DENSITY-DEPENDENCE IN SALVIA LYRATA L.

by

Ruth Geyer Shaw

Department of Botany
and
University Program in Genetics
Duke University

Date: September 12, 1983

Approved:

Janis Antonovics, Supervisor

John E. Boynton

Norman Christ

D. S. Burdick

Henry McNulty

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany and the University Program in Genetics in the Graduate School of Arts and Sciences of Duke University

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ABSTRACT

(Botany and Genetics)

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ABSTRACT

One of the central puzzles in ecology is what determines the size of populations in nature. Strident debate between those claiming precedence of density-independent factors over density-dependent ones, and *vice versa*, has subsided with the realization that both may play a role in limiting population size of any species. Nevertheless, very few attempts have been made to directly assess the effect of density on population growth in nature. This is particularly disturbing, because theoretical ecology is largely based on the density-dependent population growth routinely documented in laboratory experiments.

Similarly, explanations of the existence of variation in life history among and within species, in particular the weedy vs. non-weedy habit, have invoked density-dependent selection. Yet such selection has never been demonstrated in nature, and thus its efficacy in maintaining genetic variation is unknown. The experiments reported here were devised to determine the extent to which density limits growth of a population of *Salvia lyrata* L., an herbaceous perennial plant common in North Carolina grasslands, and whether density-dependent selection structures its genetic variation.

The local density of *Salvia* was altered by sowing in seed at different densities, and by transplanting or removing established individuals. In most cases, these manipulations elicited weak or conflicting responses; however density-dependent mortality and stunting of seedlings was evident at unnaturally high densities of sowing. Thus density is rarely sufficient to limit an individual's contribution to this population, but density effects can limit population size at extreme seedling densities.

In additional experiments, evidence of genetic variation in density response was sought by planting individuals of known genetic origin into arrangements of varying density. An experiment in protected conditions showed variation among families in the response of flowering probability to density, but not in survival nor in the number of seeds produced. A field experiment showed variation among families in the reponse of a size trait, number of leaves, to density. Given that survival and fecundity are size-dependent, as documented in observations of the natural population, this result suggests the potential for density-dependent selection in nature.

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DENSITY-DEPENDENCE IN *SALVIA LYRATA*

CHAPTER I. OBSERVATION

Introduction

The direct effect of population density on an individual's contribution to population growth is considered fundamental to population dynamics, yet the experimental methods necessary for revealing it have rarely been applied in wild populations. Experimental studies of density effects in insects (e.g., in Drosophila, Pearl, 1927; Sang, 1950; Barker, 1973; and in Tribolium, Sokal and Huber, 1963) and in plants (see Harper,1977, for review) have demonstrated major effects of density on various components of individual fitness and on population productivity. Since these experiments are executed in the relatively uniform conditions of laboratory, greenhouse, or agricultural field, they reveal idealized forms of density response. From this empirical work, mathematical functions have been derived which fit the observed density responses (e.g., May, et al., 1974; Hassell, 1975; Watkinson, 1980; White, 1981, for review of the plant literature), and concomitant theoretical work (e.g., Hassell, 1975) has employed these functions as the basis for predicting stability of population size. It remains unclear to what extent these findings apply to natural populations, because very few experimental studies of density-dependence have been carried out on wild species in natural conditions; Antonovics and Levin (1980), however, discuss some exceptions (see also, Stiven and Kuenzler, 1979; Clay and Shaw, 1981; Wise, 1981).

The preponderance of work on natural populations of plants has consisted of demographic studies of unperturbed stands. From such observational data, inferences about the effects of density have in some cases been drawn (e.g., Sarukhan and Harper, 1973; Jefferies, et al., 1981; Klemow and Reynal, 1981; Matessi and Menozzi, 1979; Barkham, 1980; Silva, et al., 1982). Other attempts to discern density effects in natural plant populations have been based on observation of overdispersed spatial patterns and of positive correlations between individual size and distance to nearest neighbor (e.g., Phillips and MacMahon, 1981; Bell, 1981; Squiers and Klosterman, 1981; Wright, 1982). Earlier studies of this kind have been reviewed by Antonovics and Levin (1980), who point out that such studies fail to separate effects of density from the effects of microenvironmental variation, since the two are confounded in unmanipulated populations.

Beyond the problem of how density effects are manifested in nature is the question of whether density-dependent selection occurs as a consequence of these density effects. Density-dependent selection is the mode of selection in which the genotype-specific fitness rankings vary with conspecific density, such that certain genotypes are favored at one density and alternative ones at different densities. Given the knowledge that increased population density frequently causes a decrease in the components of fitness of individuals, one might expect that there exists genetic variation in response of fitness traits to density. The occurrence of density-dependent selection is a possible, though not necessary, outcome of the existence of such genetically based variation.

The assumption that density-dependent selection does indeed occur in natural populations has served as the basis for theories of life history

evolution (e.g., MacArthur and Wilson,1967; Gadgil and Solbrig, 1972; Clarke, 1972; Roughgarden, 1971; Bulmer, 1974; Slatkin, 1979). Evidence of density-dependent selection is available from experiments on synthetic populations of Drosophila (e.g., Lewontin, 1955; Clark and Feldman, 1981; Marks, 1982). Moreover, variation among strains of crop plants for response to density of growth and yield characters has frequently been reported (e.g., Stivers, et al., 1971; Rumbaugh, 1970). The extent to which density-dependent selection occurs in wild species is, however, almost entirely unknown. Solbrig and Simpson (1974) and Law, et al. (1977) demonstrated genetic variation in life history traits among "biotypes" of Taraxacum officinale and among populations of Poa annua, respectively. It was suggested that density-dependent selection in the "closed" habitats led to delayed reproduction and smaller proportional allocation to reproduction relative to the life history phenotypes which were more common in the "open" environments. No studies which directly assess the extent of genetic variation in density response in a natural population have been reported. Thus, despite keen interest in the theoretical consequences of density-dependent selection, we have no direct evidence regarding the prevalence of this mode of selection in natural populations.

This dissertation addresses questions relevant to the issue of density-dependence, presenting information obtained by a variety of empirical methods applied to a population of Salvia lyrata. The primary aims of the work are 1) to assess the direct effects of density and from these results to infer their implications for population growth, using observations and experiments in the field and 2) to assess the extent of genetic variation for response to density and to infer the potential for density-dependent selection, using quantitative genetic techniques in both garden and field experiments.

The present chapter reports on the demography of a natural population of Salvia lyrata as a basis for comparison with the experimental work to be reported in subsequent chapters. Correlations of variation in life history traits with that of population density are indicated for comparison with previously reported observational studies of density effects.

METHODS

Salvia lyrata (Lamiaceae) is a perennial herb which is broadly distributed between east Texas, north Florida, Connecticut, and Illinois (Gleason and Cronquist, 1963). In the Piedmont of North Carolina, where this study was conducted, it is a common inhabitant of mown fields and roadside verges and is also found, though less frequently, in open woods. The species is apparent throughout the year as a rosette of purple-tinged leaves. In late May and rarely during the summer, the plants produce inflorescences bearing both lavender, chasmogamous and inconspicuous, cleistogamous flowers clustered in pairs of three-flowered cymules which are widely spaced along the stem. Seeds mature for approximately two weeks after anthesis and are then dispersed suddenly from the persistent, dried calyces when these are perturbed by animals or raindrops (Brodie, 1955). Seeds germinate primarily in the following March through early May; this delay in germination appears to be imposed through exogenously enforced dormancy, since the seeds germinate readily in the laboratory at all times.

The study was conducted in a mown field, approximately 40 m x 70 m, on the campus of Duke University. The field is on a gently south-facing slope and is bordered by mixed pine and deciduous woods. The University has maintained the field in its present condition for over 20 years, mowing it approximately every two weeks in the summer. The field supports a diverse assemblage of grasses and forbs, several of whose competitive interactions have been investigated in a nearby field (Fowler and Antonovics, 1981a). The most common species are grasses of the genera *Andropogon*, *Panicum*, and *Paspalum*, and the perennial dicots, *Plantago lanceolata* and *Salvia lyrata*. *Salvia lyrata* is present throughout this field and, in some areas, is the dominant species.

In June of 1979, the positions of 11 permanent plots, 50 cm x 100 cm, were located by choosing random coordinates. Environmental differences among the plots were readily apparent in both physical (e.g., soil moisture, soil texture) and biotic (e.g., shading, Salvia density, presence and density of other species) properties. The minimum distance between two plots was 3.6 m and the maximum distance was 49 m. Using a plotting frame (described in Fowler, 1978) the coordinates within the site, size (number of leaves and length of longest leaf), and flowering condition were recorded for every individual of Salvia lyrata in each plot. Every plant was marked with a toothpick, in order to facilitate identification of individuals in future censuses. Subsequent censuses were made in the same way (newly appearing individuals being recorded) in December, 1980, and then in February, May, and October for each of three years (1981-1983), concluding in May, 1983. The February census was eliminated in 1983. Over the four year period (6/79-6/83), 3248 individuals were observed, with a minimum of 600 at the December, 1980 census.

For certain statistical analyses, counts of total numbers in a plot and numbers surviving in a plot were transformed to logarithms. In these cases, tests compare proportional changes in numbers. Furthermore, in order to satisfy normality assumptions, arcsin square root transformation was used when dealing with proportions. Where comparisons of plots were of interest, replication within plots was obtained by considering the populations of the two 50 cm x 50 cm areas within plots as separate observations.

Spatial autocorrelation analysis (Sokal and Oden, 1978) was used to examine in detail the spatial association of measures of growth within plots. In particular, this method was employed to show whether a negative correlation exists between size changes of near neighbors, as might be

expected if individual growth is negatively density-dependent. The paired coordinates of all the individuals present at the February, 1981 census were used to define for each individual its nearest three neighbors (within 15 cm) and their distances. The reciprocal of the distance between each pair was used to weight the product of their deviations from the mean, because effects of neighbors are expected to decline with distance. The sum of the weighted products is the statistic, Moran's I, which measures the magnitude of the spatial autocorrelation.

All analyses, except for those of total population size and survival of established plants, employ data only from the 1980-1983 period, for which the census record is complete.

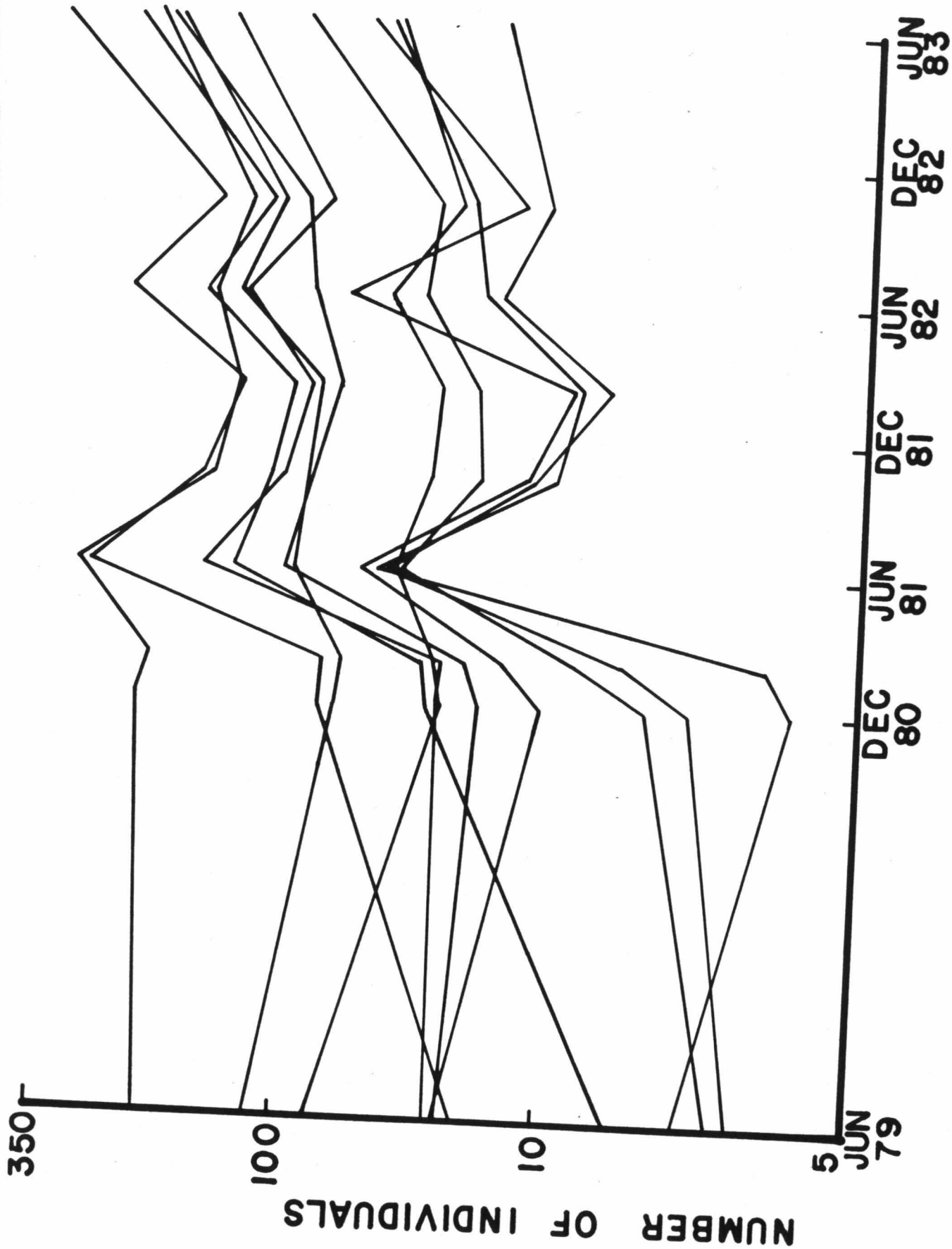
RESULTS

Population Size

Total population size (Fig. 1.1) fluctuated seasonally with peaks in May, as a result of spring recruitment, and depressions in autumn and midwinter. In addition, population sizes increased in ten of the eleven sites between May, 1979 and May, 1981, and in nine of the sites between May, 1982 and May, 1983, suggesting that environmental conditions improved in those intervals. Conversely, eight sites declined in numbers between May, 1981 and May, 1982. The variation in population size among sites is appreciable on all dates. The number of individuals in a .25 m^2 area ranges between 8 and 75 in February, 1982, for example.

There is a weak inverse relationship between population size change and site density suggested by the striking decline of the most dense site between February, 1981 and February, 1982, while most of the remaining sites were increasing. However, the overall ranking of the sites by

Figure 1.1. Population size trajectories for each of the 11 plots over the three year period, 6/79 to 6/82, log scale for number of individuals.


NUMBER OF INDIVIDUALS
350
100
10
5
JUN 79
DEC 80
JUN 81
DEC 81
JUN 82
DEC 82
JUN 83

density changed little over the whole period of observation, June, 1979 to May, 1983, with the exception that two sites, originally ranked seventh and eighth with 38 and 17 Salvia individuals respectively, were the two most populous with 351 and 239 individuals four years later.

Survival

Regressions of log numbers surviving from an initially observed group against time provide an estimate of depletion rate for the population. If the group is a single cohort, then these regressions are survivorship curves (Harper, 1977). The regressions (against time in months) were carried out for two separate groups of individuals: those observed at the second census (12/80) and those which appeared as seedlings in May, 1981 (Figs. 1.2 and 1.3). Thus, these depletion curves pertain to a mixed age group and to a single cohort, respectively. The latter is, therefore, a survivorship curve, in the strict sense. The depletion rate was higher for seedlings (b= -0.07) than for the mixed group (b=-0.02), and thus their half-life, calculated on the basis of the linear regression, was lower (10 and 35 months, respectively). In addition, when the factor, plot, is included in the model, the quadratic term ($time^2$) is positive for seedling mortality (b=.003, improving the R^2 from .865 to .891). For mortality of the mixed age group, the coefficient of the quadratic term is much smaller (b=.0003, increasing R^2 from .9401 to .9408. This suggests that the risk of mortality decreases more for seedlings with age or time of year than for adults; the effect of these factors can not be distinguished, since they are highly correlated in this study.

Regression of numbers of individuals surviving to a given time period against numbers of individuals originally present (untransformed) provides an estimate of the density-independent mortality over a given time period.

Figure 1.2. Depletion curves for the individuals observed at the December, 1980 census, log scale for number of individuals.

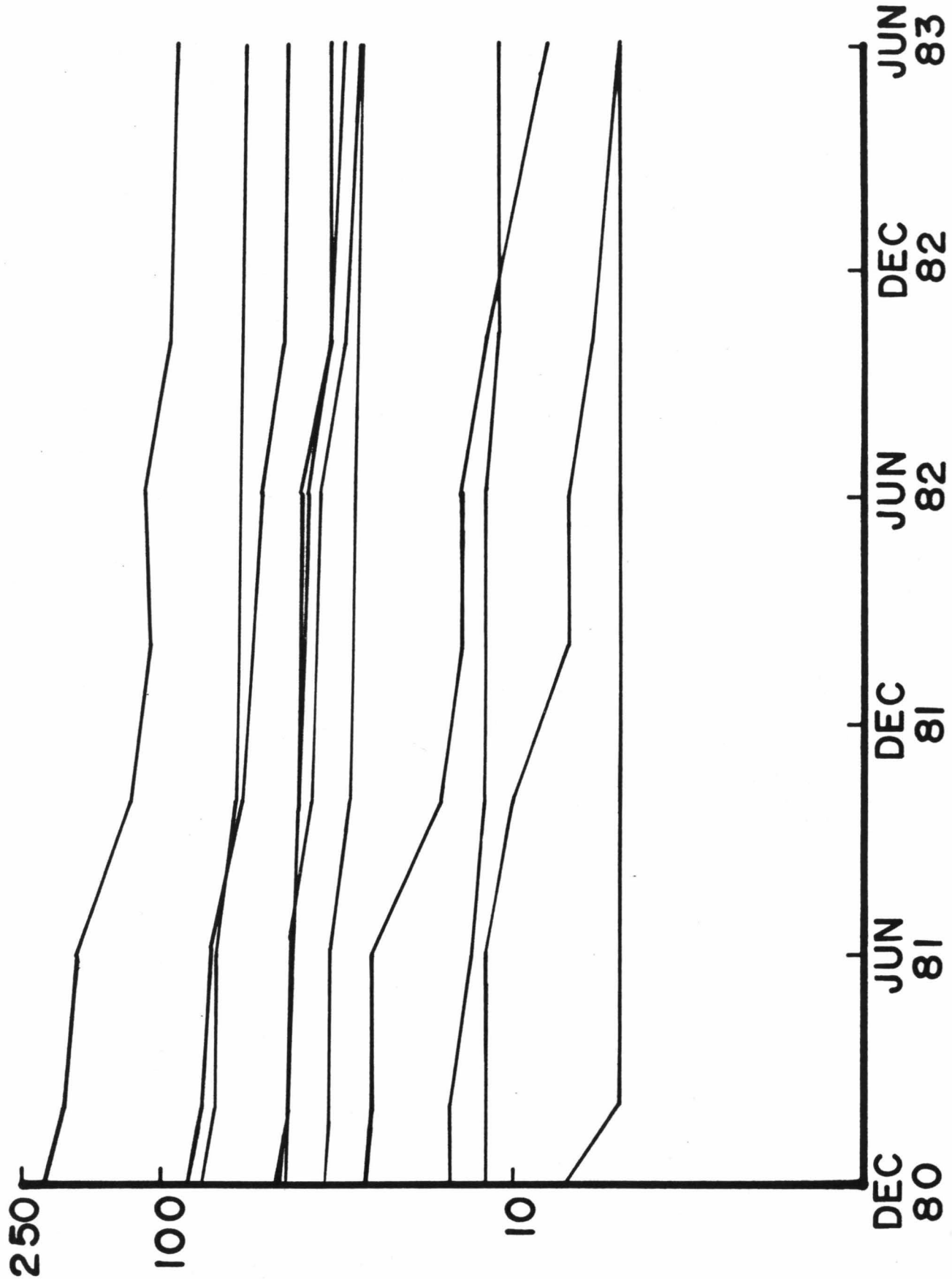
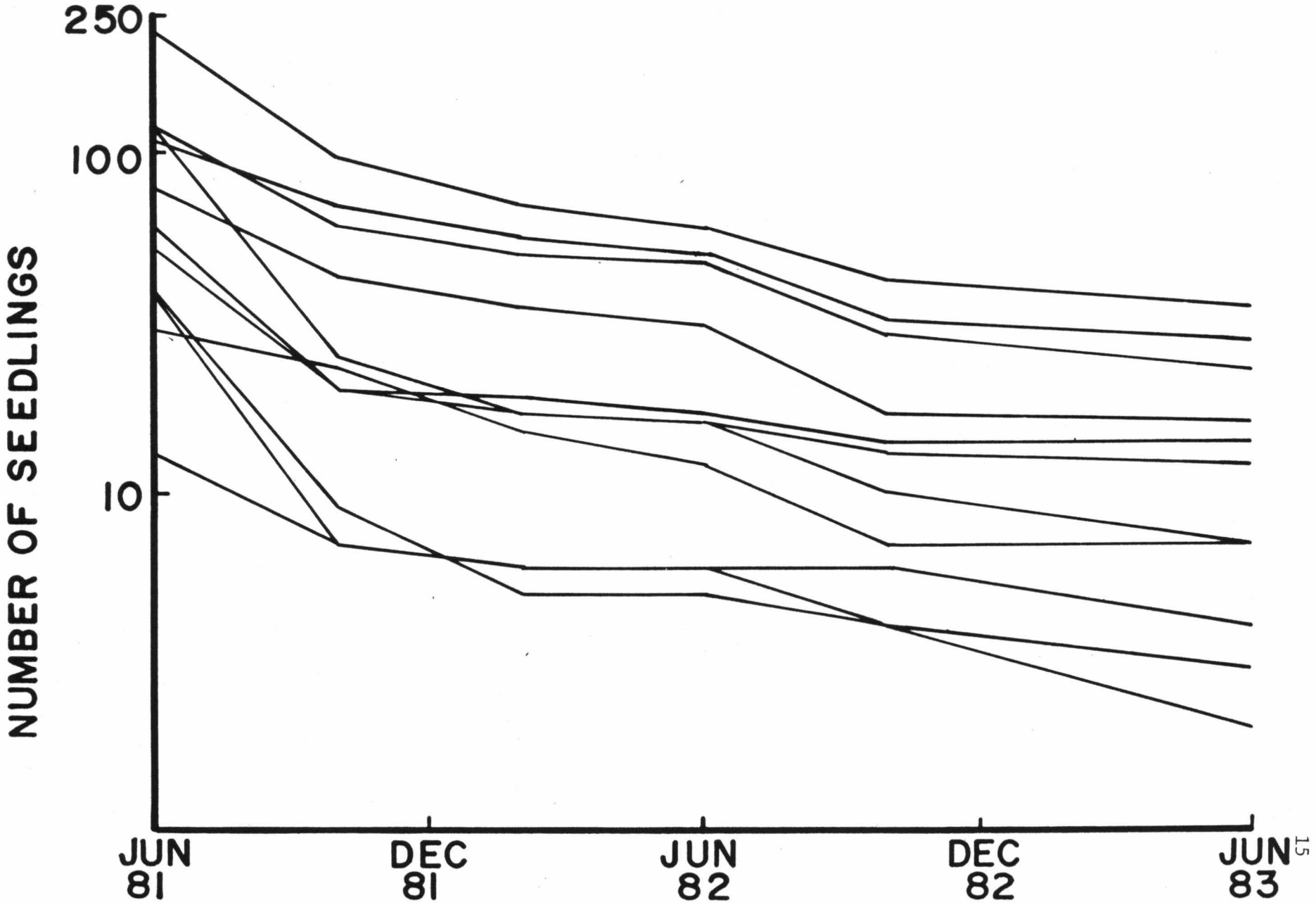

NUMBER OF INDIVIDUALS
250
100
10
DEC 80
JUN 81
DEC 81
JUN 82
DEC 82
JUN 83

Figure 1.3. Survivorship curves for the seedlings which appeared in 1981, log scale for number of seedlings.


250
100
10
NUMBER OF SEEDLINGS
JUN 81
DEC 81
JUN 82
DEC 82
JUN 83

For the 1981 seedling cohort, the estimate of density-independent mortality in the first year is 82%, whereas for the mixed age group, the value of density-independent mortality over the course of the three year period (6/79-6/82) is estimated as 36%; 235 individuals remained from the 608 originally observed. Bellows (1981) has suggested plotting residuals from these regressions against initial numbers (untransformed), as a method of detecting density-dependence in mortality. In the present study, residuals from these regressions for the 1981 cohort and the mixed 1979 group display no relationship with density (Figs. 1.4a,b).

Fecundity

In all sites taken together, 90 plants were observed to flower in 1979, 76 in 1981, 126 in 1982, and 101 in 1983. The variation among plots in the propensity of plants to flower was analysed in the following way. The number of plants flowering in May was expressed as a fraction of the total numbers of individuals present in the previous February. These values (ranging from 0 to 63%, with a mode of approximately 20%) were arcsin square root transformed and then subjected to 2-way analysis of variance with the factors, year and site, and the covariate, density, included in the model. The proportion of plants flowering showed no significant variation among years or among plots, but the proportion of flowering declined significantly with plot density (slope=-.004) (Table 1.1, Figure 1.5).

In May of 1982, more complete flowering data were collected, including the numbers of seeds produced by each plant. For each plot, the average number of seeds produced by plants which flowered was calculated. These values, which ranged from 84 to 140 seeds per plant flowering, showed no

Figure 1.4a. Scatter plot of number of individuals remaining in June, 1982 from an original number present in December, 1980, untransformed.

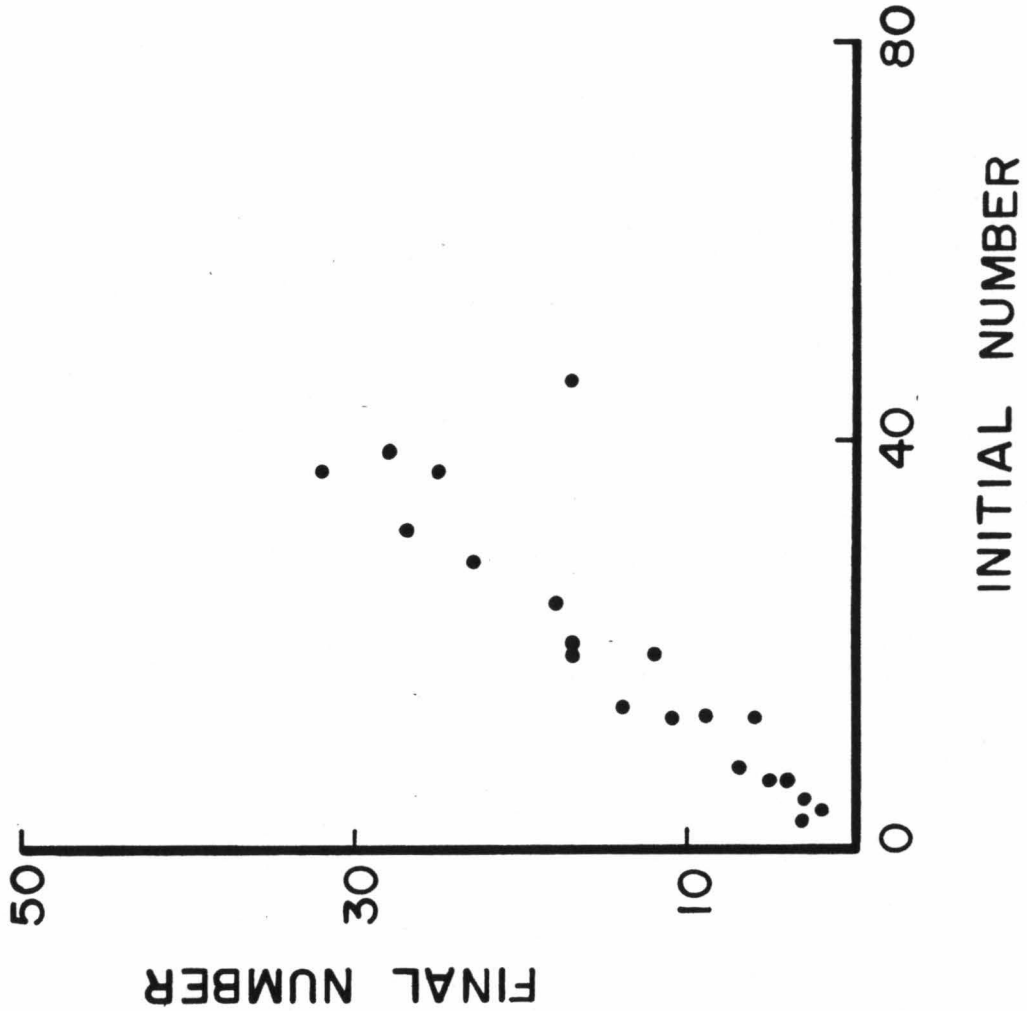

FINAL NUMBER
50
30
10
0
40
80
INITIAL NUMBER

Figure 1.4b. Scatter plot of number of individuals remaining in May, 1982 from an original number present in June, 1982, untransformed.

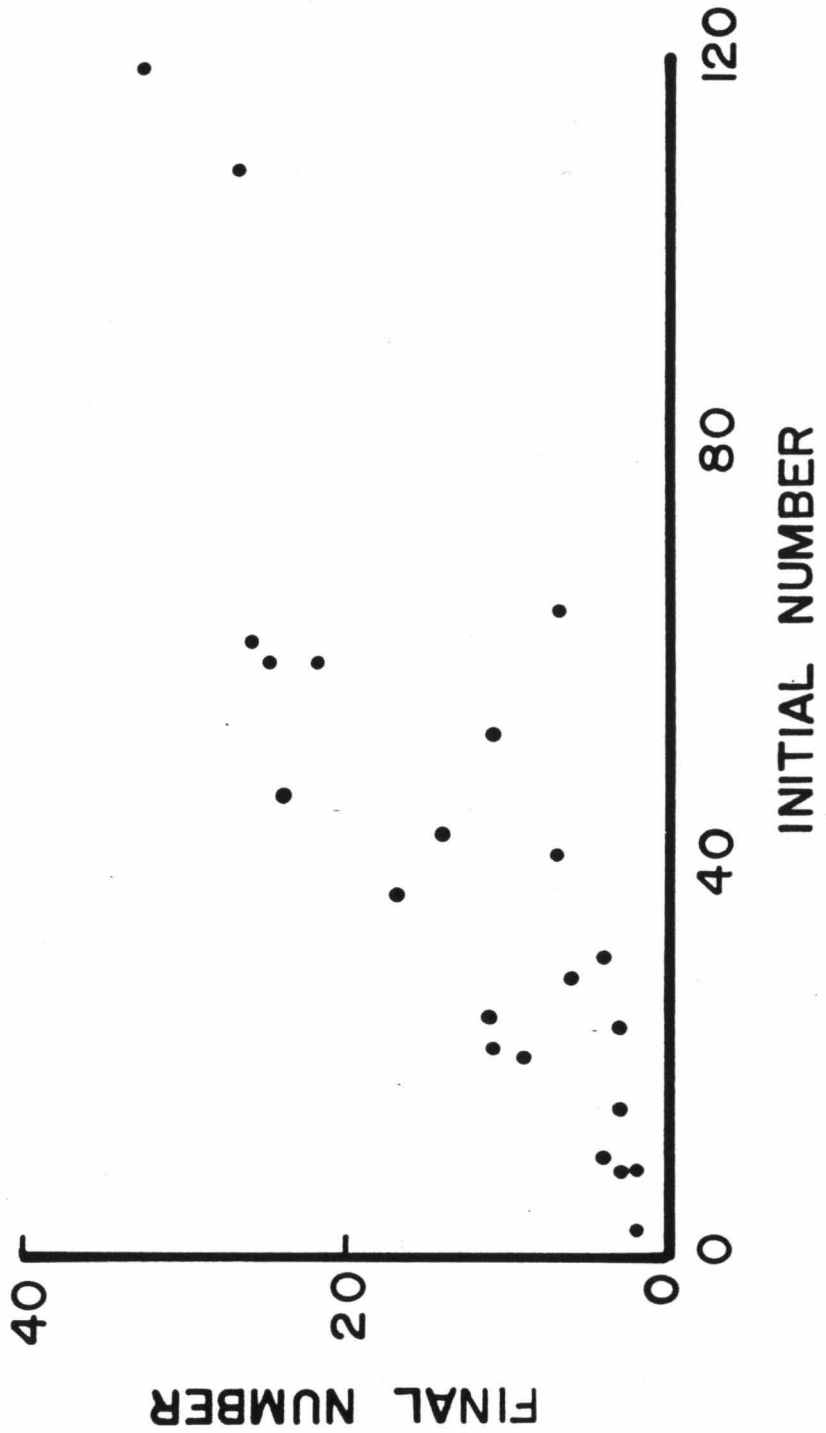

FINAL NUMBER
40
20
0
0
40
80
120
INITIAL NUMBER

Table 1.1. Analysis of variance of (number of plants flowering in May/number of plants present the previous February) for two years (1981, 1982).

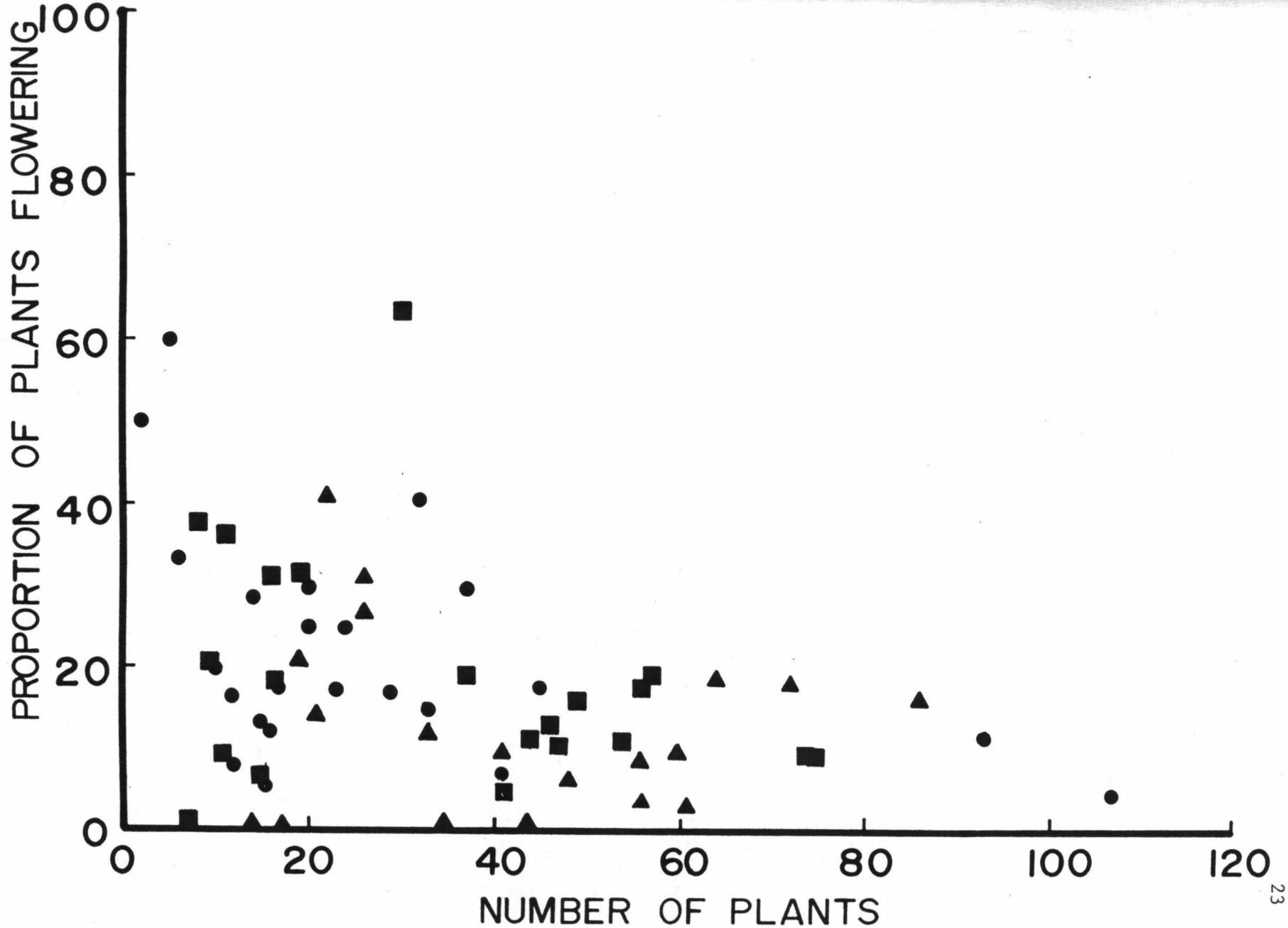
Source	df	SS	F	Pr>F	R^2
Model	12	0.53	1.7	.11	.40
Year	1	0.04	1.5	.23	
Density	1	0.17	6.6	.02	
Plot	10	0.32	1.3	.29	
Error	22	0.79			

Figure 1.5. Scatter plot of proportion of plants flowering in May of those present in February vs. numbers present in February for two years, untransformed. The years are indicated by symbols, as follows:

● 1981

■ 1982

▲ 1983


PROPORTION OF PLANTS FLOWERING
100
80
60
40
20
0
0
20
40
60
80
100
120
NUMBER OF PLANTS

significant variation among plots ($p<.93$), nor any trend with density.

Recruitment

The number of seedlings which emerged (that is, which germinated and bore cotyledons exposed above the soil surface on the dates of the censuses) varied among sites from 13 to 226 in 1981 and from 5 to 125 in 1982. The same plot gave the maximum number in both years. Overall, more seedlings emerged in 1981 (748) than in 1982 (414). This decline in seedling emergence may be due either to the overall reduction in the number of plants in flower the previous year, or to the fact that the spring census was taken several weeks later in the second year; by that time, recruitment had nearly ceased and mortality may have begun to depress seedling numbers.

The numbers of seedlings emerging was divided by the number of plants flowering in the previous year. These values were subjected to 2-way analysis of variance, with year and site and the covariate, density, in the model. There was significant variation between years in the number of seedlings emerging per flowering individual (4.7 in 1982; 10.0 in 1983), but neither of the other factors was significant (Table 1.2), indicating that the number of seedlings per plant flowering is independent of density and other variation among plots.

Growth.

Two non-destructive measures of growth were used, namely, the change in number of leaves and the change in length of longest leaf (i.e., the difference between the measures on two different dates for plants which were present on both dates). Because growth of an individual may be correlated with that of its neighbors (see below), the assumption that each

Table 1.2. Analysis of variance of (number of seedlings emerged/number of plants in flower the previous year for two years (1982,1983).

Source	df	SS	F	Pr>F	R^2
Model	12	825.4	2.0	.066	.44
Year	1	331.4	9.5	.005	
Density	1	0.2	0.01	.94	
Site	10	493.8	1.4	.22	
Error	30	1876.8			

observation is independent would be violated in an analysis of variance which used every individual as a separate observation. For this reason, a statistically conservative approach was taken, in which the growth values were averaged separately to give pairs of growth measures for each plot for a given comparison of dates. Multivariate analysis of variance for the comparison of February, 1981 with February, 1982 shows no significant variation among plots for either growth measure nor for the two considered together ($p<.31$, Wilks' criterion). Furthermore, the relationship of the growth measures with density is only weakly negative ($p<.33$, Wilks' criterion). The mean change in leaf number and mean change in length of longest leaf are positively correlated within sites.

Considering the time interval, February to May, for 1981 and 1982, however, reveals significant variation among sites ($p<.02$) for the change in length of longest leaf. Furthermore, this variation is significantly related to plot density ($b=-0.14$, $p<.0013$, $R^2=.22$). In a univariate analysis, the change in leaf number does not show significant variation among plots or with plot density, but the two growth measures are significantly correlated within plots ($r=.56$, $p<.005$). Thus, these findings suggest that, although change in leaf size from one winter to the next is not influenced by environmental variation among sites nor by density, growth during the spring flush may indeed be affected by environmental variation, including density or factors correlated with density.

The correlations among individuals' growth measures within each of the 11 plots was investigated directly by the method of spatial autocorrelation analysis (Sokal and Oden, 1979). This procedure was carried out separately for each site and for the two growth measures, defined above. Considering growth between the two winter dates (2/81-2/82), there was only one case of

significant correlation for either growth measure: one plot of intermediate density (N=37) showed a significant positive spatial autocorrelation of change in length of longest leaf. This suggests that environmental variation within that plot causes neighbors' sizes to change in the same direction, rather than in opposition to one another, as one might expect if competition among individuals were a major factor determining the magnitude of individuals' size changes. For the remaining plots, the test statistic for spatial autocorrelation ranged over negative and positive values; no trends with plot density were apparent.

In an additional set of analyses, the differences in number of leaves and in length of longest leaf were divided by initial leaf number and length, respectively, to give a measure of proportional change in the two measures. The results of the spatial autocorrelation of these values did not differ from those in the first group. Considering further the period of growth in the spring (2/81-5/81), a single plot (but a different one from before) revealed significant positive spatial autocorrelation of both growth measures. Otherwise no significant associations and no trends are revealed by this analysis.

Interrelationships of individual plant measures

The demographic data provided insight into the relationships among flowering, size, growth, and survival in the natural population. Larger plants were more likely to flower and to survive ($p<.0001$, both size measures; Kruskal-Wallis test). The mean size in winter of plants which flowered the following year was 8.9 leaves, 42.8 mm compared to that of non-flowering plants with 5.4 leaves, 24 mm. The mean size of those which survived to the following spring was 6.7 leaves, 31.4 mm; those which died in that interval averaged 5 leaves, 20.7 mm. These relationships held

true, regardless of whether the smallest size classes (number of leaves<4; length of longest leaf<20) were included. Furthermore, those plants which flowered in May, 1981, were, on average, still the largest plants the following February (number of leaves = 6.7, length of leaf = 33.5 mm vs. 4.8 leaves, 21.3 mm; $p<.0001$, both size measures, Kruskal-Wallis test). In spite of remaining the dominant plants in absolute size, those that flowered had an average of 1.5 fewer leaves, the longest of which was an average of 3.5 mm shorter at the end of the period, 2/81-2/82, ($p<.0004$, Kruskal-Wallis test), whereas those which failed to flower increased in both measures (+0.6 leaves, +4.6 mm). Nevertheless, plants which flowered one year were more likely to flower in a subsequent year than those which failed to flower (6/79-5/81, $G=4.96$, $p<.05$; 5/81-6/82, $G=7.07$, $p<.01$, G-test) (Table 1.3). Flowering individuals suffered no increased risk of mortality in 1979 ($G=.11$, ns); however, in 1981, flowering individuals showed lower incidence of mortality than expected (Table 1.4) (5/81-6/82, $G=3.98$, $p<.1$, G-test).

DISCUSSION

The demographic and life history characteristics of the population of Salvia lyrata, detailed above, resemble those previously determined for other herbaceous perennials. The following encapsulates the salient features, while drawing comparisons with demographies known for other species.

Superimposed on a relatively constant total population size are seasonal fluctuations which are the result of predominantly spring recruitment and summer mortality. Estimated population decay rates for established individuals indicate a half-life of approximately three years, which is somewhat longer than that of Anthoxanthum odoratum growing on mine

Table 1.3. Frequency table of flowering in one year vs. flowering in a subsequent year. In each cell observed values are above, expected values below.

		Flower in 1981				Flower in 1982	
		No	Yes			No	Yes
Flower in 1979	No	194 187	64 71	Flower in 1981	No	231 221	77 87
	Yes	33 41	22 15		Yes	49 59	33 23

Table 1.4. Frequency table of flowering in one year vs. survival to a subsequent year. Arrangement as in Table 5.

		Survive to 1981				Survive to 1982	
		No	Yes			No	Yes
Flower in 1979	No	12	258	Flower in 1981	No	111	308
		12	258			104	315
	Yes	2	55		Yes	17	82
		2	54			24	75

spoils (2 years, Antonovics, 1972) and within the range of Ranunculus acris populations (ca. 2 years and 4 years; Sarukhan and Harper, 1973). These grassland species show much higher turnover rates than woodland herbs, such as Narcissus pseudonarcissus (12-18 yr; Barkham, 1980) and Chamaelirium luteum (30-80 yr; Meagher, 1982). The survivorship curve for Salvia lyrata appears to assume the form of Deevy's Type III curve, with risk of mortality higher for seedlings than for older individuals. Similar survivorship curves have been noted for both grassland species mentioned above.

As a consequence of the high risk of seedling mortality, few new individuals are recruited into the flowering component of the population, and these only several years after their appearance as seedlings. No plant which was observed as a seedling flowered during the course of the study, nor did a large fraction of the total population flower in any year. Those that did flower and survived to the following spring appeared to persist as dominant individuals and flowered in subsequent years. There was, in one year, a higher chance of mortality or significant decreases in size for those plants that flowered compared with those that did not. In a study of longer duration, Antonovics (1972) similarly observed that a sizable fraction of the Anthoxanthum odoratum individuals growing on mine spoils persisted in the population for several years, failing to flower before their death.

The visible environmental heterogeneity of the field is reflected in the variation in local Salvia abundance. For most sites, the population sizes were approximately constant for the duration of the study; however two sites showed striking increases in the period. The constancy of numbers observed in the majority of cases is generated by the approximate

equivalence of recruitment and mortality rates within plots. Moreover, these rates of influx and loss of individuals show little variation among plots. This contrasts with the finding of Fowler and Antonovics (1981b) that survivorship of transplanted seedlings of S. lyrata and of Plantago lanceolata varied significantly over small distances. Apparently, the differences in abundance of S. lyrata observed in the present study were generated in the past as a consequence of intrinsic ecological differences among the site locations. Due to the persistence of the ecological differences, the spatial variation in density of S. lyrata is maintained by roughly equal proportional fluxes, as a consequence of uniform proportional birth and death rates. The two exceptions cited above may exemplify populations the quality of whose locations has improved (or which have recently been colonized) and which now experience positive rates of increase. This must remain an untested hypothesis, since the history of the plots on a fine-scale is not known, and since isolation and measurement of the discriminating ecological variables is likely to be exceedingly difficult.

The present study has revealed some circumstantial evidence of density-dependent regulation operating in this population. Trends toward reduction in proportional survival, in proportion of plants flowering, and in average individual growth with increasing density suggest a tendency of population growth rate to decline with density. Comparable investigations have shown density inhibition in dry weight per bulb of Narcissus pseudonarcissus (Barkham, 1980) and in life expectancy of ramets of Ranunculus repens (Sarukhan and Harper, 1973). Demographic studies of annuals have shown reduction in fecundity with density in Salicornia europaea (Jefferies, et al., 1981) and, in conjunction with field experiments, in Vulpia fasciculata (Watkinson and Harper, 1979) and

Diamorpha smallii (Clay and Shaw, 1981). Other investigators have discerned no density effects in Viola species (Waller, 1981), Maianthemum canadense (Silva, et al., 1982) and Melilotus alba (Klemow and Reynal, 1981). Circumstantial evidence for density-dependent mortality in S. lyrata was obtained by Fowler and Antonovics (1981b) in the study mentioned above. Seedlings transplanted into areas of high natural density had significantly higher mortality than those planted into sites at low density. As these authors noted, because local density and site locations were confounded, however, the direct effect of density remains unproven.

The present study produced conflicting findings with regard to the effect of density on individual growth. If local density, i.e., crowding by conspecific neighbors, is responsible for differences in growth among plots, as suggested by the regression of mean growth in a plot against density of the plot, this effect should be further manifested in negative spatial aurocorrelation of growth within plots. Such a finding would imply that individuals grow at the expense of their neighbors' growth. No evidence for this effect was found; instead, a few instances of positive spatial autocorrelation suggested that fine scale environmental variation has stronger effects on individual growth than does growth of neighbors.

Waller (1981) suggested several explanations for his failure to detect evidence of density effects in a demographic study of natural populations of Viola spp.: 1) lack of competition in the natural populations, 2) environmental heterogeneity (spatial or temporal), and 3) use of unreliable statistical methods (in this case, regression of number of leaves on measures of numbers, sizes, and dispersion of neighbors in observational data). But purely observational demographic methods, regardless of statistical methods, are inherently unreliable, due to their failure to

deconfound effects of density from those of other variables contributing to spatial and temporal heterogeneity in a natural population. Use of experimental alteration of field densities will lead to resolution of the questions of whether, how, and when density effects are manifested in nature. Experimental work devoted to discerning effects of density in the present population of Salvia lyrata will be reported in the subsequent chapters.

The descriptive demography of a population of Salvia lyrata has revealed that it has a life history typical of several herbaceous perennials studied previously. The population numbers are relatively constant as a consequence of the balance between recruitment and mortality. The survivorship curve is concave (Deevey's Type III), and individuals flower only several years after their recruitment. Some of the evidence is suggestive of the operation of density effects regulating the population.

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CHAPTER II. EXPERIMENTAL ALTERATION OF SEED DENSITIES

INTRODUCTION

A dependence of population growth on density is recognized as an essential element of population regulation (Murdoch 1970). Descriptive studies of natural populations (reviewed in Antonovics & Levin 1980 and Chapter I) provide only circumstantial evidence of density effects, because they fail to deconfound local density and other environmental characteristics. Only experimentation in natural populations can indicate the frequency and intensity of density effects in nature, from which predictions of the consequences of density responses can be made. Yet, despite the importance of the concept of density-dependence in understanding population regulation (see e.g., Roughgarden 1979), few studies of natural populations document direct effects of density (reviewed by Antonovics & Levin 1980). Watkinson & Harper (1978) estimated effects of density throughout the life cycle of a winter annual grass, Vulpia fasciculata, and developed a model of its population dynamics. Clay and Shaw (1981) documented density-dependent reproduction in Diamorpha smallii and used a similar model to investigate its consequences.

The present chapter reports a series of experiments designed to detect and quantify effects of conspecific density on seedling emergence and survival in the population of Salvia lyrata (Radford, et al. 1968) whose

characteristics were described in Chapter I. These experiments address the following questions:

1) Is seedling success -- either emergence, survival, or growth -- affected by seed and seedling density?

If so:

2) Over what range of densities are these effects perceptible?

3) Do these responses vary spatially or temporally within the population?

4) Do plants of different ages or sizes differ in their reponse to density?

5) Do plants of different sizes elicit different responses from their neighbors?

MATERIALS AND METHODS

The experiments were conducted in a mown field on the campus of Duke University (Chapter I). The field is inhabited by an association of numerous grass species and forbs, including Salvia, and is similar to a nearby field studied by Fowler (1978). For the present experiments, collections of naturally maturing seeds were made in June, 1980 and 1981, each seed lot being sown back in the year it was collected.

In June, 1980, forty-eight 30 cm x 30 cm plots were laid out in each of two sites in the field. One site was on a gentle slope in an open area of the field; the other was ca. 20 m distant, at a lower elevation and under four white pines. The plots were allotted to four blocks in each site, and within each block, three factors were varied in a crossed design: 1) Salvia which were already established were either removed or not, 2)

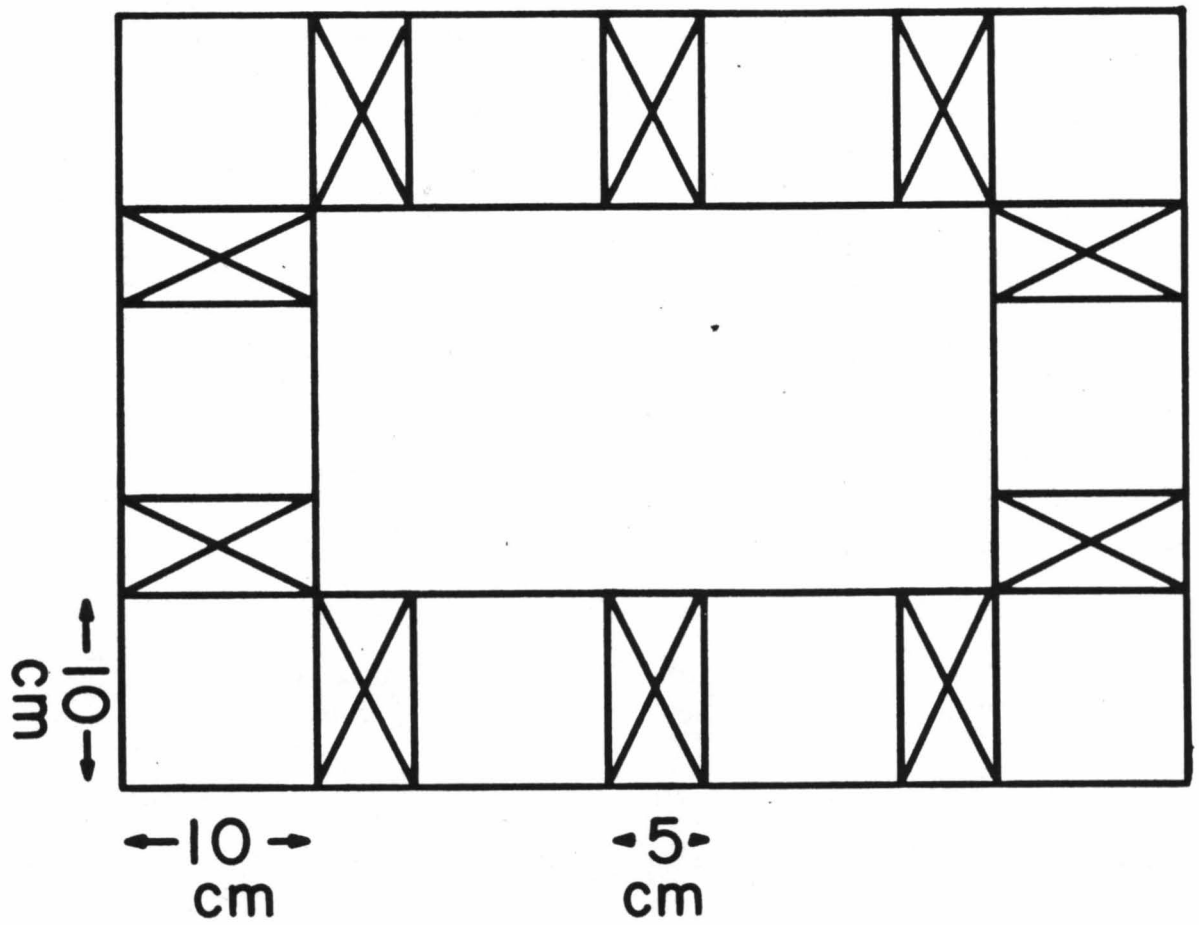
seeds were applied at three sowing densities, 0, 100, 500 seeds (by weight) per plot, 3) seedlings were either removed or not as they appeared. The twelve combinations of these factors were randomly applied within blocks. Because insufficient seed was available, the seedling removal treatment was eliminated at the highest sowing density in the second site. The plots were monitored every two weeks for germination and survival. Individuals which appeared were marked with a toothpick, and each toothpick was removed when the seedling it marked had died.

In June, 1981, inflorescences of adult Salvia occupying the sites were clipped to minimize natural seed input. The following October, the treatments were renewed as follows: pairs of adjacent blocks were aggregated such that there were twenty-four plots in each block. In the twelve plots where seedlings were absent due to the seedling removal treatment, seeds were sown at 50, 200, and 500 seeds per plot onto the plots which had received 0, 100, and 500 seeds, respectively, in the previous year. In the remaining plots, addition of either no seeds or 200 seeds was factorially crossed with the seed sowing densities of the previous year. The plots continued to be monitored for emergence and survival, as before. Analysis was based on counts of seedlings emerging and of seedlings which died, summed from June, 1980 to May, 1981, from May 1981 to November, 1981, and from March, 1982 to November, 1982.

In the second seed germination experiment, forty plots of 10 cm x 10 cm were established in the field in four adjacent rectangular blocks. Spaces 5 cm wide were left as guard areas between the plots (Fig. 2.1). All four blocks occupied a total area less than 1.5 m^2. Seed lots were made up with the following seed quantities: 30, 60, 120, 240, 480, 720, 960, 1440 (the latter six, by weight). The density treatments, including two control plots in which no seeds were sown, were assigned in four randomized blocks,

Figure 2.1. Plan for one replicate of Experiment 2. The dimensions are indicated in the diagram.

EXPERIMENTAL DESIGN



TREATMENTS

0 seeds

30

60

120

240

480

720

960

1440

and the seeds were scattered within each plot in October, 1981. In March, 1982, when emergence was beginning in Experiment 1, the newly emerged seedlings were counted and, in order to ensure accurate counts, were marked with a small dot of acrylic paint on each cotyledon. This procedure was carried out every two weeks in the spring and thereafter every six weeks until November. In addition, the individuals surviving from prior emergence were counted every four weeks through June and every twelve weeks thereafter until November. Analysis was based on counts of seedlings emerged and surviving, summed from March, 1982 to November, 1982, and recorded in April, 1983.

In May, 1983, up to sixteen individuals were chosen in each plot in a rectangular grid of approximately 3 cm spacing; their sizes (number of leaves and length of longest leaf) were recorded, and each was marked with a toothpick and ringed with plastic coated wire to identify them for subsequent measurement in August and October, 1982, and April, 1983.

The statistical analyses were carried out using the Statistical Analysis System (Helwig & Council 1979). All percent values were transformed (arcsin square root). Other transformations are noted where they were necessary. Profile analysis (Timm 1975) of seedling size trajectories provided multivariate tests of variation among groups in growth and size.

RESULTS

EXPERIMENT 1: In both years, seedling emergence began the second week in March and peaked in mid-April; by early June, about 95% of the year's total seedlings had appeared. The mean number of seedlings which emerged from

plots in which no seeds were sown were 57 (Site 1) and 52 (Site 2) in 1981 and 16 (Site 1) and 21 (Site 2) in 1982. These figures estimate the emergence from natural sowing in the previous year and from seed buried in prior years.

The total number of seedlings emerging through September, 1981, varied significantly among blocks within sites and among seed density treatments (Table 2.1). Moreover, significantly more seedlings emerged in plots from which resident Salvia had been removed prior to seed sowing (105 vs. 88 seedlings). This implies that the presence of adults reduces seedling emergence and early survival or that removal of adults created physically favorable sites. Removal of seedlings marginally reduced total emergence (91 vs. 102 seedlings), suggesting that the presence of seedlings enhances the probability of seedling emergence or survival to the time of the next census. Beyond these main effects, there was a significant two way interaction between the factors, removal of adults and seed density, such that adult removal enhanced emergence more at higher sowing densities (Table 2.1b).

Seedling mortality through November, 1981, varied significantly among sites (68% in the sunny vs. 48% in the shaded site). The number of seeds sown did not significantly affect the proportional mortality, but there was a very weak negative relationship with number of seedlings emerging (slope= -.000015, $p<.97$). There was significantly greater mortality in plots where Salvia was not removed prior to seed sowing (64% vs. 48%) (Table 2.2). Similarly, in 1982, mortality of individuals which had appeared and survived through August of the previous year was significantly increased only by the presence of resident Salvia (Table 2.3, 39% vs. 55%). Thus, as above, the principal effect of conspecifics was reduction of seedling survival by the presence of established individuals.

Table 2.1a. Analysis of variance of total numbers of seedlings emerging in 1980, Experiment 1. Type IV Sums of Squares are given in this and all the following tables.

Source	df	SS	F	p
Model	23	272557	12.6	.0001
Error	70	65813		
Site	1	6	0.01	ns
Block (Site)	6	69466	12.3	.0001
Removal of adults	1	9715	10.3	.002
Removal of seedlings	1	2702	2.9	.09
# Seeds (1980)	2	138141	73.5	.0001
Removal of adults x # Seeds	2	14982	8.0	.0008
Removal of seedlings x # Seeds	2	964	0.5	ns
Removal of adults x Removal of sdlings	1	659	0.7	ns

Means:	Site	1	98.2
		2	95.0
	Removal of adults	Yes	104.8
		No	88.4
	Number of seeds	0	58.4
		100	70.0
		500	161.4

Table 2.1b. Cell means for total emergence, Experiment 1.

Seedlings:		Removed		Not removed	
Adult *Salvia*:		Removed	Not removed	Removed	Not removed
# seeds	0	49	71	70	55
1980	100	70	63	79	74
	500	185	124	204	151

Table 2.2. Analysis of variance of percent of seedlings which died in the first year, Experiment 1.

Source	df	SS	F	p
Model	10	.54	3.1	.01
Error	35	.61		
Site	1	.31	23.5	.0001
Block(Site)	6	.08	0.7	ns
Removal of adults	1	.23	13.3	.005
# Seeds (1980)	2	.07	2.1	ns

Means:			
	Site	1	.640
		2	.481
	Removal of adults	Yes	.484
		No	.635
	Number of seeds	0	.582
		100	.598
		500	.472

Table 2.3. Analysis of variance of percent mortality of the previous year's seedlings in the second year of Experiment 1.

Source	df	SS	F	p
Model	7	0.5	1.9	.09
Error	45	1.7		
Site	1	0.09	3.3	ns
Block(Site)	2	0.06	0.7	ns
Removal of adults	1	0.25	6.5	.014
# Seeds (1981)	2	0.08	1.1	ns
# Seedlings (1981)	1	0.01	0.3	ns

Means:	Site	1	.532
		2	.487
	Removal of adults	Yes	.395
		No	.552
	Number of Seeds	0	.446
		200	.538

Slope on Number of seedlings remaining 8/81= -0.0006

Among the seedlings which emerged in 1982 where seedlings from the previous year were present, mortality was significantly higher in plots sown with more seeds (36% (0 seeds), 54% (200 seeds)). This density-dependent mortality may have been induced by the presence of the prior year's seedlings; alternatively, the difference between years may reflect variation in density response attributable to extrinsic factors (e.g., soil moisture, presence of pathogens). As in the previous year, the presence of resident Salvia significantly increased mortality (Table 2.4, 32% vs. 44%). Mortality of new seedlings was not significantly affected by the number of previous year's seedlings that were present; the slope of the regression was negative but small (-0.0004, $p<.76$). In the plots where the previous year's seedlings were removed, none of the factors significantly affected survival of new seedlings, but the trends were similar to those established above (Table 2.4b).

EXPERIMENT 2: The number of seedlings which emerged from plots in which 0 seeds were sown, ranged from 3-5 in the first year, and from 1-7 in the second spring. When calculated on equivalent areas, these values are comparable to those recorded in Experiment 1. The average numbers emerging in plots where 1440 seeds were sown was 347, or about 1/4 of the number sown.

Nonlinear regression provides a test for the null hypothesis that constant proportions of individuals germinated and emerged over the range of experimentally imposed initial densities. The model was

$$N_{out} = a(N_{in} + N_{res})^{b},$$

where N_{out} =total number of seedlings emerged in a plot and N_{in} =number of seeds sown are known values, and a, N_{res} (=number of seeds residing in the

Table 2.4. Analysis of variance of percent mortality of the second year's seedlings (1982), Experiment 1, a) in plots where the previous year's seedlings remained, b) in plots where the previous year's seedlings had been removed.

a)

Source	df	SS	F	p
Model	7	0.70	2.8	.018
Error	47	1.70		
Site	1	0.14	62.0	.0001
Block(Site)	2	0.00	0.05	ns
# Seedlings (1981)	1	0.01	0.2	ns
Removal of adults	1	0.14	3.8	.06
# Seeds (1981)	2	0.47	6.5	.003

Means:			
	Site	1	.366
		2	.491
	Removal of adults	Yes	.325
		No	.437
	Number of seeds	0	.361
		200	.537

Slope on Number of seedlings remaining, 8/81= -0.0004

b)

Source	df	SS	F	p
Site	1	.114	2.42	ns
Block(Site)	2	.094	1.13	ns
Removal of adults	1	.103	2.49	ns
# Seeds (1981)	2	.131	1.58	ns

Means:			
	Site	1	.327
		2	.443
	Removal of adults	Yes	.335
		No	.434
	Number of seeds	50	.342
		100	.327
		500	.487

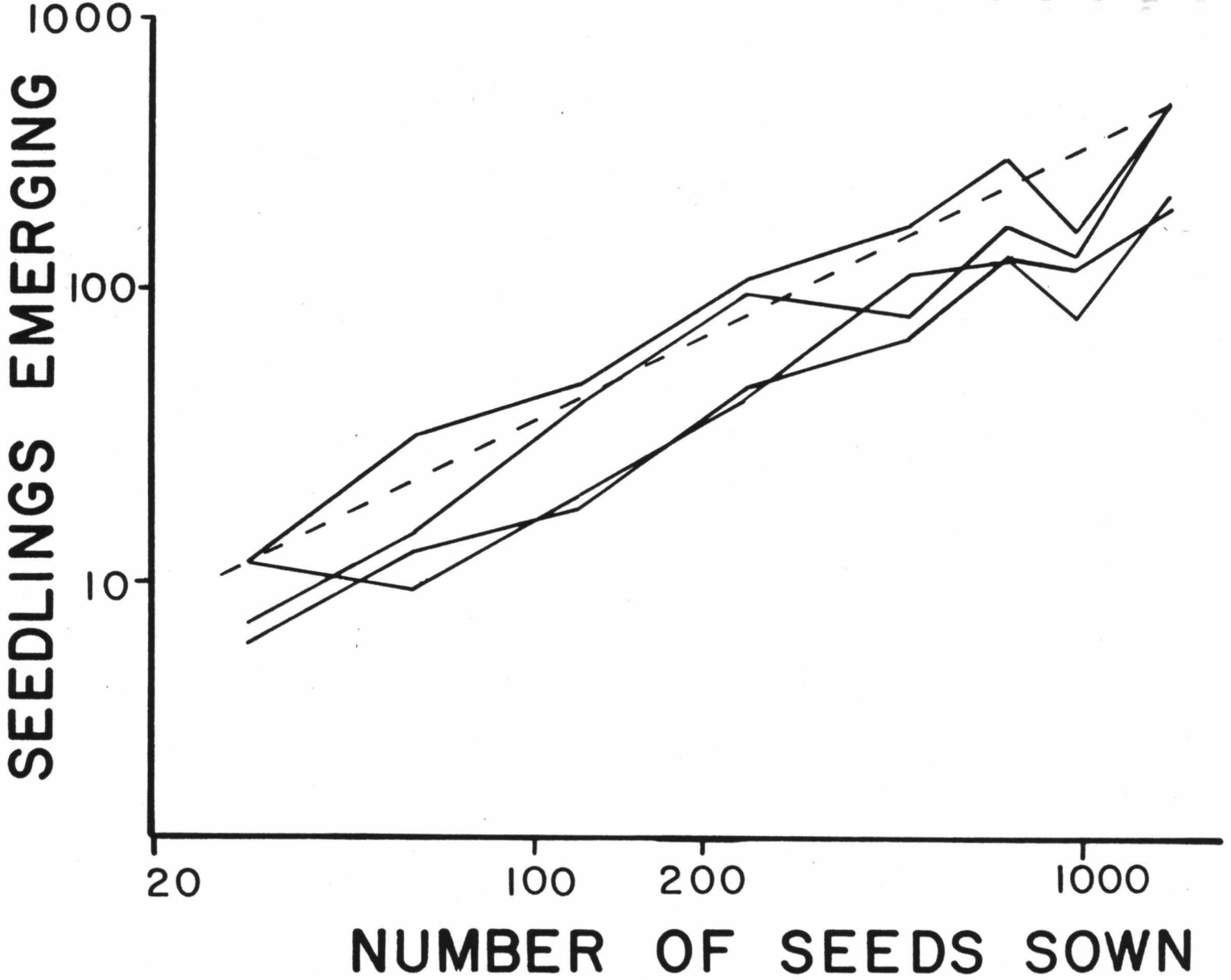
plot from natural dispersal), and b are parameters to be estimated. This model can not be transformed to one suited to the application of linear regression. Estimates of b significantly less than 1 refute the null hypothesis, implying that germination and early survival are negatively density-dependent. Nonlinear regression of total number of seedlings emerged (March through October, 1982) against the number of seeds sown reveals that seedling emergence is proportional to initial seed density throughout the range of experimental densities (slope=1.2, 95% CI=.40, 2.02). The estimate of numbers of resident seed is 91 (95% CI=-132,315). The confidence intervals of the estimates of both these parameters are large, because the estimation spaces for the three parameters are not orthogonal; a change in one in the iterative fitting of the model can be compensated by a change in the others. There is significant variation among blocks in numbers emerging (ANOVA, $p<.0001$) but the responses to density are similar in each block (Fig. 2.2). In the second year, with no additional seed application, there was, similarly, no evidence of density-dependent emergence (slope=.84, 95% CI=-2.2,3.9); an average of 3.9 seedlings emerged from plots where 0 seeds were sown, 47 where 1440 seeds were sown.

A similar model,

$$N_{surv} = aN_{germ}^{b},$$

where N_{surv} =numbers surviving and N_{germ} =totals numbers emerged, was log transformed and fitted by linear regression to test the hypothesis that constant proportions of individuals survived over the range of seedling densities. The transformed model was used in this case, because its residuals showed no trend whereas those of the untransformed model increased with the predicted value. The slope of the log regression of

Figure 2.2. Graph of the number of seedlings which emerged in Experiment 2 against the number of seeds sown (log scales). The solid lines indicate the observed values, by block; the dashed line has slope=1, corresponding to the null hypothesis.


1000
100
10
SEEDLINGS EMERGING
20
100
200
1000
NUMBER OF SEEDS SOWN

seedlings surviving in October, 1982, on total number of seedlings observed is significantly less than 1 (b=.71, 95% CI=.56,.87), indicating that seedling survival is reduced at high density. The response of mortality to density varies among blocks (block x density, $\underline{P}<.026$)(Fig. 2.3a). By April, 1983, proportional survival reflected a stronger dependence on seedling density (b=.57, 95% CI=.39,.74) (Fig. 2.3b). This relationship was, however, uniform over blocks (block x density, $\underline{P}<.6$). Inspection of Figure 3.3b reveals that the relationship of log (numbers surviving) to log (initial density) is not linear. Mortality was so severe in the four highest densities that nearly half of those plots had fewer survivors than plots in the same block at lower densities.

The changes in seedling size (log(number of leaves x length of leaf)) from May, 1982 through October, 1982, reflect suppression of growth at high seedling densities (Fig. 2.4) Profile analysis indicates significant differences in growth rates among seed densities (parallelism test, Table 5b. seed sowing treatment effect, $p<.0001$). Regression analysis reveals a significant negative relationship between size in October, 1982 and initial seed density (both log transformed, b=-.33, 95% CI=-.46,-.20; Fig. 2.5a), as do the Scheffé confidence intervals of the means for each treatment (Fig. 2.4). In general, neither growth nor absolute size varied significantly among blocks, and there was no block x density interaction (Table 2.5a,b). There was a significantly negative error correlation between growth in successive intervals ($r_{12}=-0.32$, $p<.0004$ and $r_{23}=-0.21$, $p<.025$), indicating that individuals which grew more in one interval were likely to increase proportionally less in the second interval.

Figure 2.3a. Graph of the number of seedlings which survived until October, 1982 against the number of seeds sown (log scales). Each panel represents a different block; solid lines are the fitted regression lines, and dashed lines have slope=1, as in Fig. 2.2.

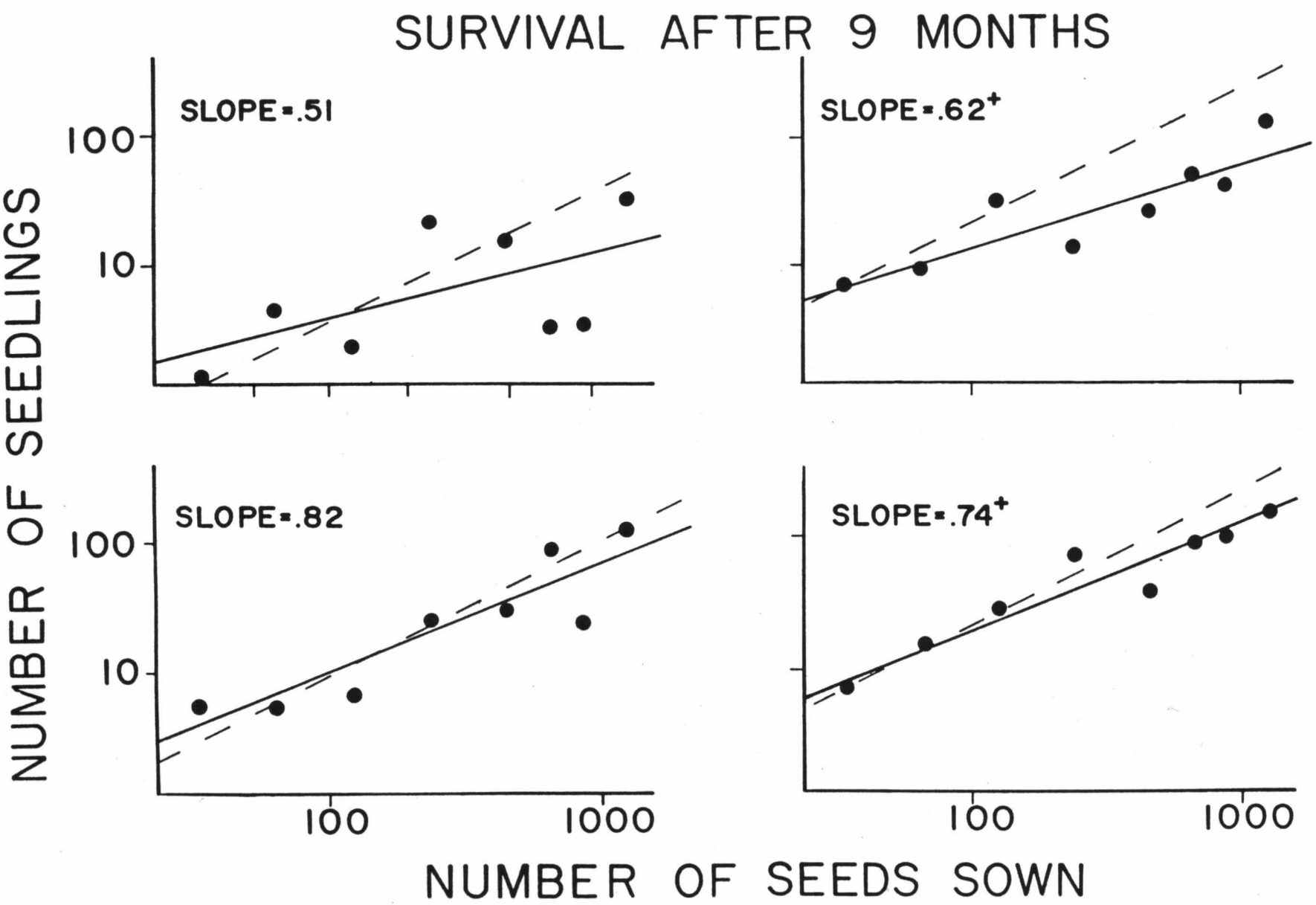

SURVIVAL AFTER 9 MONTHS
SLOPE=.51
SLOPE=.62+
SLOPE=.82
SLOPE=.74+
100
10
100
1000
NUMBER OF SEEDLINGS
NUMBER OF SEEDS SOWN

Figure 2.3b. The number of seedlings which survived until April, 1983 against the number of seeds sown.

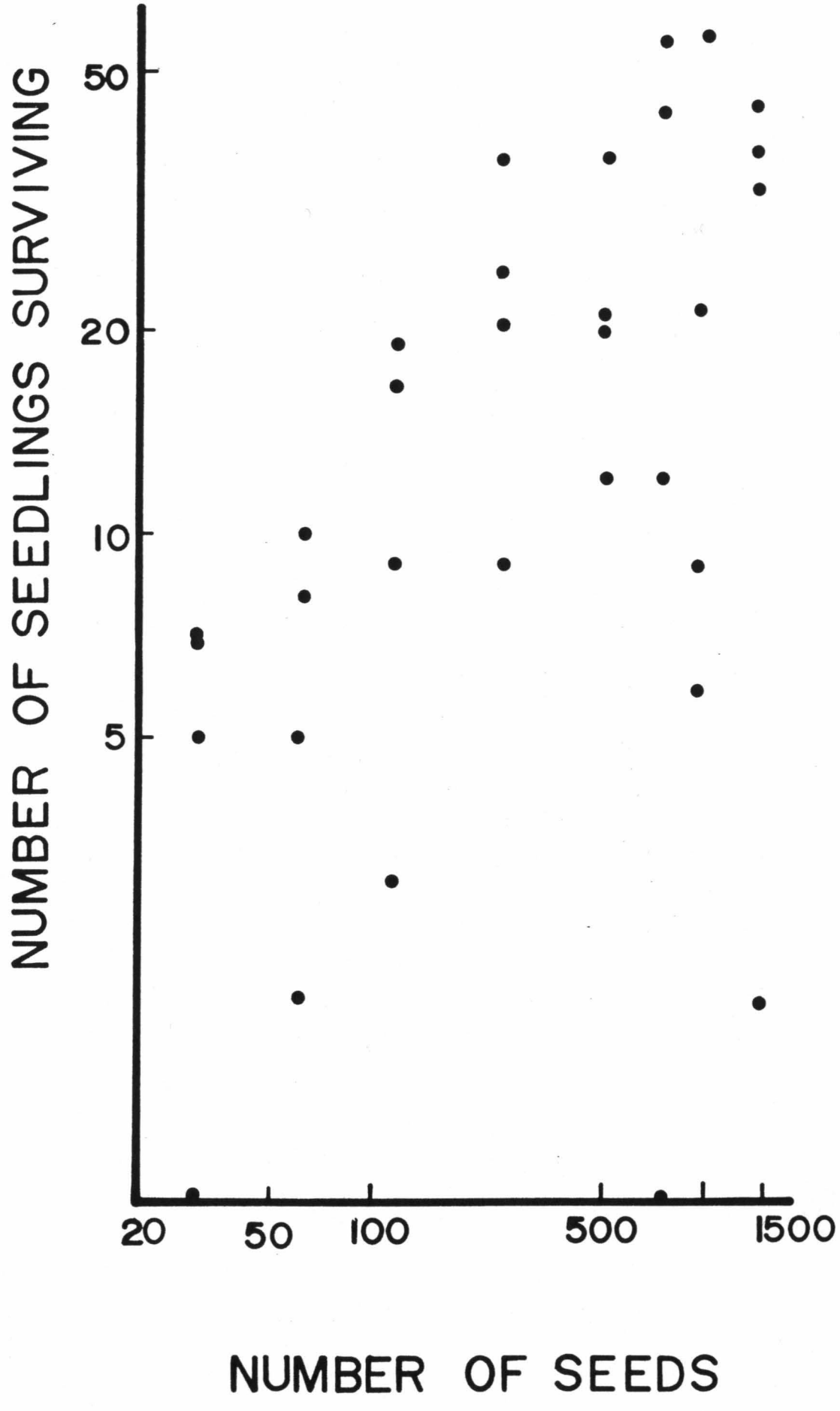

NUMBER OF SEEDLINGS SURVIVING
50
20
10
5
20
50
100
500
1500
NUMBER OF SEEDS

Figure 2.4. Graph of the size of the seedlings measured in Experiment 2 (log scale) against time. The vertical bars indicate homogeneous groups according to the Bonferroni simultaneous comparison procedure.

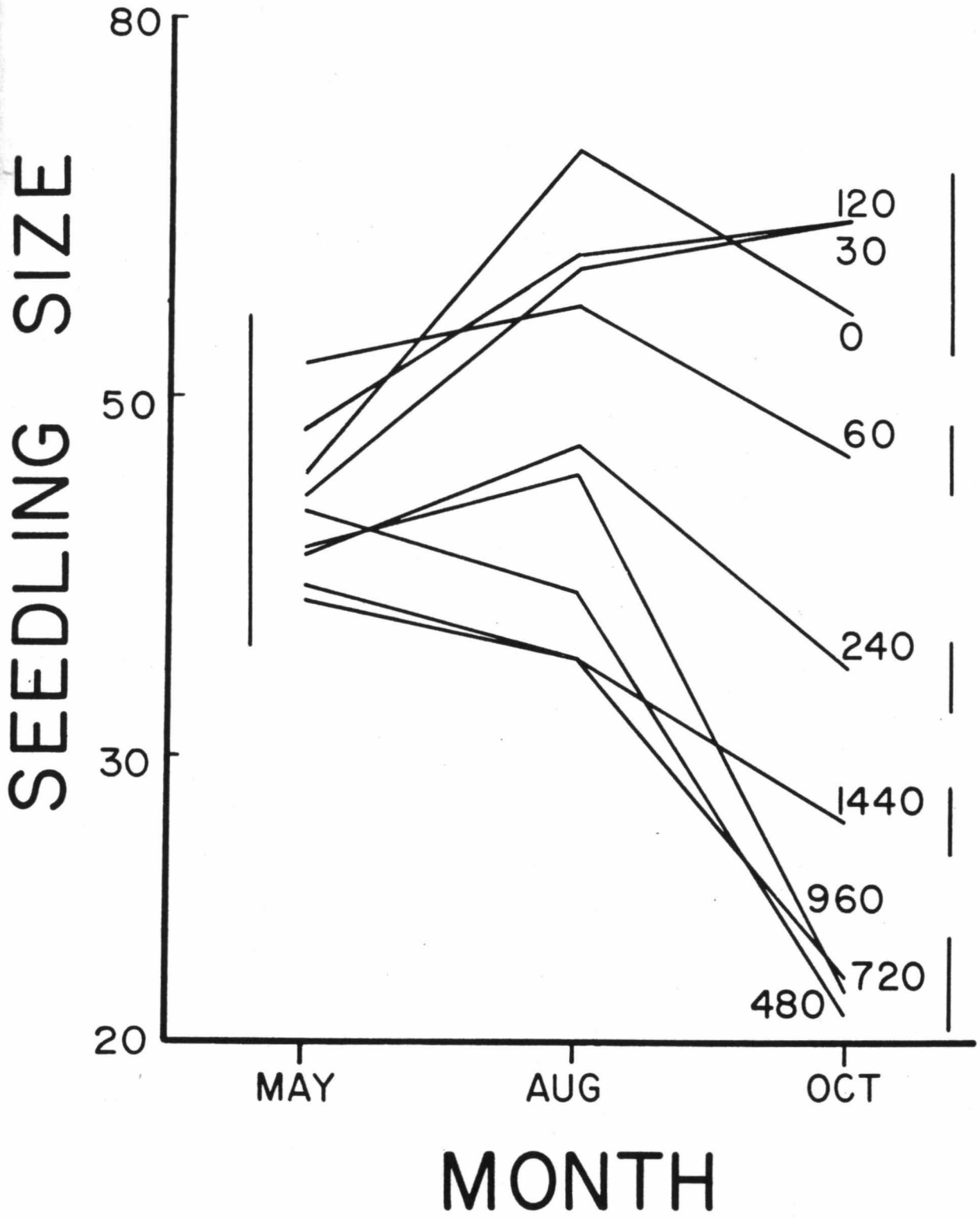
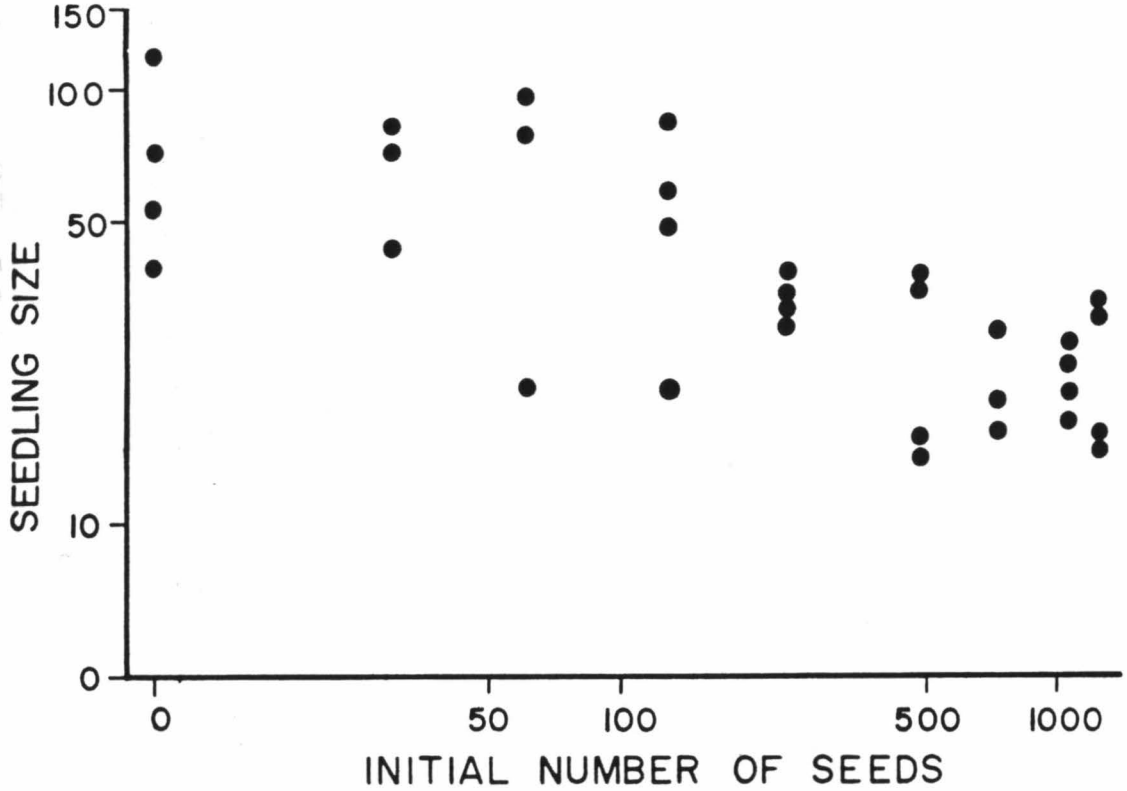

SEEDLING SIZE
80
50
30
20
120
30
0
60
240
1440
960
720
480
MAY
AUG
OCT
MONTH

Figure 2.5. Graph of the size of seedlings in October against a) initial seed input b) total seedling emergence.


150
100
50
10
0
SEEDLING SIZE
0
50
100
500
1000
INITIAL NUMBER OF SEEDS

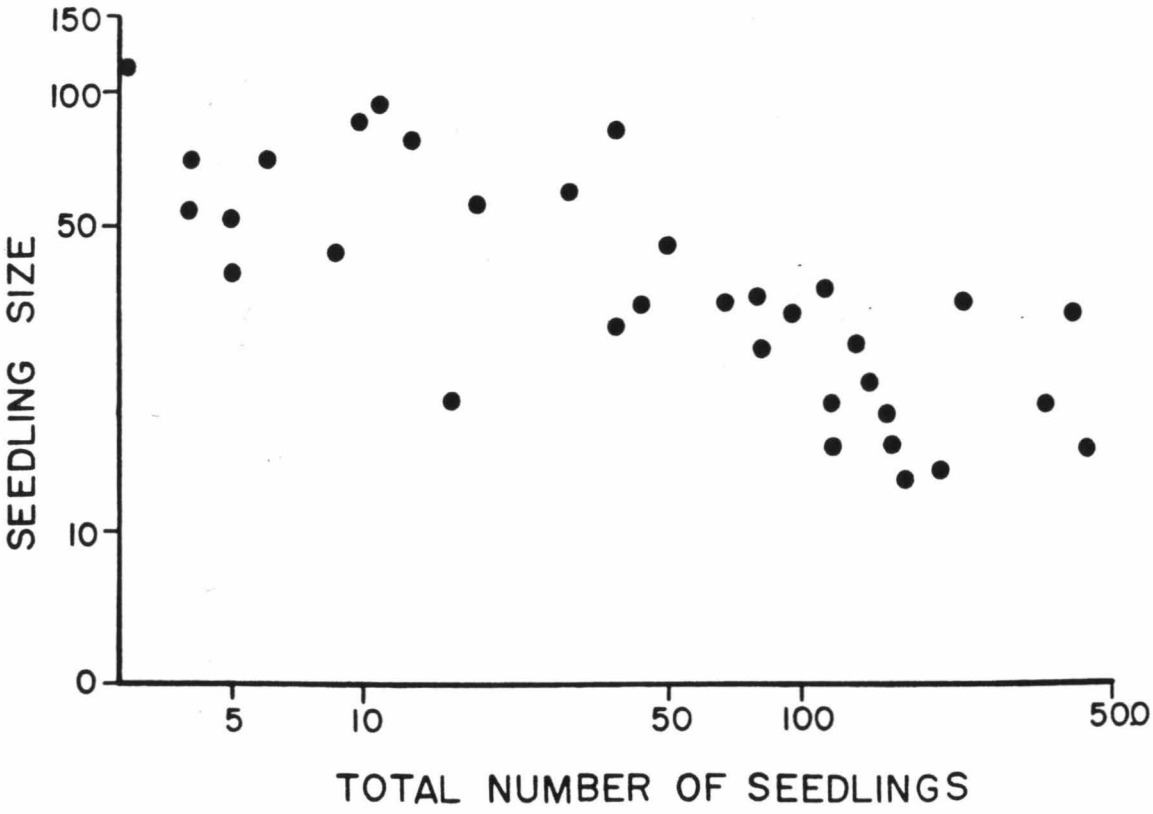

150
100
50
10
0
SEEDLING SIZE
5
10
50
100
500
TOTAL NUMBER OF SEEDLINGS

Table 2.5a. Profile analysis of the size of seedlings measured in Experiment 2.: test for differences in elevation. Size is the product of number of leaves and length of longest leaf; these values are log transformed for the analysis.

		May	August	October	April	Multi-variate
Source	df	F	F	F	F	p
Model	31	1.5 *	1.5 *	2.4 **	1.6 *	
Block	3	1.8	0.9	1.7	0.2	ns
# Seeds	9	1.2	2.3 *	3.1 **	3.2 **	**
Block x # Seeds	19	1.4	0.6	1.5	1.1	ns

Table 2.5b. Profile analysis of the size of seedlings measured in Experiment 2: test for parallelism. Growth is the difference between sizes at the beginning and end of a given interval (both log transformed).

		May-August	August-Oct	Oct-April	Multi-variate
Source	df	F	F	F	p
Model	31	1.7	1.8 *	0.9	
Block	3	0.0	2.6 *	0.7	ns
# Seeds	9	2.8 **	1.5	1.0	**
Block x # Seeds	19	1.2	1.4	1.0	ns

*, $p<.05$; **, $p<.005$

DISCUSSION

The present experiments examine the response of three traits in Salvia lyrata, germination, growth, and survival of seedlings, to variation in seed density and to the presence of established individuals. For the first trait, there is no evidence of density-dependent suppression (or enhancement) of seed germination (including seedling emergence), since the proportion of seedlings emerging is constant throughout the range of seeds sown even at densities a hundred times those of naturally emerging seedlings (Chapter I). The "safe site" hypothesis (Harper 1977), which posits that the number of sites suitable for germination and emergence is limited and predicts that these can become saturated at a high density of seeds, therefore does not apply to Salvia in this environment. Likewise, there is no indication of seed-derived chemical or physiological inhibition of germination in Salvia. Linhart (1976) has reported laboratory assays for density-dependent germination in a variety of species. On the basis of these experiments and a review of the literature, he suggested that germination in species of closed communitites is positively density-dependent and in weedy species is negatively density-dependent. However, no reports of germination responses to seed density evaluated in natural conditions are available in the literature. Consequently, the general ecological relevance of the documented germination responses to density is unknown, and the present study suggests that they may well be artifacts of the laboratory conditions in which previous studies were conducted.

Similarly, Experiment 1 produced no evidence that Salvia seedlings established earlier in the season affected the probability of germination of remaining seeds. Conversely, Inouye (1980) reported a study of desert annuals in which fewer seedlings emerged in undisturbed plots than in plots

where seedlings were removed as they emerged. However, since his data included several species lumped together (the number of species and their identities were not reported), it is unclear whether that result indicates intra- or interspecific suppression of emergence.

The local density of established Salvia seedlings did however strongly affect subsequent survival of seedlings; this effect was weak and not significant over the narrower range of densities spanned in Experiment 1, but was evident at the high densities of Experiment 2. The threshold above which density-dependent mortality occurred was about 240 seeds per dm^2 (ca. 70 seedlings per dm^2)(Fig. 2.3b). This exceeds the maximum natural seedling densities eighteen times (Chapter I) and the maximum seed density of Experiment 1 four-fold.

In the October, 1982 census of Experiment 2, there was evidence of spatial variation in response of mortality to density. In other words, the density at which mortality is induced differs among sites. Thus, control of population growth is localized, not only because of the sedentary habit of plants, which restricts the number of individuals each contacts, but also because of intrinsic variation among sites in characteristics such as availability of resources, abundance of predators, or presence of pathogens, any of which might alter density responses.

Density had the greatest effect on the size of seedlings. Suppression of plant size was evident at moderate densities by October (Figs. 2.4, 2.5) and became more severe by the following April (Fig. 2.6). Size traits are known to be highly plastic in many plant species, and responses in size are expected to precede mortality responses (Harper 1977), which ultimately reflect the loss of stunted individuals. In Experiment 2, the severity of density-dependent stunting and mortality increased with time, a phenomenon

Figure 2.6. Graph of the size of seedlings against number of surviving seedlings at each of four censuses, May, August, October, and April. The lines join observations for a given treatment through time, the arrows being directed toward the April observation. The treatments are designated by the symbols, as follows:

△ - 0 seeds

○ - 30 seeds

□ - 60 seeds

⬢ - 120 seeds

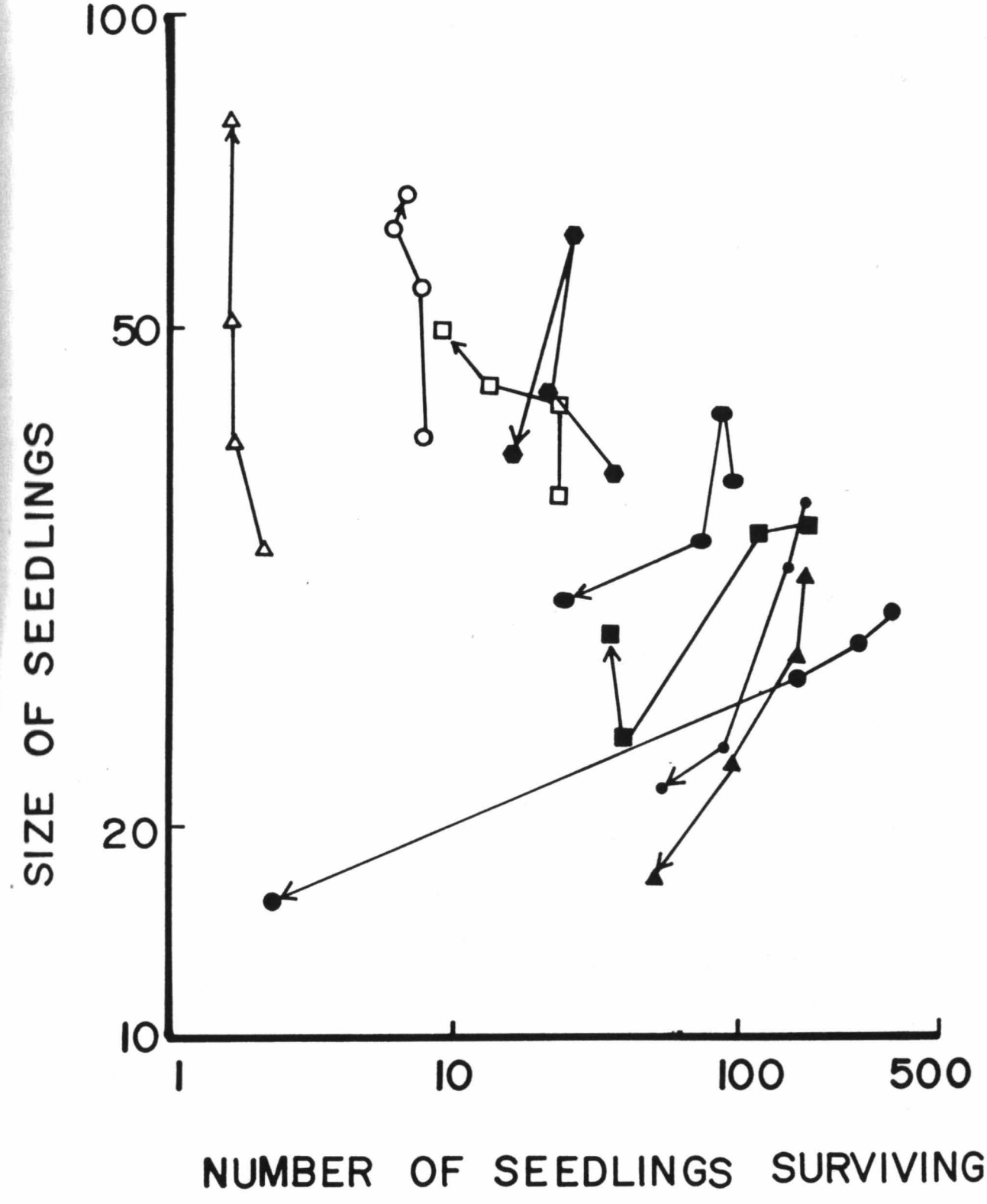
⬬ - 240 seeds

■ - 480 seeds

• - 720 seeds

▲ - 960 seeds

● - 1440 seeds


100
50
20
10
SIZE OF SEEDLINGS
1
10
100
500
NUMBER OF SEEDLINGS SURVIVING

first noted by Yoda, et al. (1963). The self-thinning rule which they propounded implies that individual size increases as numbers decline, such that total yield per plot increases despite decreasing density (see White 1981 for review). In the present experiments with Salvia, this is not case in the highest densities (number of seeds sown=120-1440) where density-dependent mortality is demonstrable (Fig. 2.6). In other words, the stunting suffered at originally high seedling densities persists after a substantial fraction of individuals has died leaving space to those remaining.

Aside from the effect of seedling density on seedling success in Salvia, Experiment 1 indicated that presence of conspecific adults reduced seedling emergence and survival. May, et al. (1974), in a model of population growth which incorporates interactions among two age classes, have shown that a strong negative effect of adults on juveniles tends to destabilize population size. However, in their model stability depends on the relationship between effects of adults and juveniles on individual fecundity and the effects of adults and juveniles on juvenile survival. Because of its restriction to two age classes, the model does not strictly apply to perennial species such as Salvia; however, assuming that individual fecundity is density-independent, it would predict that a hypothetical biennial Salvia population would be stable as long as the density effect of adults on seedling survival were less than that of seedlings on seedling survival. May, et al. (1974) defined the density effect as the regression of the change in the log of one age class on the log of another. The regression coefficient for the effect of adults on seedlings is not obtainable, since adult densities were not varied over a wide range. To ascertain to what extent interactions among age classes destabilize population size in perennial plant species will require further

experimental work and models which consider size, as well as age.

In contrast to the present study, the descriptive demography of this population of Salvia lyrata (Chapter 1) produced no evidence suggesting that survival of seedlings is density-dependent. On the basis of the experimental results, it is clear that the natural range of average densities is too low to influence these seedling traits directly. However, assuming seed is dispersed at random, clumping will occasionally produce patches of locally high densities in which density effects such as those documented may occur.

Comparison of these results with those of experiments with Salvia conducted in controlled conditions indicates that the density responses are far weaker in nature than in cultivation. In monospecific stands of hexagonal fan design, severe density-dependent mortality and fecundity were evident throughout a twenty-five fold range of densities whose maximum (385 plants per m^2) was less than the minimum density (500 plants per m^2) in the present experiments (Chapter 4). The intensity of these effects, as well as their early appearance, are certainly a result of the rapid growth rates in the fertile, protected conditions of cultivation. Differences between density responses as a consequence of variation in soil fertility (e.g., Sukatschew 1928), light (e.g., Hiroi & Monsi 1966; Lonsdale & Watkinson 1982), drought (Watkinson 1982) and association with heterospecifics (Watkinson 1981) are well documented (Harper 1977).

The two prior experimental studies of density responses in natural plant populations have employed winter annual species which naturally grow in association with few other species (Watkinson & Harper 1978; Clay & Shaw 1981). Both studies documented significant negative effects of density on fecundity. No relationship of mortality to density was observed up to 320

plants per dm^2 in the study on Vulpia fasciculata, a grass, and in Diamorpha smallii (Crassulaceae), maximum densities of 400 plants per dm^2 produced a weak mortality response. In the present study however, a similar range of densities of Salvia caused a striking mortality response within approximately the same time. This variation among species in mortality responses may be attributable, in part, to differences in plant form, the erect form of the grass minimizing the potential for interference by shading (see also Lonsdale & Watkinson 1983) and to the fact that the maximum sowing densities, while numerically comparable for the three species, are unnaturally high for Salvia, a larger herb than either of the other two.

The present experiments unequivocally demonstrate density-dependent reduction in individual size and survival in natural populations of seedlings, albeit at extraordinarily high densities. Thus, at the usual densities of Salvia in this field, population size at the seedling stage can vary widely without apparent internal control. Yet, regardless of the proximate mechanism inducing the density responses, whether competition for resources or light or density-dependent infestation by pathogens, these experiments clearly indicate that at extreme densities biotic effects come into play limiting population size.

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CHAPTER III. EXPERIMENTAL ALTERATION OF DENSITIES OF ESTABLISHED PLANTS

INTRODUCTION

The extent to which local population density influences the tendency of natural populations to increase is known for only a few plant species (Antonovics & Levin 1980), despite the central importance of density-dependent population growth in ecological theory. In particular, the study of density effects in natural populations of perennial species has been neglected, primarily because of the difficulties of experimenting with species having extended life spans and, often, delayed reproduction (Watkinson & Harper 1978). Moreover, because of the complexity of populations of perennial species, which are composed of several age and/or size classes, an understanding of their population regulation requires knowledge of the density responses of these different groups. The present chapter reports two experiments devoted to elucidating the density responses of the established members of a natural population of a perennial herb, Salvia lyrata (Lamiaceae) (Radford, et al., 1968). Here "established individual" refers to plants which have been alive for at least one year. In particular, these experiments address the following questions:

1) Do nearby conspecific neighbors affect the fitness (survival, fecundity, or size) of established Salvia individuals?

If so,

2) Over what distances are effects of neighbors manifested?

3) Do these effects vary spatially?

4) Do responses to neighbors vary with the size of individuals?

In order to answer these questions, the density of Salvia was altered in naturally established populations. In this way, present density is deconfounded from other environmental variables including previously existing density. The method of removing individuals and following the consequences for the remainder of the population was pioneered in the study of interspecific competition of sessile marine invertebrates (Connell 1961, Paine 1969, Dayton 1971) and has been extended to the study of interspecific competition in plant communities (Sagar & Harper 1961, Putwain & Harper 1970, Silander & Antonovics 1982, Fowler 1978). More recently, the method has been applied to the study of intraspecific interactions (Watson 1974, Watkinson & Harper 1978, Fonteyn & Mahall 1978, Clay & Shaw 1981).

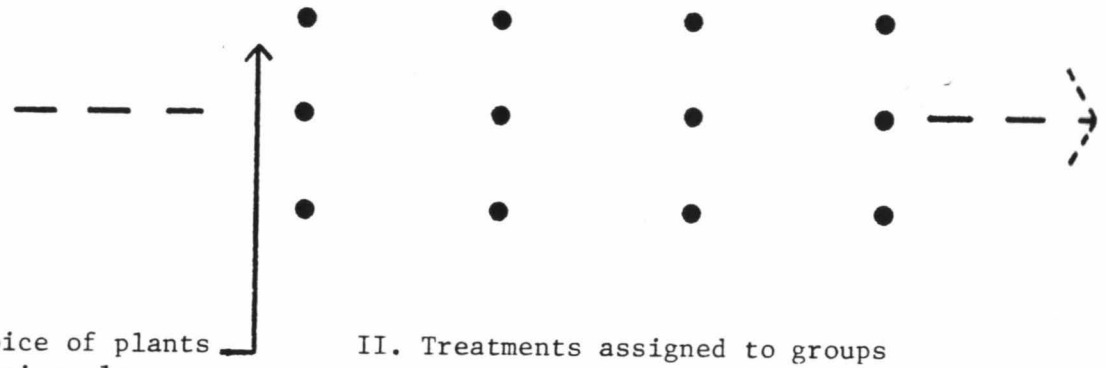
MATERIALS AND METHODS

The two experiments were conducted in a field on the campus of Duke University, Durham, N.C., and both employed Salvia lyrata as the experimental organism. The species and site were described in Chapter I. Experiment I: In February, 1980, twenty-three transects approximately 30 m long were laid such that they spanned the field, running north to south and east to west. Experimental individuals were chosen at 2 m intervals along them such that three size classes ($S<150$, $150<S<350$, $350<S$, where S=(number of leaves) x (length of longest leaf (mm)), were represented in random sequence in each successive group of three individuals; a total of three

hundred individuals was chosen. The location and size of neighboring conspecifics (within 30 cm) was mapped on a plate of clear plexiglass overlain by tracing paper. Then one of the following density manipulations was applied to each series of three plants in random sequence. In the southern, lower half of the field, surrounding Salvia individuals were removed to a distance of 10, 20 or 30 cm, or the neighborhoods were left undisturbed as controls. In the remaining half of the field, a series of addition treatments was established in which Salvia individuals which had been dug from the removal series were planted in as neighbors to the mapped individuals. In each case, six individuals were evenly spaced around the central plant at either 5, 10 or 20 cm; a fourth series where neighborhoods were left undisturbed or where soil was dug but no plants were added served as controls. The removal treatments were maintained by thinning on subsequent census dates. Transplanted individuals which died in the addition treatments were replaced once in spring, 1981. Sizes of plants (number of leaves and length of longest leaf) were recorded initially and in May, 1980, August, 1980, and May, 1981, when survival, whether the plant was flowering, and the number of seeds it was maturing were also recorded.

Experiment 2: A similar experiment was established in November, 1981-January, 1982. Eight new transects were laid in the part of the field in which removals were executed in the first experiment. The transects were deliberately placed such that the density of Salvia was uniform within transects, but a wide range of densities was sampled among transects. In rows placed 60 cm apart and perpendicular to the transect, individuals in the three size classes were chosen at distances of approximately 30 cm. Up to twelve rows of three plants were established along each transect (Fig. 3. 1). Two hundred seventy plants were chosen in all. Maps of neighboring Salvia plants were drawn, as before. In two rows of every three, all

Figure 3.1. Diagram of part of Experiment 2. Each dot represents an individual chosen for the experiment. The arrow indicates the continuation of the transect, of which 1 1/3 replicates (i.e., four rows of a maximum twelve (= 4 replicates) in each transect) are shown.



I. Choice of plants by size class

II. Treatments assigned to groups

Size class definitions:

A: $S < 150$
B: $150 \leq S < 350$
C: $350 \leq S$

where S=(# leaves) x (length of longest leaf)

0	1	2
Control: no disturbance	Removal: all *Salvia* within 20 cm removed	
	Salvia removed as it invades	All vegetation removed as it invades

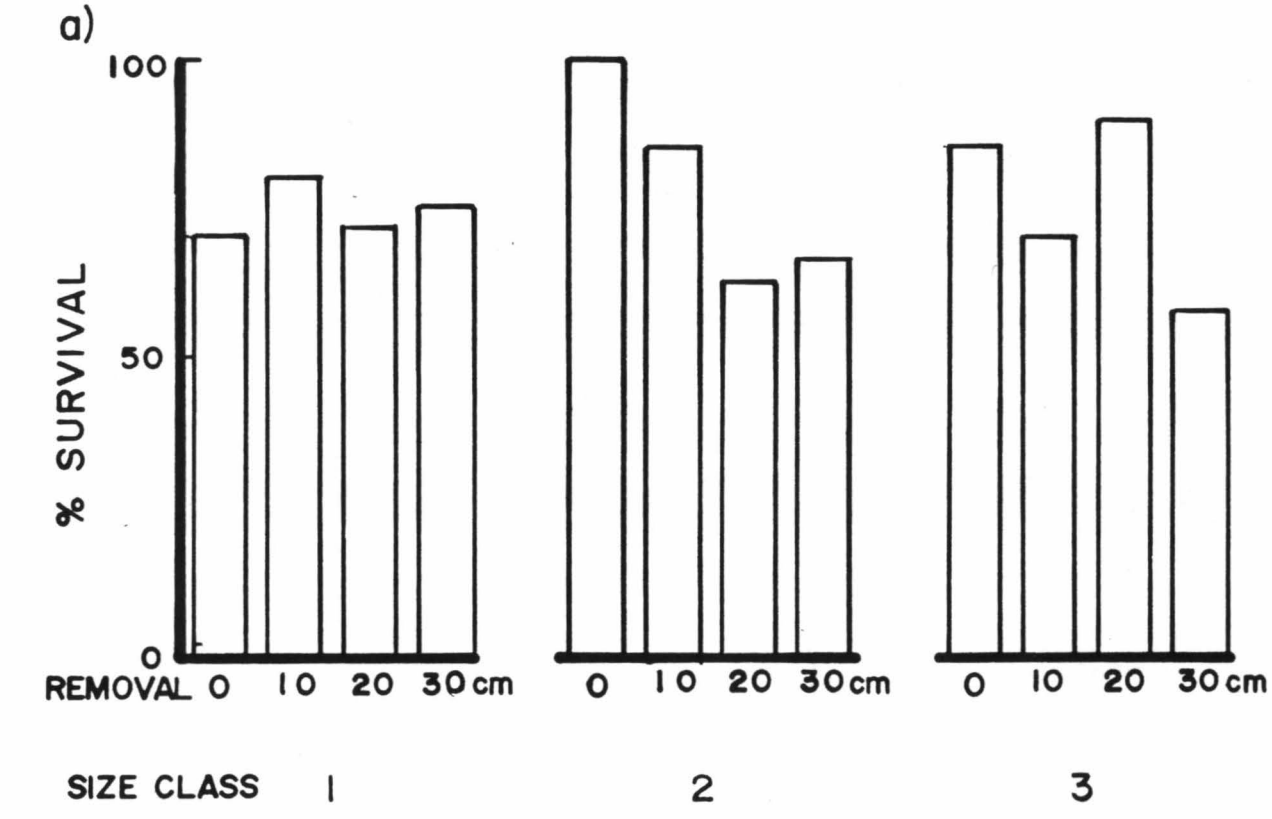
Salvia neighbors within 20 cm were removed, and the perimeters of the removed plants were marked with plastic covered wire anchored by staples. The remaining individuals were left undisturbed as controls. In May, 1982, the removal treatment group was further divided into two groups: one in which all plants invading the marked areas previously occupied by Salvia were removed, and the other in which only Salvia individuals invading those areas were removed. In this way, the effects of Salvia's presence could be distinguished from the effects of other species replacing Salvia where it was removed.

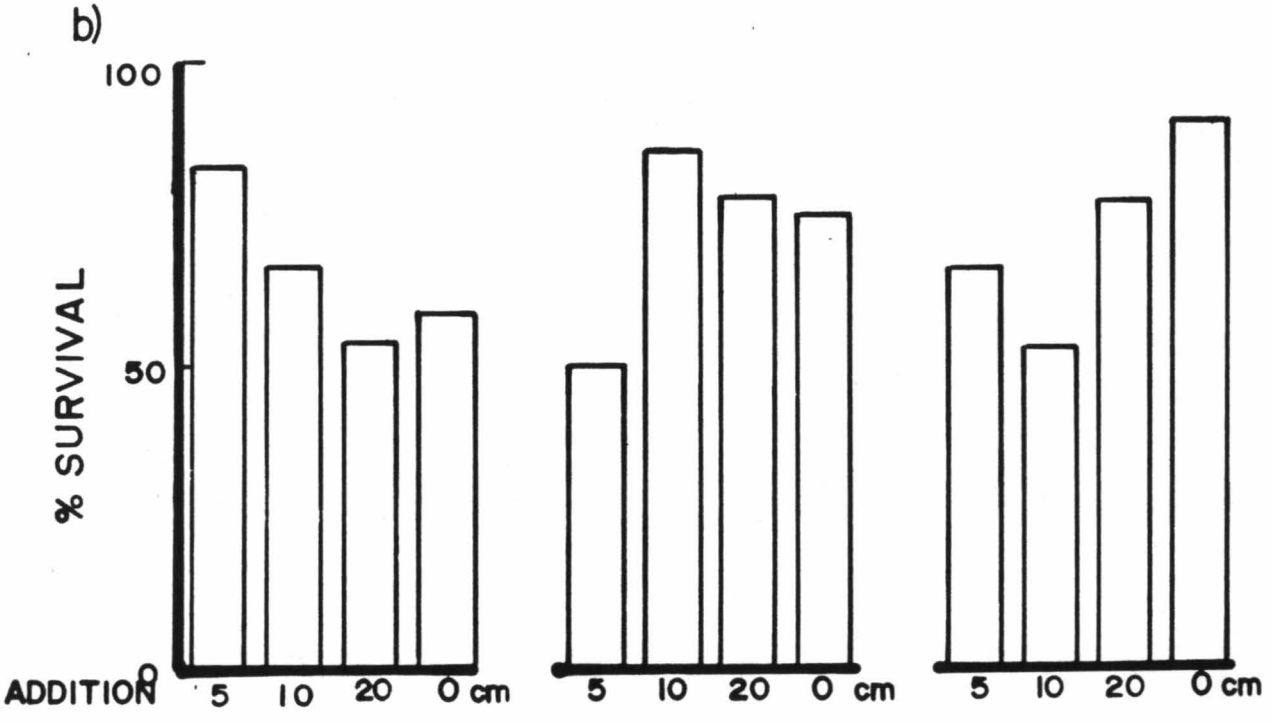
This experiment employed a split plot design with initial size as the splot plot factor and removal treatment as the whole plot factor within each replicate containing nine plants. The appropriate error term for tests of treatment effects in the analysis of variance is therefore the treatment x replicate interaction, where replicates are nested within transects. The statistical analyses were carried out using a program for the G test, written by T.R. Meagher, and the Statistical Analysis System (Helwig & Council 1979) supported by the Triangle Universities Computation Center, Research Triangle Park, N.C.

RESULTS

Experiment 1: For the duration of the experiment (February, 1980 to October, 1981), 217 out of 300 individuals survived. In the addition series, the larger two size classes exhibited the expected decrease in survival at higher densities (Fig. 3.2b). In the removal series, there was no trend or the opposite trend (Fig. 3.2a). The variation in survival was

Figure 3.2. Frequency of surviving plants against size class and treatment, Experiment 1. a) Removal series. b) Addition series. The labels on the abscissa indicate treatment levels (i.e., removal of all Salvia at 5, 10, or 20 cm, or no plants removed; addition of six Salvia at 5, 10, or 20 cm, or no plants added. Each of the three panels refers to a different size class, as defined in "Materials and Methods".


a)
100
50
0
% SURVIVAL
REMOVAL 0 10 20 30 cm
0 10 20 30 cm
0 10 20 30 cm
SIZE CLASS 1
2
3


b)
100
50
0
% SURVIVAL
ADDITION 5 10 20 0 cm
5 10 20 0 cm
5 10 20 0 cm
SIZE CLASS 1
2
3

statistically significant only for the treatment x size class x survival interaction in the addition series (Table 3.1).

In the first year, 1980, flowering was greatest among individuals which were largest initially in both removal and addition series (Table 3.1). In general, the proportion of plants flowering increased slightly with increasing distance of removal of surrounding conspecifics (Fig. 3.3); however the effect was not significant. In the second year, 1981, the probability of a plant flowering did not vary with the two experimentally altered factors.

Effects on the number of seeds produced, given that a plant flowered, differed between the removal and addition series. In the removal series, none of the effects were significant, but the trend was toward larger plants and reduced density yielding more seeds per individual. In the addition series, larger plants produced more seeds, but there was a marginally significant treatment x size interaction. Largest and smallest individuals tended to produce more seeds at lower density (largest, at 10 cm and 20 cm spacing; smallest in 20 cm spacing and no addition), but the intermediate size class produced most seeds in the lowest (no addition) and highest (5 cm distance) densities (Fig. 3.4, Table 3.2). In 1981, the trends of fecunditiy with density were very weak ($p<.5$), and no other effects were significant.

Individual growth was analysed as the change in size (log (number of leaves x length of longest leaf)) between two times. For the interval of May, 1980, to May, 1981, larger plants grew significantly less than smaller ones in both series (Fig. 3.5). The treatment effect was not significant. Growth patterns in the overlapping interval, October, 1980-October, 1981, showed the reverse trend in the addition series. The treatment effect was not significant, and the trends went opposite to expectation, with plants

Table 3.1. G test for the effects of density manipulation and initial size on survival and on flowering, Experiment 1.

		Removal Series	
		Survival	Flowering
Interaction effect	df	G	G
Removal x Size	6	7.9	9.3
Removal	9	8.5	14.9 +
Size	8	8.8	89.0 **
		Addition Series	
Addition x Size	6	11.3 +	9.8
Addition	9	12.03	13.4
Size	8	12.03	98.2 **

+, $p<.1$; **, $p<.005$.

Figure 3.3. Frequency of plants flowering against size class and treatment, Experiment 1. a) Removal series. b) Addition series. Panels are labeled as in Fig. 2.

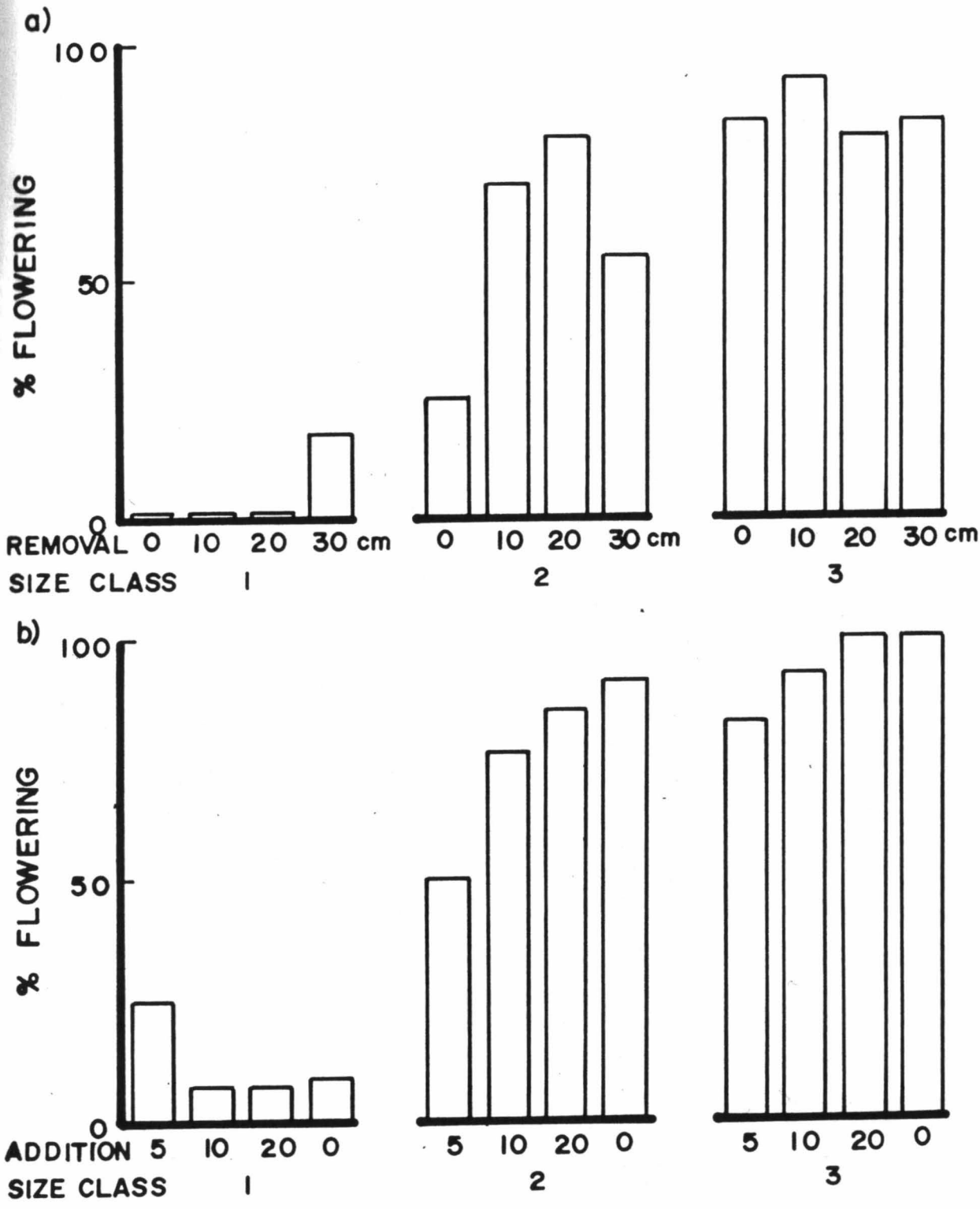
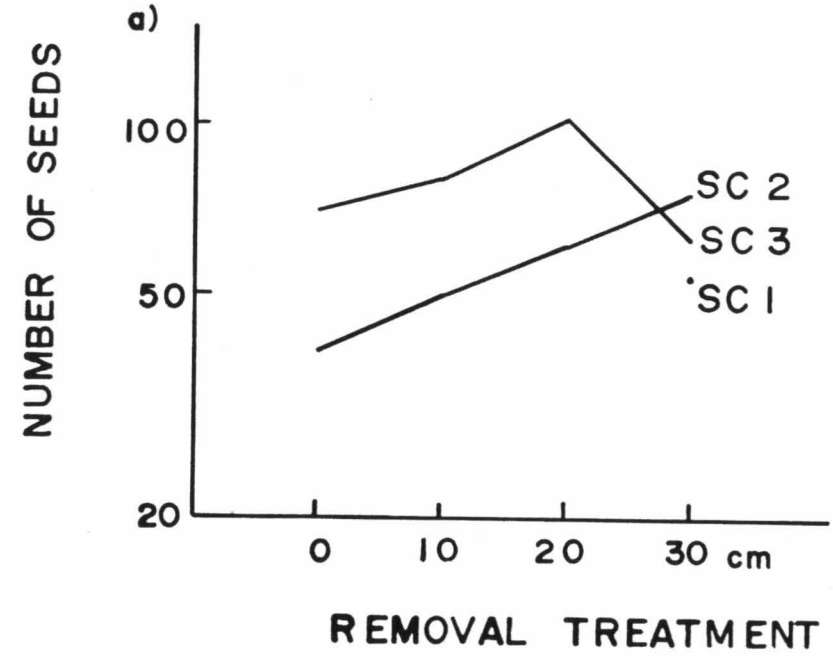

a)
100
50
0
% FLOWERING
REMOVAL 0 10 20 30 cm
SIZE CLASS 1
0 10 20 30 cm
2
0 10 20 30 cm
3
b)
100
50
0
% FLOWERING
ADDITION 5 10 20 0
SIZE CLASS 1
5 10 20 0
2
5 10 20 0
3

Figure 3.4. Number of seeds produced per individual which flowered (log scale) against treatment. The responses for the different initial size classes (SC) are plotted separately. a) Removal series. b) Addition series. Treatments are labeled as in Fig. 2.


a)
NUMBER OF SEEDS
100
50
20
SC 2
SC 3
SC 1
0
10
20
30 cm
REMOVAL TREATMENT

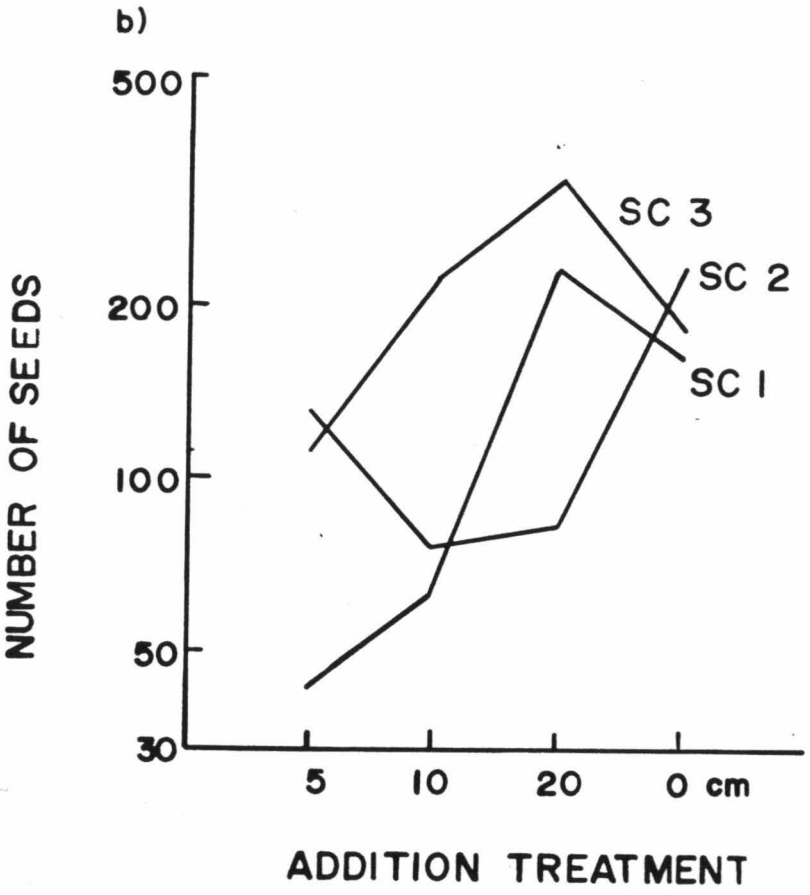
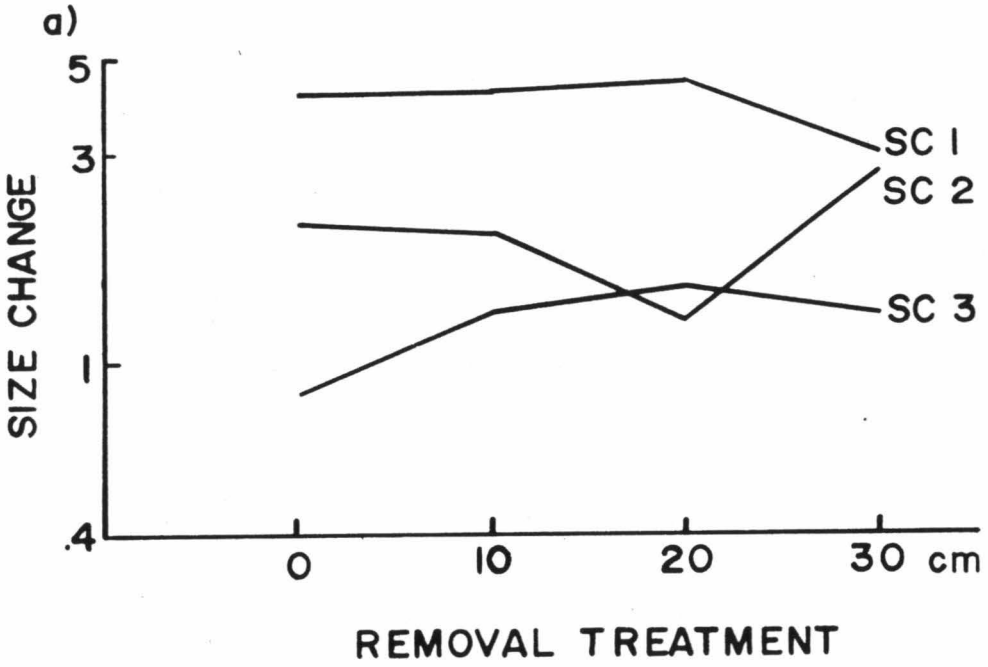
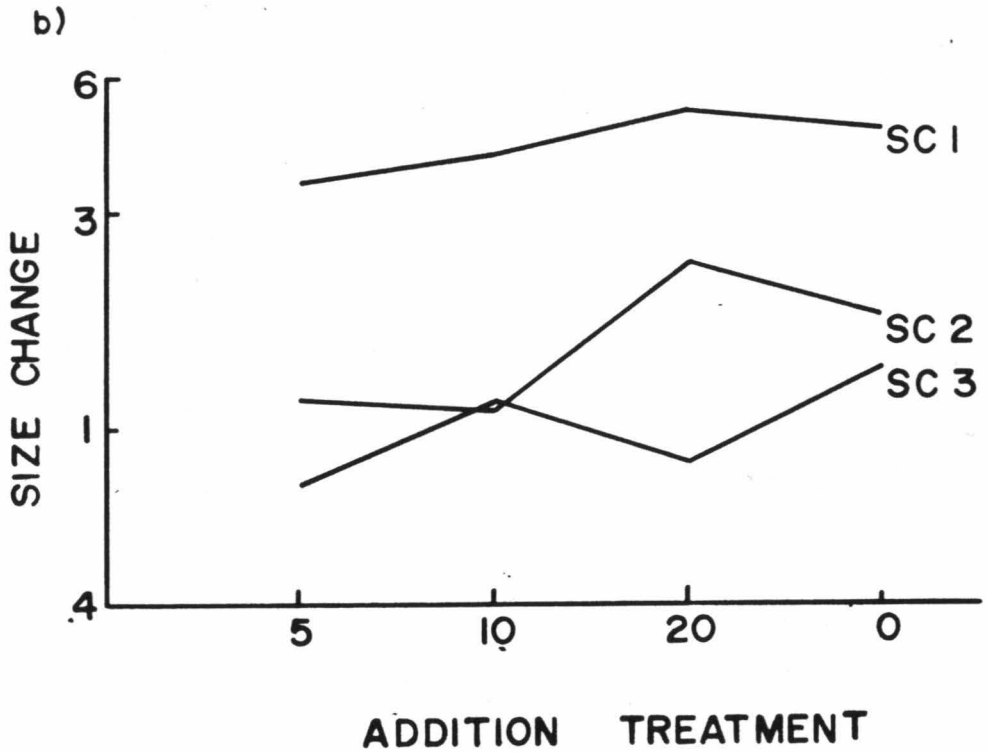

b)
NUMBER OF SEEDS
500
200
100
50
30
SC 3
SC 2
SC 1
5
10
20
0 cm
ADDITION TREATMENT

Table 3.2. Analysis of variance of number of seeds produced per plant in 1980, expt 1. The values were log transformed.

Source	df	SS	F	p
Removal Series				
Model	41	26.3	1.2	
Error	31	15.8		
Transect	10	8.2	1.3	
Initial Size	2	0.3	0.2	
Removal	3	0.8	0.4	
Transect x Removal	23	15.4	1.0	
Removal x Size	3	1.8	0.9	
Addition Series				
Model	47	46.2	1.5	.025
Error	41	27.6		
Transect	11	7.2	2.0	.1
Initial Size	2	10.5	8.5	.001
Addition	3	1.2	1.2	
Transect x Addition	25	8.5	0.5	
Addition x Size	6	5.3	2.2	.1

Figure 3.5. Change in size from May, 1980 to May, 1981, calculated as S1-S0, where Si=log(number of leaves at time i x length of longest leaf at time i), plotted against treatment. The responses for the different size classes are plotted separately. a) Removal series. b) Addition series. Treatments are labeled as in Fig. 2.


a)
5
3
1
.4
SIZE CHANGE
SC 1
SC 2
SC 3
0
10
20
30 cm
REMOVAL TREATMENT


b)
6
3
1
.4
SIZE CHANGE
SC 1
SC 2
SC 3
5
10
20
0
ADDITION TREATMENT

at lower density growing least.

Measures of local density prior to its manipulation were taken from the maps and used as covariates in additional analyses of the two response variables, number of seeds and growth. The summed areas of plants whose centers lay within 10 cm of the experimental plant measured close crowding. Similarly, the summed areas of plants occupying two larger concentric rings (10-20 cm and 20-30 cm) measured more distant crowding. None of these variables had a significant effect on the responses, nor did their inclusion in the models reveal significance of any effect which was not previously significant.

Experiment 2: Of the marked individuals, 91% survived for eighteen months (November, 1981-April, 1983). The response to the two removal and exclusion treatments differed among the size classes (removal x size class x survival $G=9.3$, 4 df, $p<.06$), but one or the other removal did improve survival over the control in each size class (Fig. 3.6). The overall effect of removal on survival was not significant (Removal x survival, $G=4.15$, 3 df, $p>.1$).

The probability of a plant flowering (Fig. 3.7) and the number of seeds produced, given that a plant flowered (Fig. 3.8, Table 3.3), both depended principally on initial size in the first year (1982) ($G=69.1$, 3 df; $p<.0001$), although there appeared to be a slight enhancement of flowering and, for the mid-size class, number of seeds produced in the two removal treatments. In the second year, the largest size class was more likely to flower in both removal treatments than in the control. Conversely, the smallest size class had a decreasing tendency to flower in removal treatments (Fig. 3.7). These trends were not significant (Removal x size class x flowering, $G=6.6$, 6 df). Moreover, the proportion of plants

Figure 3.6. Frequency of plants surviving plotted against treatment and size class, Experiment 2. Removal 1 refers to the removal treatment in which reinvasion by Salvia was prevented. Removal 2 refers to the removal treatment in which no invasion into the cleared areas was allowed.

Figure 3.7. Frequency of plants flowering plotted against treatment and size class, Experiment 2. The solid lines refer to flowering condition in May, 1982; the dotted lines refer to flowering condition in May, 1983. Removal treatments are as defined in Fig. 6.

FIG 6

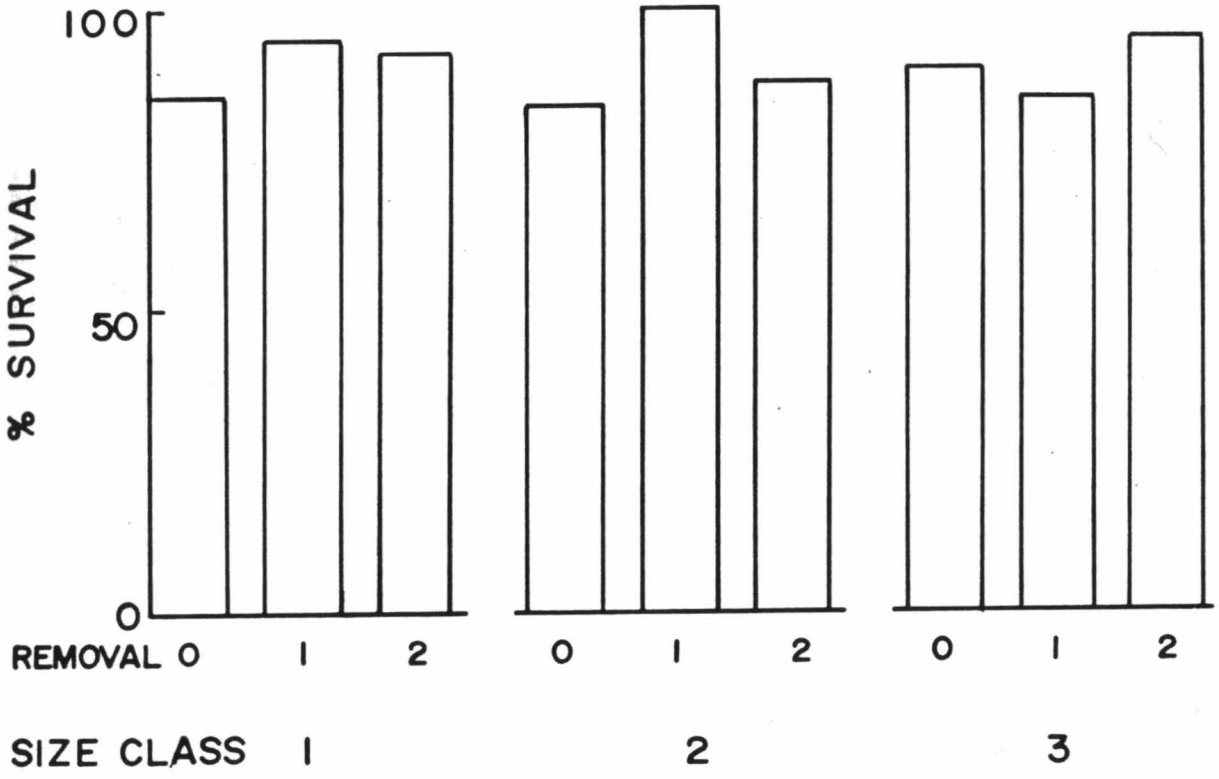

100
50
0
% SURVIVAL
REMOVAL 0 1 2
0 1 2
0 1 2
SIZE CLASS 1
2
3

FIG 7

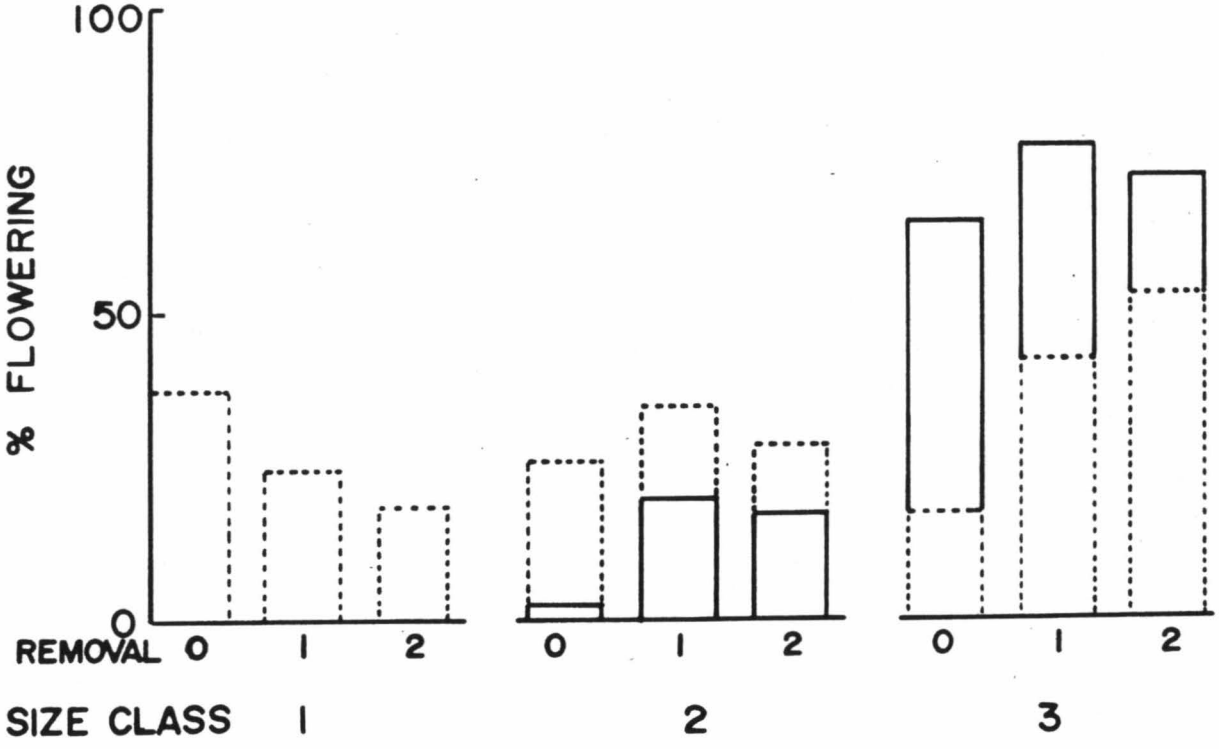

100
50
0
% FLOWERING
REMOVAL 0 1 2
0 1 2
0 1 2
SIZE CLASS 1
2
3

Table 3.3. Analysis of variance of number of seeds per plant in 1982, Experiment 2. The values were log transformed.

Source	df	SS	F	p
Model	26	61085	1.5	.1
Error	47	74638		
Transect	7	15054	1.4	
Removal	2	6989	2.3	.1
Initial Size	1	23333	15.7	.0003
Transect x Removal	14	29918	1.4	
Size x Removal	2	7015	2.3	.1

flowering in the second year did not depend significantly on their size at the beginning of the experiment. Of those plants that flowered there were no trends with removal treatment in the number of seeds produced (Fig. 3.8).

Individual size at four times (November, 1981, August, 1982, November, 1982, and April, 1983) and growth over the corresponding intervals (Fig. 3.9) were subjected to profile analysis (Timm, 1975). Only initial size significantly influenced size on subsequent dates and growth in the first interval (Table 3.4 a,b). The multivariate tests for effect of initial size on growth (slope) and size (elevation) of the plants were highly significant. No other effects were significant in either univariate or multivariate tests, although the effect of removal treatment on size at November, 1982, approached significance ($p<.06$).

The design of this experiment permitted tests of whether the growth and fecundity, as well as the response of these traits to the density manipulation, varied spatially. No substantial differences were found among the transects in plant size, growth, or fecundity (transect main effect), nor in the response of the traits to the density alterations (transect x removal interaction)(Tables 3.3 and 3.4).

DISCUSSION

The present experiments were designed to show to what extent conspecific neighbors interfere with established Salvia individuals in a natural population. Evidently, interference at natural densities is very slight. The clearest evidence that individuals can be suppressed by neighbors was found in the addition series of Experiment 1. Although individuals of the smallest size class were more successful with the addition of near neighbors, those of the two larger size classes suffered

Figure 3.8. The number of seeds produced per flowering individual plotted against treatment by size class, Experiment 2. a) 1982. b) 1983. Removal treatments are as defined in Fig. 6. Size classes are as follows:

● SC 1

■ SC 2

▲ SC 3

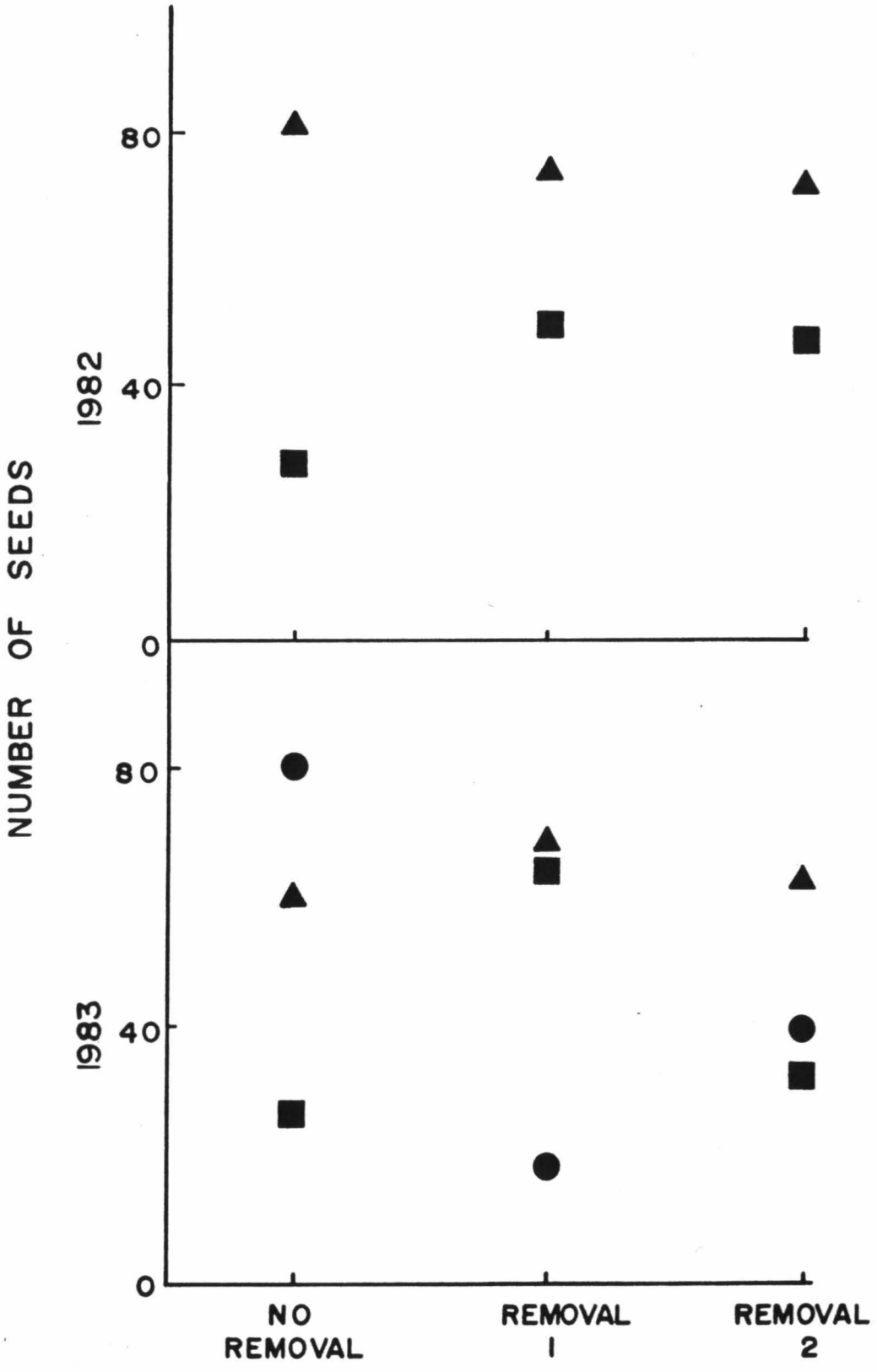

NUMBER OF SEEDS
1982
80
40
0
1983
80
40
0
NO REMOVAL
REMOVAL 1
REMOVAL 2

Figure 3.9. Size of individuals (log(number of leaves x length of longest leaf)) plotted against time by treatment, Experiment 2. Removal treatments as in Fig. 6.

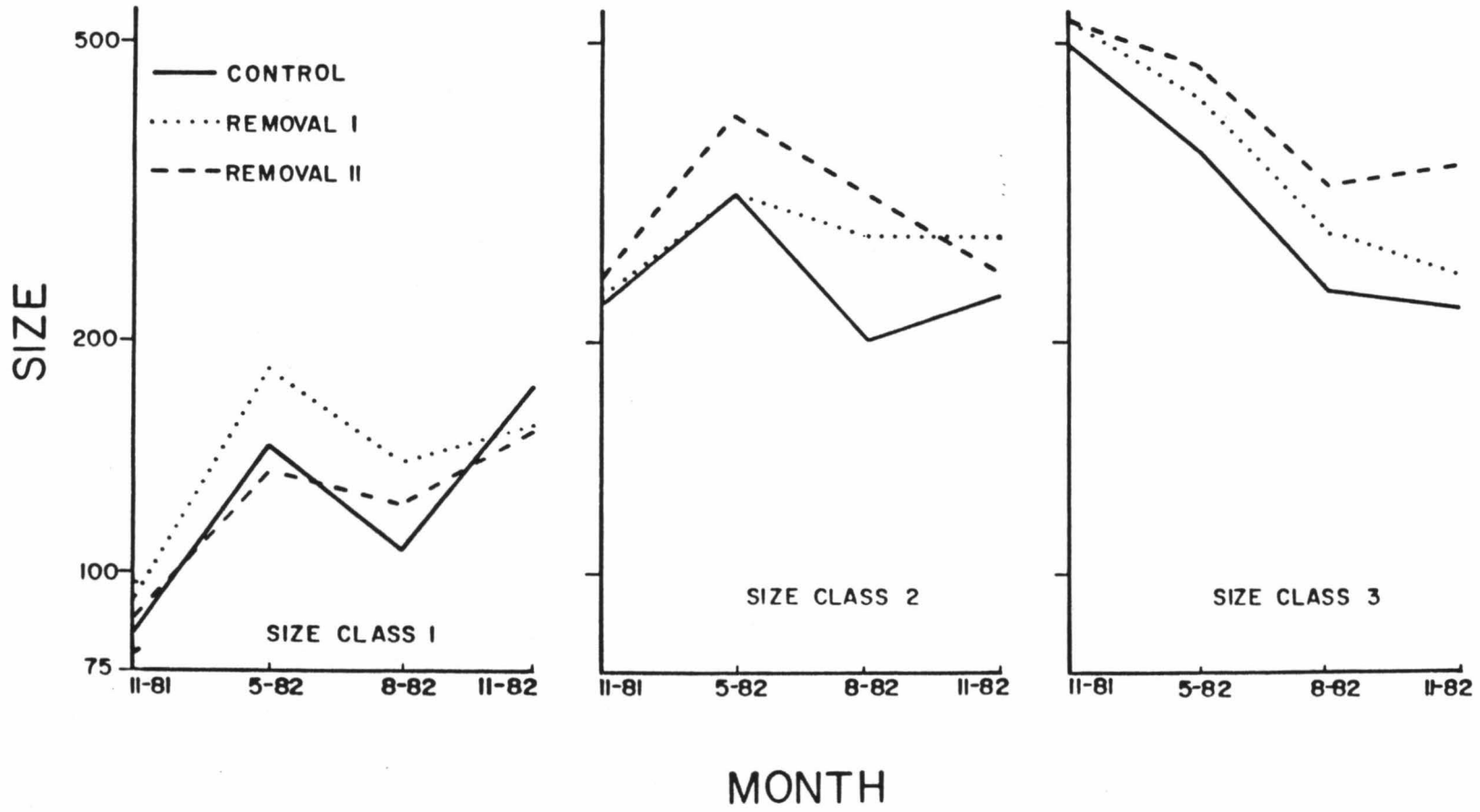

500
200
100
75
SIZE
CONTROL
REMOVAL I
REMOVAL II
SIZE CLASS 1
SIZE CLASS 2
SIZE CLASS 3
11-81
5-82
8-82
11-82
MONTH

Table 3.4a. Profile analysis of size (log transformed) from November, 1981 to April, 1983-Experiment 2.

Source	df	Nov, 81 F	Aug, 82 F	Nov, 82 F	Apr,83 F	Multivariate
Transect	7	1.4	1.2	2.3	1.1	
Replicate (Transect)	22	0.9	1.0	0.7	1.5	
Removal	2	1.1	1.0	2.7 +	1.5	
Initial Size	2	652.0 ***	12.0 ***	8.4 ***	12.0 ***	***
Transect x Removal	14	1.7	1.0	1.3	0.8	
Removal x Initial Size	4	1.6	1.9	1.0	0.6	

+, p<.06; ***, p<.005.

Table 3.4b. Profile analysis of change in size (growth) (log transformed) from November, 1981 to April, 1983, Experiment 2.

Source	df	Aug, 82-Nov, 81 F	Nov,82-Aug, 82 F	Apr, 83-Nov, 82 F	Multivariate
Transect	7	1.6	1.2	2.0	
Replicate (Transect)	22	0.9	0.6	0.9	
Removal	2	1.0	1.5	0.3	
Initial Size	2	17.7 ***	0.2	4.4 *	***
Transect * Removal	14	0.9	0.3	0.2	
Removal * Initial Size	4	1.6	0.9	1.5	

*, p<.025; ***, p<.0001

higher mortality and reduced fecundity with the addition of neighbors. This suggests that small plants gain some advantage, perhaps amelioration of the microclimate, from the proximity of conspecifics, whereas, for large plants, that advantage is negligible and is offset by concomitant interference by those neighbors. Moreover, the distance at which interference ceases to occur differs between the middle and large size classes. This difference can be easily understood; a large rosette that has neighbors centered 10 cm from its center is more likely to be overlapped than if it were smaller and had neighbors at the same distance. In other words, neighbors at a given distance crowd a larger plant more than a smaller plant. This somewhat counter-intuitive result -- one might have expected that a more robust individual would be comparatively insensitive to neighbors -- is a direct consequence of the sessile habit of plants.

The removal series (Experiment 1) gave some evidence of release from interference in the fraction of plants flowering (size class 1 and 2) and the number of seeds produced. Surprisingly, the response of the small size class was apparent only in extreme removal (30 cm), and, unlike the outcome of the addition series, the small plants did not perform better at higher densities. This disparity may be caused by differences between the two areas of the field in which the removal and addition series were conducted.

Although Experiment 2 was conducted at a level of replication double that of Experiment 1 and the compact layout of the transects allowed more precise assessment of spatial variation, responses to removal appeared weaker than in the previous experiment. This may reflect year to year variation but also strongly supports the impression that the responses of established individuals to density are, in general, very weak.

Given the weakness of the responses to removal in Experiment 2, it is not surprising that differences between the two maintenance or exclusion treatments (continued exclusion of Salvia only, compared with removal of any invading vegetation) were not apparent. If a substantial response had indicated that Salvia affect one another's growth, then responses of the same magnitude to the two treatments would suggest that invading heterospecifics do not impose an equivalent effect. Conversely, a large response in removal treatment 2 and no response in removal treatment 1 would imply that the invasion of other species imposes a competitive effect equivalent to that of Salvia. The greatest disparity between the two removal treatments was in the size of individuals in size class 3: plants in removal treatment 2 were largest, suggesting an additional competitive effect from heterospecifics. This effect was not evident in the other two size classes. The importance of the effect of heterospecifics relative to that of Salvia remains undetermined at present.

The weakness of the responses in general could be due to any one or a combination of four causes. The existing densities in nature (and even the increased densities of the addition series-Experiment 1) may be below the threshold at which individuals strongly interfere with one another. The naturally occurring range of average seedling densities falls below the range of demonstrable reduction in survival and growth of seedlings in natural conditions (Chapter II). Yet one would expect interference to occur at those densities as plants grow larger (Shinozaki & Kira 1956, Yoda, et al., 1963, Hiroi & Monsi 1966). However, if density declines constantly as a result of mortality, then the population may remain below the threshold densities thoughout the life cycle. The difficulties of acquiring, transplanting, and maintaining large numbers of mature individuals for the addition series (Experiment 1) precluded extensive testing at a wide range

of densities above those existing. But if the established population of Salvia grows at densities lower than those which cause interference, as it appears, then that threshold can only be determined by experimentally increasing density over a wide range. This has been accomplished by sowing seeds at a range of densities (Watkinson & Harper 1978; Oxley, cited in Harper 1977; Ogden, cited in Harper 1977; Chapter II), a technique which is suitable for studying density effects in seedlings and in short-lived species. Transplanting mature individuals of perennial species is a more appropriate technique for estimating density effects in populations of established perennials; however, this has apparently not been attempted, probably because of the difficulties noted above.

Salvia individuals may be rather insensitive to sudden changes in their immediate environment. The persistence of all of the vegetative parts (leaves, rhizome, roots) from year to year may buffer individuals against rapid decline in suddenly adverse (e.g, increased density) conditions. Failure to respond to newly available space would suggest, in addition, a delay in growth responses. Both of these are physiological mechanisms leading to "population inertia" (Murdoch 1970); the plant responses would then have been determined by conditions which prevailed prior to the manipulations. If such historical effects are prevalent, assessment of density reponses of mature individuals may require establishment of seedlings at a wide range of densities and following them to maturity. However this method measures the effect of density integrated over the whole life cycle, rather than at a particular time as the method of abruptly changing density can do.

The alterations of Salvia density may have produced concomitant alterations of local conditions which would not usually be related to density but which

counteracted its effects. For instance, water loss by evaporation from the exposed soil surface could exceed that which was lost from areas where vegetation remained. The problem of correlated effects of a treatment is a hazard in any experiment, but especially so when existing conditions are altered rather drastically.

Although the experiments were designed to account for "background" variation and thereby to improve the chances of detecting responses, two principal sources of variation could not be eliminated: a)spatial variation which significantly affects growth of Salvia seedlings has been documented in this field at scales finer then those spanning the replicates in these experiments (Chapter V) and b) plants within size groups varied in size over a fairly narrow range, and in other characteristics (e.g., age, flowering history) probably over a much wider range. Thus the efforts to account for variation may have been insufficient, and the weak observed responses, though not statistically significant may be of biological interest.

Few experimental studies of density responses in nature are available for comparison with the present study. Studies entailing density perturbations of annual (Watkinson & Harper 1978; Clay & Shaw 1981) and perennial (cited in Harper 1977; Chapter II) species have almost exclusively followed populations established from different deliberately sown densities of seeds and have reported striking density-dependent reduction in individual success. In addition, Watson (1974) removed all Plantago lanceolata from a strip of dune pasture and found lower mortality rates among naturally recruited seedlings in that area relative to those in the control strip. The consequences of altering existing densities by removing conspecifics from around established individuals has been reported only for Larrea tridentata and Ambrosia dumosa in the Mojave desert (Fonteyn & Mahall 1981). The response measured was pre-dawn water potential. For

Larrea, water potential was found to be significantly higher when either Larrea or Ambrosia was removed than in controls; however, only removal of Larrea caused a significant increase in water potential of Ambrosia. Unfortunately, no other response measures were reported, and drawing inferences regarding demographic consequences of the removals is difficult.

The use of removals has more often been applied to the study of interspecific interference in natural communities (Connell 1961, Sagar & Harper 1961, Colwell & Fuentes 1975, Putwain & Harper 1971, Silander & Antonovics 1982, Fowler 1978). Although intraspecific effects cannot be inferred from such studies, it is worth noting that Fowler (1978) demonstrated weak responses in per cent cover of various species to removal of individual dominant species in a field near and similar to that of the present study. She found that removal of Paspalum laeve alone or removal of all grasses caused an increase in the per cent cover of Salvia. Her general conclusion was that diffuse competition prevailed among the principal species. Conversely, in a simpler dune community, Silander and Antonovics (1982) demonstrated striking responses in per cent cover of various species to removals of individual species. The evidence of the present study indicates that intraspecific interaction of Salvia at the natural densities is also weak and, in conjunction with Fowler's work, that the diffuse competition imposed by the various grass species and Paspalum laeve in particular may be the principal determinant of individual Salvia phenotypes and, hence, the demography of the species.

Experimentalists investigating density response in nature have, to date, chosen to study simple communities and their members. In such situations the focal species are the dominant members of their communities, a fact that may enhance the chances of detecting density reponses.

Experiments conducted in artificial conditions have demonstrated that the density reponse of a given species is stronger when its own frequency is high than when a second species is present at high frequency (Harper & McNaughton 1962, Watkinson 1981, Antonovics, et al. 1983); thus, the existing literature of density responses in nature is biased toward detection of severe density effects more frequently than they actually occur.

The responses of mature individuals documented here are far weaker than those manifested in an experiment in which densities were established at the seedling stage in garden conditions (high soil fertility, regular spacing, pure culture). Density-dependent reduction in fecundity and survival was apparent at spacings as great as 15 cm (Chapter IV). As mentioned above, this discrepancy may be due, in part, to persisting effects of earlier conditions in the present experiments; obviously, effects of previous conditioning by density and of present density can not be disentangled from the garden experiment. The discrepancy is due also, almost certainly, to the difference between rates of individual growth and limits to maximum size in field and garden conditions, such that, at a given spacing, individuals grow large enough to interfere with one another at wider spacings.

In summary, the two experiments presented here have revealed only slight, if any, suppression of established Salvia individuals by conspecific neighbors at naturally occurring densities. This finding implies that internal control of population growth at the adult stage, as at the seedling stage (Chapter II) is either very weak or quite delayed. In either case, population numbers may be expected to fluctuate without internal control. Thus, local extinctions followed by recruitment by seed from neighboring areas may be common in this field, whereas attainment of

very high densities at which self-regulation would occur appears to be precluded usually by density-independent effects. Since studies of density effects in nature have been restricted primarily to inhabitants of open communities, it is yet unclear whether density reponses should be expected to be weak in diverse communities or whether this is typical of perennial species relative to annual. Generalities of this kind must await future studies.

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CHAPTER IV. GENETIC EXPERIMENTS IN ARTIFICIAL CONDITIONS

INTRODUCTION

The realization in the middle of this century that genetic variation within species is the rule rather than the exception (Lewontin 1974), and that evolution is an ongoing process subject to experimental investigation (Antonovics 1976) motivated efforts to understand the maintenance of this variation. Among the many mechanisms, some were based on environmental effects, others on genetic effects, and still others were based on interactions between genetic and environmental effects. Density-dependent selection is an example of the latter. In density-dependent selection, the fitness rankings of genotypes in a population vary as population density varies. (Sometimes the term is used more loosely for any situation in which selection intensity varies with density). This mode of selection is of particular interest, because it is driven by variation in the biotic environment, and because it is thought to be particularly powerful in maintaining genetic variation in life history traits (MacArthur and Wilson 1967; Roughgarden 1979; Stearns 1976).

The occurrence of density-dependent selection depends on the existence of genetic variation for response to density, as would be indicated by a statistical interaction of genotype x density in fitness or its component traits. Such evidence was first detected in Drosophila by Timofeev-

Ressovsky (1934) and by Dobzhansky and Spassky (1944) and then more fully investigated by Lewontin (1955). Since then, numerous examples of genetic variation in sensitivity to density have been documented for a variety of fitness traits in laboratory populations of insects (e.g., Birch 1955; Sokal and Sullivan 1963; Sokal and Huber 1963; Sokal and Karten 1964; DeBenedictis 1977; Marks 1982) and for yield characters in populations of crop species (Baker and Briggs 1982; Campbell and Kern 1982; Talukdar and Bains 1982).

Given the amount of effort that has been applied to the experimental study of genotype x density interactions and to the theoretical investigation of their consequences in natural populations, it is surprising that very little information is available which bears directly on whether density-dependent selection occurs in natural populations. Previous studies fail to address this point on two accounts. First, with the exception of Marks (1982), all the studies have compared strains which were previously subjected to artificial selection. The variation detected in any trait is, thus, unlikely to resemble that of any wild population. Second, these studies have all been conducted in artificial conditions. As a result, the responses measured may differ from those that would be displayed in a natural background environment. Because of these limitations in the previous work, and because prior work on natural populations has merely correlated life history characteristics of species with the densities of the environments they occupy (e.g, Gadgil and Solbrig 1972), we remain ignorant of the process of density-dependent selection in nature, even though its significance has been repeatedly emphasized.

In view of this, the experiments of the present chapter were designed to reveal genetic variation in density response in a wild population of

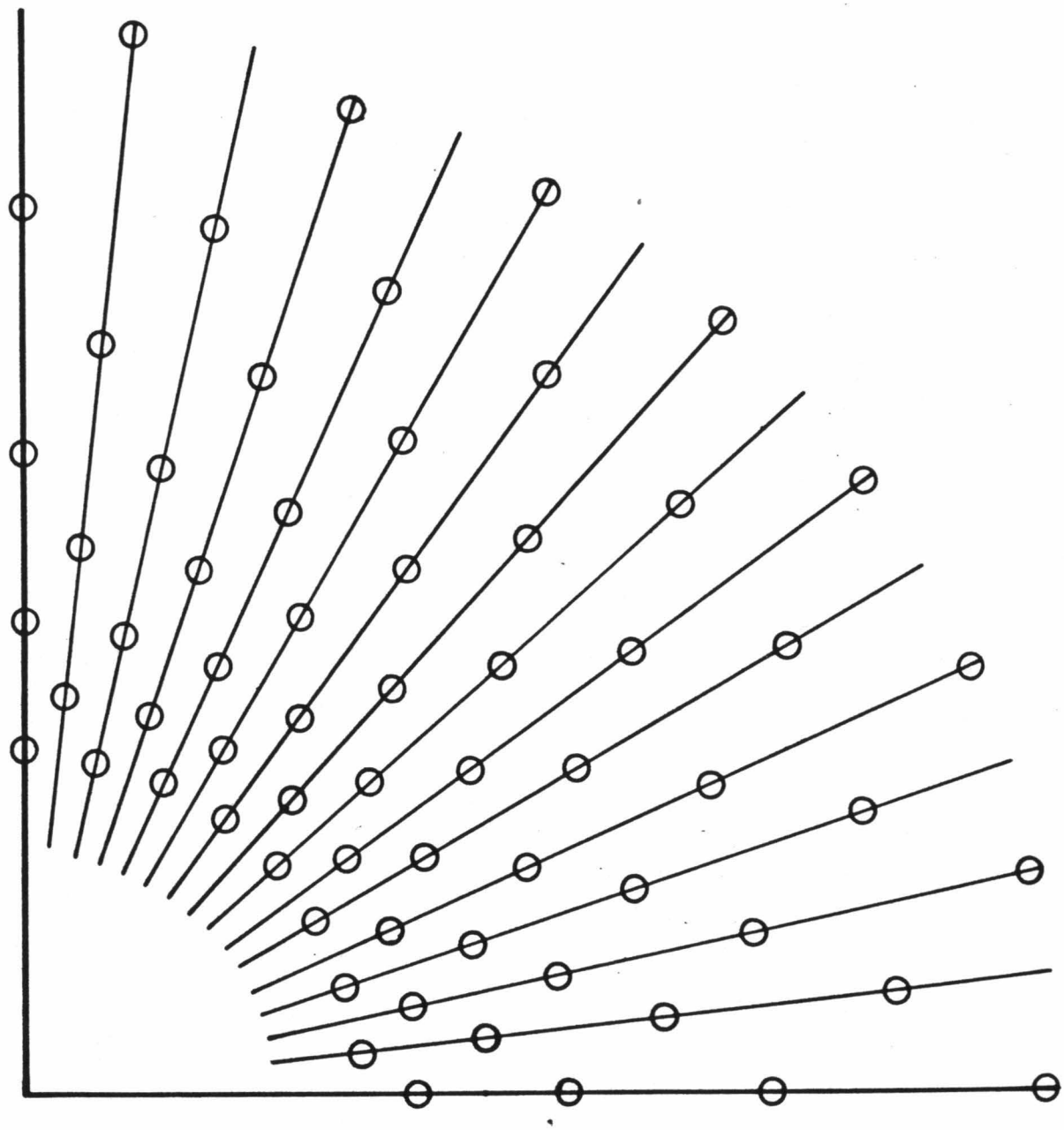
Salvia lyrata. The experiments were conducted under somewhat artificial conditions (i.e., high soil fertility, low background species diversity) with the intention that these would most clearly reveal the variation in the wild collected population of Salvia. The experiment described in the final chapter was similar in design but was conducted in the field which was the source of the experimental population. This last experiment thus reveals the density responses evinced in the natural environment.

MATERIALS AND METHODS

Experiment I: In May, 1979, seeds of Salvia lyrata were collected in lots according to common female parent from plants growing in a wild population on the campus of Duke University (for description of the field site, see Chapter 1). Flowering plants were chosen at 2 m intervals along a 50 m transect running from west to east and a 70 m transect running from north to south. The plants were visited on several occasions during one week in order to provide as many mature seeds as possible from each parent plant. Since Salvia is self-compatible, and cross-pollination is apparently infrequent in nature, each seed collection should be considered more nearly a self-sibship than a half-sibship.

On November 21, 1979, twelve sibships having at least 35 seeds were planted at even spacing into soil in flats in the Duke University Botany Department Greenhouses and raised for four weeks. The seedlings were then planted into cold frames in a fan-shaped array (Fig. 4.1), in which spacing between plants varied in a geometric series from 1 cm to 18 cm. The families were randomized into the six postions of the 16 arcs (=spacings) such that each family occurred in each successive pair of arcs within each fan. One row of extra individuals was planted along the edge but were

Figure 4.1. Planting design for Experiment I. Each circle represents an individual plant. Only eight arcs (spacings) are shown; sixteen were used in the experiment.



from the analysis. The fan design was replicated four times with orientation of the fan randomly determined for each replicate. Every occurred in every spacing twice over the whole experiment.

During the winter, the cold frames were covered at night and during with plastic stretched across a wooden frame. The plants flowered in 1980. At that time and in each subsequent month through November, and the following May, data on survival and flowering, including whether plant flowered and the number of maturing seeds, were recorded.

The highest six densities were excluded from the analysis, because were too crowded to allow certain identification of individuals, very which survived for the duration of the experiment. The arcs were to render the design balanced. Thus, all subsequent references to "density" indicate pairs of spacing treatments in each of every family occurs once in every replicate.

In May, 1980, seeds were collected by maternal parent from growing in the same field referred to above. The plants were chosen intervals along a series of twenty-three transects which spanned the each about 40 m long. Twenty families from among those obtained chosen at random and from each of the twenty families, five seeds were into soil in the greenhouse in November, 1980. The plants flowered following March. At that time, one plant from as many of the twenty as possible was chosen for a series of crosses, according to its flowering was synchronized with other individuals. This obtaining the maximum number of crossed seeds. Half the plants designated to serve as egg parents and half as pollen parents to yield factorial crossing design.

Pollen transfer was effected in the following way. Prior to

dehiscence, anthers were removed from large, closed buds by piercing the corolla at the attachment of the filament. The stigma was then exposed and pollen from a dehisced anther of the designated male parent was deposited on its surface. For each combination of male and female, nine flowers were pollinated in order to provide a maximum number of thirty-six seeds per cross.

Asynchrony in flowering time limited the number of crossed progenies to forty-one from eight male and eight female parents. Seeds produced by selfing were also collected from each individual. The seeds obtained in this way were weighed individually on a Cahn microbalance and then planted individually in soil into compartments of "Rootrainer" inserts in July, 1981, in a randomized block design. The date of emergence was recorded for each of the seedlings during the emergence period which lasted six months.

Following their emergence and early growth in the greenhouse, the progeny were transplanted outside to an experimental garden in the "Duke University Botany Experimental Plot". The area had previously been cleared of vegetation by treatment with methyl bromide. Stolons of reinvading Cynodon dactylon were removed during planting, and afterwards Poa pratensis was sown to produce conditions of interspecific competition somewhat resembling those in the field. Regular hexagonal arrays of two different spacings (7.5 cm and 15 cm between neighbors) were established in eight replicate blocks with each individual of known parentage surrounded by six Salvia plants obtained from a random seed collection from the field. These latter were measured to permit a nearest neighbor analysis, but are not considered in the present paper. Because emergence was protracted, individuals representing each family were chosen according to their date of emergence, such that all individuals within a block were, as nearly as

possible, the same age and, hence, the same size. Each family was represented once in each plot (=block x spacing combination). Full-sib families in which too few seeds or seedlings were available were eliminated from the experiment. Thus, a total of twenty-three families from five male and five female parents (two crosses missing from the factorial), as well as selfed progeny of each parent were planted in split plot design. Spacing was the whole plot factor and full-sib family was the split plot factor. Individuals were transplanted by block from September through November, 1981. The last three blocks planted were eliminated from the analysis due to severe mortality.

The size of each individual (number of leaves and length of longest leaf) was measured in October, 1981, and in May, 1982, when the height of inflorescence, number of flowering nodes, number of mature flowers and number of seeds maturing were also recorded.

The data were analysed using the GLM procedure of the Statistical Analysis System (Helwig and Council, 1979) and using programs for the G test (Fienberg 1970), written by Dr. T.R. Meagher for a Hewlett-Packard 9820 and for an IBM 5100. Variance component correlations and mean square correlations among full-sib families were calculated from hypothesis and error matrices obtained in multivariate analysis of variance. The former were occasionally undefined due to estimates of negative variance components, and since significance tests are not at present available for them (Tallis 1959), the discussion of correlations employs the values obtained by both methods.

RESULTS

Initial survival of seedlings in Experiment I was high. After one month, 369 remained from 384 seedlings planted. In the first growing season, through September, 1980, the probability of survival depended significantly on an individual's family identity ($G=28.4$, $df=11$, $p<.005$) and on the density in which it grew ($G=19.8$, $df=4$, $p<.005$). The mortality which occurred in the ensuing eight months was more highly dependent on density ($G=69.4$, $df=4$, $p<.001$) and less so on family identity ($G=22.7$, $df=11$, $p<.025$). In both intervals, the interaction of family x density x survival was not significant ($G=42.5$, $G=45.0$, $df=44$, $p<.5$), indicating that the density response of mortality was similar for all twelve families (Fig. 4.2).

In May, 1980, the fraction of plants flowering at each density varied among families ($G=67.13$, $df=44$, $p<.05$) (Fig. 4.3). Two-way G tests of family x flowering considering each density separately and of density x flowering considering each family separately revealed the sources of this interaction (Tables 4.1 and 4.2). At the three lowest densities, the fraction of plants flowering was uniform among the families. Disparities among the families appeared only at the two highest densities. Five of the twelve families declined significantly in fraction of plants flowering with increasing density (Table 4.2, Fig. 4.3), whereas the remaining seven showed less (Families 9 and 10) or no (Families 4 and 6) variation among densities.

The number of seeds produced per plant in the initial flowering episode, May, 1980, was much higher in comparison with plants growing in the natural population and, in the low densities, fecundity remained very

Figure 4.2. Histograms of numbers of individuals surviving in each density for each family. The initial number of individuals in every family x density combination was four.

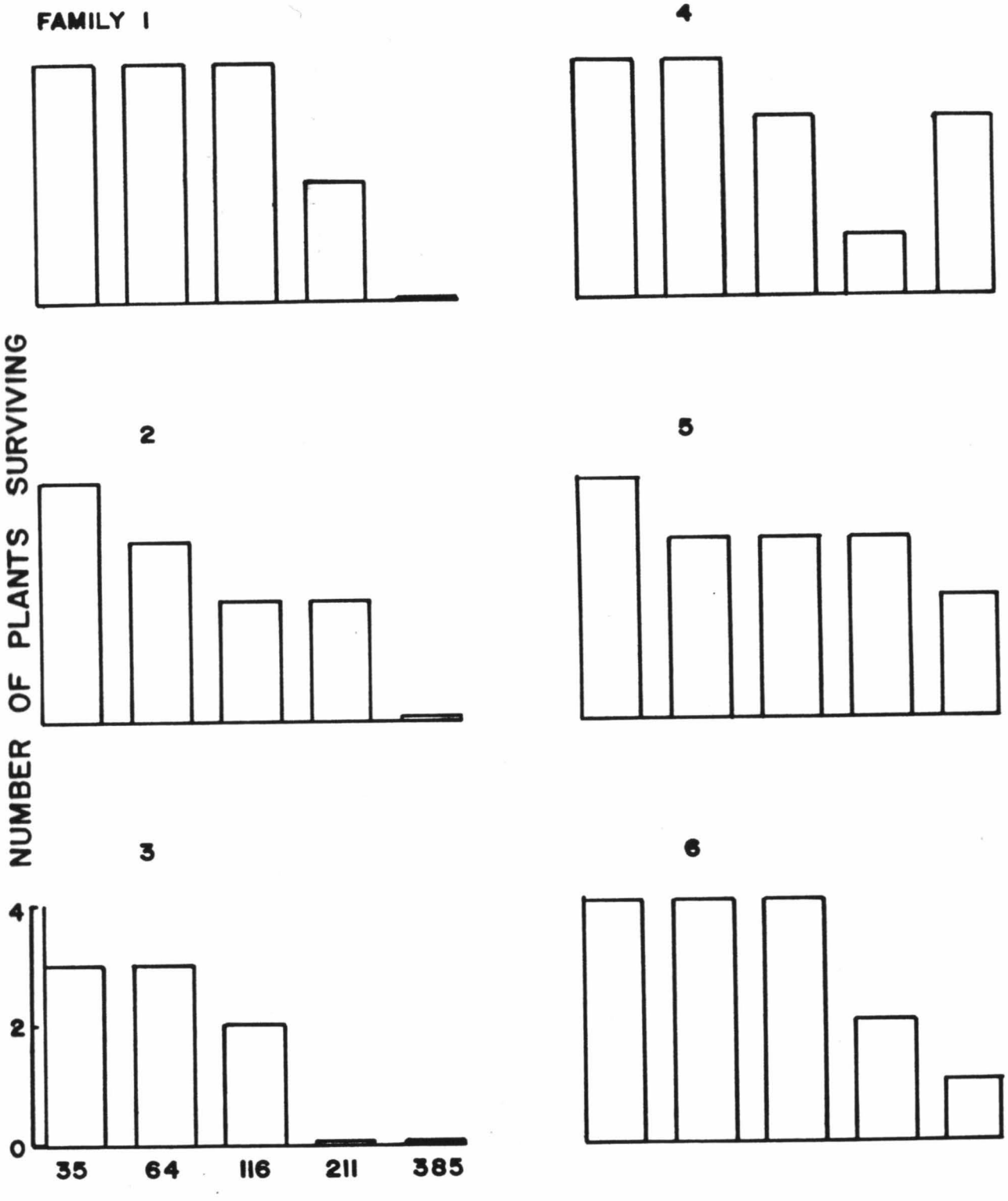

FAMILY 1
4
2
5
3
6
NUMBER OF PLANTS SURVIVING
0
2
4
35
64
116
211
385
DENSITY
(plants / m2)

Figure 4.2. continued.

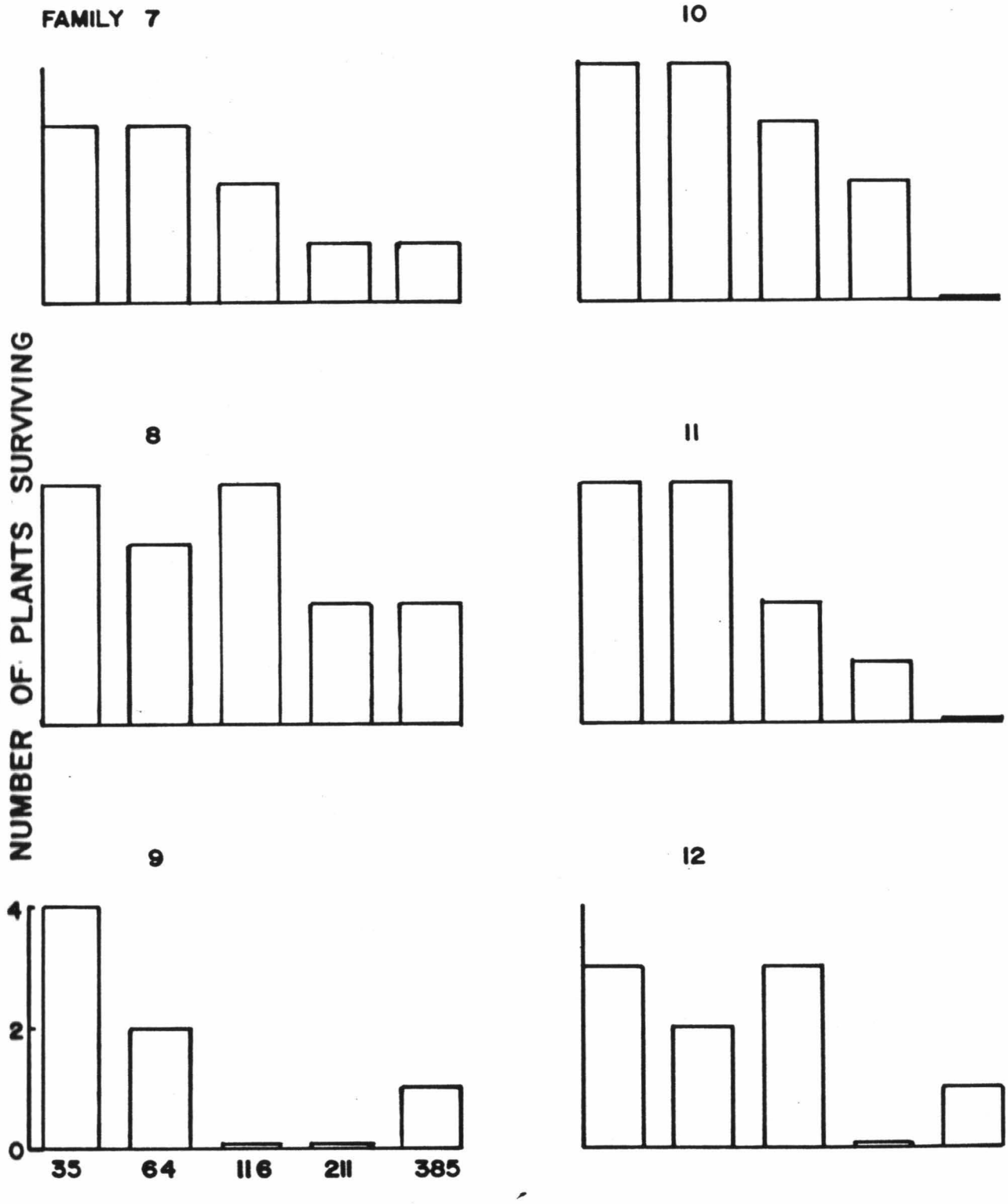

FAMILY 7
10
8
11
9
12
NUMBER OF PLANTS SURVIVING
4
2
0
35
64
116
211
385
DENSITY
(plants / m2)

Figure 4.3. Histograms of numbers of individuals flowering in each density for each family.

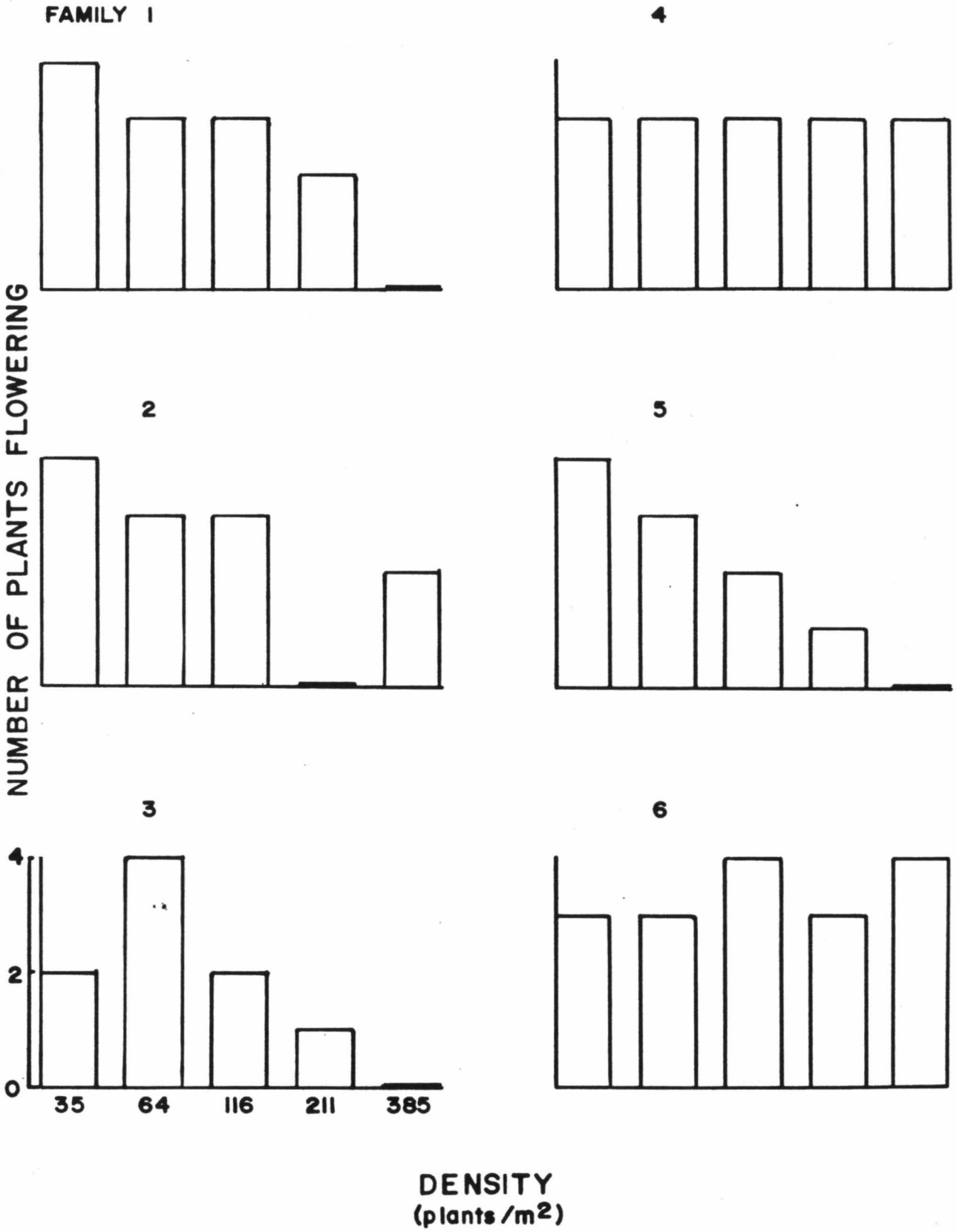

FAMILY 1
4
2
5
3
6
NUMBER OF PLANTS FLOWERING
0
2
4
35
64
116
211
385
DENSITY
(plants /m2)

Figure 4.3. continued.

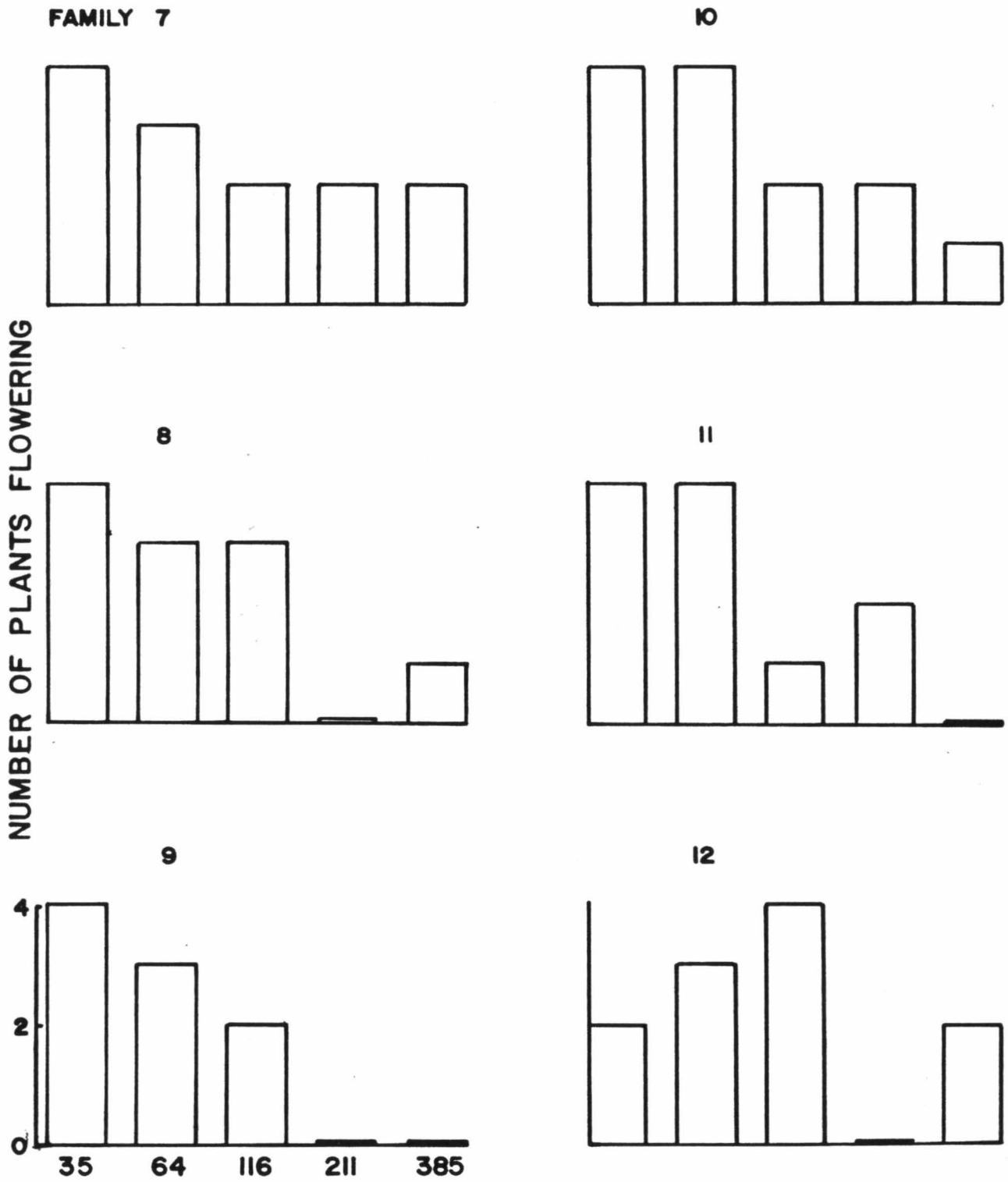

FAMILY 7
10
8
11
9
12
NUMBER OF PLANTS FLOWERING
4
2
0
35
64
116
211
385
DENSITY
(plants / m²)

Table 4.1. Tests of heterogeneity among families in flowering response by density, Expt I.

Density plants/m2	Family x Flowering G	p<
35	16.1	ns
64	6.9	ns
116	12.2	ns
211	20.8	.05
383	29.4	.005

Table 4.2. Tests of heterogeneity among densities in flowering response by family, Expt I.

Family	Density x Flowering G	p≤
1	17.3	.005
2	14.6	.01
3	12.5	.025
4	1.6	ns
5	12.4	.025
6	3.8	ns
7	4.7	ns
8	12.6	.025
9	6.5	ns
10	5.2	ns
11	8.4	ns
12	10.2	ns

high throughout the experiment. For individuals growing at the lowest four densities, the average seed output in the first flowering episode (May, 1980) was 283, compared with 110 for comparably sparse areas in the natural population, in which a far lower proportion of plants flowered.

The number of seeds produced per planted individual varied significantly among densities in May 1980 (Table 4.3). At that time, there was no significant variation among families in seed number nor in the response of that trait to density. The total seed output per individual summed from May, 1980, through May, 1981, varied among densities and families (Fig. 4.4, Table 4.4), but there was no significant interaction.

Virtually all individuals planted into the earlier five blocks of Experiment II survived. Four size traits (number of leaves and length of longest leaf, measured in October, 1981 and in May, 1982) and one fecundity measure (number of seeds produced in May, 1982) were analysed.

Comparison of individuals produced by selfing with those derived from crosses revealed substantial differences between them (Table 4.5). The mean for crossed plants was higher for each of the five traits, regardless of density. There was significant variation among densities in the number of leaves per plant in May, 1982. For that and every other trait the plants were smaller and less productive at high densities. The interactions of breeding type with density and with block were not significant.

Within the class of plants produced by selfing, there was significant variation among the progenies in the two early size traits (number of leaves and length of longest leaf in October, 1981) and also in the number of seeds produced (Table 4.6). For this group as well, a density effect was apparent only in the number of leaves in May, 1982, but there was a consistent tendency toward reduction in size and fecundity with increasing

Table 4.3. Analysis of variance of number of seeds borne in May, 1980 (log transformed), Expt I.

Source	df	SS	F	
Block	3	21.5	1.5	
Density	2	129.8	7.6	$p<.05$
Family	11	37.3	0.7	
Family x Block	33	158.9	0.9	
Family x Density	22	137.9	1.2	

Figure 4.4. The number of seeds produced per planted individual over the period, May, 1980 - May, 1981 plotted against density by family. The dashed lines indicate families with significantly lower average seed output relative to the families shown in dotted lines, according to Bonferroni confidence intervals.

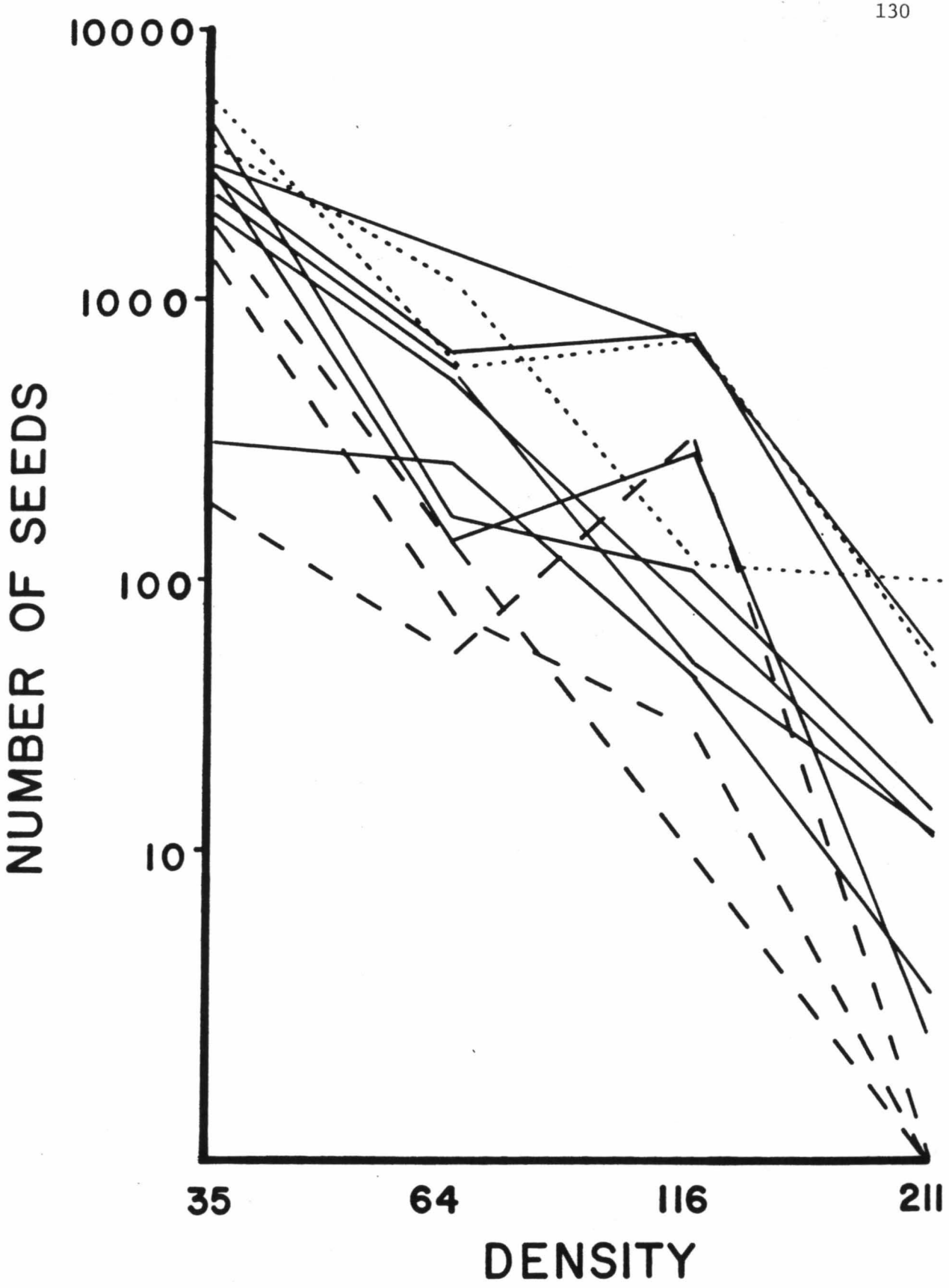

10000
1000
100
10
NUMBER OF SEEDS
35
64
116
211
DENSITY

Table 4.4. Analysis of variance of total seed output over one year (May, 1980-May 1981), (log transformed), Expt I.

Source	df	SS	F	
Block	3	1.3	.1	
Density	3	678.6	70.1	p<.0001
Family	11	194.9	4.0	p<.001
Family x Block	33	145.9	0.9	
Family x Density	33	106.9	0.7	

Table 4.5. Comparison of progeny resulting from selfing and outcrossing in two densities for each of five traits. Values sharing the same letter horizontally are not significantly different between densities; values sharing the same letter vertically are not significantly different between breeding systems.

		Low Density		High Density
Number of Leaves 10/81	Selfed	11.0	a	10.0
		a		a
	Crossed	11.3	a	10.1
Length of Leaf 10/81	Selfed	60.4	a	59.5
		a		a
	Crossed	62.7	a	62.4
Number of Leaves 5/82	Selfed	15.7	a	9.8
		a		b
	Crossed	18.8	b	11.8
Length of Leaf 5/82	Selfed	66.3	a	62.7
		a		a
	Crossed	74.8	b	67.2
Number of Seeds 5/82	Selfed	126.8	a	88.2
		a		a
	Crossed	163.6	a	106.4

Table 4.6. Multivariate analysis of variance of size and fecundity, selfed progeny alone, Experiment II. The factor, Mother, is considered random. Tests are based on Type IV Sums of Squares.

Source	df	Number of Leaves 10/81 F	Length of Leaf 10/81 F	Number of Leaves 5/82 F	Length of Leaf 5/82 F	Number of Seeds 5/82 F	Multivariate
Block	4	1.10	7.75 *	6.51 *	3.90	6.06 +	
Density	1	4.09	0.64	30.86 ***	2.19	2.09	
Mother	8	8.80 ***	3.56 **	0.99	1.01	5.37 **	
B x M	31	1.64	1.35	0.68	0.80	0.70	
D x M	8	1.95	0.31	0.58	0.96	0.69	

+, $p<.07$; *, $p<.05$; **, $p<.01$; ***$p<.0001$

density. There were no significant interactions of family x density or family x block. Thus, among the inbred individuals, the responses to environmental variation, both in density and among blocks, were relatively uniform.

The size and growth of outbred individuals were analysed with two measures of size prior to planting (number of leaves and length of longest leaf in August, 1981) included in the model. A reduction in size and fecundity with increasing density was observed, though this effect was not, in general, significant (Table 4.7). There was no other significant variation among maternal half-sibships, nor any among paternal half-sibships when individual size prior to planting was included in the model.

Graphs of the traits against density by half-sibship and by full-sibship (Figs. 4.5-4.9) reveal that the density responses are not uniform and that the response lines cross. The shifts in rankings are slight, however, and the interactions are not statistically significant. The interaction of full-sibship x density (father x mother x density) is marginally significant for the trait, length of longest leaf in May, 1982 (Fig. 8b). Extreme shifts in ranking are apparent, although significance tests for the shifts of rank are not available. This three-way interaction in the absence of father x density and mother x density interactions implies that dominance or epistatic effects, rather than additive genetic effects, influence the response of this size trait to density. Tests for overall significance of the effects in the model in the multivariate sense are all non-significant.

Differences in individual growth due to the factors in the experiment were investigated using the parallelism test of profile analysis (Timm 1975). The difference between size in May, 1982 and size in October, 1981

Table 4.7. Multivariate analysis of variance of size and fecundity, outbred individuals alone, Experiment II. The factors, Father and Mother, were considered as random effects. Tests are based on Type IV Sums of Squares. +, $p<.1$; *, $p<.05$; **, $p<.01$.

Source	df	Number of Leaves 10/81 F	Length of Leaf 10/81 F	Number of Leaves 5/82 F	Length of Leaf 5/82 F	Number of Seeds 5/82 F	Multivariate
Block	4	2.17	19.97 **	2.50	13.91 *	3.57	
Density	1	0.12	10.13 *	0.33	0.68	0.01	
Father	4	2.65 +	1.46	1.89	0.45	1.58	
Mother	4	4.22 *	4.56 *	0.51	0.83	0.78	
Number of Lvs 8/81	1	0.50	3.22 +	0.91	0.00	0.34	
Length of Lf 8/81	1	11.34 **	1.02	0.73	0.51	1.47	+
B x F	15	0.68	1.42	1.22	0.98	0.66	
B x M	16	0.87	1.33	0.91	1.33	0.44	
D x F	4	1.52	0.05	2.55	0.50	0.48	
D x M	4	0.59	0.93	2.59 +	0.47	0.85	
NL x D	1	0.26	1.64	0.36	0.42	0.52	
LL x D	1	0.38	0.96	5.49 *	2.13	0.02	
M x F	13	1.26	0.87	2.00 *	1.13	1.36	
D F M	13	1.20	1.39	0.54	1.75	1.30	
B F M	33	1.85 *	0.84	1.81 *	1.32	1.40	

Figure 4.5. Number of leaves, 10/81, vs. density. a) by half-sibship. Maternal families are indicated by an asterisk on the right margin. Paternal families are unmarked. b) by full-sibship.

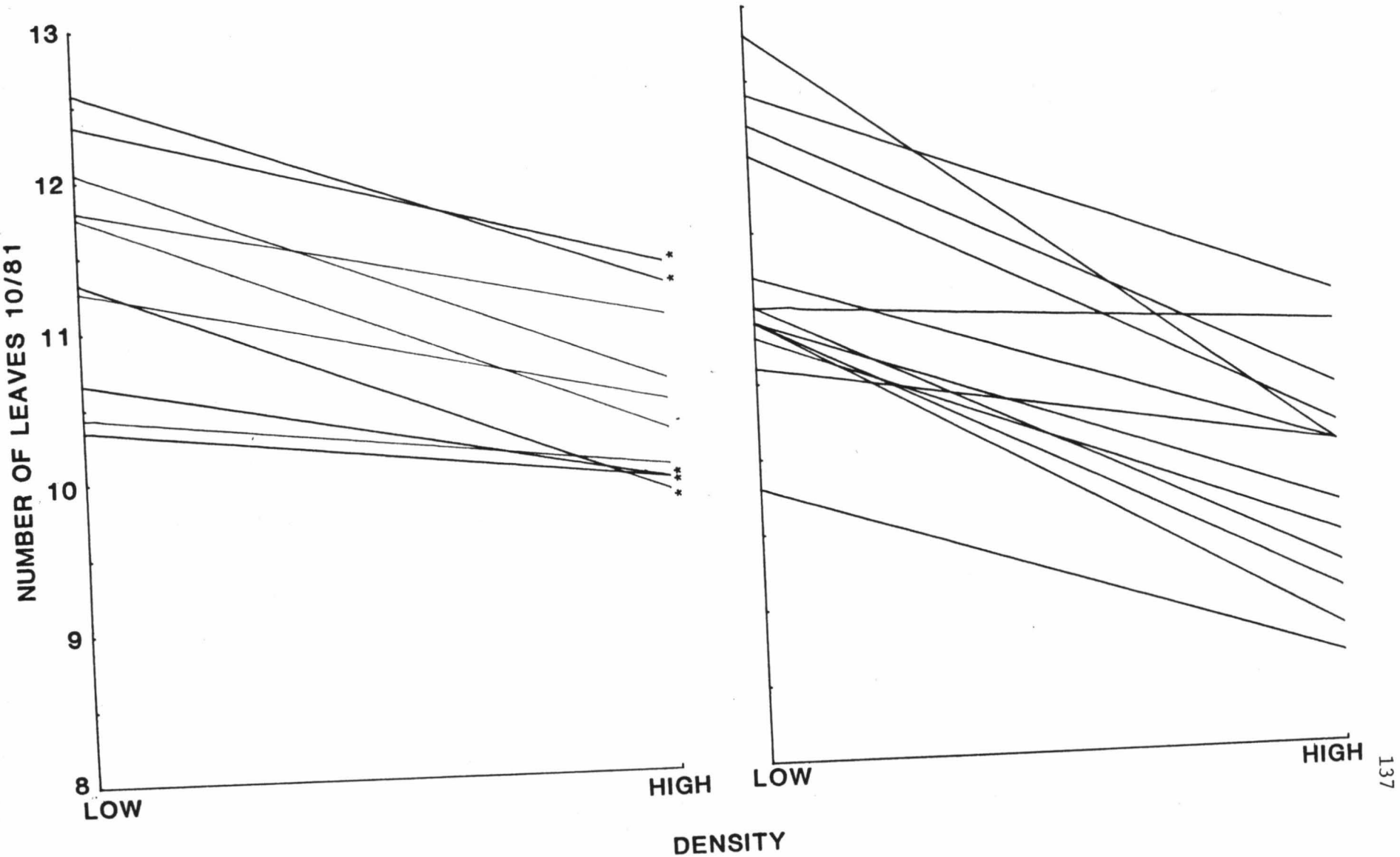

13
12
11
10
9
8
NUMBER OF LEAVES 10/81
LOW
HIGH
LOW
HIGH
DENSITY

Figure 4.6. Length of longest, leaf 10/81, vs. density. a) by half-sibship. Maternal families identified as in Fig. 5. b) by full-sibship.

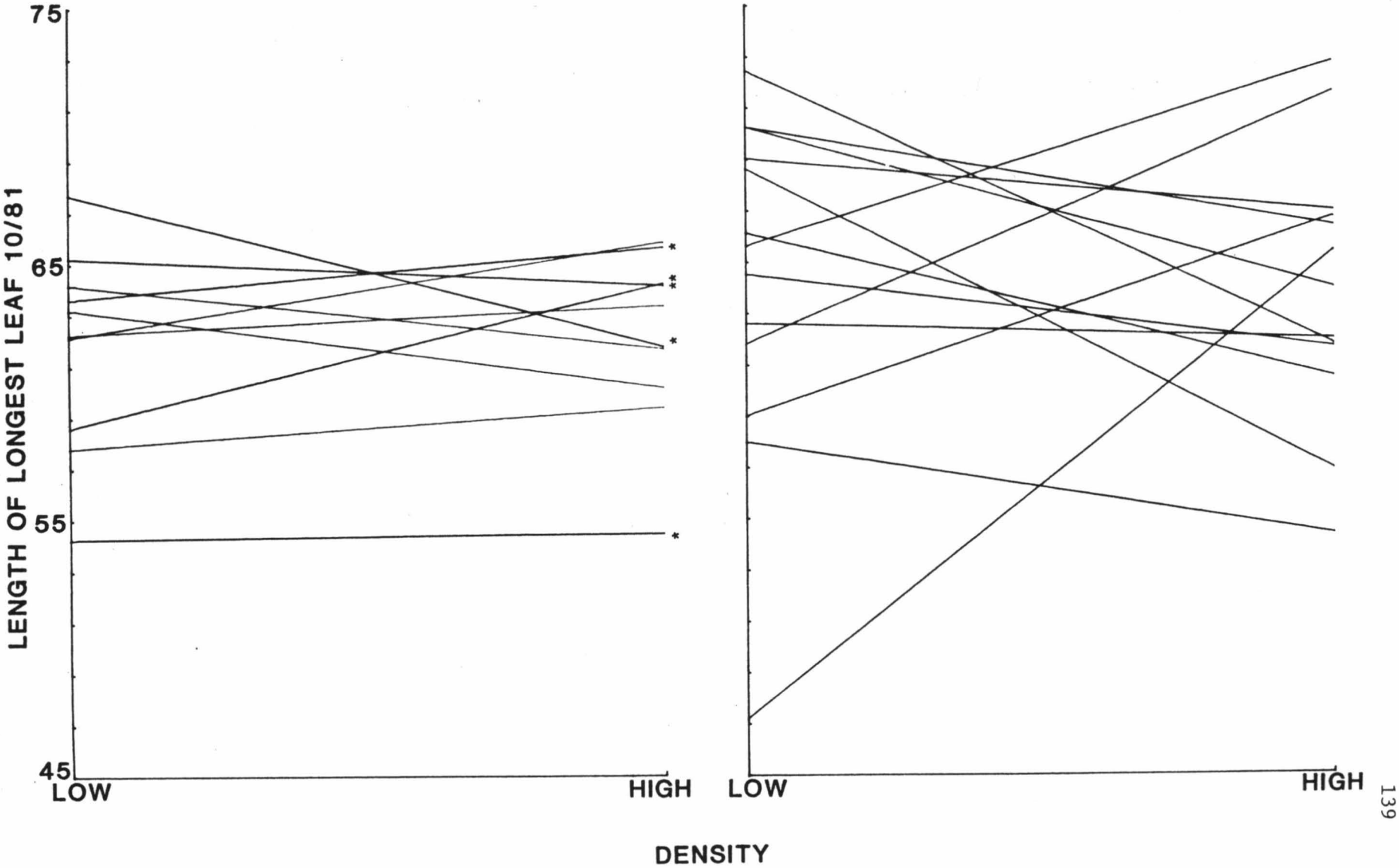

75
65
55
45
LENGTH OF LONGEST LEAF 10/81
*
**
*
*
LOW
HIGH
LOW
HIGH
DENSITY

Figure 4.7. Number of leaves, 5/82, vs. density. a)by half-sibship. Maternal families identified as in Fig. 5. b) by full-sibship.

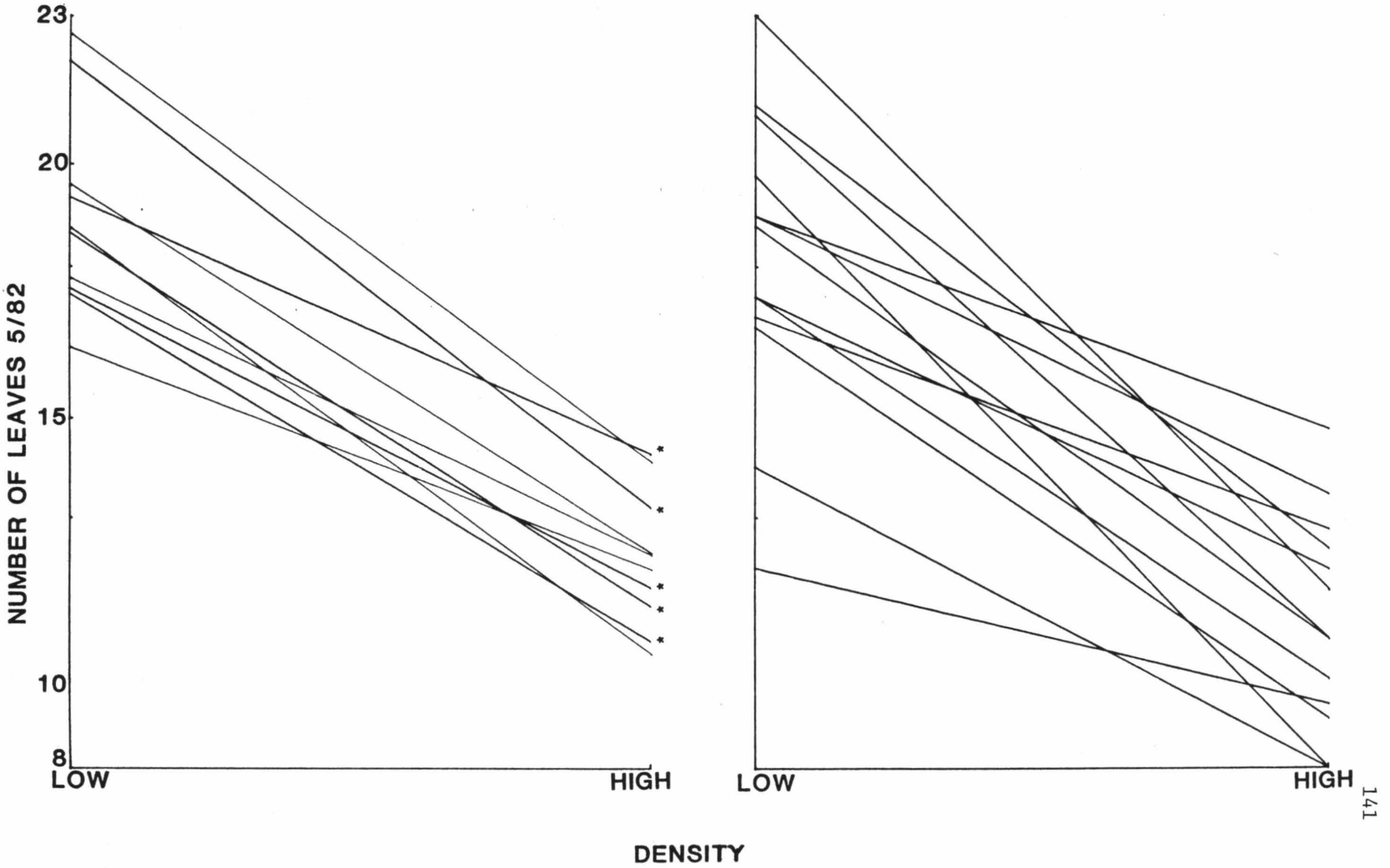

23
20
15
10
8
NUMBER OF LEAVES 5/82
LOW
HIGH
LOW
HIGH
DENSITY

Figure 4.8. Length of longest leaf, 5/82, vs. density. a)by half-sibship. Maternal families indicated as in Fig. 5. b) by full-sibship.

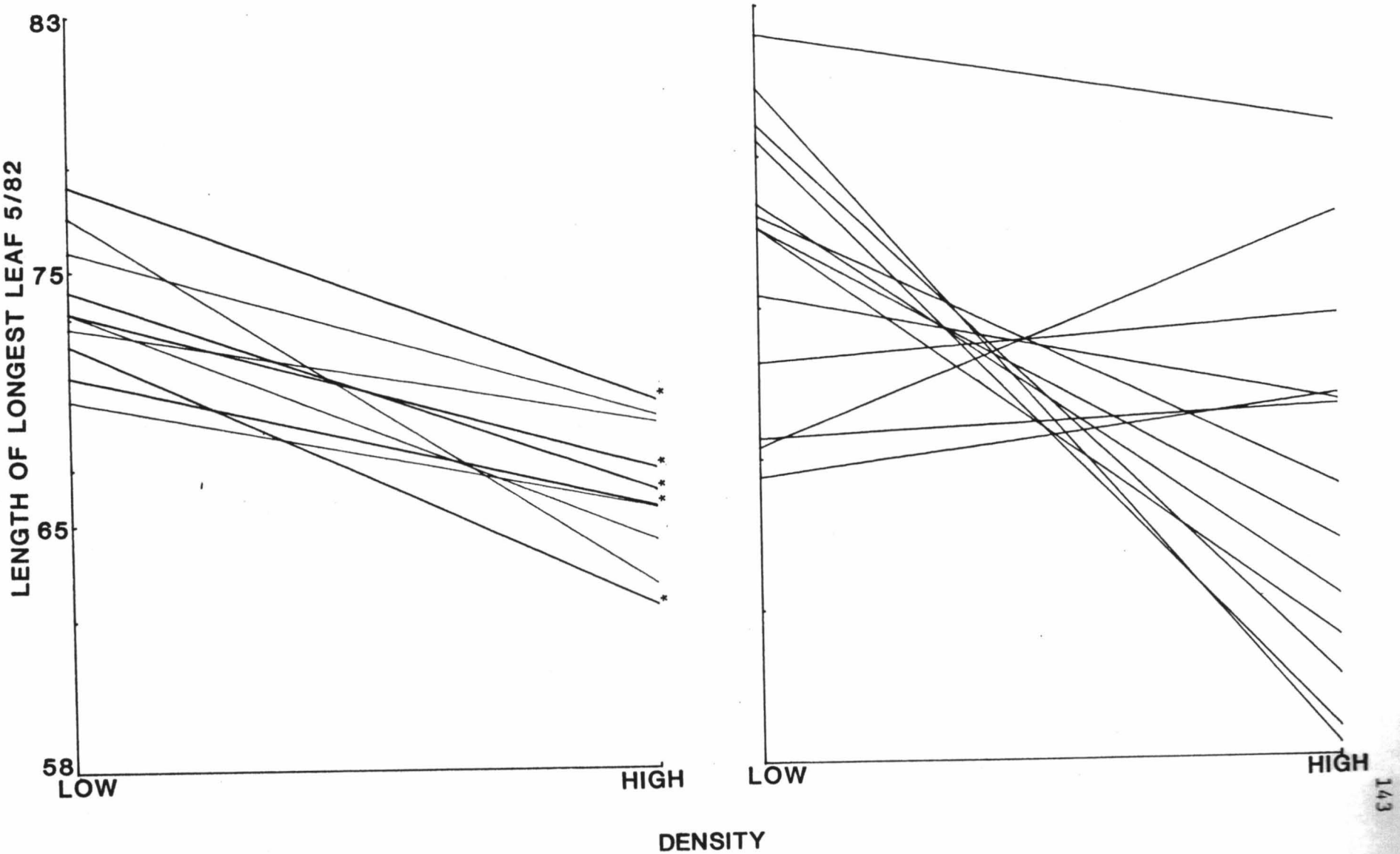

83
75
65
58
LENGTH OF LONGEST LEAF 5/82
LOW
HIGH
LOW
HIGH
DENSITY

Figure 4.9. Number of seeds, 5/82, vs. density. a) by half-sibship. Maternal families identified as in Fig. 5. b) by full-sibship.

both in number of leaves and in length of longest leaf were the response variables in the multivariate analysis of variance (Table 4.8). There was a significant effect of the interaction, density x mother, both on the univariate analysis of change in leaf number and in the overall multivariate test. Thus the response of that growth trait to density varies among maternal half-sib families. In addition, the three way interaction, block x mother x father is significant for the change in leaf number, implying that variation exists among full-sibships in the response of this growth trait to background environmental variation.

The correlations among full-sib families for eight traits related to size and fecundity are presented for the two density treatments (Table 4.9a,b). The causes of these correlations include genetic ones (i.e, pleiotropy and linkage) and environmental ones (i.e, maternal effects). Statistical tests for comparing whole correlation matrices are not available, nor are tests for the significance of individual variance component correlations. However, element by element comparison, considering variance component and mean square correlations together, can reveal differences among matrices which are of interest.

There are conspicuous similarities in correlations of the traits of plants grown at different densities. Among these are the strong positive correlations with number of flowers (with number of seeds, inflorescence height, number of leaves in October and May and length of leaf in May). Strong negative genetic correlations at the low density (for example, length of leaf in October with number of leaves in October, inflorescence height, number of flowers, and number of seeds; seed weight with number of flowers; and number of seeds with inflorescence height) are not apparent in the high density. The negative correlations suggest a genetically based constraint, either through pleiotropy or linkage, to increasing both of

Table 4.8. Multivariate analysis of variance of two growth traits, change in number of leaves 10/81-5/82 (DNL) and change in length of longest leaf 10/81-5/82 (DLL). The effects, Father and Mother, are considered random. Tests are based on Type IV Sums of Squares.

		DNL	DLL	Multivariate
Source	df	F	F	
Block	4	1.19	4.22 +	
Density	1	0.14	2.70	
Father	4	1.61	0.59	
Mother	4	0.32	1.99	
Number of Lvs 8/81	1	0.39	1.85	
Length of Lf 8/81	1	0.31	0.03	
B x F	15	1.34	0.77	
B x M	16	0.69	0.99	
D x F	4	3.32	0.57	
D x M	4	5.09	1.45	*
NL 8/81 x D	1	0.61	2.29	
LL 8/82 x D	1	3.98 +	0.91	
M x F	13	1.35	0.91	
D x F x M	13	0.34	1.07	
B x F x M	33	1.69 *	1.51 +	

+, $p<.07$; *, $p<.05$.

Table 4.9. Genetic correlations based on full-sibships, Experiment II. The top value, where present, is the variance component correlation; the lower value is the correlation of family means, for which significance tests can be made. The variance component correlation is occasionally undefined, because negative values for the variance components are estimated. a) Correlations in low density.

a)

	Seed weight	Leaf # 10/81	Leaf Length 10/81	Leaf # 5/82	Leaf Length 5/82	Infl. height	# of Flowers	# of Seeds
Seed weight		-.22 -.13	.27 .14	-- -.28	-- -.30	-.71 -.23	-1.01 -.54**	-.23 -.09
Leaf number 10/81	-.22 -.13		-.98 -.19	-- .31	-- .28	.27 .34	.49 .51*	-.03 .30
Leaf length 10/81	.27 .14	-.98 -.19		-- -.10	-- .03	-1.48 -.26	-2.06 -.46*	-3.09 -.42
Leaf number 5/82	-- -.28	-- .31	-- -.10		-- -.02	-- .00	-- .51*	-- .56**
Leaf length 5/82	-- -.30	-- .28	-- .03	-- -.02		-- .28	-- .53*	-- .34
Inflorescence height	-.71 -.23	.27 .34	-1.48 -.26	-- .00	-- .28		-.07 .42+	-1.68 .29
Number of flowers	-1.01 -.54**	.49 .51*	-2.06 -.46	-- .51*	-- .53*	-.07 .42+		.22 .75**
Number of seeds	-.23 -.09	-.03 .30	-3.09 -.42+	-- .56**	-- .34	-1.68 .29	.22 .75**	

Table 4.9b. Genetic correlations based on full-sibships, Experiment II. Correlations in high density.

	Seed weight	Leaf # 10/81	Leaf Length 10/81	Leaf # 5/82	Leaf Length 5/82	Infl. height	# of Flowers	# of Seeds
Seed		-.91	-.03	-.44	-.20	--	-.32	.52
weight		-.34	-.07	-.31	-.05	-.32	-.17	-.27
Leaf number	-.91		-.41	1.95	1.20	--	1.47	.66
10/81	-.34		-.32	.65**	.23	.14	.56**	.27
Leaf length	-.03	-.41		.08	2.88	--	-.34	-.04
10/81	-.07	-.32		-.04	.57**	-.10	-.15	-.06
Leaf number	-.44	1.95	.08		1.05	--	.91	-.12
5/82	-.31	.65**	-.04		.38	-.28	.36	-.09
Leaf length	-.20	1.20	2.88	1.05		--	1.07	2.00
5/82	-.05	.23	.57**	.38		-.40	.05	.04
Inflorescence	--	--	--	--	--		--	--
height	-.32	.14	-.10	-.28	-.40		.43*	.43*
Number of	-.32	1.47	-.34	.91	1.07	--		.78
flowers	-.17	.56**	-.15	.36	.05	.43*		.76**
Number of	.52	.66	-.04	-.12	2.00	--	.78	
seeds	.27	.27	-.06	-.09	.04	.43*	.76**	

the traits or of their density responses, in the strict sense, because the design employed field collected maternal sibships, probably largely selfed seed. The differences among sibships revealed by the experiment are attributable to environmentally induced variation among the collections, maternal genetic effects, dominance and epistasis, as well as additive genetic effects. If there is a negligible contribution of the former effects to the density responses however, the experiment suggests that under the environmental conditions employed density-dependent selection on the basis of these fitness traits fails to maintain genetic variation in the population.

The planting design of Experiment I is an efficient one for estimating responses over a wide range of densities, since far fewer individuals are required to establish the array of densities than in a split plot design in which each plot is established at a different uniform density (see Antonovics, et al. 1983 for full discussion). The condensed form of the fan design may, however, be a drawback, when the density response of a large number of groups (genotypes or families) is to be assayed. The number of positions in an arc is fixed by the size of the interior angles, which in turn determines the factor of the geometric series by which the spacing increases, and by the angle including the whole fan (here 90^{o}, but may be up to 360^{o}). If the number of genotypes to be tested exceeds the number of positions per arc, then an incomplete design must be used. The compact design precludes replication of genotypes within density x fan combinations, reducing statistical power and rendering mortality a severe problem. Thus, the fan is most appropriate for ascertaining average and genotype-specific responses of a limited number of genotypes (e.g., Antonovics, et al. 1983).

Several flaws in Experiment II preclude making any but weak inferences from it. The major problem of small sample size was a consequence of 1) Salvia's floral structure which sets a strict limit of four seeds per flower (i.e. per pollen transfer), 2) the failure of all individuals to flower simultaneously and to continue flowering until all proposed crosses were completed, and 3) germination delay and failure. Had the latter problem been anticipated, it could almost certainly have been minimized by germinating seeds in water in individual vials and then transplanting the seedlings to soil. In a more recent experiment using this method (Chapter 5), germination was nearly synchronous and exceeded 95%. The lack of synchrony in germination and emergence led to large variance in the size of plants at the start of the experiment. Moreover, blocks planted with the latest emerging seedlings had to be eliminated due to severe mortality, presumably because winter weather began before the plants had acclimated sufficiently. In addition to the problem of sample size, the two spacings which were chosen on the basis of Experiment I were not sufficiently extreme to produce a strong main effect of density. Apparently, in the conditions of the Botany Plot, somewhat higher densities are necessary to cause a reduction in fecundity. This is certainly due to the poorer soil, lack of protection, and the deliberately sown background of Poa pratensis. These characteristics of the Botany Plot reduced growth relative to that in the cold frame and therefore retarded expression of density effects.

These flaws severely weaken the conclusions that can be drawn from this experiment. The graphs of family-specific density responses of size and fecundity traits (Figs. 4.5-4.9) show some variation in slopes. The genotype x density interactions are, however, not statistically significant. The significant mother x density effect on change in leaf number is suggestive of the potential for density-dependent selection, if

differences in growth rate are eventually converted into differences in survival or fecundity. An experiment of longer duration might reveal variation among families in response of fecundity and survival to density as a consequence of this interaction in growth. However, the interaction appears to be exclusively a maternal effect and, as such, may or may not be heritable.

Thus, in the background of the Botany Plot, given the two spacings chosen, the genotypes employed, and the size of the experiment, density-dependent selection may occur, though interactions in fitness traits appear slow to develop. However, since statistical interactions reflect the extent to which the effects of the particular levels of the main effects are non-additive, inferences must be limited to the density levels as well as to the genoypes used in the experiment. Moreover, even with these restrictions, extrapolation of these results to inference of the prevalence of genotype x density interaction in the wild population is inappropriate, since the extent to which differences between the field and the Botany Plot alter density responses is unknown.

The experiment did yield evidence that the density of the Salvia population can influence the genetic correlations of a set of traits. This had been shown previously by Khan, et al. (1976) for crosses of cultivars of Linum usitatissimum. In a different context, Lanza, et al. (1982) demonstrated the effect of environment on genetic correlations in that inclusion of aflatoxin in the diet of chickens altered the genetic correlations between body size and blood characters, relative to controls. Since prediction of correlated responses to selection depend on heritabilities of the traits and their genetic correlations (Falconer 1976), and all these can vary with environment, clearly such predictions

will apply only in the environment in which the genetic parameters are estimated. Thus, Lande's (1975) model of multivariate response to selection, which assumes that the matrix of genetic variances and covariances is independent of environment, is likely to be overly simplistic in a large number of cases. Heritability estimates have long been known to be very sensitive to environmental conditions, however, this has apparently not been widely noted for genetic correlations.

The present experiments were conducted in relatively controlled and, hence, artificial conditions in the belief that the uniformity of the background environment, against which density was deliberately varied, would render genetic and genotype x environment effects more prominent. Experiment I may have accomplished this aim, but the initial variability in the experimental material in Experiment II, due to the causes named above, almost certainly masked genetic variation. Moreover, Travis (1980) previously noted that, contrary to intuition, genetic variance may be greater and, hence, more easily detected in natural environments than in artificial ones. In addition, the responses in the natural environment are of principal interest in the present case. It is therefore appropriate to seek evidence of density-dependent selection employing experimental designs similar to the present ones in the natural population itself, as reported in the following chapter.

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CHAPTER V. GENETIC EXPERIMENTS IN THE FIELD

INTRODUCTION

The discovery of genetic variation in response to conspecific density in laboratory populations of Drosophila (Timofeev-Ressovsky, 1934; Dobzhansky and Spassky, 1944) led to the recognition that density-dependent selection (that is, selection of different genotypes as density varies) could be a powerful force maintaining the observed diversity of life history phenotypes in nature (see Roughgarden, 1979, for review). More recent experimental work (e.g., insects, Lewontin, 1955; Bentvelzen, 1963; Sokal and Huber, 1963; Sokal and Karten, 1963; Clark and Feldman, 1981; Marks, 1982; in crop species, Stivers, et al., 1971; Khan, et al., 1976; Baker and Briggs, 1982) has confirmed the existence of genetic variation in density response, and theoretical work (Slatkin, 1979; Turelli and Petry, 1980; Asmusson, 1983) has examined its evolutionary consequences. However, despite keen interest in causes maintaining life history variation in nature (Stearns, 1976), we as yet lack direct evidence of density-dependent selection in wild species.

Evidence from experiments conducted in artificial conditions cannot bridge this gap for two reasons. 1) Frequently, such experiments seek genetic variation in density response among artificially selected strains (e.g., Khan, et al. 1976); therefore the genotypes do not represent a

random collection from a wild population. 2) The studies are executed in artificial background environments; to the extent that the background environment influences both general (e.g., Watkinson, 1981,1982) and genotype specific responses to density, that is, to the extent that staistical interactions of environment x density and genotype x environment x density exist, results of these studies may not apply to populations in nature.

Moreover, as has been pointed out (Wilbur, et al., 1974; Stearns, 1976; Law, et al., 1977), correlation of life history attributes with habitat characteristics cannot be taken as evidence that these characteristics were the selective cause of the presently observed life history phenotypes.

Direct evidence of the potential for density-dependent selection consists of a shift in the fitness rankings (to the extent that these can be evaluated) of genotypes with densities or, in the statistical sense, a genotype x density interaction in fitness. Although this criterion has frequently been applied in studies of evolution in laboratory populations of insects and in studies of yield in crop species, it has not been applied to document the potential for density-dependent selection in a wild species. Moreover, only a few studies have directly demonstrated effects of density on components of individual fitness in a wild population (in plants, Watkinson and Harper, 1978; Clay and Shaw, 1981; Chapters II and III; Antonovics, in prep.; in animals, Brockelman, 1969; Wilbur, 1972, 1976).

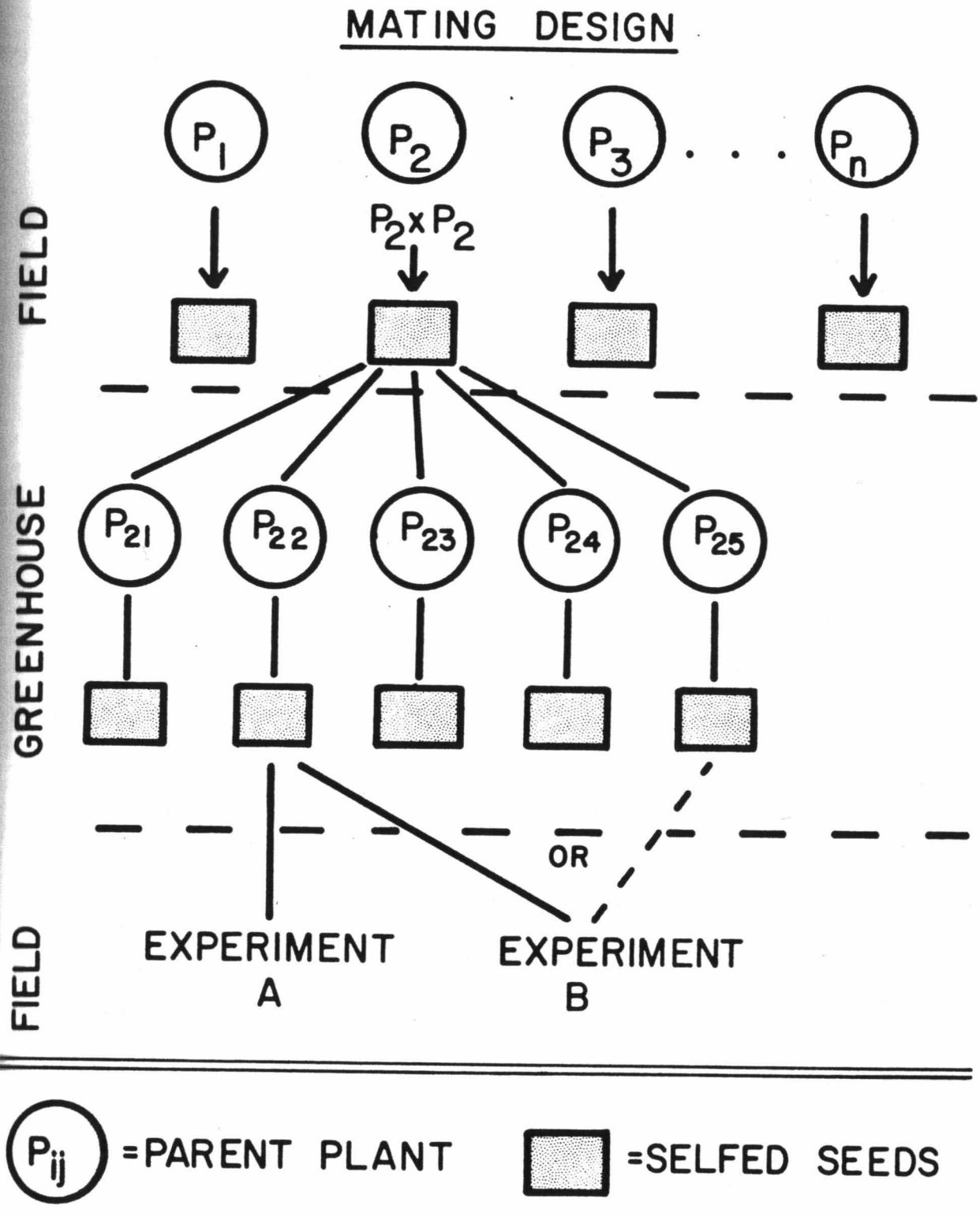
In view of this gap, the present study was designed to provide evidence regarding the extent to which the potential for density-dependent selection (and also density-dependent regulation) exists in a wild

population of Salvia lyrata (hereafter, "Salvia") growing in its natural circumstances. The study was conducted in the field from which the source population was collected. By experiments carried out using transplants directly into the field, it was intended to gain more realistic estimates of the density response and of its variation. Experiments which were conducted under more controlled conditions (Chapter III) suggested the potential for density-dependent selection with regard to one trait, flowering tendency, but not for the traits, probability of survival and number of seeds.

METHODS

The field collections used in Experiment 2 of Chapter IV provided seeds for the present experiment. A subsample of field-collected individuals was raised in the Duke University Department of Botany Greenhouses (see Chapter IV). In May, 1981, the plants were allowed to self. The resulting seeds were collected according to maternal parent. Salvia selfs readily, and, moreover, emasculation experiments demonstrated that pollen transfer between flowers was not occurring in the greenhouse. Therefore, the progeny of each individual was a set of self-sibs (Fig. 5.1). Thus, to the extent that the original collections differed genetically, the families of the next generation differed to approximatedly the same extent. Moreover, because the second generation was obtained from parents grown in common conditions, environmentally induced differences among the progenies are expected to be somewhat reduced compared to those among the first generation collections. Although "grandmother" effects, i.e., carryover of environmental effects to the second generation, are known (citations in Van Vleck, 1976), these are expected to be weaker than effects induced on the first generation. The second generation families were two orders of

Figure 5.1. Design for collecting and culturing individuals for the experiment. The circles denote flowering plants from which seeds were collected. The boxes denote seed collections. Panel I, Field; Panel II, Greenhouse; Panel III, Field.


MATING DESIGN
P1
P2
P3
Pn
P2xP2
FIELD
GREENHOUSE
P21
P22
P23
P24
P25
OR
FIELD
EXPERIMENT A
EXPERIMENT B
Pij =PARENT PLANT
=SELFED SEEDS

magnitude larger than the families collected in the field, and this was a considerable advantage in the design of the field experiment. Of the seeds obtained in this way, 30 from each of 20 unrelated families were watered and germinated in Petri dishes under 12 hours of light per day (Sears' Easy-gro plant light, 40 W) in the laboratory. Eleven such germination blocks were established at approximately 4 day intervals. At the time of planting into the field (see Chapter I for description of the site) approximately 3 weeks later, the seedlings bore fully expanded cotyledons and radicles between 5 and 10 cm long.

Planting Design and Procedure

The field study was partitioned into two experiments to reveal trends over a wide range of spacings, while providing sufficient statistical power at a few spacings to make significance tests reliable. Experiment A employed three planting densities with 35 individuals in each combination of density and family, whereas Experiment B employed 5 planting densities, with 12 individuals per combination. With a few exceptions, the same seed families were used in both experiments; when insufficient seed was available to complete the design, seed from plants which were sibs of the intended parent (see Fig. 5.1) were used. These substitutions were made only in Experiment B.

For Experiment A, 7 blocks were laid out in the field in an area which was chosen for its apparent uniformity and which was the minimum size to accomodate both experiments. The site was on a gentle, south-facing slope in full sun throughout the summer. Within the blocks, locations were randomly allotted to each spacing treatment plot. From these plots, resident Salvia individuals were removed, and other vegetation was clipped to within 1/2 inch of the soil surface. One hundred points were arrayed in

each plot in uniform hexagonal design; the spacing treatments were 1 cm, 2 cm, and 8 cm between individuals, corresponding to densities of 11765, 2940, and 184 plants per m^2, respectively. The family identities were assigned to these points with an additional level of blocking: there were 5 subblocks of 20 individuals each (one from each family) in each plot, the 20 points were grouped to occupy minimum areas. This lower level of blocking was used to preclude effects of differences in planting time biasing the results. Thus, the experiment was designed as a split plot, with spacing treatment as the whole plot factor and family as the split plot factor.

Experiment B was executed similarly: 5 spacings were used, these being 3 cm, 4 cm, 6 cm, 12 cm, and 16 cm between individuals (equivalently, 1307, 735, 327, 82, and 46 individuals per m^2, respectively). These were planted in 4 out of the 7 blocks allotted to Experiment A. In each plot, 60 seedlings were planted; they were randomized in three strips of 20 points hexagonally arranged (i.e., 1 row of 6 flanked by 2 rows of 7 points).

The planting of the seedlings which was done in April and May, 1982 required six weeks. A plastic toothpick marked the location of each individual and indicated its family identity, according to a color and symbol code. Individuals which died before June were replaced with their respective sibs remaining in the Petri dishes from the same germination group. During this period, the plots were watered about once a week if they appeared dry, and this combined with an unusually wet spring was responsible for high survival. After mid-May, supplemental watering ceased. Twice during the summer, the plots were mown with a Stihl FS80 Brush Cutter, rather than a rotary mower, in order to minimize damage to the toothpicks. No other care was applied. In September, 1982, the survival and size (number of leaves

and length of longest leaf) of each individual, except those in the 1 cm spacing treatment, were recorded. Due to the difficulty of working at the most extreme density, the plants in the 1 cm spacing were not measured, but were reserved for destructive sampling at a later time.

Data Analysis

Experiment A and Experiment B were analysed separately using programs for conducting G tests, written by T. R. Meagher and M.D. Rausher and using the Statistical Analysis System (Helwig and Council, 1979), maintained at Triangle Universities Computation Center, Research Triangle Park, N.C. Plants which died were included in the analysis of size. This practice preserves the near balance in the design and permits one to draw unconditional conclusions. When this might obscure trends among the survivors, the results of analyses from which dead plants were excluded are also reported. Because computer funds were limited, these latter analyses were carried out on cell means, a procedure which is not strictly valid, since it artificially imposes balance and homogeneity of variance. For this reason, the p-values of the reported analyses of unbalanced data should be taken as a rough guide to the actual significance levels. This caution also applies to analyses where edge plants were eliminated.

The factors, blocks, families, and subblocks within plots, were considered random effects and spacings as fixed in a mixed model analysis of variance. Thus, the inferences can be extended to a wider population of families and areas in the field, and components of variance attributable to each factor can be calculated. However, neither the field locations nor the timing of planting the blocks were randomly chosen. The effect this model has on the hypothesis tests, relative to considering blocks fixed, will be noted and discussed.

Genetic correlations of the two traits within each spacing (e.g., length of leaf in 2 cm with number of leaves in 2 cm) were carried out using the Nested procedure of SAS. Correlations of traits between spacings (e.g., length of leaf in 2 cm with length of leaf in 8 cm, or with number of leaves in 8 cm) were obtained by considering each trait, measured in different spacings as different traits, and by calculating correlations of family means for each experiment. Falconer (1952) originally suggested this approach, but it has been used little (for an exception see, Khan, et al., 1976).

RESULTS

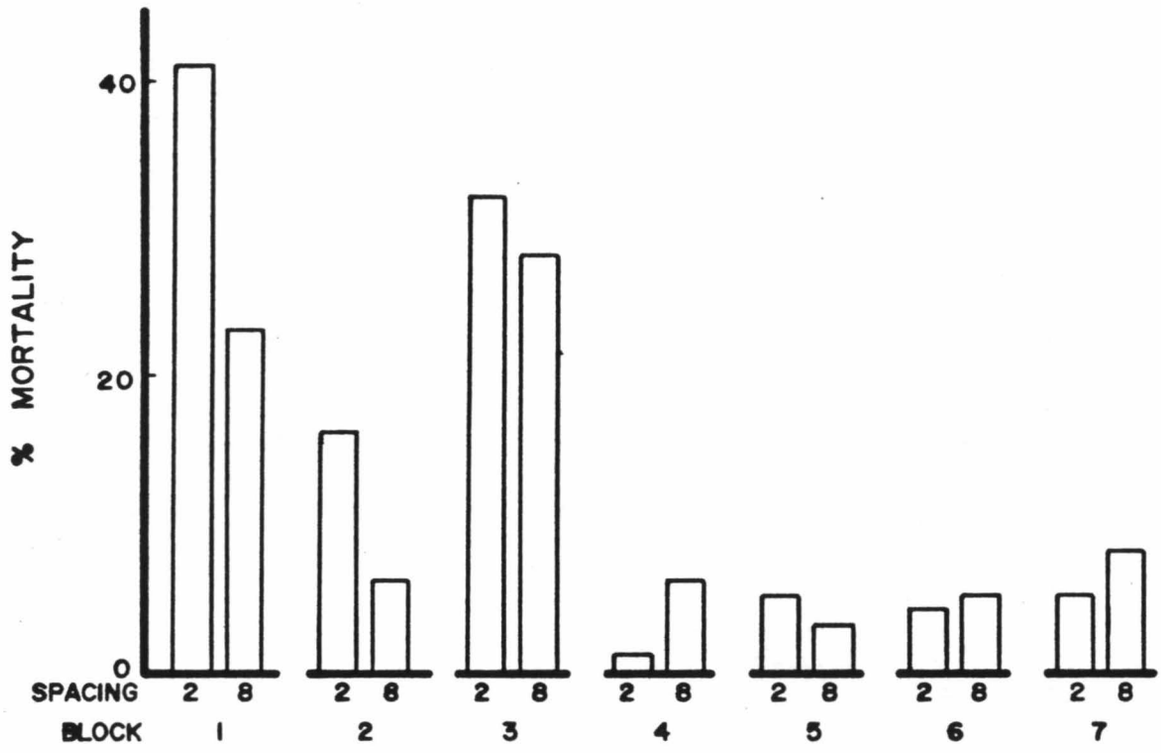
Mortality

For both experiments taken together, the survival of plants during the first month's establishment was 97% (2701/2795). Of the 94 dead individuals which were replaced, only 16 survived to the September census, probably because the individuals held over for replacement were weaker than those planted in the normal sequence.

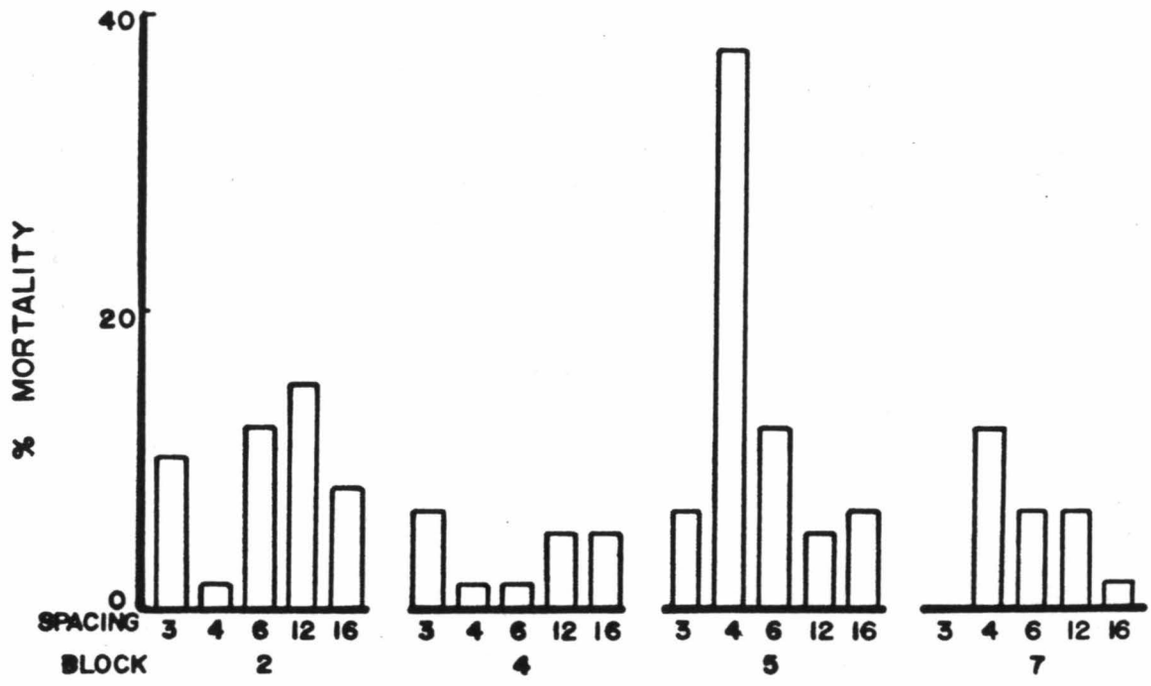
In Experiment A, four months later, 87% (1196/1376 plants) survived. The mortality was distributed randomly among the families (G test, $p<.95$). The overall trend was toward higher mortality in the 2 cm spacing than in the 8 cm spacing (Fig. 5.2a), but this trend was dependent on the particular block (block X spacing X mortality, G test, $p<.05$). Three of the blocks showed far higher mortality than the remaining four (G test, $p<.0001$); these were the three earliest planted blocks, and, hence, their high mortality may be due to environmental factors confounded with earliness (e.g., occasional frost, relative drought at that time, the inexperience of the investigator in the early stages of the planting procedure) or to spatial variation.

Figure 5.2. Number of plants which died by September, 1982, plotted against spacing and block treatment. a, Experiment A; b, Experiment B.

FIG 2 a)


% MORTALITY
40
20
0
SPACING 2 8 2 8 2 8 2 8 2 8 2 8 2 8
BLOCK 1 2 3 4 5 6 7

b)


% MORTALITY
40
20
0
SPACING 3 4 6 12 16 3 4 6 12 16 3 4 6 12 16 3 4 6 12 16
BLOCK 2 4 5 7

In Experiment B, 76% (1081/1419) survived to September. The variation in this mortality is confined to a single aberrant plot (Fig. 5.2b; Block 5, Spacing=4 cm), whose unusually high mortality appeared to be due to disturbance by moles, rather than to density or block dependent effects. This mortality renders the block x spacing x mortality interaction significant (G test, $p<.0001$). Neither the heterogeneity among families ($p<.1$), nor that of any other higher order interaction was significant ($p<.5$).

Size Distributions

The size distributions of surviving plants were similar for all spacings. They all differed significantly from a normal distribution (Kolmogorov-Smirnov test, $p<.01$) with positive coefficients of skewness and frequently positive coefficients of kurtosis. The variance of number of leaves per plant ranged from 1.6 in the 2 cm spacing to a maximum of 2.6 in the 3 cm spacing, that of length of longest leaf from 11.6 in the 6 cm spacing to 21.7 in the 8 cm spacing. In summary, the variances were relatively homogeneous across spacings; most of the distributions were more peaked and with more individuals in the smaller classes than is expected of a normal distribution.

Effects on mean size

In Experiment A, there was significant variation among the blocks, the spacings, and the subblocks within plots (Table 5.1a, Figs. 5.3a, 5.4a in leaf number and length). The estimated components of variance (Table 5.2a) indicate the relative magnitudes of these effects. The means for the blocks somewhat reflect their spatial and temporal arrangement (Table 5.3a); Blocks 1-3 have plants with smaller leaves and 1 and 3 have plants with fewer leaves; these observations partially reflect the higher

Table 5.1a. Analysis of variance of number of leaves in September, 1982, Experiment A. The F ratios are calculated according to a mixed model with block, family, and subblock random and spacing fixed.

Source	df	SS	F	Pr>F	R^2
Model	335	1833.9	2.13	.0001	.407
Error	1040	2676.0			
Block	6	398.5	14.04	.0001	
Spacing	1	298.5	56.75	.0001	
Block*Spacing	6	21.6	.67	ns	
Subblock (Block*Spacing)	56	328.6			
Family	19	117.3	1.93	.05	
Block*Family	114	363.9	1.55	.025	
Spacing*Family	19	70.8	1.81	.05	
Block*Space*Family	114	234.7	0.8	ns	

Analysis of variance of length of longest leaf in September, 1982, Experiment A.

Model	335	24866.8	1.72	.0001	.356
Error	1040	44969.5			
Block	6	3787.1	19.05	.0001	
Spacing	1	1413.5	137.47	.0001	
Block*Spacing	6	127.0	.15	ns	
Subblock (Block*Spacing)	56	8146.3			
Family	19	1807.6	2.06	.05	
Block*Family	114	5253.2	1.35	.05	
Spacing*Family	19	441.4	.68	ns	
Block*Space*Family	114	3890.5	.79	ns	

Multivariate tests

Source	Wilks' L	p
Block	.0093	.0015
Spacing	.1839	.0001
Family	.4665	.0001
Block*Family	.7248	.012
Spacing*Family	.6719	.12
Block*Spacing*Family	.8368	.945

Table 5.1b. Analysis of variance of number of leaves in September, 1982, Experiment B, carried out as explained in Table 5.1a.

Source	df	SS	F	Pr>F	R^2
Model	439	1929.2	1.85	.0001	.518
Error	751	1788.5			
Block	3	397.9	6.85	.01	
Spacing	4	12.4	.16	ns	
Block*Spacing	12	227.5	4.96	.001	
Subblock (Block*Spacing)	40	146.6			
Family	19	224.8	4.01	.001	
Block*Family	57	168.1	1.15	ns	
Spacing*Family	76	172.1	.88	ns	
Block*Space*Family	228	579.6	1.07	ns	

Analysis of variance of length of longest leaf, in September, 1982, Experiment B.

Model	439	20556.8	1.66	.0001	.493
Error	751	21158.0			
Block	3	1995.8	2.43	.1	
Spacing	4	819.9	.81	ns	
Block*Spacing	12	3001.7	7.57	.0001	
Subblock (Block*Spacing)	40	1441.1			
Family	19	2725.9	2.94	.005	
Block*Family	57	2784.9	1.94	.001	
Spacing*Family	76	2051.5	1.07	ns	
Block*Space*Family	228	5736.0	.89	ns	

Multivariate tests

Source	Wilks' L	p
Block	.2500	.0112
Spacing	.6380	.694
Family	.1976	.0001
Block*Family	.5603	.02
Spacing*Family	.5748	.63
Block*Spacing*Family	.5466	.023

Table 5.1c. Analysis of variance of number of leaves in April, 1983, Experiment A.

Source	df	SS	F	Pr>F	R^2
Model	335	4150.8	2.03	.0001	.395
Error	1040	6351.1			
Block	6	912.2	6.88	.025	
Spacing	1	537.6	24.32	.0001	
Block*Spacing	6	132.7	1.59	ns	
Subblock (Block*Spacing)	56	780.2			
Family	19	337.1	2.77	.0001	
Block*Family	114	732.2	1.05	ns	
Spacing*Family	19	132.1	1.35	ns	
Block*Space*Family	114	586.7	0.84	ns	

Analysis of variance of length of longest leaf, in April, 1982, Experiment A.

Model	335	83923.7	1.91	.0001	.380
Error	1040	136421.6			
Block	6	13069.7	5.05	.05	
Spacing	1	12036.0	27.89	.0001	
Block*Spacing	6	2591.8	1.21	ns	
Subblock (Block*Spacing)	56	19907.0			
Family	19	7715.5	3.07	.0001	
Block*Family	114	15110.8	1.01	ns	
Spacing*Family	19	2347.7	1.25	ns	
Block*Space*Family	114	11144.8	.75	ns	

Multivariate tests

Source	Wilks' L	p<
Block	.00008	.001
Spacing	.00733	.002
Family	.23507	.0001
Block*Family	.61919	.02
Spacing*Family	.51392	.34
Spacing*Block*Family	.68940	.95

Table 5.1d. Analysis of variance of number of leaves in April, 1982, Experiment B, carried out as explained in Table 5.1a.

Source	df	SS	F	Pr>F	R^2
Model	439	4856.1	1.71	.0001	.499
Error	751	4868.7			
Block	3	1038.5	15.43	.0001	
Spacing	4	173.6	1.94	ns	
Block*Spacing	12	268.9	1.70	ns	
Subblock (Block*Spacing)	40	525.9			
Family	19	437.5	2.79	.001	
Block*Family	57	468.7	1.27	ns	
Spacing*Family	76	460.2	.93	ns	
Block*Space*Family	228	1482.6	1.00	ns	

Analysis of variance of length of longest leaf, in April, 1983, Experiment B.

Model	439	101229.2	1.79	.0001	.512
Error	751	96534.3			
Block	3	19533.4	12.14	.0001	
Spacing	4	3875.3	1.80	ns	
Block*Spacing	12	6430.3	1.90	.1	
Subblock (Block*Spacing)	40	11219.9			
Family	19	9740.4	3.00	.0001	
Block*Family	57	9779.0	1.33	.054	
Spacing*Family	76	8969.2	0.85	ns	
Block*Space*Family	228	31681.6	1.08	ns	

Multivariate tests

Source	Wilks' L	p<
Block	.0219	.0001
Spacing	.1823	.26
Family	.0831	.0001
Block*Family	.6805	.001
Spacing*Family	.3057	.39
Spacing*Block*Family	.3185	.056

Figure 5.3a. Length of longest leaf plotted against block for each family, Experiment A. The blocks are plotted along the abscissa in order of increasing block mean.

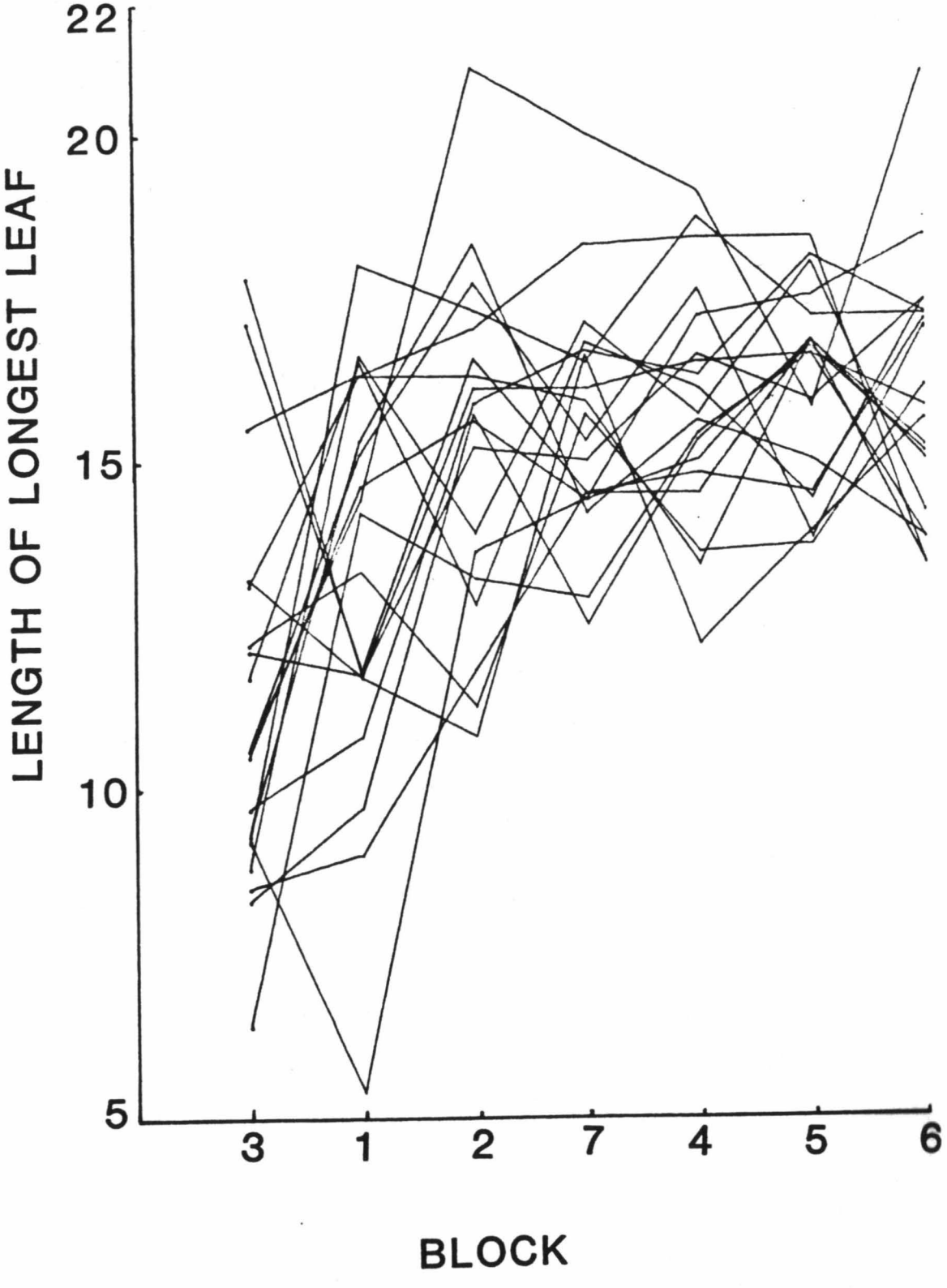

LENGTH OF LONGEST LEAF
22
20
15
10
5
3
1
2
7
4
5
6
BLOCK

Figure 5.3b. Length of longest leaf plotted against block for each family, Experiment B.

ly,

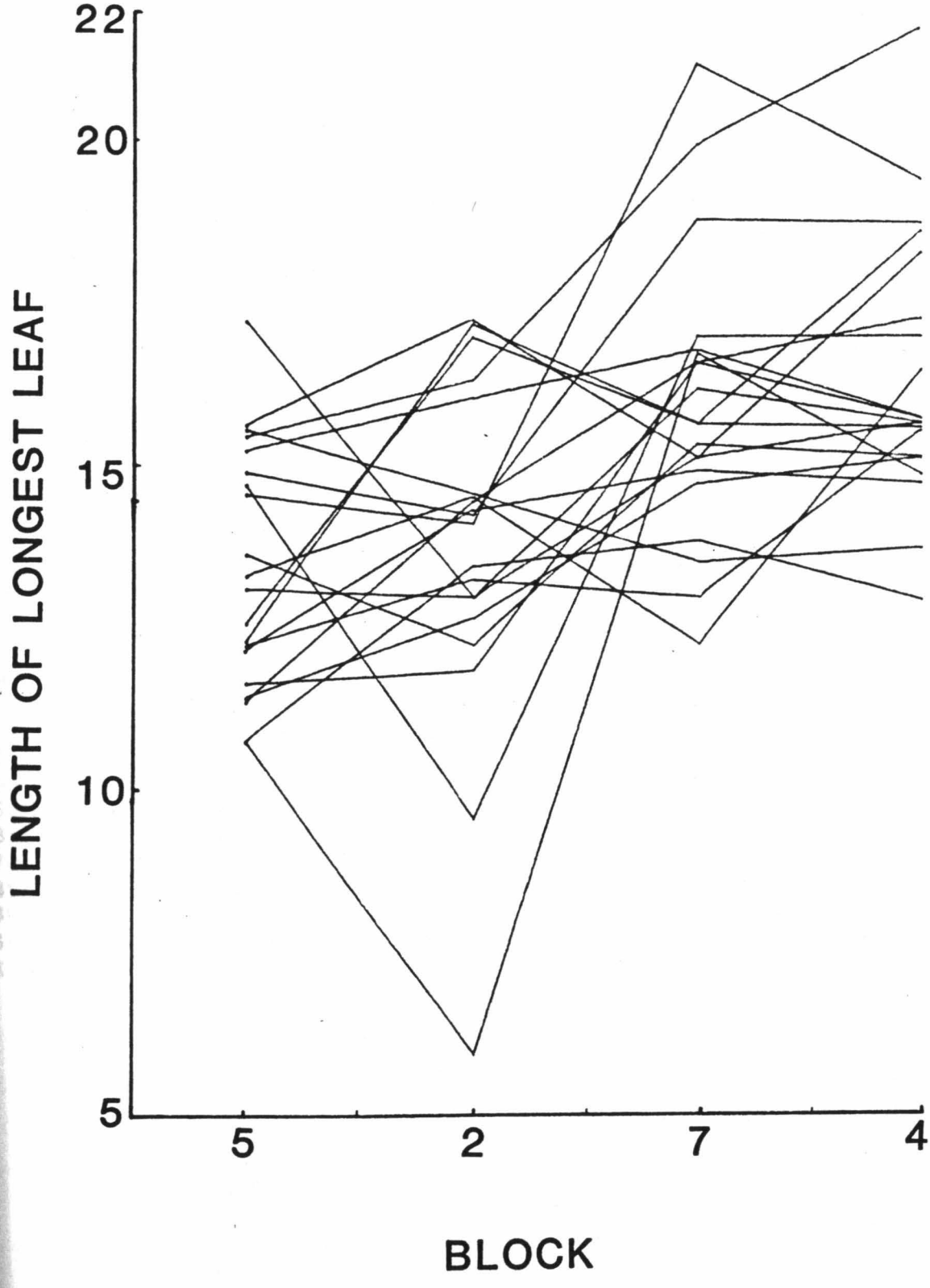


Table 5.2a. Estimates of the variance components from the analyses of variance, September, 1982 data, Experiment A.

		Number of Leaves		Length of Leaf	
Source	df	Variance Component	Standard Error	Variance Component	Standard Error
Block	6	.327	.166	3.168	3.159
Spacing	1	.446	.348	2.12	1.649
Subblock	56	.165	.055	5.112	1.35
B*S	6	-.019	.021	-1.21	0.294
Family	19	.043	.033	0.701	0.445
B*F	114	.119	.050	1.259	0.753
S*F	19	.050	.033	-0.330	0.241
B*S*F	114	-.108	.059	-1.93	0.973
Error	1040	2.57		43.24	

Table 5.2b. Estimates of the variance components from the analyses of variance, Experiment B.

		Number of Leaves		Length of Leaf	
Source	df	Variance Component	Standard Error	Variance Component	Standard Error
Block	3	.378	.281	1.305	1.438
Spacing	4	-.065	.031	-0.196	0.631
Subblock	40	.427	.040	2.617	2.66
B*S	12	.25	.120	3.61	1.582
Family	19	.173	.062	1.98	0.757
B*F	57	.054	.039	1.58	0.617
S*F	76	-.046	.036	0.153	0.409
S*B*F	228	.053	.089	-1.003	0.919
Error	751	2.38		28.173	

Table 5.3a. Means by block, spacing, and family for Experiment A, September,1982. Values followed by the same letter are not significantly different ($p<.05$), according to the Bonferroni criterion.

Mean Number of Leaves	Block	Mean Length of Leaf	Block
3.7	6 A	16.0	6 A
3.5	7 A	16.0	5 A
3.3	2 A	15.9	4 A
3.2	4 AB	15.7	7 A
3.2	5 AB	15.2	2 A
2.6	1 BC	13.1	1 B
2.0	3 C	11.4	3 C
	Spacing		Spacing
3.6	8 A	15.8	8 A
2.6	2 B	13.7	2 B
	Family		Family
3.6	6 A	17.4	15 A
3.5	15 A	16.4	16 AB
3.5	8 A	16.3	12 AB
3.4	19 A	15.7	4 ABC
3.4	17 A	15.6	18 ABC
3.3	5 A	15.3	20 ABCD
3.3	12 A	15.2	8 ABCD
3.2	20 A	14.9	6 ABCD
3.1	16 A	14.9	17 ABCD
3.1	11 A	14.9	10 ABCD
3.0	18 A	14.8	3 ABCD
2.9	3 A	14.6	5 BCD
2.9	1 A	14.5	13 BCD
2.9	4 A	14.5	7 BCD
2.9	2 A	13.7	9 BCD
2.8	14 A	13.7	19 BCD
2.8	9 A	13.5	14 CD
2.8	13 A	13.4	1 CD
2.7	10 A	13.3	2 CD
2.6	7 A	12.6	11 D

mortality in those blocks, as noted above.

There is far less variation among families, and, in univariate tests, it is significant only for the trait, length of longest leaf ($p<.025$, Tables 5.1a, 5.3a); the multivariate test, however, indicated that the pair of traits varies significantly among families ($p<.0001$). The variance component for the family effect on length of leaf is the same order of magnitude as those of the environmental causes, whereas the family component for number of leaves is approximately an order of magnitude smaller.

The effects of these factors are not purely additive, as reflected by their statistical interactions. There was no evidence that the effect of spacing on size varied from block to block, but a significant block x family interaction (multivariate test, $p<.003$) indicates that the relative mean sizes for the families differed among blocks (Fig. 5.3b). The variance components for this interaction are larger than the components due to families. This effect is particularly dramatic in comparisons of Blocks 4-7, whose means and variances are homogeneous.

In addition to the block x family interaction, there was a significant spacing x family interaction for number of leaves ($p<.05$, Table 1a. Fig. 5.4a); this interaction was not significant for the trait, length of longest leaf, and its variance component was negative, probably due to sampling error. There was no evidence of three-way interactions, and their variance components were negative.

The auxiliary analysis from which dead plants were eliminated gave similar results to those reported. The main exception was that the family and block x spacing effects were highly significant for both traits. A further analysis showed a significant interaction between spacing and the location of an individual in the plot (i.e. edge vs. interior; $p<.011$).

Figure 5.4a. Number of leaves plotted against spacing treatment for each family, Experiment A.

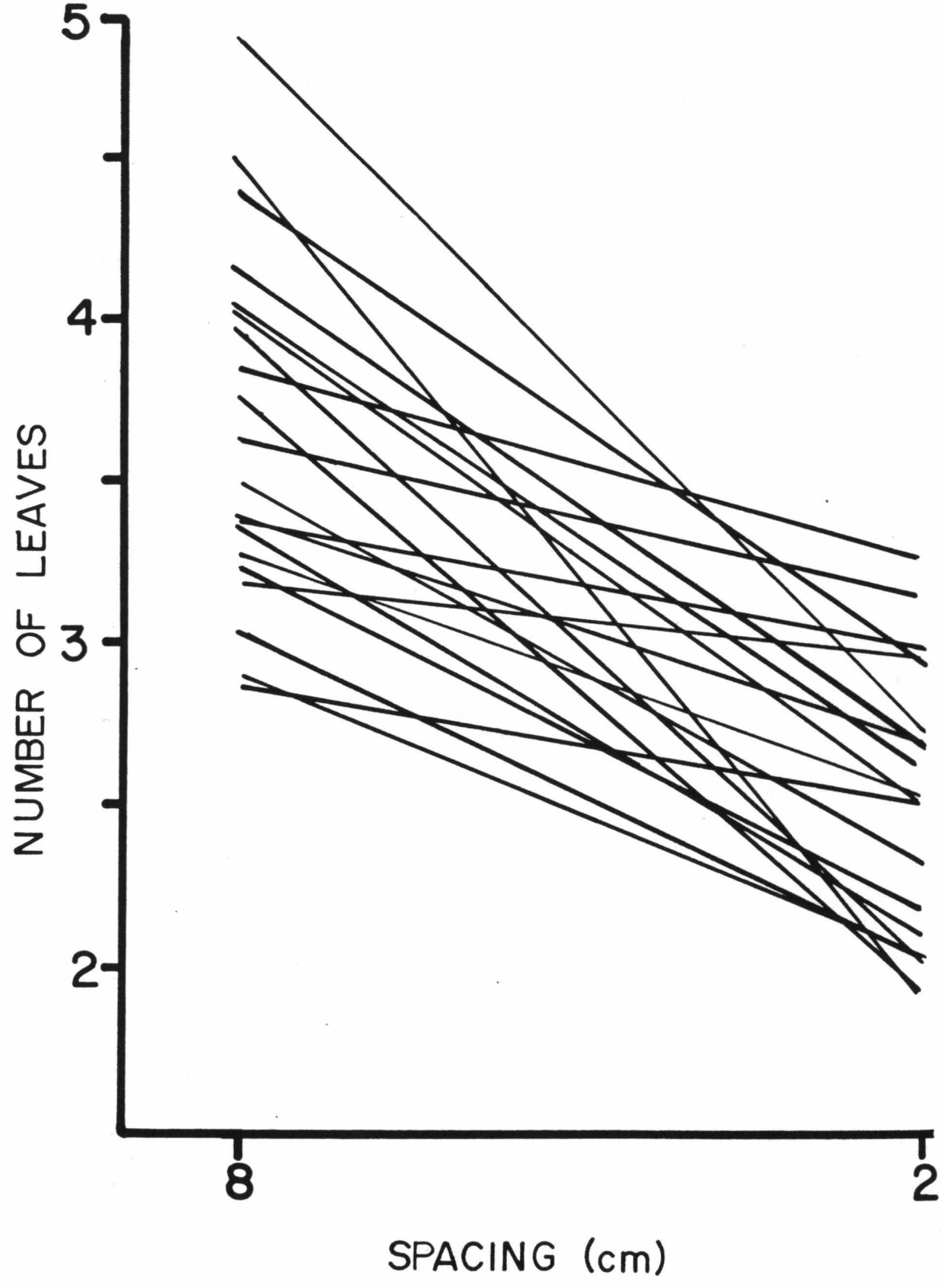
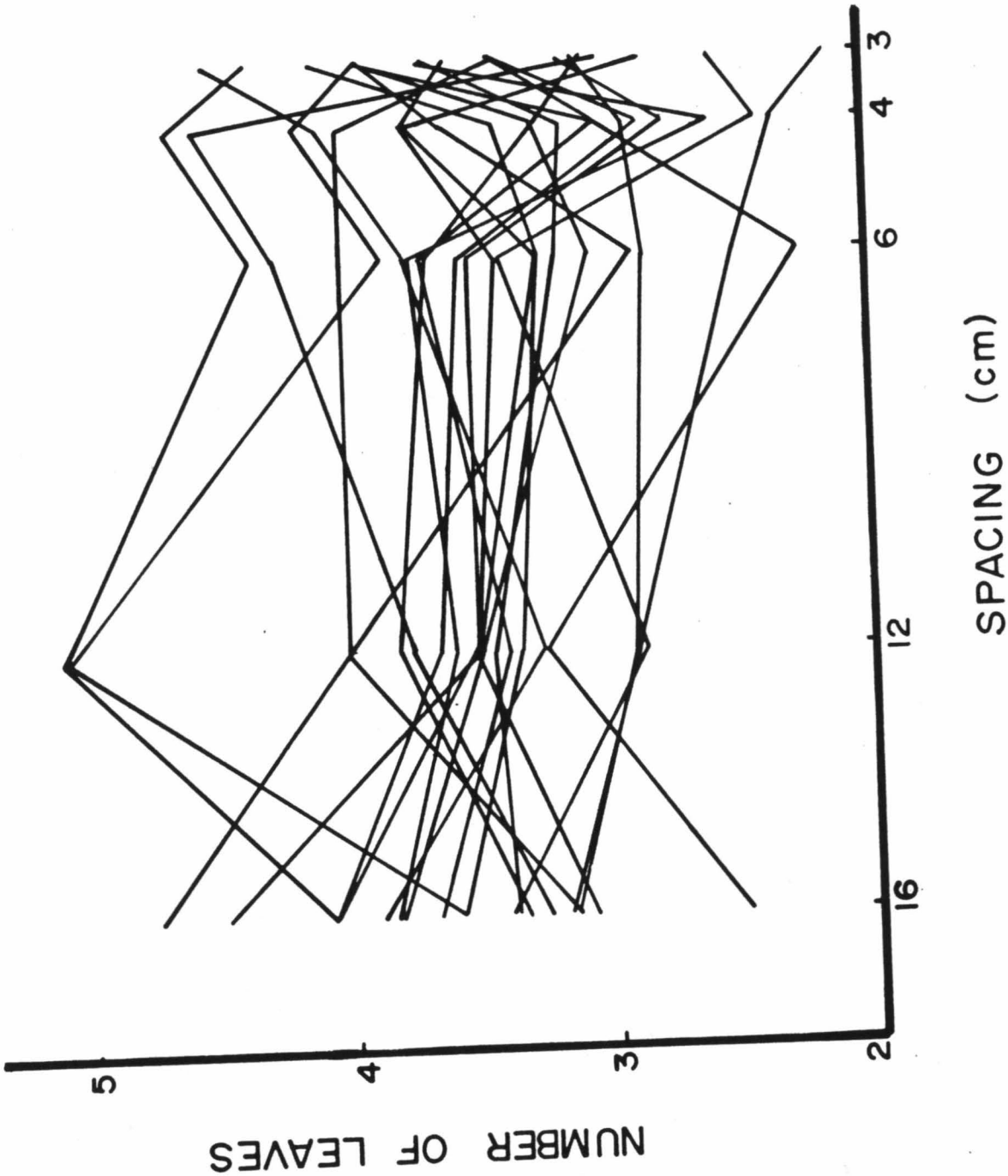

5
4
3
2
NUMBER OF LEAVES
8
2
SPACING (cm)

Figure 5.4b. Number of leaves plotted against spacing treatment for each family, Experiment B.


NUMBER OF LEAVES
5
4
3
2
SPACING (cm)
3
4
6
12
16

When edge plants were consequently eliminated from the main analysis, the conclusions were not substantially altered, although the spacing x family interaction was stronger in the multivariate sense ($p<.02$).

The results based on the April, 1983 census were similar to those from the September, 1982 census (Table 5.1c), with the exception that, in the univariate tests, the main effect of families was stronger and the two way interactions were no longer significant. In the multivariate test, the block x family interaction was significant. The spacing x family interaction was marginally significant ($p<.06$) for number of leaves in the auxiliary analysis of means with edge plants and dead plants excluded.

The results of Experiment B differ from those of Experiment A in one notable respect. The variation due to environmental factors is apportioned principally to the spacing x block interaction (Tables 5.1b, 5.2b); the blocks (Table 5.3b) and subblocks varied in mean number of leaves per plant, but there is no evidence of variation among the spacing treatments. Note that the variance components for block, block x spacing, and subblock are comparable, whereas those for spacing are negative (Table 5.2b).

The family main effect (Tables 5.1b, 5.3b) and the block x family interaction (Fig. 5.3b) account for most of the remaining variation. There is no evidence that the families differ in their response to the range of spacings employed in Experiment B (Fig. 5.4b). Surprisingly, the block x spacing x family interaction, however, is significant in the multivariate test ($p<.028$). The conclusions based on the April, 1983 census do not substantially differ from those of the September, 1982 census (Table 5.1d), with the exception that the block x spacing effect is not significant at the later date.

To summarize the effects on size, the effects of spacing are only

Table 5.3b. Means by block, spacing, and family for Experiment B.

Mean Number of Leaves	Block	Mean Length of Leaf	Block
4.2	7 A	16.3	4 A
3.9	4 AB	15.9	7 A
3.0	2 B	13.7	2 A
2.8	5 B	13.4	5 A
	Spacing (cm)		Spacing (cm)
3.6	16 A	15.6	3 A
3.6	12 A	15.5	12 A
3.5	3 A	15.4	16 A
3.4	6 A	15.9	4 A
3.4	4 A	15.7	6 A
	Family		Family
4.5	15 A	18.4	15 A
4.1	17 AB	17.3	10 AB
4.0	8 ABC	16.6	8 ABC
3.9	6 ABCD	16.5	12 ABC
3.8	1 ABCD	15.9	17 ABCD
3.7	19 ABCDE	15.9	20 ABCD
3.7	12 BCDEF	15.5	18 ABCDE
3.5	3 BCDEF	15.2	9 ABCDE
3.5	11 BCDEF	14.5	3 BCDE
3.5	5 BCDEF	14.5	14 BCDE
3.5	20 BCDEF	14.3	16 BCDE
3.4	10 BCDEF	14.2	6 BCDE
3.4	14 BCDEF	14.2	13 BCDE
3.4	9 CDEFG	14.1	5 BCDE
3.3	13 DEFG	14.0	19 CDE
3.2	18 DEFG	14.0	1 CDE
3.0	2 EFG	13.4	7 CDE
2.9	7 FG	13.4	11 CDE
2.9	16 FG	12.7	2 DE
2.6	4 G	12.2	4 E

apparent in comparisons with the most extreme treatment. No trend of the means with spacing is revealed by the graph of size against spacing for Experiment B (Fig. 5.4b). Moreover, variation among families in their performance at particular spacings is discernible only in Experiment A. Conversely, other environmental effects are considerable in both experiments, and variation among families in their performance in different blocks or plots is evident.

Heritabilities and Family Correlations

The broad sense heritabilities of the two size measures and their variance component correlation attributable to covariation among families were calculated from the full analysis (Table 5.4). The heritabilities are generally low as might be expected of size traits in a field experiment. The family correlation of the two traits calculated for Experiment A is also small, whereas that for Experiment B is large.

The family component correlations between the two size traits within spacing treatments and within dates, computed separately for the two experiments (Table 5.5a,b), are significantly positive in most of the spacing treatments. Frequently, the correlations within spacings and between dates are still larger, indicating a strong influence of family identity on both size traits through time in the first year. Similarly, the correlation of the same trait measured in two different spacings is frequently positive and significant, while the correlations of the opposite traits in different spacings are positive but less often significant. There is no evidence of negative genetic correlations between traits, whether within or between spacings and within or between dates.

DISCUSSION

These experiments have revealed the relative magnitudes of

Table 5.4. Family variance component correlations of number of leaves and length of longest leaf, based on estimates from multivariate analysis of the full model (see Tables 5.1a,b). Diagonal elements are the heritabilities of the traits.

Experiment A			Experiment B		
	NL	LL		NL	LL
NL	.024	.062	NL	.096	.659
LL		.027	LL		.105

Table 5.5a. Correlations among families of the two traits within and between spacing treatments and census dates, Experiment A. Where two correlation coefficients are reported, the variance component correlation is above and the mean square correlation is below. Where one coefficient is given, it is the correlation of family means.

	September, 1982		April, 1983		September, 1982		April, 1983	
	NL 2	LL 2	NL 2	LL 2	NL 8	LL 8	NL 8	LL 8
NL 2	.100							
LL 2	.676 .555 *	.116						
NL 2	1.081 .703 **	.903 .633 *	.080					
LL 2	,685 .570 *	.891 .750 **	.891 .770 **	.096				
NL 8	.355	.023	.164	.157	.120			
LL 8	.273	.687 **	.262	.428 +	-.640 -.172	.086		
NL 8	.316	.131	.144	.067	.654 .504 *	-.733 -.040	.027	
LL 8	.311	.495 *	.034	.417 +	.360 .447 *	.317 .461 *	.033 .502 *	.026

+, $p<.07$; *, $p<.05$; **, $p<.001$.

Table 5.5b. Correlations among families of the two size traits within and between spacing treatments and census dates, Experiment B. The organization is as in Table 5.5a.

	9/82		4/83		9/82		4/83		9/82	
	NL 3	LL 3	NL 3	LL 3	NL 4	LL 4	NL 4	LL 4	NL 6	LL 6
N 3	.073									
L 3	-.073 .398	.114								
N 3	-.259 .264	.504 .537 *	.111							
L 3	.775 .673 **	1.282 .739 **	-.384 .498 *	.018						
N 4	.490 *	.382	.470 *	.259	.198					
L 4	.337	.385	.563 **	.386	1.203 .495 *	.024				
N 4	.231	.317	.563 **	.385	.803 .602 **	.826 .459 *	.156			
L 4	.446 *	.511 *	.563 **	.617 **	.839 .656 **	1.106 .721 **	.783 .727 **	.120		
N 6	.223	.384	.365	.085	.579 **	.359	.614 **	.512 *	.207	
L 6	.275	.546 *	.637 **	.312	.579 **	.712 **	.519 *	.647 **	.842 .623 **	.334

N 6	-.000	.193	.324	.122	.400	.139	.395	.499 *	1.097 .789 ***	.871 .661 **
L 6	.098	.437 +	.368	.254	.462 *	.414 +	.315	.578 **	.919 .739 **	1.027 .822 ***
N12	.494 *	.580 **	.613 **	.439 +	.672 **	.573 **	.856 ***	.715 **	.601 **	.550 *
L12	.210	.630 **	.758 **	.459 *	.350	.459 *	.635 **	.574 **	.560 *	.765 **
N12	.004	.125	.497 *	.449 *	.470 *	.386	.779 ***	.497 *	.478 *	.414 +
L12	.274	.500 *	.480 *	.709 **	.404	.398	.611 **	.648 **	.470 *	.566 **
N16	.345	.247	.270	.236	.355	.063	.342	.342	.530 *	.439 +
L16	-.097	.359	.229	.363	-.218	-.091	.240	.208	.411	.320
N16	.216	.323	.478 *	.766 ***	.462 *	.413 +	.516 *	.528 *	.275	.320
L16	.335	.466	.335	.665 **	.369	.528 *	.477 *	.625**	.336	.446 *

	4/83		9/82		4/83		9/82		4/83	
	NL 6	LL 6	NL12	LL12	NL12	LL12	NL16	LL16	NL16	LL16
N 6	.190									
L 6	.974 .796 ***	.170								
N12	.272	.285	.160							
L12	.217	.396	.603 .497 *	.374						
N12	.313	.265	.917 .732 **	.587 .568 **	.280					
L12	.428 +	.532 *	.834 .690 **	.956 .778 **	.812 .735 **	.136				
N16	.358	.222	.309	.353	.250	.436 *	.037			
L16	.271	.372	.168	.495 *	.179	.457 *	-.268 .188	.190		
N16	.154	.173	.621 **	.550 *	.638 **	.534 * .518 *	-- .367	--	--	
L16	.198	.363	.548 *	.456 *	.350	.507 *	-- .465 *	-- .485 *	-- .626	--

+, p<.07; *, p<.05; **, p<.01; ***p<.0001

environmental and genetic effects on several measures of individual success in a natural population of Salvia lyrata. Although interest in the combined effects of genotype and density provided motivation for the pair of experiments, the effect of blocks, that is, patches of approximately 6 m^2, or its interaction with spacing by far outweighed those of the remaining factors. The block x spacing interaction (which might be interpreted as variation in response to spacing among blocks) can not be distinguished from a simple reflection of environmental differences among plots, since each spacing treatment is represented by only a single plot in each block. Furthermore, because the decision to employ a balanced design dictated the use of plots of varying area, the spatial scale of Experiment A is small relative to that of Experiment B; this fact could easily account for the significant spacing x block interactions in the latter. In other words, the spatial scale which reflects variation among blocks in Experiment A is that which yields significant plot effects (=space x block interactions) in Experiment B. Indeed, since there is no space x block interaction in Experiment A, indicating neither environmental variation among plots nor in density response (or that these two effects, if present, cancel each other, an unlikely event), consideration of the spacing x block interaction in Experiment B as a plot effect most simply explains these findings.

Thus, in both experiments, the greatest contribution to variance in size was spatial heterogeneity on a moderate scale of a few meters. In addition, spatial heterogeneity on the smaller scale of subblocks within plots contributed to variation in size, the effect being stronger in Experiment A, possibly because more compact areas served as subblocks compared to the strips used in Experiment B. Together, heterogeneity on

these two spatial scales far surpasses that of spacing or family in the magnitude of its effect on the individual plant's phenotype. The whole experiment occupied a minimum area chosen for its apparent homogeneity. For this reason, the finding of large block and subblock differences was unexpected.

In an experiment where plots were chosen to represent biotically different backgrounds, Fowler and Antonovics (1981) also found significant differences among plots in survival and leaf number for both Salvia lyrata and Plantago lanceolata in a field near and similar to that of the present study; on the smaller spatial scale of replicates within plots, no significant variation was detected. On a larger scale, Antonovics and Primack (1982), demonstrated significant variation among widely separated sites in their effects on life history characteristics of P. lanceolata. Experimentally using the response of the organism itself as a measure of general environmental heterogeneity, as the latter authors advocated, has not found wide application. Therefore assessing the commonness of spatial heterogeneity as it affects plants in natural populations is difficult.

The effects of crowding on size were perceptible only in comparisons of the closest spacing (2 cm) with a wide spacing (8 cm). It is perhaps remarkable that any effect of spacing appeared at the stage of growth presently being considered; at the September census, four months after planting, the average size of the plants was 3.25 leaves with the longest leaf measuring 1.48 cm. Assuming a uniform size distribution and that the leaves lie flat on the ground (as they normally do), then in the 2 cm spacing there is potential for pairs of adjacent plants to overlap leaves by 7 mm, roughly one third their length; conversely, such overlap is not possible for the average plant at any wider spacing. As the plants grow larger, however, one would expect the area of overlap to increase, with a

resulting manifestation of competition at wider spacings. This phenomenon, already amply demonstrated in experiments under controlled conditions (see Harper, 1977, for review), illustrates the observation that the number of individuals per unit area is an inadequate measure of density in plants, due to their plasticity in size. Effective plant population densities vary with plant age and size and, hence, with time, even when the number of individuals does not change. Thus, the expectation that density effects may, with time, become evident in the wider spacings hinges on the notion that effective density increases as the plants grow. Verification of this trend awaits future censuses.

In spite of major effects of environmental variation, family differences were nevertheless apparent. The difficulty of obtaining sufficient seed from formal genetic crosses dictated the use of self-sibships in these experiments. As a result, components of genetic variance are confounded with maternal effects to make up variation among families; precautions to minimize the contribution of environmental effects to seed quality were noted above. The broad sense heritabilities, which represent upper bounds to the proportional genetic determination of traits, indicate that small but significant proportions of the phenotypic variation in early size are attributable to family effects. Since growth is a multiplicative process, small differences in size at early stages become augmented in later stages, as has often been shown in controlled experiments (Harper, 1977). Density can increase this effect still further (Koyama and Kira, 1956; Ford, 1975). Thus, one might expect that the small size differences observed at the early stage of the present study are likely to be compounded in the future and thus lead to differential success.

The establishment and consequences of size hierarchies have rarely

been documented experimentally in natural plant populations. Fowler and Antonovics (1981) showed that size (i.e., leaf number) of Salvia and P. lanceolata on certain dates was significantly positively correlated with size and fecundity as much as six months later. Clay (1982) reported that the initial weight of tillers of Danthonia spicata planted into the field was a highly significant determinant of certain flowering characteristics a year later. But, in general, the relevance of the phenomenon of compounding of size advantage in natural populations is not known. To the extent that this effect can be observed in later censuses, the family based size differences may ultimately confer differences in fitness in this experimental population.

Both experiments uncovered block x family interactions. Their variance components were, in some cases, equal in magnitude to those of the environmental main effects and appreciably larger than those for the family main effect. This finding indicates that the response to the spatial and temporal heterogeneity varies among families. Moreover, the block x spacing x family interaction in Experiment B, for which the multivariate test was significant, can be considered a plot x family interaction by the argument given above (first paragraph, Discussion). This may be taken as further evidence of family differences in response to the spatial heterogeneity of the field. If the size measures are correlated with fitness, such variation may provide the potential for differential success of individuals depending on whether their genotype "matches" the patch in which they become established.

Dickinson and Antonovics (1973) showed theoretically that, even with a large number of niche types in the environment and random fluctuation in the selection pressure in each niche, variation in the environment can greatly influence the genetic structure of the population it supports. Few

experiments have been designed to reveal such potential for multiple niche selection in natural populations, but those mentioned earlier (Fowler and Antonovics, 1981; Antonovics and Primack, 1982) gave evidence in P. lanceolata that survival and size were significantly affected by either plot x family or site x population effects. On the other hand, Clay (1982) demonstrated no significant family x block interactions in the study mentioned above. In each of these examples, including the present one, the relationship of the measured traits with fitness remains to be determined. But the studies suggest the occurrence of multiple niche selection and exemplify approaches to its further study.

The experiment revealed weak differences among the families in their response to the range of spacings (number of leaves in Experiment A) and, hence, only slight potential for density-dependent selection. Density-dependent selection, for which genotype x density interaction in fitness is a necessary condition, can be viewed as a special case of multiple niche selection, which depends on genotype x niche interactions. The two modes of selection are driven by variation in the environment: in the first case, by variation in conspecific density and in the second, more generally, by any variation to which genotypes respond differently. The expected outcome of density-dependent selection is that populations growing at different densities will favor different genotypes with distinct life histories. This process would maintain genetic variation among populations. This result depends on the existence of "tradeoffs", or negative genetic correlations between fitness in the different densities, a condition which has often been assumed (e.g., Gadgil & Solbrig 1972), but not demonstrated.

From the present study, only suggestive evidence of the potential for density-dependent selection is available in the genotype x density

interactions for number of leaves per individual. The genetic correlations of the size traits between densities are occasionally significantly positive; in particular, the size traits in the 12 cm spacing are significantly correlated with those in each other spacing treatment. Correlations of size between the remaining densities are, in general, not significant, suggesting genetic independence of size in these different densities. In neither experiment is there any evidence of genetically based tradeoffs between growth in one density and growth in another. More definitive evidence on this phenomenon must await later censuses when 1) the density effect may be anticipated to spread to more of the spacing levels (see above) and 2) more reliable fitness traits, such as seed number, may be measurable, or selective mortality may have occurred.

It is of interest to consider how the inferences from this pair of experiments would differ if the block factor were considered fixed and only the families were random. Accordingly, instead of referring our conclusions to the population of patches similar to the ones used, we restrict them to the finite population of patches actually used in the experiment. The hypothesis mean squares for the within plot effects and their interactions are then tested over the residual mean square rather than a linear combination of interactions. We find that for the present experiment, the family effect is in every case highly significant, whereas the first and second order interactions are not, as a rule; only the block x family interaction is significant for length of leaves in Experiment B. Thus, when the hypotheses are restricted, we find differences among families in their average size, but little indication that they differ in their response to environment. These two sets of conclusions can be reconciled by noting that the residual mean square is that of the interaction of subblocks and families, because each family is represented

once in a single subblock. If the families show significant variation in response to small scale spatial variation, then the subblock x family effect does not estimate the true error, and the latter tests are vitiated. The general conclusions about block and spacing effects are not altered when blocks are considered fixed.

The overall conclusions from this pair of experiments are that naturally occuring spatial heterogeneity on at least two scales strongly influences the size of individuals within their first four months of growth. Moreover, the imposed variation in density also has a detectable effect on size, albeit only at the highest measured density (2940 plants/m^2). Variation among families in average size and in their site specific size rankings, while substantial, accounts for much less of the total phenotypic variance than does spatial heterogeneity. The indication that families perform differently at different densities is also very weak. Assessment of future survival and reproduction of the experimental individuals will provide stronger evidence of whether natural selection (density-dependent, niche-dependent, or independent of environmental influences) molds the observed variation among families. To date, however, the experiment reveals environmentally and genetically induced variation in a natural population grown in the field and exemplifies approaches to ascertaining the evolutionary significance of this variation.

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CONCLUDING REMARKS

The results of the experiments reported here indicate that density has only a weak or delayed effect in limiting population growth in this natural population of *Salvia lyrata*. Manipulation of seed densities to extreme levels, nearly forty times the natural level of seed input, revealed the only striking evidence of density-dependent limitation at the seedling level. The awkwardness of enhancing population density of mature individuals to such extremes precluded determination of whether similarly striking responses occur among larger individuals. However, at densities similar to those found in the natural population, density responses are weak and frequently undetectable. Thus, the population of this moderately long-lived perennial is not self-regulated, as one might have expected on the basis of traditional concepts of ecology. It persists with little change from year to year at numbers well below its "carrying capacity". It seems likely that addition of individuals or change in environment could boost the population to higher densities and still no limitation by density would curtail growth or reduce numbers. Therefore, the population is limited by low seed input or by high density-independent mortality of seedlings and juveniles. At presently observed densities, this population is governed by inertia (*sensu* Murdoch, 1970), rather than self-regulation.

The extent to which this view of dynamics may apply to other natural populations, especially of perennials growing in mixtures, is unknown. However, it suggests that traditional views of population growth warrant revision. In particular, if few plant populations persist at an experimentally definable carrying capacity, and do not appear to exhibit dynamics dictated by it, then that parameter is of little value as an indicator of the degree to which density influences the trajectory of the

population. More useful in prediction of population change would be a measure of a population's location along an input/output curve experimentally determined over a wide range of densities. For perennial species, such a representation is not a trivial matter, since the frequency of different size classes, all of which differ in survival, fecundity, sensitivity to density, and force of density they impose, must be taken into account. Nevertheless, evaluation of density responses in this way would represent more realistically the force of density effects in populations such as this one.

The slow growth rate of seedlings in natural conditions impeded definitive assessment of the efficacy of density-dependent selection in the field population of Salvia lyrata. However, the initial results suggest the potential for density-dependent selection on the basis of variation among families in response of one size trait to density. Information from future censuses will more definitively address this issue. Perhaps most critical in the theoretical discussions of density-dependent selection and its consequences for life history evolution is the assumption that genetically based tradeoffs impose conflicts between fecundity in low density and competitive ability in high density. If this is true, then alternative genotypes should be selected in densities at the opposite extremes. It is hoped that forthcoming information will provide a test of this assumption.

BIOGRAPHY

Ruth Geyer Shaw
Department of Botany
Duke University
Durham, N.C. 27706

Born: June 10, 1953. Bryn Mawr, Pa.

Education:

1976 B.A. Oberlin College, Oberlin, Ohio. Biology.
1978- Department of Botany, Duke University, Durham, N.C.
Minor field: Mathematics
Member, University Program in Genetics

Positions Held:

1977-1978 Research assistant, Microbial Ecology Laboratory, Division of Engineering and Applied Sciences (Ralph Mitchell), Harvard University.

1978-1979 Teaching assistant, Duke University. Genetics courses.

Academic Honors and Fellowships:

1976 Phi Beta Kappa

1979-1982 National Science Foundation Predoctoral Fellowship

1982-1983 National Institutes of Health Traineeship administered by the University Program in Genetics, Duke University

1984-1986 National Institutes of Health Postdoctoral Fellowship

Professional Affiliations:

Genetics Society of America
Society for the Study of Evolution

BIOGRAPHY (continued)

Invited Seminars:

Density-dependence: experimental studies in a natural population of Salvia lyrata. University of Michigan, March 15, 1983.

Genetic variation for response to density in Salvia lyrata. Harvard University, April 7, 1983.

Genetic variation for response to density in Salvia lyrata. West Virginia University, May 5, 1983.

Conference Presentations:

Genetics of life history variation; response to density. Poster session, Symposium on genetics of life history. University of Iowa, October, 1980.

Genotype-specific responses to density in Salvia lyrata, a perennial herb. Southeastern Ecological Genetics Group meeting. Fontana Dam, N.C., August, 1981.

Genetic and phenotypic variation in response to density in Salvia lyrata. Meeting of the Society for the Study of Evolution and the American Society of Naturalists. SUNY, Stony Brook, N.Y., June, 1982.

Evidence of density-dependent selection under natural conditions in Salvia lyrata. Joint meeting of Genetics Society of America, Society for the Study of Evolution, American Society of Naturalists, and the Stadler genetics Symposium. Washington University, St. Louis, Mo. June, 1983.

Publications:

1981 (with K.Clay) An experimental demonstration of density-dependent reproduction in a natural population of Diamorpha smallii, a rare annual. Oecologia 51: 1-6.