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EFFECTIVE POPULATION SIZE AND GENETIC DRIFT IN TRISTYLOUS *EICHHORNIA PANICULATA* (PONTEDERIACEAE)

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Abstract.—Populations of the tristylous, annual *Eichhornia paniculata* are markedly differentiated with respect to frequency of mating types. This variation is associated with evolutionary changes in mating system, from predominant outcrossing to high self-fertilization. To assess the potential influence of genetic drift acting on this variation, we estimated effective population size in 10 populations from northeastern Brazil using genetic and demographic methods. Effective size (N_e) was inferred from temporal changes in allele frequency at two to eight isozyme loci and also calculated using five demographic variables: 1) the number of flowering individuals (N); 2) temporal fluctuations in N; 3) variance in flower number; 4) frequency of mating types; and 5) selfing rate. Average N_e based on isozyme data was 15.8, range 3.4–70.6, and represented a fraction (mean $N_e/N = 0.106$) of the census number of individuals (mean $N = 762.8$; range: 30.5–5,040). Temporal variation in N and variance in flower number each reduced N_e to about a half of N, whereas mating type frequencies and selfing rate caused only small reductions in N_e relative to N. All estimates of N_e based on demographic variables were considerably larger than those obtained from genetic data. The two kinds of estimates were in general agreement, however, when all demographic variables were combined into a single measure. Monte Carlo simulations indicated that effective size must be fewer than about 40 for drift to overcome the frequency-dependent selection that maintains the polymorphism for mating type. Applying the average N_e/N value to 167 populations censused in northeastern Brazil indicated that 72% had effective sizes below this number. This suggests that genetic drift is likely to play a dominant role in natural populations of *E. paniculata*.

Key words.—Effective population size, *Eichhornia paniculata*, electrophoresis, genetic drift, simulation, tristyly.

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Measures of effective population size (N_e) are necessary for evaluating the mechanisms of genetic differentiation in natural populations. The magnitude of N_e determines the amount of sampling error between generations that causes genetic drift or random fluctuations in allele frequency. Wright (1931, 1938) developed the concept of effective population size for application of population genetic theory to natural populations. In theoretical models, population size refers to the number of breeding individuals in an idealized population, in which individuals mate at random; the number of progeny per parent has a Poisson distribution and size is constant through time (Fisher, 1930; Wright, 1931). Natural populations, however, seldom satisfy these criteria. Rather, mating often involves more inbreeding than a random expectation, populations fluctuate in size and some individuals contribute gametes disproportionately to the offspring pool. Effective population size represents the number of individuals in a theoretical population that would have the same variance in allele frequencies or level of inbreeding observed in the actual population (Kimura and Crow, 1963). Measures of effective population size often deviate from the actual count of individuals, reflecting the disparities between natural and theoretically "ideal" populations.

Most studies of effective population size have relied on ecological estimators formulated in terms of the demographic and reproductive features of populations that cause departures from panmixia (Wright, 1938; Crow, 1954; Crow and Morton, 1955; Kimura and Crow, 1963; Nei and Imaizumi, 1966; Felsenstein, 1971; Hill, 1972; Heywood, 1986; Crow and Denniston, 1988). These have included estimates of N_e based on variation in reproductive effort and overlapping generations (Wood, 1987), sex ratios (Berven and Grudzien, 1990), fluctuations in population size (Jain and Rai, 1974; Berven and Grudzien, 1990; Fenster,

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1991), and variation in plant size (Heywood, 1986; Fenster, 1991). While such measures often are considerably less than a census of individuals would indicate, each one usually represents only a single stage in the organism's life history. Because many features of the life history can influence the magnitude of indeterminate changes in the gene pool, estimates of N_e based on single traits are likely to be inaccurate. The combined effect of these components of N_e and their relative importance has not been investigated in any detail (although see Wood, 1987).

An alternative to ecological approaches to measuring N_e is estimates based on the analysis of genetic data. Such methods stem from the direct relationship between N and genetic variability, described in models of the genetics of finite populations (Crow and Kimura, 1970). Estimators have been formulated based on measures of allele number (Chakraborty and Neel, 1989), linkage disequilibrium (Laurie-Ahlberg and Weir, 1979; Hill, 1981), allelism of lethal mutations (Dobzhansky and Wright, 1941), fixation rates of chromosomal rearrangements (Lande, 1979), geographic variation in gene frequencies (Dobzhansky and Wright, 1943), and temporal variation in allele frequencies (Krimbas and Tsakas, 1971). These methods are particularly appealing because the estimates are inferred from the genes themselves in a retrospective manner and, therefore, represent realized rather than potential measures of N_e.

The measurement of N_e based on temporal variation in allele frequencies has received considerable attention (Yasuda, 1969; Krimbas and Tsakas, 1971; Pamilo and Varvio-Aho, 1980; Nei and Tajima, 1981; Pollak, 1983; Waples, 1989). Unlike other genetic methods, N_e is inferred from observed changes in gene frequency through time rather than from the existing patterns of genetic diversity. As a result these estimates are not confounded by historical processes and require no assumptions about previous gene flow and selection. While these methods are not without their difficulties, particularly with respect to large sampling errors, some estimators show promise for studies of small populations (Waples, 1989). Despite the importance of genetic estimates of N_e for evolutionary studies of natural populations, relatively few are available in the literature (Begon et al., 1980; Loukas et al., 1980) and none exist for plant populations (although see Cheliak et al., 1985).

We estimated effective population size in the tristylous, annual, *Eichhornia paniculata* (Pontederiaceae) to evaluate the role of genetic drift in the evolution of its mating system. Tristylous populations contain three mating types, primarily distinguished by style length (long-, mid- and short-styles, hereafter L, M, and S morphs). Theoretical models indicate that all three morphs should occur at equal frequency within populations, because they are maintained by frequency-dependent selection due to strong disassortative mating among the morphs (Charlesworth, 1979; Heuch, 1979; Barrett et al., 1987; Barrett et al., 1989). However, *E. paniculata* exhibits considerable heterogeneity with respect to both the frequency and number of morphs that occur within natural populations. This variation is associated with changes in mating system from predominant outcrossing to high levels of self-fertilization (Barrett and Husband, 1990).

It has been proposed that genetic drift may account for unequal frequencies of morphs and their loss from certain populations of *E. paniculata* (Barrett, 1985). Simulations by Heuch (1980) indicate that drift in tristylous populations containing fewer than about 30 individuals may be sufficiently strong to overcome selection maintaining the morphs. Surveys of population size in northeastern Brazil have indicated that the majority of populations contain fewer than 100 individuals with many having considerably fewer (Barrett et al., 1989). However, effective population sizes in *E. paniculata* may be smaller than these estimates would indicate. Population sizes fluctuate widely in time, owing to the ephemeral nature of aquatic habitats in which the species occurs, and individuals vary greatly in reproductive output because of high phenotypic plasticity.

To assess the likelihood of genetic drift in populations of *E. paniculata,* we estimated the variance effective population size, N_e, from temporal variation in allele frequencies at isozyme loci from 10 popula-

TABLE 1. Summary of demographic and reproductive measures in 10 populations of *Eichhornia paniculata* from northeastern Brazil. N_0 and N_t are the population sizes in the first and last sampling periods. Multi-locus selfing rates (s) are the mean of estimates from both sampling years (when available). Style morph frequencies are based on a random sample of inflorescences in the first year sampled.

Population	Sampling interval (years)	N_0	N_t	s	Style morph frequencies		
					L	M	S
B34	2	2,500	120	0.16	0.40	0.34	0.26
B58	2	1,400	256	0.15	0.36	0.46	0.17
B59	1	22	51	0.45	0.45	0.55	0.00
B63	1	10,000	80	0.59	0.00	1.00	0.00
B69	7	33	200	0.54	0.00	1.00	0.00
B72	2	53	8	0.74	0.23	0.77	0.00
B75	1	50	35	0.43	0.56	0.44	0.00
B78	1	30	250	0.29	0.17	0.63	0.20
B81	2	54	80	0.20	0.55	0.21	0.24
B85	2	85	15	0.19	0.48	0.36	0.15

tions in northeastern Brazil. In addition, ecologically based estimates of N_e for these populations were obtained from measurements of five demographic and reproductive characteristics. These data were compared to assess the congruity between genetic and ecological methods for the estimation of N_e. The role of genetic drift in the breakdown of the mating system polymorphism in *E. paniculata* was then evaluated using Monte Carlo simulations incorporating empirical data on mating parameters from natural populations and estimates of effective population size.

MATERIALS AND METHODS

Genetic Measures of Effective Population Size

We estimated effective population size (N_e) from temporal changes in allele frequency in 10 populations of *Eichhornia paniculata* from northeastern Brazil. Populations were chosen from a survey of 167 populations, and comprised a representative sample of sizes, mating type frequencies, and selfing rates from the region (Table 1). We collected seed families randomly from each population at the beginning and end of a sampling interval, which ranged from one to seven years. In small populations ($N < 25$) all maternal parents bearing seed were sampled. Seeds from all families were germinated on moist soil and after about three weeks, up to 10 seedlings per family were transplanted into pots and grown until flowering.

Populations were assayed for variability at 25 isozyme loci using horizontal starch gel electrophoresis. Flower buds were extracted in a 0.02 M Na_2HPO_4 buffer (pH = 7.4) containing DL-dithiothreitol (1 mg/ml); the homogenate was absorbed onto chromatography-paper wicks (Whatman 3) and loaded onto 11% horizontal starch gels. The enzymes (number of loci in brackets) AAT (3), DIA (1), GDH (1) and TPI (3) were resolved on a lithium-borate buffer system (pH = 8.0), while ACO(2), ACP(2), IDH(2), MDH(3), PER(1), PGD(2), PGI(2), and SkDH(2) were resolved on a histidine-citrate buffer system (pH = 6.2). Running conditions and buffer systems used on flower bud extracts have been described in detail by Glover and Barrett (1987). To supplement these data, a subset of the polymorphic loci scored on flower bud extracts (*AAT-3, ACP-2, PGI-2, TPI-2*) and one additional locus not scored on flowering individuals (*PGI-3*; lithium-borate buffer system) were assayed on seed. Seeds, soaked in distilled water for about one week were ground in a 0.02 M Na_2HPO_4 buffer (pH 7.4) containing diethyldithiocarbamate, EDTA, Bovine Serum Albumin, and Tween 80 (see Kohn and Barrett, 1992 for details on grinding buffer). The homogenate was absorbed onto chromatography-paper wicks (Whatman 17). The running conditions and staining procedures were the same for flower buds.

From the electrophoretic analyses, we estimated allele frequencies for each population-locus-year combination. Frequencies

were estimated from all progeny genotypes to make use of the additional allele frequency information provided by the pollen pool. One difficulty with this approach, however, is that progeny from the same family are not statistically independent. To adjust for this, all calculations were based on an "effective" sample size (S_t) for each population sample using the formula

$$S_t = \frac{2 + n_f(1 - s)}{2} \cdot \text{no. of families} \quad (1)$$

where s is the mean population selfing rate and n_f is the mean number of progeny per family. The effective sample size indicates the number of independent samples upon which each estimate of allele frequency is based, assuming that all outcrosses are random. Low estimates of the correlation among paternal gametes in outcrossed progeny sampled between fruits (mean $f_{rs} = 0.13$) from a single population are consistent with this assumption (Morgan and Barrett, 1990). Population selfing rates are summarized in Table 1. For comparison, allele frequencies based on inferred maternal genotypes were also used to calculate N_e.

Before estimating N_e from electrophoretic data, we determined whether allele frequencies changed significantly through the time interval sampled with chi-square tests on each population-locus combination. Fifty-four separate tests were conducted at an experimentwise error rate of 0.05 (Sokal and Rohlf, 1981).

Effective population size was then estimated using temporal changes in allele frequency. The method assumes that allelic variation is near selectively neutral, migration between populations and mutation is negligible and generations are discrete. The estimator is based on the premise that the variance of allele frequencies [$V(P_t)$] after t generations is inversely proportional to effective population size, as described by

$$V(P_t) = P(1 - P)\left[1 - \left(1 - \frac{1}{2N_e}\right)^t\right] \quad (2)$$

where P is the allele frequency in the initial generation. Effective population size, therefore, is approximated by,

$$N_e = \frac{t}{2F} \quad (3)$$

where t is the time interval, in generations, separating allele frequency estimates and F is a standardized variance, used to compensate for differences in initial allele frequency. This relationship forms the basis of several different formulations of the temporal method (Krimbas and Tsakas, 1971; Pamilo and Varvio-Aho, 1980; Nei and Tajima, 1981; Pollak, 1983; Waples, 1989). Waples (1989) developed a general model for estimating N_e and evaluated its potential use in natural populations. He described two estimators (see Equations 11 and 12 in Waples, 1989), which correspond to different sampling schemes, originally suggested by Nei and Tajima (1981). Effective population size based on allele frequency changes in progeny was estimated using,

$$N_e = \frac{t}{2\left(F - \frac{1}{2S_0} - \frac{1}{2S_t}\right)} \quad (4)$$

where S_0 and S_t represent the effective sample sizes at time zero and time t. (Eq. 11, Waples, 1989). This formula provides the best estimate of N_e when individuals are sampled from early in the life cycle, before they have reproduced. With this sampling scheme, gametes approach a binomial distribution and N_e is independent of N. Because sample sizes varied among loci, we used the harmonic mean of S_0 and S_t. In addition, the 95% confidence limits were also calculated for each estimate of effective population size following Waples (1989). Effective population size based on inferred maternal genotypes was calculated using Waples' Equation 12 (1989) and estimates of mean population size from the census data.

For all estimates, we used Pollak's (1983) formulation of the standardized variance,

$$F_k = \frac{1}{k - 1} \sum_{i=1}^{k} \frac{(x_i - y_i)^2}{\frac{(x_i + y_i)}{2}} \quad (5)$$

where k is the number of alleles, and x and y represent the allele frequency estimates for

time 0 and t, respectively. This measure allows a locus to be fixed for a single allele in one of the samples, is robust to loci having uneven allele frequencies, and tends to compensate for the general overestimate of N_e by the temporal method (Waples, 1989). For multilocus data, we calculated a mean F, weighted by the number of alleles at each locus.

Demographic Measures of Effective Population Size

We measured five demographic and reproductive characteristics of *E. paniculata* that could cause a departure from the idealized population structure and examined their individual and combined effects on effective population size. Specifically, we estimated N_e for all 10 populations in terms of a simple count of individuals (N), temporal changes in the number of individuals (N_p), variance in flower number (N_v), the frequency of mating types (N_{lms}), and selfing rate (N_s). For the remainder of the paper, effective sizes calculated using different demographic factors are distinguished by their subscripts. All of these characteristics, except mating type frequency, have previously been characterized in terms of their theoretical effect on N_e (summarized in Crow and Kimura, 1970; Wright, 1969). Because populations in *E. paniculata* exhibit no systematic changes in size, we assumed that the effects of fluctuating population size were negligible and based each N_e estimate on the arithmetic mean of population size estimated at the beginning and end of the sampling interval.

Number of Individuals.—The number of breeding individuals (N) within a population, measured by census, provides a rough approximation of the effective population size. At best, it is an upper limit, below which N_e will decrease depending on departures from idealized structure. An estimate of the number of reproductive individuals in each of the 10 *Eichhornia paniculata* populations was calculated from at least two independent estimates per census period. A complete census was possible in populations with fewer than about 250 individuals. To summarize N for each population, we used the arithmetic mean over the first and last census periods.

Size Fluctuations.—When a population varies in size in different generations, the lowest number of individuals, or the most severe bottleneck, largely determines the effective population size. When N varies, the variance effective size is best represented by the harmonic mean of the population sizes observed through time (Kimura and Crow, 1963). For *E. paniculata,* this measure (N_p) was calculated from values of N estimated throughout the sampling interval (N estimates from a maximum of four different years).

Variance in Flower Number.—Kimura and Crow (1963) developed an expression that describes N_e as a function of the distribution of progeny among parents. When there is a random contribution of gametes to progeny among parents, then $N_e = N$. Heywood (1986) reformulated their analysis in terms of size variation, which in annual plants is often highly correlated to fecundity, and easier to measure in plants. The general expression for the variance effective population size as a function of variance of plant size is

$$N_V = \frac{1}{(1 + F)\dfrac{varb}{xb^2} + 1} \cdot N \qquad (6)$$

where xb and $varb$ are the mean and variance in a plant size variable, F is the inbreeding coefficient of the population and N is the arithmetic mean of size for the two sampling periods. The plant size variable used was the number of flowers per individual, estimated from the number of inflorescences present and the number of flowers per inflorescence. Variance in flower number was estimated for 2 of the 10 populations in this study (B63, B85). The remaining eight were assigned the species mean, which was based on estimates of flower number for the aforementioned populations and seven additional Brazilian populations (Table 2). The sample included estimates from one population (B56) in two consecutive years. Inbreeding coefficients were estimated from the population selfing rate (Table 1), assuming populations were at inbreeding equilibrium.

Number of Mating Types.—Because most outcrossed matings (≈90 percent) occur be-

TABLE 2. Mean and variance of flower number in 10 populations of *Eichhornia paniculata* from northeastern Brazil. Measures of flower number were used to predict the effect of variation in reproductive capacity among individuals on effective population size (see Materials and Methods).

	Number of flowers			
Population	Mean	Variance	Sample size	Variance / Mean^2
B42	2,150.28	1,435,635.04	25	0.310
B45	46.84	1,848.56	25	0.843
B46	287.22	55,394.09	25	0.671
B56 (1987)	145.92	19,009.33	25	0.893
B56 (1988)	308.54	85,035.92	79	0.893
B63	308.44	131,285.26	25	1.380
B71	103.56	18,092.26	25	1.687
B79	87.12	3,331.03	25	0.439
B85	305.90	190,770.26	82	2.039
B142	339.88	28,856.42	45	0.250

tween morphs (Barrett et al., 1987; Kohn and Barrett, 1992), the contribution by each morph to the gamete pool may be asymmetrical when morph frequencies are skewed. To account for the effects of the tristylous breeding system on effective population size, we formulated the effective population size as a function of morph frequency. We assume that all matings occur between morphs and the success of two morphs siring ovules of the third is in direct proportion to their frequencies in the population. Here we define the inbreeding effective population size of tristylous populations by determining the sum of the probabilities that two uniting gametes originated from the same L, M, and S morphs (inbreeding effective size). This quantity is approximately the inverse of the effective size, N_{lms}. In abbreviated form,

$$\frac{1}{N_{lms}} = p(L)^2 \cdot \frac{1}{N_L} + p(M)^2 \cdot \frac{1}{N_M} + p(S)^2 \cdot \frac{1}{N_S}. \quad (7)$$

where N_L, N_M, and N_S are number of L, M, and S morphs in the populations and $p(x)^2$ is the probability of drawing two alleles from a parent of morph x in generation $t - 1$. To estimate N_{lms}, the initial morph frequencies from the first sample of each population were used. For details on the derivation of this equation and clarification of terms, see the Appendix. Assuming population size and morph frequencies are stable, the variance effective size produces the same result (Appendix).

Selfing Rate.—The amount of self-fertilization can also influence effective population size. Effective size, as a function of selfing rate is

$$N_S = \frac{N(2 - s)}{2} \quad (8)$$

where s is the proportion of progeny derived from self-fertilization and N is the average number of individuals censused. Complete selfing ($s = 1$) will decrease N_e to $1/2N$ because the loss of variation is similar to that in a haploid population of size N. Selfing rates in the 10 populations of *E. paniculata* in this study ranged from 0.15 to 0.74 (Table 1). Each estimate of selfing was calculated from electrophoretic variation in open-pollinated progeny arrays using Ritland and Jain's (1981) multi-locus estimation procedure (for further details see Barrett and Husband, 1990).

Joint Effects of Demographic and Reproductive Factors.—The models above consider the individual effects of each demographic and reproductive factor on effective population size. The joint effect of these factors was also considered. We derived the combined N_e (N_{comb}) by calculating each individual N_e in sequence. Starting with the census of individuals in each population, we calculated the N_e value based on variation in population size (N_p). We then used the N_p value in the calculation of N_e based on selfing rate (N_s), the N_s value to calculate N_{lms}, and finally N_{lms} to calculate N_v. Effective population size, based on each demographic factor was calculated in turn, each time incorporating the N_e value from the previous calculation. Because individual estimates of selfing rate, and variance in flower number were not available for each sampling period and population, the combined measure was calculated by estimating N_p first and then incorporating the effects of the other demographic variables. Because the effects of the individual variables are multiplicative, the sequence in which the remaining variables were incorporated had

no effect on N_{comb} estimates. Values based on the joint effects of all five demographic characteristics were compared to genetic estimates of N_e for each population using product moment correlations and a paired t-test.

Effect of N_e *on Loss of Style Morphs*

We used Monte Carlo simulations to determine the effective population size below which genetic drift would overcome the effects of frequency-dependent mating on the maintenance of trimorphic population structure. The primary feature distinguishing our simulations from those of Barrett et al. (1989) is that, rather than explore an arbitrary combination of parameter values, we used values derived from studies of natural populations of *E. paniculata* (selfing rate $s = 0.15$, frequency of outcrosses between morphs $d = 0.95$, frequency of random outcrosses $r = 0.05$; Barrett et al., 1987; Barrett and Husband, 1990).

Each simulation comprised 100 independent populations of size N. The populations were initially established by randomly selecting style morph genotypes (Long: *ssmm*; Mid: *ssMm, ssMM*; Short: *Ssmm, SsMm, SsMM, SSmm, SSMm, SSMM*) from an infinite population at genotypic equilibrium (Heuch and Lie, 1985). Each generation, N new zygotes were chosen from the gametes of the previous generation. The maternal parent of each offspring was chosen at random from the population. The paternal parent was either the same as the maternal parent or a different individual depending on the probability of self fertilization (s) versus outcrossing ($1 - s$). Within the outcross pool, mating was either disassortative (paternal and maternal parents not the same morph) with probability d, or random with respect to morph (r, where $d + r = 1$). For each disassortative mating event, the paternal parent was selected randomly from all parents that were a different morph than the maternal parent. If there was no available parent, the simulation returned to the mating step until either a random or self-fertilization pathway was chosen. As a consequence, the actual rate of disassortative mating decreases below d when populations have lost two morphs. This is likely to occur in natural populations when morph ratios are uneven.

The loss of morphs from simulated populations due to genetic drift was examined for a range of population sizes (N = 5, 10, 20, 30, 40, 50, 60, 70, 80). We assessed the strength of drift in the simulations by monitoring the proportion of the 100 trimorphic populations that remained trimorphic versus nontrimorphic after 100 generations of mating. Each simulation of 100 populations was replicated 10 times to generate standard errors of the percent of trimorphic populations for each estimate.

RESULTS

Genetic Measures of Effective Population Size

We assayed a total of 3,721 progeny from 440 families in 10 populations of *Eichhornia paniculata* to determine changes in allele frequency between generations. Of the 25 loci scored, 18 were polymorphic (frequency of common allele <0.95 in at least one sample period) in at least one population; two to eight loci were polymorphic in each population (Table 3). Allele frequencies changed significantly from the first to the last sampling period in 17 of 54 possible population-locus combinations, based on a chi-square test. The percentage of tests that are significant (31.5%) is considerably greater than the nominal alpha level for the test (5%). Of the 17 significant cases, six exhibited an increase in frequency of the most common allele while 11 exhibited a decrease. With a population experimentwise error rate of 0.05, four population-locus combinations exhibited significant changes in allele frequency.

Effective population size (N_e), as estimated from temporal changes in allele frequency, was low for this species (Table 4). The mean effective population size for the 10 populations was 15.8 (geometric mean = 10.8; range 3.4–70.6) and ranged from 0.2 to 17.4% (mean = 10.6) of the actual count of reproductive individuals in each population. Population estimates were similar when based on allele frequencies derived from the inferred maternal parents rather than the progeny (arithmetic mean N_e = 11.8, range 0.6–25). The N_e/N ratio

TABLE 3. Significance levels for single-locus χ^2 tests of temporal variation in allele frequencies in 10 populations of *Eichhornia paniculata* from northeastern Brazil. Only loci that were polymorphic in at least one year are reported. Dashed lines indicate that a locus was not polymorphic in a given population. With an experimentwise error rate of 0.05, only loci with a significance level of 0.001 remained significant.

	Population									
Locus	B34	B58	B59	B63	B69	B72	B75	B78	B81	B85
AAT-2	—	—	—	—	0.05	—	—	—	—	—
AAT-3	NS	0.05	—	NS	NS	—	NS	NS	NS	0.025
TPI-2	NS	NS	NS	NS	—	—	—	—	NS	0.005
TPI-3	NS	—	—	—	—	—	—	—	—	—
PER-1	—	NS	—	—	NS	—	0.025	NS	—	—
GDH-1	NS	—	NS	NS	0.001	—	NS	NS	—	0.025
PGI-2	NS	NS	0.025	—	—	NS	—	—	—	NS
PGI-3	—	—	—	—	NS	0.05	—	—	—	0.025
PGD-1	0.05	—	—	NS	—	—	—	—	—	—
ACP-1	NS	—	—	—	—	—	—	NS	—	—
ACP-2	—	—	—	—	—	—	—	NS	NS	NS
MDH-2	—	—	—	—	—	—	—	NS	—	—
MDH-3	—	—	—	—	—	—	—	0.001	—	—
DIA-1	—	NS	—	0.05	—	—	—	—	—	—
IDH-1	—	NS	0.001	—	—	—	NS	—	—	—
IDH-2	0.025	—	—	—	—	—	NS	—	—	—
ACO-1	—	—	—	—	—	—	—	—	—	—
ACO-2	—	0.001	—	0.025	—	—	—	—	—	—
All loci	0.05	0.001	0.001	NS	0.001	0.05	0.05	0.001	NS	0.001

was particularly small (0.002–0.086) in populations with more than 100 individuals compared to populations with fewer than 100 (0.093–0.174). This may be a function of the increased number of loci involved in the analysis of larger populations. In all but one population, the upper confidence limit was less than about half of the census number of individuals. In only one population (B72) did the upper 95% confidence limit exceed the actual count of individuals.

Ecological Measures of Effective Population Size

Demographic Variables.—Estimates of population size over the two sampling periods ranged from eight to 10,000 individuals (Table 1). Average size, based on the mean number of individuals sampled in the first and last census periods for each population was 766.1 (range 30.5–5,040; Table 5). These results reflect the skewed distri-

TABLE 4. Effective population size (N_e) inferred from temporal variation in allele frequencies for 10 populations of *Eichhornia paniculata* from northeastern Brazil. N_e values are based on the estimator described in Waples (1989) for a sample of genotypes taken before reproduction (sampling scheme II). S_0 and S_t represent the harmonic mean of the number of independent genotypes sampled across loci for time zero and t. Also given are the number of families and progeny sampled at each sampling period, the number of polymorphic loci examined, and the lower and upper 95% confidence limits for N_e.

	Number of families/progeny						Confidence limits	
Pop.	0	t	Number of loci	S_0	S_t	N_e	Lower	Upper
B34	20/161.7	59/595.3	8	88.1	309.1	70.6	15.56	349.53
B58	17/149.7	23/185.1	7	80.9	101.7	12.1	3.32	30.07
B59	23/177.5	10/70.2	4	72.1	29.3	3.4	0.62	10.64
B63	42/270.2	17/115.3	6	97.3	40.5	11.2	1.40	73.75
B69	10/147.9	37.2/389.7	5	44.0	124.4	17.0	3.31	45.74
B72	16/127.0	7/56.0	2	32.5	14.3	5.0	0.10	56.76
B75	10/104.3	18/174.6	6	39.7	67.8	7.4	1.45	27.01
B78	16/137.0	11/69.0	7	64.5	35.5	12.1	2.38	71.83
B81	24/174.1	27/273.2	3	94.1	136.5	10.7	1.20	36.04
B85	39/177.2	14/166.2	6	111.2	81.8	8.4	1.90	21.74

TABLE 5. Comparison of genetic and ecological measures of effective population size (N_e) in 10 populations of *Eichhornia paniculata* from northeastern Brazil. The measures are based on 1) temporal variation in allele frequencies (N_e), 2) the census number of individuals (N), 3) fluctuations in number of individuals (N_p), 4) variation in flower production among individuals (N_v), 5) morph frequency (N_{lms}), and 6) variation in selfing rate (N_s). The combined effect of all five demographic and reproductive features on N_e is presented as N_{comb}. Asterisks indicate the populations for which the average variance in flower number for the species was used to estimate N_v.

Population	N_e	N	N_p	N_v	N_{lms}	N_s	N_{comb}
B34	70.6	1,310.0	328.5	647.9*	1,305.4	1,205.2	148.9
B58	12.1	828.0	145.5	410.7*	816.7	765.9	66.5
B59	3.4	36.5	30.7	16.5*	36.2	28.2	10.6
B63	11.2	5,040.0	158.7	1,704.2	5,040.0	3,540.6	37.8
B69	17.0	116.5	69.6	50.9*	116.5	84.9	22.2
B72	5.0	30.5	11.2	12.2*	21.4	19.2	2.0
B75	7.4	42.5	41.2	19.3*	41.9	33.4	14.5
B78	12.1	140.0	53.6	66.7*	123.8	119.4	19.4
B81	10.7	67.0	30.7	32.8*	63.5	60.1	12.8
B85	8.4	50.0	33.4	15.4	49.1	45.3	9.2

bution of population size in the species (see Fig. 5 in Barrett et al., 1989), with a few large populations and many smaller ones.

Population size in *E. paniculata* was not constant from year to year. When fluctuations in population size were weighted using the harmonic mean, mean effective size (N_p) was reduced to 90.3, which was on average 0.47 of N.

Variance in flower number among individuals in *E. paniculata* populations also caused a substantial reduction in estimates of effective size relative to N. On average, N_v was 297.7 or an average of 0.42 of N. The value of N_v is higher than N_p, yet comprises a smaller fraction of N. This is because fluctuations in population size were particularly large in B63, the largest population. The arithmetic mean N_p was biased downward as a result of this population.

Mating System.—Our analytical results of the influence of tristyly on N_e indicated that the frequencies of the three mating types reduced effective population size most when they were uneven. To illustrate this effect, N_e/N values for a range of morph structures are reported in Figure 1. For populations in which the morphs occurred at equal frequency, disassortative mating had no effect on N_e and therefore $N_e/N = 1$. Moreover when one morph became rare or absent, N_e was not affected as long as the remaining morphs were near equally frequent; however, morph frequency had a noticeable effect on N_e when one morph increased in association with the decline of the other two (Fig. 1). As the frequency of the most common morph increased, N_e became increasingly small relative to N. For example, in a population in which a single morph comprised 0.98 of all individuals, N_e was 0.15 of N.

Morph frequencies in the 10 populations examined were highly variable (Table 1). Five populations contained three morphs, three populations had two morphs, and two populations were fixed for a single morph; however, because frequencies within each population were not strongly skewed, morph frequency resulted in only small reductions in N_{lms} relative to N. On average, N_{lms} was 761 (21–5,040), 95% of the value of N (Table 5). In the two monomorphic populations $N_{lms} = N$ because of the absence of disassortative mating.

The selfing rate of populations also had a small effect on estimates of N_e. Selfing rates ranged from 0.15 to 0.59 among the 10 populations. The mean estimate of N_s was 590 (19–3,540) or 0.81 of N (Table 5). Populations with one or two morphs had the largest disparities between N_s and N because these populations exhibited the highest selfing rates.

Combined Effects.—Values of N_{comb} were not influenced by the order in which each of the demographic and reproductive factors was incorporated. Measures of effective size for each population were similar to values based on genetic data (Table 5). In 9 of

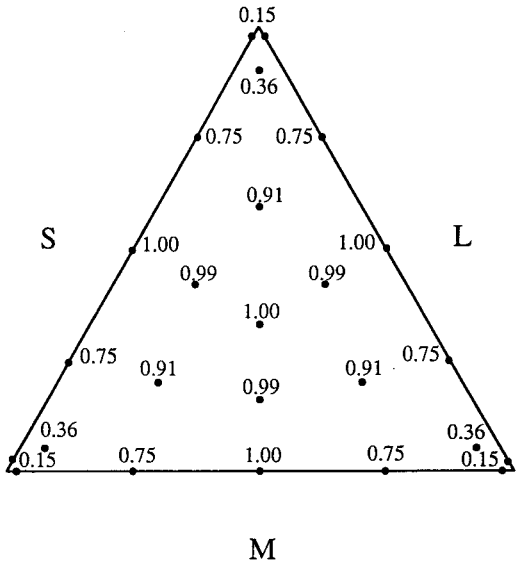

FIG. 1. The ratio of effective population size (N_e) to the census number (N) as a function of the frequency of style morphs in a tristylous species. The values were calculated assuming complete disassortative mating among style morphs. The axes represent the L, M, and S morphs. Each point on the triangle plot represents a different population morph structure, where the frequency of each morph is proportional to the distance from its respective axis. N_e/N values were derived from the analytical formulation of N_e in terms of morph frequencies (see Appendix).

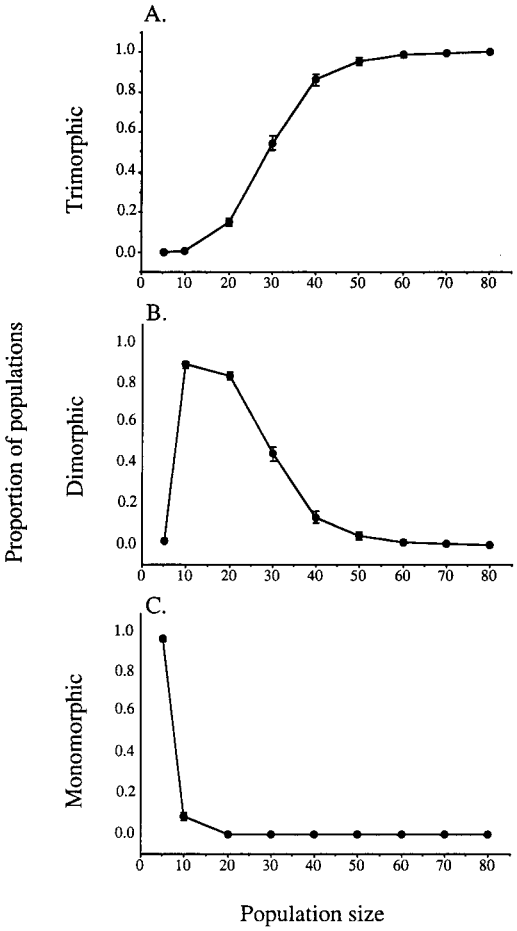

FIG. 2. The effect of population size on the loss of morphs from simulated trimorphic populations. Graphs show the proportion of populations with A) three, B) two, and C) one morph after 100 generations. Each simulation comprised 100 populations and was replicated 10 times. Bars represent two standard errors.

10 populations, N_{comb} occurred within the 95% confidence intervals for N_e. The two measures were significantly correlated (r_p = 0.93, $P > 0.05$), indicating that the population ranking for N_{comb} and N_e was similar and their means were not significantly different (Paired t test; $t = 2.2$ $df = 9$ $P > 0.05$).

Monte Carlo Simulations

Many of the estimates of N_e presented above are sufficiently low to suggest that drift plays an important role in influencing morph frequencies in natural populations of *E. paniculata*. Simulations involving populations of 50 or more individuals exhibited little morph loss after 100 generations. In contrast, populations with fewer than 40 individuals were most likely to lose morphs, despite strong levels of disassortative mating. For example, nearly 50% of populations at $N = 30$ had lost a single morph after 100 generations. At $N = 10$, no trimorphic populations remained, and nearly 10% of populations had lost two morphs. These values were consistent among replicate simulations, as the small standard error in Figure 2 indicates.

While the values for mating parameters were set similarly for all population size treatments, actual levels of disassortative mating monitored during the simulations were correlated with population size. With population sizes of 20 or more, 95% of all outcrosses were disassortative as expected. When population size was fewer than 20, the actual percentage of outcrosses that were disassortative dropped to 87 and 2% at $N = 10$ and 5, respectively. As a proportion of the total matings, about 83% were be-

tween different morphs (intermorph), except in populations of $N = 10$ and 5, where intermorph mating made up 75 and 2%, respectively.

Discussion

Considering the importance of population size to population genetic theory and the ease with which some plants can be censused, it is surprising that data on size are so infrequent in the evolutionary literature on plants. The census number of individuals likely varies from thousands, in species that occur in continuously distributed habitats, to a few plants in many rare species and those restricted to specialized habitats. From a genetic viewpoint, however, plants probably exchange genes with a relatively small number of individuals, regardless of whether they are distributed continuously or in discrete patches. Studies of pollen and seed dispersal have indicated that many continuously distributed species consist of small breeding units, or neighborhoods, within which mating is considered to be random (Wright, 1978; Crawford, 1984; Golenberg, 1987; Smyth and Hamrick, 1987; Levin, 1988; Campbell and Waser, 1989; Fenster, 1991). The census size of neighborhoods or of discrete populations may be regarded as an upper limit below which the effective number will occur, depending on deviations from idealized structure.

Estimates of effective population size in *E. paniculata,* based on temporal variation in allele frequency, are much smaller than a simple census of individuals would indicate. Effective size varied from 3.4 to 70.6 among the 10 populations examined, and on average, comprised about 11% of the census number of reproductive individuals. These values are difficult to evaluate because there are few published estimates of effective population size from genetic data for plant populations. Moreover, the few published estimates that are available have been obtained using different methods.

Schoen and Brown (1991) reported estimates of N_e for 17 plant species with contrasting mating systems using data on allele richness from the literature. The values they obtained were considerably higher than in this study, with the mean N_e for inbreeding and outbreeding species being 3,557 and 6,990, respectively. It is difficult to compare these values with those from *E. paniculata* because estimates of N were not available for the species surveyed and the emphasis of their study was on measuring the distribution of N_e and not its absolute value. There are only two comparable studies of plant populations involving the measurement of N_e using temporal variation in allele frequencies. An estimate of N_e for a spruce (*Picea mariana*) seed orchard reported by Barrett et al. (1987), using maternal and filial allele frequencies was 17, or 28% of N. In contrast, Cheliak et al. (1985) found the variance effective size to be 1.4 times larger than the number of parents in a stand of white spruce. The sampling interval used in both these studies was from maternal parents to seed progeny; thus the N_e estimates represent sampling error that occurs only during the mating cycle. Because this method does not include allele frequency variation during recruitment and seedling establishment, these estimates likely overestimate the true value of N_e.

Our values of N_e in *E. paniculata* are substantially less than those estimated by genetic methods for animal populations. In *Drosophila subobscura* populations, N_e estimates based on the frequency of allelism among lethal mutants (Loukas et al., 1980) and temporal changes in allele frequency (Begon et al., 1980) were effectively infinite. High values of N_e have also been reported for *Dacus olea* based on changes in allele frequency ($N_e = 722$; Waples, 1989, data from Krimbas and Tsakas, 1971) and for *Drosophila pseudoobscura* populations using the allelism method ($N_e = 500$–1,000; Dobzhansky and Wright, 1943). Lande (1979) estimated N_e to be 30–200 in mammals, 40–300 in lower vertebrates and 200–800 in dipterans, based on rates of chromosomal rearrangements. Effective population size may be low in *E. paniculata,* in comparison with the above estimates, because of the few individuals within populations and the restricted rates of migration that occur among them (B. C. Husband and S. C. H. Barrett, unpubl. data).

Estimates of N_e based on temporal variation in allele frequencies assume that the genetic variation used is selectively neutral. The impact of selection on estimates of N_e

will depend on the number of loci affected and the robustness of the method to departures from neutrality. Waples (1989) suggested that weak selection would not have a significant effect on estimates because selection is often locus-specific, and therefore will have relatively minor effects on multilocus methods. Moreover, while strong selection can alter estimates of N_e, this effect is likely to be of minor significance when the interval between samples is relatively small (Nicholas and Robertson, 1976; Pollak, 1983).

A second concern in using the temporal method for estimating N_e is the large variances in F, the standardized variance in allele frequency, that are often obtained (Pamilo and Varvio-Aho, 1980). Allele frequency variances due to sampling error will often exceed and, hence, obscure any variation due to genetic drift in the population. Therefore, estimates are reliable only with large sample sizes from populations which are likely to have small N_e values. Furthermore, the precision of the estimates is improved by sampling intervals greater than one year (Waples, 1989). These two criteria were met in some *Eichhornia paniculata* populations. Sample sizes averaged 83.3 per generation, populations were generally small and the sampling intervals were greater than one year in six of the 10 populations investigated. Despite this, confidence intervals averaged 69.2, range 10.02–333.97, suggesting that considerable scope still exists for increasing the precision of the estimates of N_e based on temporal changes in allele frequency.

Ecological methods based on various demographic and reproductive variables have been used more frequently for estimating effective population size. Compilations of these estimates have been undertaken for various animal groups (Crawford, 1984 and references therein) and plants (Heywood, 1986). Similar methods can be used to estimate effective neighborhood number, although this is rarely done (Crawford, 1984; Fenster, 1991). How accurately these measures predict the genetic behavior of populations is difficult to evaluate because of the limited number of studies involving both ecological and genetic approaches (although see Begon et al., 1980). In our results, demographic and reproductive factors led to estimates of effective population size that were between 2 and 500 times larger than genetic estimates of N_e. Moreover, these estimates varied widely depending on the particular attribute that was used (Table 5). Variance in reproductive capacity and fluctuations in population size had the largest individual effects on N_e. The importance of variance in reproductive capacity among individuals in reducing N_e has also been demonstrated in several other herbaceous plant species. In an analysis of seed set data in the annual Poppy (*Papaver dubium*), N_e was approximately 0.07 of the census number (N) (data of MacKay, 1980 in Crawford, 1984). Based on a survey of variation in fruit number in 27 species, Heywood (1986) found variance effective population numbers were, on average, 0.417 of the actual census of individuals. Large variation in reproductive output also resulted in a 7–22% reduction in the effective size of neighborhoods of *Chamaecrista fasciculata* relative to the census count (Fenster, 1991). Clearly, the high phenotypic plasticity in reproductive output of many plants will be a major factor leading to a reduction of N_e from N.

Estimates of N_e in *E. paniculata* using a genetic approach were generally smaller than those based on individual demographic and reproductive characteristics. While average N_e values based on the combined effects of all demographic attributes were more similar they were still approximately two times greater than the genetically based measures. Differences between the two kinds of estimates may result from a number of factors relating to the methods of estimation. For example, our estimate of variance in progeny number was based on the distribution of flower number among individuals, which may be only a crude approximation of the contributions through male and female function to the next generation. A comparison of estimates using plant size and the actual distribution of progeny number has not been made. Moreover, the variance in reproductive effort for eight populations in this study was represented by a species mean, derived from seven populations that were otherwise not examined in this study. While these populations were representative with respect to habitat, population size, mating

type frequency and selfing rate, bias in estimates of reproductive effort may have occurred.

Differences between ecological and genetic measures of N_e may also arise because all demographic estimates of N_e were based on expected deviations from the arithmetic mean (N) for each population and hence were not influenced by systematic changes in population size throughout the sampling interval. In contrast, N_e based on variation in allele frequencies is influenced by any unidirectional change in population size. As a result, if population sizes in the last sampling period are consistently smaller than in the first sampling period, N_e estimated from genetic methods could be considerably less than demographic estimates. This seems unlikely, however, because changes in population size were not consistent in direction and there was no relationship between the direction of change in N over the sampling period and the deviation between genetic and demographic estimates of N_e (Mann Whitney comparison of two samples: $U = 14$, $P > .50$).

Finally, differences between genetic and demographic estimates of N_e may result from the effects of additional demographic features not taken into account. One feature that has not been fully investigated but which likely influences many plant species is the contribution to the gene pool from a seed bank (Templeton and Levin, 1979). Glasshouse experiments and field observations suggest weak seed dormancy in *E. paniculata*; however, the contribution to the breeding population by seed from different year classes is not known for natural populations. If the strength of seed dormancy has been underestimated, estimates of N_e from demographic methods would be biased downward. If seed dormancy is strong, estimates of N_e using genetic methods will represent the effective number of breeding individuals each year, but will be an underestimate of the effective size per generation of a population (Waples, 1990).

Our genetic estimates of N_e, in conjunction with Monte Carlo simulations of finite, tristylous populations, indicate that genetic drift likely plays a major role in causing the loss of mating types from natural populations of *E. paniculata* (and see Husband and Barrett, 1992). In simulated populations, morph loss occurred only in populations of fewer than 40 to 50 individuals (Fig. 2). Estimates of N_e were fewer than 20 in 9 of 10 populations examined and were on average, 10.6% of N. If we assume that this value is representative, then 120 of the 167 populations we have surveyed in northeastern Brazil would be equal or less than the critical size below which genetic drift will have a dominant influence on morph frequency variation.

Wright (1948, 1982) emphasized that genetic drift may cause significant population differentiation, but alone, is not likely to bring about important "evolutionary progress." The loss of style morphs from populations of *E. paniculata* through drift does not appear to result in significant evolutionary changes in the mating system, unless also accompanied by selection favoring the spread of selfing variants (Barrett et al., 1989; Barrett and Husband, 1990; J. R. Kohn and S. C. H. Barrett, unpubl. data). The interplay between drift and selection in *E. paniculata* appears to be favored during periods of small effective population size. Genetic estimates from this study suggest that a significant number of populations in northeastern Brazil meet this condition.

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APPENDIX

Like species with separate sexes, the frequency of mating types in tristylous populations may influence the relative contribution of gametes among individuals because matings primarily occur between different types or morphs. To account for the effects of the tristylous breeding system on effective population size, we formulated the inbreeding and variance effective population sizes as a function of mating type frequency. We assumed that all mating occurs between different morphs, i.e., is disassortative, and that morphs sire seeds in proportion to their frequency in the population.

Inbreeding Effective Population Size.—The inbreeding effective population size is defined by the probability that two genes sampled from the same individuals are identical by descent. In a population with L, M, and S morphs, it is easiest to formulate this in two components by first determining the probability that two genes are derived from the same morph and second, the probability that they are identical by descent (from the same individual within that mating type). For example, the probability that a gene sampled at time t is from an L parent at time $t - 1$ is

$$\begin{aligned}\text{P(allele from a L morph)} = \text{p}(L) &= \frac{1}{2}\left(\frac{N_L}{N_L + N_M + N_S}\right) \\ &+ \frac{1}{2}\left(\frac{N_M}{N_L + N_M + N_S}\right)\left(\frac{N_L}{N_L + N_S}\right) \\ &+ \frac{1}{2}\left(\frac{N_S}{N_L + N_M + N_S}\right)\left(\frac{N_L}{N_L + N_M}\right)\end{aligned}$$

where N_L, N_M, and N_S are the number of L, M, and S morphs in the population. The first term in this equation represents the probability of an L morph contributing to the gamete pool through female function while the second two terms represent the contribution by the L morph through male function on M and S morphs, respectively.

It follows that the probability that two genes chosen in generation t are from an L parent in generation $t - 1$ is $\text{p}(L)^2$. Finally, the probability that both genes are derived from the same individual of the L morph is,

$$\text{p}(L)^2 \cdot \frac{1}{N_L{}^2} \cdot N_L \qquad \text{(A1)}$$

Using this method, similar probabilities can be calculated for contributions from M and S parents. In an idealized population the chance of two gametes coming from the same parent is simply the reciprocal of the number of individuals in the parent generation. Here we define the inbreeding effective number by equating the sum of the probabilities of two uniting gametes being identical by descent for each morph to $1/N_{\text{Ims}}$. In an abbreviated form, this is

$$\frac{1}{N_{\text{Ims}}} = \text{p}(L)^2 \cdot \frac{1}{N_L} + \text{p}(M)^2 \cdot \frac{1}{N_M} + \text{p}(S)^2 \cdot \frac{1}{N_S}. \qquad \text{(A2)}$$

Variance Effective Population Size.—The sampling variance, V(P), of allele P in 2N gametes that are drawn at random is

$$\text{Var}(P) = \frac{P(1 - P)}{2N}. \qquad \text{(A3)}$$

Therefore, the variance effective number is the value that most accurately replaces N in the formula (Wright, 1969). The variance of an allele P in a tristylous species with disassortative mating consists of the variance of P in each morph, weighted by their respective contributions to the 2N gamete pool,

$$\mathrm{Var}(P) = (W_L)^2\frac{P(1-P)}{2N_L} + (W_M)^2\frac{P(1-P)}{2N_M} + (W_S)^2\frac{P(1-P)}{2N_S} \quad \text{(A4)}$$

Each morph will be weighted according to its frequency in the population as

$$W_L = \frac{1}{2}\left(\frac{N_L}{N_L+N_M+N_S}\right) + \frac{1}{2}\left(\frac{N_M}{N_L+N_M+N_S}\right)\left(\frac{N_L}{N_L+N_S}\right) + \frac{1}{2}\left(\frac{N_S}{N_L+N_M+N_S}\right)\left(\frac{N_L}{N_L+N_M}\right)$$

$$W_M = \frac{1}{2}\left(\frac{N_M}{N_L+N_M+N_S}\right) + \frac{1}{2}\left(\frac{N_L}{N_L+N_M+N_S}\right)\left(\frac{N_M}{N_M+N_S}\right) + \frac{1}{2}\left(\frac{N_S}{N_L+N_M+N_S}\right)\left(\frac{N_M}{N_L+N_M}\right)$$

$$W_S = \frac{1}{2}\left(\frac{N_S}{N_L+N_M+N_S}\right) + \frac{1}{2}\left(\frac{N_L}{N_L+N_M+N_S}\right)\left(\frac{N_S}{N_M+N_S}\right) + \frac{1}{2}\left(\frac{N_M}{N_L+N_M+N_S}\right)\left(\frac{N_S}{N_L+N_S}\right)$$

where $W_L + W_M + W_S = 1$. Using these weightings and solving for N_e gives,

$$N_e = \frac{N_L \cdot N_M \cdot N_S}{(W_L)^2\cdot(N_M\cdot N_S) + (W_M)^2\cdot(N_L\cdot N_S) + (W_S)^2\cdot(N_L\cdot N_M)} \quad \text{(A5)}$$

The analytical results of N_e based on the sampling variance of alleles (variance effective number) in tristylous populations are in agreement with those based on the levels of inbreeding (inbreeding effective number). For populations with two morphs all parameters pertaining to the missing morph should be omitted from the equations. The effect of morph frequency on N_e/N is reported in Figure 1.