segregating progeny of a male-sterile by hermaphrodite cross should yield 50% each of male-steriles and hermaphrodites. In the single locus dominant restorer model, selfing a hermaphrodite from one of these segregating progeny arrays should again yield equal numbers of hermaphrodites and male-steriles (Model 7, Table 3). However, one such cross produced all hermaphrodites (Generation 2 of Cross 5). A single locus, recessive restorer model could conceivably explain the results of Cross 5, since in this model all hermaphrodites are expected from the aforementioned self progenies (Model 8). Two other combinations (Crosses 6 and 7), however, reject the recessive restorer model. Three selfed hermaphrodites, derived from the segregating progenies, were also found to segregate. Thus, a single-locus, fertility-restorer model also did not account for all data.

In addition to understanding the mode of inheritance of male sterility in gynodioecious populations, other crosses aimed at detecting the restorer alleles in hermaphroditic populations were initiated. Fifteen different pollen parents from seven different hermaphroditic populations failed to restore male fertility when crossed with the male-steriles taken from segregating progenies. This limited sample suggests that fertility restorers are not widespread.

To this point we are unable to establish or eliminate any other models of male sterility inheritance in *Limnanthes*. We do not know whether there are several mechanisms producing and restoring male fertility in different genic backgrounds, or whether there is a single universal multilocus model which satisfactorily explains our results. The former situation has been reported repeatedly in maize (Duvick,

TABLE 3. *Segregation patterns in selected crosses or selfed progenies*[a]

Name of model	Genotype ♀	Genotype ♦	Diagnostic crosses	Progeny ratio expected ♦:♀	Crossed parents (gen 1) female × male	Progeny ratio observed ♦:♀	Crossed parents (gen 2) female × male	Progeny ratio observed ♦:♀
1) Single locus dominant	Mm	mm	Mm × mm	1:1	1) 528-8S × 528-20F	0:9		
2) Single locus recessive	mm	MM Mm	mm × Mm	1:1	2) 528-9S × 528-24F	0:6		
			mm × MM	1:0				
3) Two locus dominant		(would degenerate to Model 1)			3) 529-3S × 529-31F	0:10		
4) Two locus recessive	$m_1m_1m_2m_2$	(others)	$m_1m_1m_2m_2$ × $M_1m_1m_2m_2$	1:1	4) 528-1S × 528-29F	0:18	528-1:1S × 528-20:1F	0:8
			$m_1m_1m_2m_2$ × $M_1m_1M_2m_2$	3:1			528-1:10S × 528-24:2F	0:20
			$m_1m_2m_2m_2$ × $M_1M_1m_2m_2$	1:0				
5) Two locus epistatic	M-hh	(others)	MMhh × mmhh	0:1	5) 529-7S × 529-20F	13:7	529-7:19F selfed	30:0
			Mmhh × mmhh[b]	1:1			529-7:17F × 529-7:19F	6:6
6) Cytoplasmic, no restorers	(S)	(F)	(S) × (F)	0:1	6) 529-15S × 529-22F	1:17	529-15:4S × 529-15:9F	7:5:3 int
			(F) × (F)	1:0			529-15:9F selfed	17:6
7) Cytoplasmic, single locus dominant restorer	(S) rr	(others)	(S) rr × (−) Rr	1:1	7) 528-7S × 528-35F	7:19	528-7:1F selfed	2:2
			(S) Rr (selfed)[b]	1:1			528-7:2F selfed	2:1
8) Cytoplasmic, single locus recessive restorer	(S) R−	(others)	(S) Rr × (−) rr	1:1				
			(S) rr (selfed)[b]	1:0				

[a] F = male fertile plant; S = male sterile plant; M, m, h, R and r = nuclear alleles defined by the models; (S) = sterile cytoplasm; (F) = fertile cytoplasm.
[b] Second generation cross using the progeny of the previous cross.

TABLE 4. *Comparison of flower number per plant between male-steriles and hermaphrodites, sampled from ten subsites in 1980*[a]

Population	Sample	Mean (± S.E.) no. of flowers per plant ♀	Mean (± S.E.) no. of flowers per plant ♂	Ratio[b] of means for ♂/♀
Pasture	Quadrat 1	9.4 ± 0.78	9.3 ± 1.11	0.99
	Quadrat 2	7.1 ± 0.94	7.3 ± 0.65	1.03
	Quadrat 3	10.2 ± 2.7	8.8 ± 1.21	0.86
	Quadrat 4	30.0 ± 5.4	23.6 ± 2.60	0.79
	Quadrat 5	3.0 ± 0.0	3.0 ± 0.0	1.00
	Quadrat 6	8.9 ± 2.9	4.9 ± 0.88	0.55*
	Transect 7	21.3 ± 3.0	18.7 ± 3.60	0.88
Lakeshore	Quadrat 1	16.1 ± 1.5	16.1 ± 2.40	1.00
	Quadrat 2	19.9 ± 2.4	10.3 ± 0.69	0.52*
	Quadrat 3	8.6 ± 2.4	8.4 ± 1.10	0.98

[a] Sample sizes per transect or quadrat averaged 10 to 12 plants, varying with plant densities.
[b] * *t*-test comparing means, $P < 0.05$.

1965), *Plantago* (van Damme, 1982), and *Origanum* (Kheyr-Pour, 1980). Ross (1982) discussed alternative evolutionary origins of subdioecy and gynodioecy, and emphasized that potentially several genetic mechanisms may exist among populations of the same species. Thus, although we cannot yet develop a population genetic model, we shall describe some ecogenetic and breeding system aspects. The key issue at this stage is the basic choice among the nuclear, cytoplasmic or nucleo-cytoplasmic inheritance mechanisms since this defines the general framework of fitness requirements needed for the maintenance of gynodioecy. Further details of the number of loci, dominance, and multiple allelism would be needed only as we develop evolutionary theory and the fitness analyses required for the detection of gametophytic selection.

Female fertility and plant biomass as fitness components—An increased female fertility as compensation for the lack of male contribution in male-steriles appears partially evident here. In order to eliminate major microhabitat differences between sites, male-steriles and hermaphrodites sampled within the ¼-m^2 plots as well as along one linear transect were compared (Table 4). Although only two of the samples gave a significant difference (*t*-test, $P < 0.05$). Fisher's combined test (see Sokal and Rohlf, 1969) showed that, overall, male-steriles had a highly significant increase in flower number/plant ($P < 0.01$). This fitness difference, which appeared patch-specific and possibly density- and year-dependent, was not sufficiently large per se to maintain male sterility since the relative fitness of hermaphrodites was only 0.81 (male-steriles with 1.0 value) and not the needed 0.50 or less, as predicted theoretically.

To investigate further the female-fertility and viability differences between male-steriles and hermaphrodites, we conducted a greenhouse experiment (Table 5). In a sample of 114 plants containing 40% male-steriles, male-steriles had larger total plant biomass ($P < 0.05$) and produced more flowers ($P < 0.001$) than the hermaphrodites. The increase in flower number was not simply a function of an increased plant size as the percent reproductive allocation (estimated from flower number per unit biomass) was also greater in male-steriles ($P < 0.01$). Thus, female fertility as well as plant growth rate were both higher for male-steriles; the latter might also result in a better survivorship but we have no data on the emergence or early seedling deaths. The cumulative advantage of male-steriles (computed from mean flower number) showed hermaphrodites to have a relative fitness of 0.76.

A third experiment estimated the relative reproductive fitnesses of the progenies of male-steriles and hermaphrodites from a three-way comparison among the hermaphroditic progeny of five hermaphrodite mothers, and the

TABLE 5. *Estimates of relative growth rate and fertility differences between male-steriles and hermaphrodites (sample from Pasture population grown in a greenhouse)*

Class	No. of plants	Mean dry weight (gm) $\bar{x}$ ± S.E.	Mean flower no./plant $\bar{x}$ ± S.E.	Mean no. flower/gram pl. wt. $\bar{x}$ ± S.E.
♀	46	0.87 ± 0.03	33.2 ± 1.67	37.9 ± 1.06
♂	68	0.79 ± 0.03 *	25.3 ± 1.16 ***	32.2 ± 1.27 **

t-test comparing means; * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

TABLE 6. *Comparisons of flower number per plant in the progenies of segregating male-steriles and non-segregating hermaphrodites*

Sample and family		No. of plants	Flower number $\bar{x} \pm$ S.E. ♀	Flower number $\bar{x} \pm$ S.E. ♂
PATCH 1				
Segregating family	1	10	78.7 ± 11.1	70.3 ± 5.5
	2	10	54.2 ± 8.2	43.0 ± 1.2
	3	14	75.0 ± 4.2	50.6 ± 3.4***
	4	10	75.7 ± 12.2	74.7 ± 11.8
Non-segregating family	1	10		44.2 ± 5.0
	2	6		39.5 ± 3.8
	3	6		28.0 ± 1.2
PATCH 2				
Segregating family	1	14	51.4 ± 6.0	46.3 ± 3.2
	2	16	28.1 ± 0.6	53.4 ± 3.4***
	3	23	41.8 ± 3.6	49.9 ± 2.5
Non-segregating family	1	10		42.1 ± 4.3
	2	10		42.1 ± 4.4

*** t-test comparing means of ♀ and ♂ individuals within families gave $P < 0.001$.

hermaphroditic and male-sterile individuals taken from the segregating progenies of male-sterile mothers (Table 6). All parental plants had been sampled from two adjacent 1-m^2 quadrats. Both classes of progeny from the male-sterile mothers had greater number of flowers per plant than the progeny of hermaphrodites (t-test for combined classes, $P < 0.01$). The overall relative fitness of the progeny of hermaphrodites was 0.68. Within the segregating families, hermaphrodites and male-steriles were generally not significantly different. One family (Patch 1, segregating family no. 3) had a highly significant ($P < 0.001$) increase in flower number for the male-steriles; another family (Patch 2, segregating family no. 2), conversely, had hermaphrodites with a higher number of flowers ($P < 0.001$). It is not known whether this variation is patch- or plant-specific, or whether there are specific loci affecting reproductive fitness that are linked to a restorer locus.

Mating success—In the schematic representation of the life cycle the transition between gamete and zygote production (seed set) is termed mating success. A combination of events including the rate of self-pollination, degree of inbreeding depression among those that self, pollination efficiency (amount of pollen and activity of pollinators) associated with those that outcross, and gametophytic competition, all occur here. It has been suggested that male sterility has evolved as an outbreeding mechanism (Baker, 1959; Lewis, 1942; Lloyd, 1974). Two assumptions are necessary in order to arrive at this conclusion: 1) there must be significant amounts of self-pollination in hermaphrodites occurring in a gynodioecious population (see Charlesworth and Charlesworth, 1979, for an excellent review of population genetic aspects), and 2) there must be a fitness differential between the inbred and outbred progenies, resulting in heterosis, or conversely, inbreeding depression. These parameters are estimated next.

Outcrossing rates and levels of heterozygosity—A total of 740 seedlings in 61 hermaphrodite families were scored for 11 polymorphic allozyme loci. Outcrossing rate estimates for the Lakeshore and Pasture populations were virtually identical, viz. 0.75 ± 0.030 and 0.77 ± 0.038 respectively, with the estimates for individual mothers ranging from 0.21 to 1.00. The outcrossing rate in the Maughn population (UCL 511) which contains no male sterility, was significantly higher (0.95 ± 0.03) and the plant-to-plant variation was much reduced, ranging from 0.88 to 1.00. Thus, hermaphrodites in the two gynodioecious populations indeed gave a higher selfing rate than those in the population lacking male-steriles. Pooled allozyme variation data from both Lakeshore and Pasture populations (Table 7) showed that the progeny of male-steriles had more heterozygosity and significantly lower fixation indices than the progeny of hermaphrodites (t-test, $P < 0.05$). Linkage relationships among these loci are not yet known but, apparently, selection favoring heterozygotes might involve only a small proportion of loci.

TABLE 7. *Estimates of common allelic frequency, observed heterozygosity and fixation index*

Parameter[a]	Allozyme loci[b]									
	Pgm-2	Got-2	Got-3	Pgi-1	Pgi-2	Mdh-2	Sdh-1	Prx-1	Prx-2	$\bar{F}$
Hermaphrodites										
N	40	40	40	34	40	40	38	22	22	
p	0.88	0.91	0.93	0.79	0.99	0.76	0.64	0.86	0.98	
H_{obs}	0.15	0.08	0.08	0.35	0.03	0.23	0.39	0.27	0.05	
F	0.32	0.50	0.38	−0.06	−0.50	0.39	0.20	−0.13	−0.25	0.21
Male-steriles										
N	21	21	21	17	21	21	20	10	10	
p	0.73	0.77	0.95	0.75	0.98	0.61	0.65	0.69	0.85	
H_{obs}	0.36	0.45	0.09	0.39	0.05	0.68	0.50	0.63	0.10	
F	0.10	−0.29	0.10	0.00	−0.25	−0.21	0.01	−0.34	0.62	−0.05

[a] *N* = number of individuals sampled; p = frequency of the most common allele; H_{obs} = observed frequency of heterozygotes; F = fixation index; $F = 1 - H_{obs}/pi^2$; and $\bar{F}$ = mean of the single locus estimates weighted by inverse of their variances.

[b] Locus designations are the same as in Kesseli and Jain (ms in review).

Inbreeding depression—Inbreeding depression may cause reduced seed viability, slower growth rates and reduced fertility. As suggested earlier, it may be a principal source of the fitness disadvantage observed in hermaphrodites. The results of the ANOVA comparing inbreeding effects in three hermaphroditic and one gynodioecious population (Table 8) showed: 1) highly significant differences between populations for flower number and plant biomass ($P < 0.001$); 2) a highly significant population × breeding treatment difference between the selfs vs. outcrossed bulks for plant biomass ($P < 0.001$); and 3) a highly significant interaction term for flower number ($P < 0.001$) and a marginally significant interaction one for plant biomass ($P < 0.10$). This implies variation between populations for inbreeding depression, and while there were significant between-replication differences, the rep × breeding treatment interaction term was not significant.

Least significant differences were used to evaluate the characters, populations and replicates separately (Table 9). Outcrossed bulks had significantly more flowers/plant than selfs in three of the four Pasture site reps ($P < 0.05$). In no other population were there significant amounts of inbreeding depression for this character. For plant biomass, six reps gave a significantly higher value for the outcrosses ($P < 0.05$, or $P < 0.01$); three of these reps are those of the Pasture site. In all cases, the outcrossed individuals had a higher mean plant biomass. This is in sharp contrast to the flower number character with significant heterotic advantage only in one population.

Per cent survival and phenodeviants were also scored for each population (Table 10). The Maughn population, showing a small difference between the selfs and the outcrosses in other fitness comparisons, showed a significantly lower survival rate in the selfed progeny (91 vs. 100%, $\chi^2_{(1)} = 9.5$, $P < 0.005$). Phenodeviants were particularly numerous in the selfs of populations Shippee and Vina, but not in the Pasture site which had registered the greatest amount of inbreeding depression for flower number and plant biomass. For each component of inbreeding depression, we calculated a relative value for the selfed progeny of each population; the value of outcrossed progeny was set at 1.0.

Finally, outcrossed bulks and selfs were analyzed for allozyme variability. The outcrossed bulks of all four populations had similar levels of observed heterozygosity (0.15; Table 10). Half as many heterozygotes are expected in the progenies of selfed plants, and this is generally

TABLE 8. *F-values from ANOVA to compare the effects of inbreeding*

Source of variation	df	No. of flowers per plant[a]	Plant biomass
Population (P)	3	54.15**	12.49**
Rep (R)	3	17.37**	30.00**
Breeding treatment (BT)	1	1.10	43.14**
P × BT	3	7.38**	2.11
R × BT	3	0.71	1.19
Error	925		

[a] Analysis of actual data is given here; log transformations did not change any of the results.

** $P < 0.01$.

TABLE 9. *Within-population comparisons between selfed and outcrossed progenies*

Population	Flower number[a]				Plant biomass[a]			
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4
528	5.04*	5.24*	6.50**	1.77	0.069	0.093*	0.137**	1.08*
516	1.83	2.56	−4.13	−0.6	0.109*	0.152**	0.053	0.060
512	−0.53	−1.64	−2.32	−1.18	0.009	0.111*	0.018	0.041
511	−1.20	−1.86	1.76	−2.42	0.023	0.061	0.053	0.037

[a] Positive values indicate outcrossed progenies have more flowers or plant biomass than the selfs. For flower number, the L.S.D. for $P = 0.05$ and 0.01 are 4.17 and 5.62 respectively, and for plant biomass, the L.S.D. for $P = 0.05$ and 0.01 are 0.088 and 0.119 respectively; * $P = 0.05$, ** $P = 0.01$.

realized in all but the Maughn population. The significant number of deaths among the selfs of the Maughn population could bias upward the proportion of heterozygotes found in those that survived and were analyzed. Curiously, of the populations in which the outcrossing rates were estimated, the one which had the highest rate (viz. 95% in Maughn population), had the largest deficiency of heterozygotes ($F = 0.31$). Thus, various measures of inbreeding effects (reproductive fitness, viability, growth rate, phenodeviants, heterozygosis) appeared to be uncorrelated among different populations.

DISCUSSION—*Genetic system: its nature and stability*—Our results conclusively showed that both cytoplasmic and nuclear restorer factors are involved in the control of male sterility in *Limnanthes*. For the origin of nucleo-cytoplasmic male sterility in agricultural plants, two models have been proposed: 1) Forced inbreeding of allogamous crop species can allow rare, recessive, nuclear alleles sensitive to different plasmatypes to be expressed. Clear evidence for multiple plasmatypes retained within populations comes from the work of Gracen and Grogen (1974) who screened 38 different plasmatypes from 28 inbred lines of maize; these formed 8 different groups defined in terms of restorer loci (three of these groups are the well known T, C and S cytoplasms). 2) Introduction of alien cytoplasm into a stock through wide hybridization, i.e., crossing diverse taxa or varieties, can combine sterile (S) cytoplasm of one line with the susceptible nuclear alleles of another "normal" (N) cytoplasm line. Plant breeders have used this technique in several autogamous crops. Within a given cultivar one would not generally retain sterile plasmatypes since they are often derived from single lines and since any male sterility, containing a new plasmatype with susceptible alleles, would be quickly lost as pollen for outcrossing is often limited in a selfing species (e.g., *Nicotiana*, see Edwardson, 1970).

Both models are relevant as alternative hy-

TABLE 10. *Effects of inbreeding estimated in terms of survival, percent of phenodeviants, relative fitnesses and level of heterozygosity*

Population and progeny class	Phenodeviants (%)	Survival after germination (%)	Relative biomass	Relative flower no.	H_{obs}[a]	$\bar{F}$[b]
UCL 528 (Pasture)						
Selfed	9.1	98.3	0.86	0.84	0.07	0.80
Outcrossed	1.7	100.0	1.00	1.00	0.15	−0.01
UCL 516 (Vina)						
Selfed	35.8	98.3	0.89	0.99	0.06	0.73
Outcrossed	1.7	100.0	1.00	1.00	0.14	0.11
UCL 512 (Shippee)						
Selfed	30.0	99.2	0.94	1.07	0.10	0.67
Outcrossed	1.7	100.0	1.00	1.00	0.15	0.16
UCL 511 (Maughn)						
Selfed	9.1	90.8**	0.94	1.02	0.13	0.50
Outcrossed	0	100.0	1.00	1.00	0.15	0.31

** = $P < 0.005$ that selfed equals outcrossed, based on χ^2-test of heterogeneity.

[a] H_{obs} = Mean observed heterozygosity per locus.

[b] $\bar{F}$ = Wright's (1965) fixation index, mean of single locus values weighted by the inverse of the variance at each locus.

potheses for the origin of male sterility in natural populations of *Limnanthes douglasii*. Our results provide mixed support for each of the alternative hypotheses. A comparison of outcrossing rates shows that the hermaphrodites in the gynodioecious "Pasture" population do indeed have a significantly higher selfing rate than those in the hermaphroditic populations. Morphological data (Kesseli and Jain, in review), however, seem to support the hybrid origin hypothesis. The Pasture and Lakeshore sites are both highly polymorphic for flower color, anther color and pubescence suggesting some gene exchange among different *L. douglasii* varieties. This is not typical as several populations of *L. d.* var. *rosea* are nearly monomorphic for these traits. The two gynodioecious populations reported here are in the same drainage system, albeit at a lower elevation, as the taxon *L. d.* var. *nivea*, and they share several morphological characters with the typical *L. d.* var. *nivea*. The hybridization hypothesis has the added advantage of allowing the rapid introduction of "alien" cytoplasm in high frequencies without invoking the need for strong selection favoring the newly-arisen male-steriles. Artificial hybridization and inbreeding studies are needed to test whether or how rapidly one can induce male sterility experimentally.

The exact genetic make-up of the nuclear fertility restorer system in *Limnanthes* is uncertain, and clearly, more sampling and further diagnostic crosses are needed. Note that difficulties in obtaining clear segregation patterns are not unique to *Limnanthes*. Kheyr-Pour (1980), Ganders (1978), Ross (1982) and others have cited similar complexities in nature, which perhaps parallel the situation in certain agricultural species with multiple, non-allelic restorers of cytoplasmic male sterility (Duvick, 1965). Even more extreme is the case of tomato in which more than 30 recessive nonallelic male sterility genes have been found (Clayberg et al., 1966). A variety of genetic systems controlling male sterility have been reported within the same crop species as, for example, in cotton: single recessive allele, single dominant allele, two-locus double recessive, and cytoplasmic control of male sterility have all been found (Weaver, 1968; Kohel and Richmond, 1963; Murthi and Weaver, 1974).

Evolutionary pathways to and from gynodioecy can be postulated and a population can be moved by natural selection to any state. There are no apparent strict evolutionary dead ends (Delannay Gouyon and Valdeyron, 1982). We postulate three evolutionary stages leading to gynodioecy in *Limnanthes*: First, hybridization of *L. douglasii* races initially introduced a foreign plasmatype into target populations and yielded cytoplasmically inherited male sterility. Next, the frequency of male sterility increased in the populations since the relative fitness of hermaphrodites (W_h) is less than the 1.0 of male-steriles (W_{ms}). Finally, the introduction of male fertility restoring (R_f) alleles moved the population toward either a polymorphic equilibrium containing male sterility (if $W_h < 0.5\ W_{ms}$) or a trivial equilibrium lacking male sterility (if $W_h > 0.5\ W_{ms}$) The alternatives of the third point are discussed below. The restricted distribution of gynodioecy could be explained by failures at either of the first two stages. Seed flow between populations with different plasmatypes may be rare. Also, since populations differ substantially in their outcrossing rates and levels of inbreeding depression, it is possible that male-steriles may have no advantage in some populations and be readily eliminated.

Models for the maintenance of male sterility—Numerous models have been proposed for the maintenance of male sterility (Lewis and Crowe, 1956; Lloyd, 1974; Ross and Weir, 1975), all of which acknowledge the strong selection against male-steriles, since they produce no pollen and therefore produce only half as many gametes as hermaphrodites. The selective advantages for male-steriles must encompass some combination of an increase in ovule production for male-steriles or a decrease in some fitness component for hermaphrodites. The increased ovular output of male-steriles may result from resource reallocation (Darwin, 1877). A decrease in hermaphrodite fitness components results if significant selfing and inbreeding depression are involved. This argument emphasizes the obligate outcrossing role of male sterility, also recognized originally by Darwin (1877). Thus, both genetic and ecological propositions are old; recent authors (e.g., Givnish, 1980; Willson, 1979; and others) have attempted to emphasize the dichotomy of the arguments in relation to the evolution of dioecy.

The argument of resource reallocation, independent of the genetic consequences caused by inbreeding, was not strongly supported by our data. No consistent differences in fertility (flower or ovule number) were found between male-sterile and hermaphroditec siblings of segregating families. This implies that the similar genetic backgrounds, not the sex, of the progeny bestows the advantages and thus the fertility differences that we do detect may be tied to the inbreeding depression in hermaph-

rodites. Our experiments showed that this inbreeding depression can occur at several stages in the life cycle. However, the inbreeding depression measured thus far cannot entirely account for the fitness differences between male-steriles and hermaphrodites. With a relative fitness for selfed progeny of 0.82 (survival multiplied by relative flower number) and an outcrossing rate of 0.75, the fitness of the progeny of a hermaphrodite would be only 0.95. Of course, along with the survival and reproductive components of inbreeding, we must further consider the phenodeviants and growth rates which would certainly lower the relative fitness of hermaphrodites.

Resource reallocation may be more likely in a species with an indeterminant ovule number per flower (e.g., *Hirschfeldia incana*, Horovitz and Beiles, 1980) in which lowered male function might directly partition resources toward an ovule number increase. However, resource reallocation may still be found in *Limnanthes* since our analyses only examined the fitnesses through fertility stages (ovule, production) and have not addressed actual fecundity (seed production). Indeed energy savings associated with male sterility may translate into fewer seed abortions. This and similar ecogenetic observations suggest that attempts to separate the outbreeding advantage hypothesis from the so-called non-genetic (ecological) ones would be preemptive for evolutionary thinking and may be fallacious. As noted succinctly by Ross (1982), most genetic models invoke the ecological parameters anyway.

A final question needs addressing. Are the observed advantages great enough for male sterility to evolve and reach equilibrium frequencies such as those observed (10–20%) in nature? If, as we postulated, cytoplasmic male sterility was the initial state, and since hermaphrodites do have relative fitnesses of 0.68 to 0.88, then male sterility could spread in a population until pollen becomes limiting. The equilibrium frequency of male-steriles, p, depends on the overall relative fitness of hermaphrodite, W_h (fitness of male-steriles W_{ms} = 1.0) and the pollinator activity, x (ranging from 0 to ∞) that corresponds to fertilization probability such that $p = (x - W_h)/x$ (Lloyd, 1974). As nutlets begin to swell after pollination, x was crudely underestimated by scoring the proportion of flowers, among a total of 128 flowers on 26 male-sterile plants, which had at least one enlarged nutlet (Kesseli, unpubl. data). With x approximately 0.90 and W_h 0.68 to 0.81, p could range from 0.08 to 0.23 which encompasses the ranges found in nature. Once Rf alleles and a nucleo-cytoplasmic mode of inheritance have been introduced, it is clear that the W_h must be lower (< 0.50) than the values detected in this study (Charlesworth and Ganders, 1979). On the other hand, several observations indicate that we have not yet exhausted the search for fitness differences. First, a higher growth rate resulting in larger plant size during early growth not only accounts for the increase in flower number per plant among male-steriles but it could also have a major effect on a greater competitive ability; under a sib-competition model, this would assume a special population genetic role of heterozygosity in favor of the male-steriles. Second, the high frequency of male steriles (40%) in our two-year old bulk seed collections also implies that male-steriles may have greater longevity in seed bank; H. G. Baker (unpubl. data) had also observed the stored seed to yield increasingly higher frequencies of male-steriles in *L. douglasii*. Hence, a wider variety of fitness differences might be detected in the experiments involving further ecological genetic analyses of life cycle stages. Male sterility, clearly, offers an interesting trait for which genetic control, dynamic role in population structure, and resource allocation features can be documented unequivocally. It represents a major gene polymorphism that should appeal to the ecologists for its rather obvious natural selection components.

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