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The University of Arizona, 1991

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DEMOGRAPHIC CHANGES AND GENETIC VARIATION OF AN ALFALFA (Medicago sativa L.) POPULATION

by

Niu Shi

A Thesis Submitted to the Faculty of the

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1991

STATEMENT BY AUTHOR

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ABSTRACT

Substantial mortality appears to be common during establishment of an alfalfa (Medicago sativa L.) stand. However, studies on demographic changes of alfalfa populations are scarce. Mortality may be random with respect to genotype or the result of selection acting on genetic variation. The objectives of this study were to describe demographic change in an alfalfa population and determine whether genetic changes were associated with stand loss during the year of establishment. A nondormant composite population (AZ-88NDC) was sown at 1944 seeds per square meter in Oct. 1988 at Tucson, AZ. Only 29% of the seeds sown germinated and emerged. The survivorship curve of the emerged seedling population had some characteristics of Deevey type III. Two heavy mortality periods were observed; the first in mid-November, 1988 and the second in late Feburary, 1989. During these two periods, stand losses were 40 and 29%, respectively. Low temperatures might be the major cause of the first heavy stand loss. The second significant stand loss could be explained by increased density stress.

Isozyme profiles were produced from 60-day survivors (=" 60-d") dug from the field, unselected greenhouse-grown plants (="Unsel") and 300-d progenies produced by intermating plants surviving 300 days. Significant differences were observed in overall level of population heterozygosity of 60-d and unselected populations based on fixation indices of six isozyme loci. This

suggests that genetic changes may be associated with establishment of an alfalfa stand.

INTRODUCTION

Plant Demography

Value of Plant Demography

Plant demography is the study of changes in population size with time. Plant demographic studies can provide information on the dynamics of populations, including the rate at which one individual is replaced by another. They can also supply information on the relative replacement rate of different individuals. Demography forms the basis of much of the research in plant population genetics and evolution (Silvertown, 1982). Plant demography is of great importance in agricultural production especially with perennial pastures. For example, in the production of pasture legumes, understanding demographic change can help to predict the effect of management and climate on persistence, aid in breeding and selection and in quantitative and qualitative modeling of production (Jones and Carter, 1989).

Plant Demographic Models

One approach in studies of plant demography is to describe various stages of the life history of a plant population by quantifying the number of individuals present at each stage. In the last two decades, extensive research on plant demography has been conducted and has led to the creation of several population growth models, among them the continuous-time and the matrix models. Continuous-time models are used for populations of individuals

whose growth is continuous and whose birth, death, and size are correlated with age. Using a continuous-time model, the instantaneous rate of change in population number can be determined by the equation $dN/dt = rN (K - N/K)$, where N is the number of individuals in the population at time t; r is the instantaneous rate of increase per individual in the population; and K is the carrying capacity of a population, which is related to population size that will be allowed by resource availability.

Matrix models deal with discrete time periods and use an identifiable phase of the plant's life history, rather than age, as the basis for prediction of demographic change. The matrix model is useful when the population units are moving from one identifiable stage of growth to another and when the researcher is interested in the effect of different transition probabilities such as would be present in contrasting habitats or in changing environments.

The data from demographic studies can be organized into cohort or time specific life tables, depending on the life-span of the plants. A life table is a concise summary of certain vital statistics of a population. Beginning with a cohort, whose members start life together, the life table states for every interval of age the number of deaths, the survivors remaining, the rate of mortality, and the expectation of further life. Therefore, life tables can provide information on age-specific mortality rate, survivorship, fecundity, and net reproduction rate of a population (Silvertown, 1982).

Demography of Forage Plant Populations

Plant demography has never received as much attention from researchers as has animal demography. Therefore, plant demography is still considered to be relatively poorly understood (Harper, 1977). Nevertheless, extensive data on demography of different forage plant species have been collected during the last three decades (Silvertown, 1982).

Forage grasses

A long series of observations was made on grasses on rangeland in Arizona (Canfield, 1957). Over a period of 17 years the position of grass clumps was mapped in areas subject to year-long grazing and in enclosures that restricted grazing. Two species of Bouteloua (B. filfformis and B. rothrockii) had a constant annual death risk with half-lives of 9 to 10 months and 4.5 to 6 months, respectively. The remaining species exhibited remarkable patterns of survivorship with a low risk of death in middle age followed by a high death risk as they grew older.

Another group of perennial grasses was studied in a semi-arid environment in Australia. Williams (1970) followed the survivorship of cohorts of seedlings that established in different years. The survivorship curves appear to be negatively skewed. The most intriguing aspect of the dynamics of these populations was that the survivorship curves were clearly different for different cohorts. Death risk to the population was apparently determined at the time it was formed rather than by the characteristics of the years in which its

members died.

A long-term study of the survivorship of perennial grasses was made at Deniliquin, Australia in the middle of the geographical range of Danthonia caespitosa. Life tables were prepared for three species, Stipa variabilis, Enteropogon acicularis, and D. caespitosa, in plots grazed by sheep. The three species illustrated markedly different age structures. The population size of E. acilcularis were small but the individuals were often very long lived. The population present at the beginning of the experiment was depleted with a half-life of 4 to 5 yr. Some large individuals outlived other plants in their cohorts by up to 10.8 to 17.5 yr. This species was favored by enclosure but nevertheless had a very high life expectancy in the grazed plots. Stipa variabilis, in contrast, was a short-lived perennial with a half-life measured in months. There was frequent recruitment of large numbers of individuals in which each cohort moved rapidly to extinction. There were no individuals that had long survivorship as was the case with Enteropogon. Danthonia caespitosa was intermediate in behavior with a half-life of slightly less than one yr but the recruitment of seedlings was often on a massive scale. Williams (1970) suggested that death was due to the severity of the climate, but when data are plotted on appropriate logarithmic scales there is remarkably little difference in mortality risk between years. The differences between species are much greater than the differences between cohorts over years or between years over cohorts in this research.

Forage Legumes

Chapman (1987) studied rates of Trifolium repens seedling appearance and survival, and plant establishment, following natural seed deposition in Palmerston North, New Zealand. Results of this work could be used to validate descriptive models and assumptions involved in plant breeding programs and growth dynamics studies of T. repens. These results could also be used to improve understanding of the ecology of and genetic diversity of T. repens within grazed swards. Permanent 1 m^2 quadrats were marked on four farms, representing all combinations of two grazing management systems (set stocking and rotational grazing with sheep) and two super phosphate fertilizer input levels (low: 11 kg P $ha^{-1}year^{-1}$ and high: 50 kg P $ha^{-1}year^{-1}$). Observations began at slightly different times during the three years of study (1982-85) depending on fall rainfall patterns. Observations of seedling survival continued until a seedling died, or had reached a morphological stage considered to represent successful establishment, i.e. with two or more healthy stolons.

When averaged across years and treatment combinations, a mean of six seedlings per square meter appeared from naturally deposited seeds each year. Most seedlings appeared during late fall to early winter. A small secondary peak of appearance occurred in early spring each year, but by the end of October further seedling appearance was negligible. Recorded seedling densities showed that an average of 4.2% of seeds deposited appeared later as

seedlings, But this value differed between years. Of a total of 788 seedlings appearing during the 3 yr of observations, only 35 (4.4%) survived to form established, stolon-bearing plants. This represented one seedling per 5.5 m^{-2}. The competition for resources such as light and nutrients accounted for 72 to 95% of seedling losses. Failure of the radicle to penetrate soil caused 1.2 to 6% of seedling loss. Burial or treading and insect predation resulted in 2.4 to 12 and 1.2 to 8.4% of seedling losses, respectively.

Factors Affecting the Demography of Alfalfa Populations During Establishment

Demographic variation in alfalfa stands is not easy to predict because of complex and often transitory interactions of genotype, environment, and management (Rowe, 1988). Plant death is usually substantial during establishment of any alfalfa stand. The number of plants surviving the first year after seeding is generally in the range of 40 to 50 % of the seeds sown. Frequently, plant survival can be less than 20 % and this percentage can further decrease in subsequent years (Veronesi and Lorenzetti, 1983).

Because of their small initial size, alfalfa seedlings are especially vulunerable to highly variable environmental conditions on the soil surface. Any factors reducing seedling size and growth rate may therefore affect the chance of survival. Alfalfa plants under intensive management are potentially under selection pressures due to biotic and abiotic stress and competition among individuals. These selection pressures can be considered as either

density dependent or density independent. Each of these are discussed separately below.

Density dependent selection

From a purely ecological point of view, few causes of mortality in alfalfa stands are likely to be wholely free of some influence of density. A density of 150 to 250 plants m^{-2} is probably optimum for maximum yield in the year after seeding (Tesar and Marble, 1988). Farmers' seeding rates usually can be as high as 20 kg ha^{-1} or even higher. If one assumes that the weight per 1000 alfalfa seeds is about 1.5 g, potential densities can be as high as 1000 plants m^{-2} with a seeding rate of 20 kg ha^{-1}. This is far beyond the optimum. However, when plant population density is high, some individuals will inevitably die (Harper, 1977).

The term "self-thinning" has been used to describle density dependent mortality in plant populations. The process of "self-thinning" was studied initially by Yoda et al. (1963). They developed a mathematical relation between density and number of plants in a dense population. This relation, "the 3/2 thinning law", is $W = cp^{-3/2,}$ where W is the mean dry weight per plant, p is the density of plants remaining in the community and c is a constant that varies among species. This relation can be explained that as log mean plant weight is plotted against log density of survivors, the line has a slope of 3/2. In terms of plant population demography, this formula can be explained by the following (Harper and White, 1974):

1. The rate of mortality of plants is directly related to the stress caused by the pressure of population density.

2. The rate of elimination of plants is related to the rate of growth of the survivors. If the growth rate increases (e.g. by adding nutrients, water, etc.) death rate increases.

3. Mortality risk tends to remain constant over time.

4. In density stressed populations, the frequency distribution of individual plant weight becomes strongly skewed over time, approaching the log of mortality. Thus, a population of even-aged individuals develops a hierarchy of size with a few dominant and a large class of suppressed plants, mortality is largely concentrated in the suppressed class.

Even though the mechanisms of the self-thinning phenomenon are not well understood and the 3/2 thinning law is still open to debate (Harper, 1977), it has been shown that the plants that are most likely to die in natural thinning process are the smallest and weakest. For example, Black (1968) planted a population of Trifolium subterraneum using a mixture of small and large seeds. Self-thinning occurred in the seedling population after about 40 d of growth. Black found that the mortality was concentrated almost exclusively among the plants derived from the small seeds. Since small seeds produce small plants, small plants and weak plants are the primary victim of self-thinning pressure.

Density dependent selection can also be considered in terms of

competition among individual plants in populations. Ecologists today still do not have a complete definition of competition. Grime's (1977) definition is probably adequate and states that competition is the tendency of neighboring plants to utilize the same quantum of light, ion of a mineral nutrient, molecule of water, or volume of space. The effects of competition on the survival of alfalfa plants have been studied extensively. Competition for light among alfalfa seedlings would result in the lack of light energy for less competitive seedlings and would decrease their ability to survive (Fick et al., 1988). Bula and Mott (1957) studied the effects of light including various light intensities on the growth of alfalfa seedlings. He found that dry weight accumulation was essentially proportional to light intensities when grown under 696.7, 1393.5, and 2687 lux and a 15 h photoperiod. Gist and Mott (1957) found that under the three light intensities (1114.8, 557.4, 185.8 lux), both seedling top and root dry weight decreased with response of top being curvilinear and roots being linear. Matches et al. (1962) examined alfalfa seedlings of three cultivars under three levels of shading (100, 69 and 49% of full sunlight). Top dry weight tended to decrease with increasing shade, particularly when light was reduced 69%, root dry weight under full light was significantly greater than those under the 69% light treatment.

The competition for water may reduce the amount of available water for each plant and also can greatly affect the growth of alfalfa seedlings (Fick et al., 1988). Gist and Mott (1957) found that both top and root growth of

seedlings was reduced with increasing moisture stress. Cowett and Sprague (1962) observed that the number of stems and buds per plant, plant height and that root :shoot weight ratio decreased with increasing moisture stress. Janson (1975) found that both root and top growth increased with greater amount and frequency of irrigation and that root:shoot weight ratio decreased with higher available moisture. Nutrient deficiencies caused by competition among plants can also result in alfalfa seedling abnormalities or reduction in seedling growth (Fick et al., 1988).

Density independent selection

During establishment, alfalfa seedlings are not only subjected to the pressure of density dependent selection but also that of density-independent selection. Biotic and abiotic stresses are the sources of density independent selection. Grime (1977) defined stress as "the external constraints that limit the rate of dry matter production". Stress can be due to abiotic and biotic factors. Abiotic stress includes such factors as water stress (excess or deficiency), temperature and salinity. Biotic stress can result from factors such as diseases, insects or nematode attack.

McElgunn (1973) found that a 13/2° C alternating temperature treatment (12/12 h) reduced final germination percentage of alfalfa seeds compared with constant temperatures. As seedlings grew, Garza et al. (1965) showed that growth rate was greatly reduced outside the temperature range of 10 to 37° C. Salinity can be another factor affecting the establishment of alfalfa.

Germination may be reduced by toxic effects of ions and salt also has a deleterious effects on the growth of seedlings (Fick et al., 1988). Excess soil moisture may decrease the growth of alfalfa seedlings directly through the development of anaerobic conditions in the root zone (Barta, 1980). Wet soil conditions may also lead to the development of fungal diseases, especially Pythium (damping off) and Phytophthora root rot complexes (Fick et al., 1988).

Biotic stress may also contribute to the death of alfalfa seedlings. Seedling diseases can affect the plant both before and after emergence. Several fungi such as Pythium ultimum and P. debaryanum have been found associated with seedling diseases in alfalfa (Leath et al, 1988). Nematodes may affect the growth and establishment of alfalfa seedlings. For example, even though Meloidogyne hapla is considered a mild parasite on alfalfa, severe invasion of alfalfa seedlings from highly infested soil is capable of causing high mortality in young plants (Leath et al, 1988). Numerous insects can attack and damage alfalfa seedlings. For example, the alfalfa or lucerne bud mite, Eriophyes medicaqinis, and spotted alfalfa aphid, Therioaphis maculata may cause damage to alfalfa by reducing the growth of seedlings (Lehman and Flock, 1970).

Research on the Demography of Alfalfa Populations

The latest research on the demography of alfalfa populations was conducted by Rowe (1988). He studied demographic variation in three alfalfa

populations (two germplasms, Bic6-Cls5 and NCMP3d, and 'Lahontan'). The populations were grown under three density conditions. The significance of the differences in rates of thinning for the different densities were tested by comparing the error terms from regression fitted to pooled and unpooled data. First a single regression was fit for all data at all densities. Next a regression was fit for the data pooled from any two densities, and finally a third regression was fit to data from each density separately. In Rowe's research, the loss of plants over time was presented as the cumulative percentage of loss averaged over the three populations at each density. Rates of stand thinning were very different for the three populations. But their relative rankings were consistent over densities. Lahontan always thinned the slowest while the NCMP3d lost plants most rapidly. On average, the percent of stand that survived from one harvest to the next was 90.7% for the high density plots, 96.3% for the medium density plots, and 98.2% for the low density plots. The percentage of loss of stand over the winters (harvest 0 to 1 and 4 to 5) was greater than that between consecutive harvests within a growing season. But the difference was not striking for a 210-day period. Slopes of each regression for each density were significantly different from each other.

Survival of alfalfa plants and changes in the row cover of alfalfa in relation to grazing management were studied by Leach (1979) in southeast Queensland, from 1971 to 1977. There were 6 grazing treatments (rest for 32 or 44 d combined with grazing for 4 or 16 d; rest for 56 d with grazing for 4

d; and rest for 40 d with grazing for 8 d) and three mown treatments (every 36, 48, or 60 d). The population used was the cultivar Hunter River. Five permanent quadrats, each including 4 rows of 0.5 m length, were marked in each plot when the experiment began. Alfalfa crowns in each quadrat were counted in 1971, 1972 and 1974 and then at twice yearly intervals to the end of the experiment in Oct. 1977. The data showed that treatment effects were small and the alfalfa density declined at a relatively uniform rate. There were about 42 plants m^{-2} in Nov.1971 and about 13 m^{-2} in Oct.1977. Thus, survival was good in all treatments, with stands losing about five plants m^{-2} annually. The author reasoned that the good survival was probably a consequence of the moisture regime experienced or of the constraints on the range of grazing and mowing systems included.

Compared to the demographic studies done with other plant species, alfalfa demographic research seems to have been less sophisticated. No detailed life tables of populations were provided. In most studies with alfalfa, the time intervals between stand counts have been too long so that it may have been easy to have missed some critical time periods.

Genetic Consequences of Demographic Variation of Plant Populations

The genetic consequences of demographic variation of plant populations have been studied with several species and different conclusions were drawn

from these studies. Following are some examples.

Pasture

Rossiter (1966) studied genetic changes in a mixture of 51 lines of Trifolium subterraneum over time. He found that over 10 yrs, 10 lines eventually dominated the sward and most changes took place in the first two to three years. Rapid initial reduction in the plant density of a sown pasture were also recorded by Charles (1961). He found that mortality was not random, but when different cultivars of Lolium, Dactylis and Phleum were sown in mixtures, large changes in the proportion of cultivars occurred in the first year.

Like alfalfa, seedlings of Lolium perenne (ryegrass) in pure stands exhibit high mortality during establishment (Hayward, 1978). Up to 75% of viable seedlings died during the first two months, with 90% eliminated in the first year in Hayward's research. In order to determine whether seedling mortality during establishment was a non-random process with respect to the initial genetic variability, Hayward compared the genetic structure of a series of 3-yr-old swards of L. perenne with that of the original seed stocks. His data showed that for one of the three swards, the frequency of various enzyme genotypes in the surviving population differed from that in the original unselected sample. But for the other two swards, the enzyme genotype frequencies in surviving populations were the same as those of their respective original populations. Based on this, Hayward concluded that survival in the

sward was genetically random with no apparent selective difference among the genotypes examined over the 3 yr period.

Annual Crops

Allard et al. (1972) used Composite Cross V (CCV), an experimental population developed by intermating 50 barley cultivars representing all of the major barley growing regions of the world, to determine the relative effects of mutation, selection, migration, population size and mating system on the single locus population dynamics of four esterase loci. The population had been allowed to reproduce by natural self-pollination for 25 years. Each year a random sample of seeds were harvested and grown the next year. Therefore, only natural selection could be acting on this population. The data showed that highly significant changes in allelic frequencies occurred in a number of single generation transitions. Long term changes in allelic frequency also occurred. The data also showed that the proportion of heterozygotes decreased rapidly in early generations (F_4 to F_6). However, the proportion of heterozygotes showed no further consistent decrease but fluctuated about apparent equilibrium values thereafter. This was confirmed by the calculation of inbreeding coefficients. Based on this, Allard claimed that observed genetic changes in CCV were not due to genetic drift as sample sizes were too large. Mutation also did not have a significant effect since mutation rate was too low to have affected the short term dynamics of the population. Migration was also not a factor since no alleles not present originally were

found in any later generations. Thus, among the remaining established evolutionary forces, only selection was left to explain the change in gene and genotypic frequencies that occurred in CCV. Allard et al. (1972) explained that selection in CCV took two forms. First, directional selection was represented by the observed changes in gene and genotypic frequencies. Second, balancing selection lead to an excess of heterozygotes over expectations based on consideration of mating system alone.

Allard (1988) summarized the results of a series of experiments with barley that had been a central activity in his research group for more than forty years. In all experiments, Allard related observed phenotypic changes in adaptedness to genetic changes, specifically to changes in allelic frequencies at single Mendelian loci as well as to changes in frequencies of multilocus complexes of pairs, triplets and larger number of loci. Overall, the results with regard to adaptive characters were: (i) large and clear-cut directional changes toward higher and more stable grain yield and somewhat more compact and heavier spikes with larger number of seeds, and (ii) smaller directional changes leading to larger leaves, thicker culms and longer awns. Allard also noted that three genetic changes occurred. First, esterase allele 1.0 increased rapidly in frequency in the early generations of Composite Cross II (CCII). After decline in frequency from generation 9 to generation 17, allele 1.0 again increased rapidly to reach a frequency of 0.95 in generation F_{53} Besides this long term trend, statistically significant changes in allele 1.0

frequency also occurred in many single generations. Highly significant compensating changes occurred in the frequencies of alleles 1.8 and 0.2. The frequency of allele 1.2 decreased from 0.12 in the parent to about one third of its original frequency in F_6 after which its frequency fluctuated over a narrow range into the latest generations. Allard considered this as a case of strong frequency-dependent selection. Other types of allele frequency changes were observed in the experimental populations. Allele 2.7 of esterase-2 was present in the parents and early generation of CCII at a frequency of 0.98. Eleven other alleles of this locus were present in low frequency. Statistically significant changes occurred for all the alleles in some single generation transitions in CCII and the change was significant overall for allele 2.7. Allard reasoned that alleles of this locus were under strong directional selection.

Trees

Substantial research on the relationship between demographic and genetic changes has been conducted by forest geneticists primarily with Pinus sylvestris (Scots pine). Scots pine has a mixed mating system and individual trees produce a very large number of seeds, many of which may be inbred. Severe mortality can also occur among scots pine seedlings. Muona (1987) suspected that even though much of this mortality was probably random under natural conditions, some could be due to selection resulting in genetic changes between different life stages. She reasoned that unfit plants such as those suffering from inbreeding depression may be removed from the population at a

early stage. Muona compared the genetic structure of an original seed population with that of a surviving population in the field after three years. The fixation index (a measure of deviation from random mating equilibrium in plant populations) in the original seed population was 0.12 but was substantially lower (0.0006) in the 3-yr-old population. Since higher fixation indices indicate higher levels of homozygosity, according to Muona's data, inbred individuals were eliminated as the stand developed.

A similar study with scots pine was completed by Yazdani et al.(1985) in Sweden. These researchers observed that regeneration under seed trees of scots pine was affected by many factors. Among them were potentially strong selective factors that could cause large decreases in plant number. They also observed that most mortality appeared in early stages of development. They investigated the genetic changes in three different life stages. Again an excess of homozygosity was found in the embryos at most loci compared to the young and adult populations of the seed-tree stand. Yazdani et al. proposed that homozygosity may been due to partially self-fertilization, which gave rise to these inbred embryos.

Shaw and Allard (1982) used eleven electrophoretically detectable loci as markers to examine patterns of heterozygosity in both adults and open-pollinated progenies of Douglas fir (Pseudotsuga menziesii var. menziesii). They also found that heterozygotes were less frequent in samples of embryos than expected under panmixia. Conversely, a slight excess of heterozygotes

was found in adults relative to expected panmictic proportions. They concluded that most of the differences between the adult and the offspring genotypic distributions probably resulted from viability selection favoring outcrosses, i.e. by selective removal of selfed offspring prior to reproductive maturity.

Perennial Herbs

Schaal and Levin (1976) attempted to relate demographic and genetic features in the perennial herb, Liatris cylindracea. The population studied was divided into six age classes. The mean allele frequency of each age class was determined based on the average frequency per age class for five loci, MDH (malic dehydrogenase), EST_2 (esterase), PGI (phosphaexose isomerase), ALP(alkaline phosphatase), and Est_3. For each locus, age class was correlated with allele frequency to determine if there was a systematic change in allele frequency with age. The results of these correlations were nonsignificant. However, the authors commented that the absence of gene frequency changes between age classes does not precluded changes in mean heterozygosity. The latter could be determined by calculating the F_{is} population structure mean, which is related to the proportion of heterozygotes by the formula $F_{is} = 1 - ($ observed heterozygotes / expected heterozygotes). The use of F_{is} to measure change in frequency of heterozygotes has an advantage over using only genotype frequencies since F_{is} is weighted by the expected frequency of heterozygotes and thus samples from areas with different gene frequencies may

be compared. The F_{is} was determined for each age class for 15 polymorphic loci. A general trend of decreasing F_{is} with increasing age class occurred at the polymorphic loci. F_{is} values suggested that mortality was genotype dependent. A heterozygote advantage was the most plausible explanation for the observed increase in heterozygote frequency.

Isozyme Markers: Tools for Detecting Genetic Variation in Plant Populations Over Time

Many different genotypes may produce the same or similar phenotype in a single environment or, conversely, the same genotype may produce different phenotypes in the different environments. Thus, experiments based on phenotypic data may provide little information relative to the question of how phenotypic or demographic changes are associated with genetic changes in plant populations (Allard, 1988). To answer this question it is necessary to measure purely genetic changes. Fortunately, the development of biological techniques such as protein electrophoresis present an opportunity for addressing such problems.

Protein electrophoresis is a biochemical technique by which proteins present in tissue extracts are separated in a gel matrix according to their electrostatic charge. If a protein has an amino acid substitution that leads to a difference in the overall ionic charge of the molecule, then the protein will

have a somewhat altered electrophoretic mobility, and will move at a different rate in gel. Amino acid sequences in proteins are coded by nucleotide sequences in DNA. Any change in amino acid sequence will reflect change in nucleotide sequence. Protein electrophoresis can therefore be used to detect genetic changes that result in differences in electrophoretic mobility of the corresponding protein.

Existence of extensive genetic variation within most natural populations has been revealed since the first systematic application of protein electrophoresis during the 1960s (Hartl, 1988). In most species, 15 to 50% of genes coding for enzymes were observed to include two or more widespread, polymorphic alleles. The polymorphic alleles occurred with frequencies too high to result from equilibrium between opposing selection and mutation (Hartl, 1988). These observations raise several questions. For examples, what are the forces responsible for the evolution of protein diversity in nature? And, what is the relationship between morphological and physiological change and the variation in protein polymorphism? These questions in turn lead to a fundamental question that is important to answer in this study: Can genetic changes detected at the molecular level be related with plants' survival ability under natural selection pressures?

Selectionists and Neutralists would give diametrically opposed answers to these questions. Selectionists claim that protein polymorphisms are actively maintained by some form of balancing selection such as heterozygote advantage

or frequency-dependent selection that favors the minority. Many experiments have been done to support these hypothesis (Nevo and Beiles, 1989; Allard et al., 1972; Allard, 1988; Muona et al., 1987; Yazdani et al., 1985; Pipkin et al., 1973; Clarke, 1975; Kojima, 1967). On the contrary, neutralists claim that most protein polymorphisms are selectively neutral and maintained in the population through mutational input and random extinction (Kimura, 1968). As an extension of this theory, Neutralists believe that protein markers are not the right tools to detect genetic changes associated with demographic changes in populations (Harris, 1977; Nei et al., 1976; Koehn and Eanes, 1977; Somero, 1974; Yamazaki, 1982; Milkman, 1973; Mukai et al., 1980).

The debate between Neutralist and Selectionist theories continues. There is no complete agreement regarding the legitimate use of protein polymorphisms as a tool to detect relationship between genetic variation and improved survival ability of a plant population.

Research Objectives

Although the demography of alfalfa populations has been studied before, those studies were not highly detailed and researchers failed to provide detailed information on survivorship. Natural selection acting on an alfalfa population may result in significant changes in the genetic structure of the population. It is not known whether the relationship between demographic change and genetic variation is a random process or not.

The objectives of this study were to

1. Describe demographic change in an alfalfa population in a detailed manner; and

2. Investigate the relationship between demographic and genetic changes using isozyme markers.

This research could provide information useful to alfalfa breeders. For example, if a particular genetic constitution (e.g. high levels of heterozygosity) is associated with improved survival in alfalfa, then it may be useful to eliminate particularly unfavorable genotypes from breeding populations based on mean heterozygosity before beginning field evaluation. This could greatly improve the efficiency of expensive field testing procedures. Moreover, it may be advantageous to modify selection environments so that selection against inbred individuals is intensified during pasture establishment.

MATERIALS AND METHODS

Demographic Study

Field Trial

An alfalfa stand was established at the Campus Agricultural Center, Tucson, AZ on 29 Oct. 1988. The experimental area consisted of four 4.4 m^2 blocks (1 x 4.4m). One-meter long rows were seeded 0.15 m apart. The population sown was the Arizona Nondormant Composite (AZ-88NDC), which is syn-1 seed of a nondormant composite produced by bulking equal amounts of certified seed of 13 elite nondormant alfalfa varieties (Smith and Fairbanks, 1989). Before planting, germination of the AZ-88NDC was tested under laboratory conditions as well as in a greenhouse trial. The seeding rate was 1938 seed m^{-2} ($\approx$46.8 kg ha^{-1}). After planting, the field was flood irrigated so that minimum soil moisture could be guaranteed for germination and seedling growth. The plot was harvested five times during the first 550 days after planting. However, forage yield data were not collected.

In each block, four 0.25 m^2 quadrats were randomly selected and remained throughout the whole experiment. During the first 218 days after planting, the number of plants was counted in all 16 quadrats. Thereafter, counting was carried out only in the 12 quadrats in replications 1 to 3 since a beecage was placed over replication 4 for seed production. The time interval between any two counts ranged from 3 to 40 d depending on the rate of

demographic change in the population stand; Initially, counts were made frequently since rapid change in population density were observed. After about 60 d, the number of plants in the population tended to stabilize and the counting interval was increased from 14 to 40 d for the remainder of the study.

The number of plants in each quadrat was estimated by crown count on the soil surface. This method may have underestimated the actual number of plants. However, Lodge and Gleeson (1984) showed that if large amounts of seeds were sown, crown counts on the surface of the soil in a small permanently located quadrat would be a reliable method of measuring relative change over time. Two other methods, frequency measurement, and tap root and crown counting could be used to measure forage plant density. However, the use of frequency measurement was ruled out in this experiment because this method is sensitive only when population density is in the range of 0 to 80 plants m^{-2} (Lodge and Gleeson,1984); during the first 60-days after planting in this study, the population density was around 300 plants m^{-2}. Tap root counting is the most accurate way to estimate the density of a forage plant population. Unfortunately, this method requires that plants be excavated.

Data analysis

A " life table " is probably the most concise and efficient way to present certain vital statistics of populations over time (Deevey, 1943). A population is divided into age classes, each of which has an age-specific mortality risk. In

this study, the number of plants that survived to each counting day, the number of plants that died during the time interval, and average mortality per day were recorded.

A survivorship curve was drawn to describe general demographic trends in the population. The curve was drawn by plotting the logarithm of the number of survivors against the days after planting.

Genetic Study

Populations surveyed

To detect any genetic change that might be associated with demographic variation, population samples were taken at three dates (60-d selected, control or unselected, and 300-d progeny). A sample of AZ-88NDC was sown in the greenhouse at the same time as seeds were planted in the field. These plants was grown under near optimum conditions in the greenhouse and subjected to no apparent selection pressures. Emergence rate of the seeds sown was about 85% and survival rate of this population was above 80% after one year. This population served as an unselected or control population that could not have been subjected to selection.

Sixty days after planting, a tremendous drop in population density had been observed in the field. The drop could have been due to either random processes or strong natural selection acting on the population. At this time, 123 surviving plants from throughout each replication were randomly selected,

dug and transferred back to the greenhouse. These plants represented the "60-day survivor population".

Seeds resulting from intermating among plants surviving 300-d after planting in the field were collected and planted in the greenhouse too. These plants would be the progeny population and used for the study on the effects of natural reproduction on the changes of the population genetic structure.

Electrophoretic Procedures

Making Gels

1. Making Gel Cassette

Glass plates were put into Chromerge solutions for 5 min. and removed to water in a 2L beaker to wash the Chromerge from the surface of the plates. Plates then were soaked in undistilled tap water for 2 h. Holding plates with gloves, plates were rinsed with distilled water carefully and air dried overnight.

Each of two spacers was put in the middle of a pair of plates lengthwise with yellow tape. Two pieces of glass were put on tape, one on each side of the spacer. A comb was added to one end of a cassette with teeth facing in.

2. Pouring Gel

Stock solution recipe:

Gel Buffer:

10.75 g Tris

5.04 g Boric Acid

0.93 g Sodium EDTA

Bring to 1 L with double distilled water and store in refrigerator.

Solution A, for 30% end:

28.8 g acrylamide

1.2 g bis acrylamide (N.N methylene bis-acrylamide)

Bring to 50 ml with gel buffer and store in refrigerator.

Solution B, for 4% end:

4.608 g acrylamide

0.192 g bis acrylamide

Bring to 60 ml with gel buffer and store in refrigerator.

TEMED Solution (N,N,N,N tetramethyl-ethylenediamine):

0.15 ml brought to 50 ml with gel buffer.

Ammonium Persulfate Solution:

0.13 g brought to 100 ml with gel buffer. It should be made within 2 h before pouring gel.

30% mixture:

25 ml of solution A

12.5 ml TEMED

12.5 ml Ammonium Persulfate

4% mixture:

30 ml of solution B

15 ml TEMED

15 ml Ammonium Persulfate

Acrylamide and the TEMED are mixed before pouring. Ammonium persulfate is mixed only at the last minute before pouring.

A gradient maker (Apparatus GE-2/4, Pharmacia), a peristaltic pump and a casting chamber (Apparatus GSC-8, Pharmacia) are set up on a table and linked together with micropipet tip and plastic tube. With the outlet of gradient maker closed, 100ml gel buffer is poured into right (4%) tank of the gradient maker. After the pump is turned on, pump speed is increased slowly to avoid bubbles. Right before all the buffer has left the tank, the pump is turned off and the front outlet left open.

30% mixture was poured into left tank first, then 4% mixture into right. Blade of stirrer is put into the right tank and turned on. Pump is turned on again and valve between the two tanks opened. When the solutions in the tanks get low, the back end needs to be lifted up slightly to prevent air bubbles. When the end of the gel flow in the hose was close to the outlet of the chamber, the flow is stopped by clamping the tube with a hemostat and turning the pump off.

Running Gel

Tank Buffer:

60.6 g tris

285.2 g Glycine

Bring to 2 L. Mix 1 L of this stock with 4 L distilled water to use.

About 30 mg healthy, white root tissue is harvested from individual

plants. The root tissue is put in an ice-cold mortar with 150 ul tank buffer and ground very hard. Everything in the mortal was sucked up with a pipet and kept in a 1 ml microfuge tube on ice. The mortal and pestle are cleaned before root tissues from another plant are ground. The microcentrifuge tubes were spun in a microcentrifuge for 15 min. 100 ul supernatant in a tube was removed and put into another tube with 20 ul sampling buffer. They were mixed well and loaded into wells on top of a gel. The voltage and running time varied with different enzyme systems: 125v, 20h for APS (Acid Phosphatase), and EST (Esterase); 175v, 22h for LAP (Leucine Amino Peptidase), PRX (Peroxidase).

During gel runing, the whole system is kept at 6° C.

Staining Gels

1. Peroxidase (for two gels):

pH 5.0 Sodium Acetate Buffer:

Solution A: 0.2 M acetic acid, start with 11.55 ml glacial acetic acid and bring to 1 L with distilled water.

Solution B:

5.44g sodium acetate trihydrate, bring to 200 ml with distilled water.

Mix 30 ml solution A and 70 ml solution B, add 100 ml distilled water for total volume of 200 ml. Dissolve 0.1 g 3-amino-9-ethylcarbazole in 10 ml N,N dimethyl formamide and add this to 200 ml sodium acetate buffer.

Mix up 3% hydrogen peroxide by diluting 30% H_2O_2 storing in a

refrigerator in a 1:10 ratio. Add to above. Put gels into pyrex dish and put on shaker, set on slow. Allow to stain for 20 to 30 min. Rinse and store gels in refrigerator in distilled water.

2. Leucine Amino Peptidase (for two gels)

   pH 6.0, 0.2 M Sodium Phosphate Buffer:

   Solution A: 2.4 g monobasic anhydrous sodium phosphate, Bring to 100 ml with distilled water, or 5.5 g monobasic monohydrate sodium phosphate.

   Solution B: 2.84g dibasic anhydrous sodium phosphate, bring to 100 ml with distilled water.

   Mix 87.7 ml solution A and 12.3 ml solution B. Add 100 ml distilled water for final volume of 200 ml. Dissolve 0.05 g L-leucine ß-napthylamide in 2.0 ml absolute methanol. Add to phosphate buffer while stirring. Add 0.1 g Fast Black K Salt. Put gels into pyrex dish and put on a shaker, set on slow.Allow to stain for 1-2 h. Rinse and store gels in refrigerator in distilled water.

3. Acid Phosphatase Stain (for two gels):

   Prepare 200 ml pH 5.0 sodium acetate buffer as above (peroxidase stain).

   Weigh out:

   0.15 g Fast Garnet GBC salt, add to buffer while stirring in the dark about 30 minutes before needed. Lastly, add 0.04 g ß-naphyl acid phosphate and mix well. Stain gels without shaking in the dark for 2 to 3 hr. Rinse gels with distilled water, fix in 3% acetic acid and store in

refrigerator covered with foil.

4. Esterase Stain (for two gels):

   pH 7.1, 0.1 M Sodium Phosphate Buffer:

   Solution A: 0.2 M monobasic sodium phosphate (2.78 g anhydrous or 3.614 g dihydrate). Bring to 100 ml with distilled water.

   Solution B: 0.2 M dibasic sodium phosphate (2.661 g anhydrous or 5.365 g 7-hydrate or 7.17 g 12-hydrate). Mix 30 ml solution A and 67 ml solution B.bring to 200 ml with distilled water.

   In beaker:

   Add 0.2 g Fast Blue RR Salt to 200 ml buffer.

   In stoppered vial:

   Mix 0.06 g α-naphyl acetate and 0.06 g ß-naphylacetate. Add 3 ml acetone. When dissolved, add 3 ml distilled water. Mix beakers 1 and 2 just before staining. Stain gels without shaking in the dark 1 hr. Rinse gels, fix in 7% acetic acid and store in refrigerator covered with foil.

Sample size

Gorman and Renzi (1979) argued that a sample of 8 to 12 individuals yielded heterozygosity estimates within 1% of the percent heterozygosity calculated for larger samples. However this is true only when the number of enzyme loci analyzed is larger than 20. In this experiment, only four enzymes were evaluated for a total of six loci. Therefore, approximately 60 individual plants would be evaluated per isozyme for each population.

Data Analysis

Genetic differences between populations can be detected by comparing the genetic structure such as overall level of heterozygosity. Two major methods can be used to analyze genetic structure of plant populations based on isozyme data. One of them involves calculation of average heterozygosity (gene diversity), the other calculation of the fixation index. Nei (1973) defined gene diversity for a single locus as the heterozygosity (frequency of heterozygotes) expected at Hardy-Weinberg equilibrium. The average of heterozygosity (h) of all loci in a population would be the average heterozygosity (H) of that population and represents the percentage of genes that are heterozygous. H can be calculated as:

$$H = \Sigma\, h_i / r$$

where x_j is the frequency of jth allele in a locus, r is the number of loci, and h_i is the heterozygosity in the ith locus ($h_i = 1 - \Sigma\, x_j^2$) . Obviously, the more loci investigated, the more precise the estimation of average heterozygosity of a population will be (Nei, 1975).

The idea of the fixation index was developed by Wright (1951). It is primarily an estimate of the inbreeding coefficient and is intended to measure deviation of genotype frequencies from Hardy-Weinberg equilibrium expectations. According to Wright, the fixation index is defined as:

F=1-(observed heterozygosity/expected heterozygosity)

where observed heterozygosity is the proportion of heterozygotes in the

population, and expected heterozygosity equals Nei's definition of gene diversity at a locus. A negative F value indicates an excess of heterozygosity while a positive F value shows an excess of homozygosity, both relative to equilibrium expectations. When estimates are made from small samples, F can be biased downward. But the fixation index can be corrected for this bias by multiplying the expected heterozygosity by [1+1/(2N-1)], where N is the sample size (Shaw and Allard, 1982).

Average heterozygosity and the fixation index are closely related statistics. But motivations for using these statistics are different. Average heterozygosity was developed primarily to estimate the inter- or intra-population genic variation with respect to the entire genome of the organism. Alternatively, the fixation index is used primarily to understand the relationship between genotype frequencies in the total population at a single locus (Nei, 1977).

Besides average heterozygosity and the fixation index, the coefficient of inbreeding can sometimes be used to measure the genetic structure of plant populations. A formula for estimating the coefficient of inbreeding in autotetraploids was developed by Gallis (1968). However, this method was not used in this study because it could not reflect the proportion of genotypes with different level of heterozygosity in the average heterozygosity.

Nei's definition of average heterozygosity was developed based on a diploid model. Although he claimed that this definition was valid at all ploidy

levels and regardless of allele number per locus, it is apparently not valid for autotetraploid organisms like alfalfa since if two alleles in a locus of a tetraploid plant are identical, it does not necessarily guarantee that the locus is homozygous. Only when all four alleles are identical will a locus be homozygous in an autotetraploid. For a autotetraploid organism, therefore, average heterozygosity can be defined as:

$$H = 1 - \Sigma\, h_i / r, \quad \text{where } (h_i = 1 - \Sigma\, x_j^4)$$

Heterozygosity of autotetraploids is also different from that of diploids in that heterozygous autotetraploids can have any one of the three allelic structures (di-, tri-, and tetra-allelic) while a diploid heterozygote has only a diallelic structure. For autotetraploids, it may be more meaningful to partition average heterozygosity based on the allelic structure and measure the proportion of each different heterozygote in the average heterozygosity.

When an autotetraploid population is at random mating equilibrium , the proportion of different allelic structures (assuming four alleles at a locus) can be defined as:

$$1x(a^4+b^4+c^4+d^4)+4x(a^3b+ab^3+a^3c+ac^3+a^3d+ad^3+b^3c+bc^3+b^3d+bd^3+c^3d+cd^3)+6x(a^2b^2+a^2c^2+a^2d^2+b^2c^2+b^2d^2+c^2d^2)+12x(a^2bc+ab^2c+abc^2+a^2cd+ac^2d+acd^2+b^2cd+bc^2d+bcd^2+a^2bd+ab^2d+abd^2)+24x(abcd),$$

where a, b, c, d represent the frequencies of the four alleles.

Based on the above expansion of $(a+b+c+d)^4$ and Nei's (1977) definition of heterozygosity, the diallelic heterozygosity of a tetraploid organism

at a locus(h_{di}) can be defined as:

$h_{di} = 4x(a^3b+ab^3+a^3c+ac^3+a^3d+ad^3+b^3c+bc^3+b^3d+bd^3+c^3d+cd^3)+6x(a^2b^2+a^2c^2+a^2d^2+b^2c^2+b^2d^2+c^2d^2)$

The average diallelic heterozygosity of a tetraploid organism H_{di} can be defined as:

$$H_{di} = \Sigma h_{di}/r$$

and the proportion of average heterozygosity explained by diallelic heterozygote can be defined as:

$$P_{di} = H_{di}/H$$

Likewise the triallelic heterozygosity of a tetraploid organism at a locus (h_{tri}) can be defined as:

$h_{tri} = 12x(a^2bc+ab^2c+a^2cd+ac^2d+acd^2+b^2cd+bc^2d+bcd^2+a^2bd+ab^2d+abd^2)$

and

the average triallelic heterozygosity (H_{tri}) can be defined as:

$$H_{tri} = \Sigma h_{tri}/r$$

The proportion of average heterozygosity explained by triallelic heterozygosity is defined as:

$$P_{tri} = H_{tri}/H$$

Finally, the tetraallelic heterozygosity of a tetraploid organism at a locus can be defined as: $h_{tetra} = 24(abcd)$

and the average tetrallelic heterozygosity (H_{tetra}) can be defined as:

$$H_{tetra} = \Sigma h_{tetra}/r$$

and the proportion of average heterozygosity explained by tetrallelic heterozygosity is defined as:

$$P_{tetra} = H_{tetra} / H$$

The sampling properties of average heterozygosity has been worked out by Nei (1975). The theoretical variance of the estimate of heterozygosity at a locus is shown to be:

$$V(h) = 2(n-1)[(3-2n)j^2 + 2(n-2)\ x_j^3 + j)]/n^3$$

where $j = 1-h$ and n is the number of loci sampled.

Heterozygosity, however, generally varies considerably among loci and thus the variance of average heterozygosity of a population includes the interlocus variance. If gene frequency for r loci is studied, sampling variance of the average heterozygosity (H) can be estimated by:

$$V(H) = \ (h_l - H)^2/r(r-1)$$

where subscript l refers to the lth locus (Nei, 1975).

Like average heterozygosity, the fixation index of an autotetraploid organism can be partioned so that the deviation of di-, tri-, and tetra-allelic heterozygosity from equilibrium can be estimated. Based on Wright's definition of the fixation index and the formulae developed above, the fixation index of a heterozygote at a locus with diallelic structure (F_{di}) can be defined as:

$$F_{di} = 1-(\text{observed diallelic heterozygosity}/h_{di})$$

The fixation index of heterozygotes at a locus with triallelic structure can be defined as:

$$F_{tri} = 1\text{-(observed triallelic heterozygosity}/h_{tri})$$

and the fixation index of heterozygotes at a locus with tetraallelic structure can be defined as:

$$F_{tetra} = 1\text{-(observed tetraallelic heterozygosity}/h_{tetra})$$

RESULTS AND DISCUSSION

Demographic Pattern of the Alfalfa Population and Causes of Its Mortality

Survivorship curves are often used to present an overview of demographic patterns in plant populations (Silvertown, 1982). The curves are obtained by plotting the logarithm of the number of survivors against time. Survivorship curves can be crudely classified into three types depending on the distribution of mortality risk with age. These three types of curves are often referred to as Deevey types I, II and III. Type I, the negatively skewed rectangular, is shown by members of a cohort which, having started life at the same time, die more or less simultaneously after an esentially uniform life span. Type II is diagonal, implying a constant mortality rate for all age groups with no one age favored for mortality. Type III, the positively skewed rectangular, shows extremely heavy mortality early in life, while the few individuals that survive to an advanced age having a relatively high expectation of further life (Silvertown, 1989). Starting with the first seedlings that emerged after planting, the survivorship curve of the alfalfa population reveals some characteristics of the type III curve, such as heavy mortality for newly emerged seedlings and low mortality at middle and later stages of the establishing year (Fig.1).

Detailed demographic data of the population is summarized in Table.1.

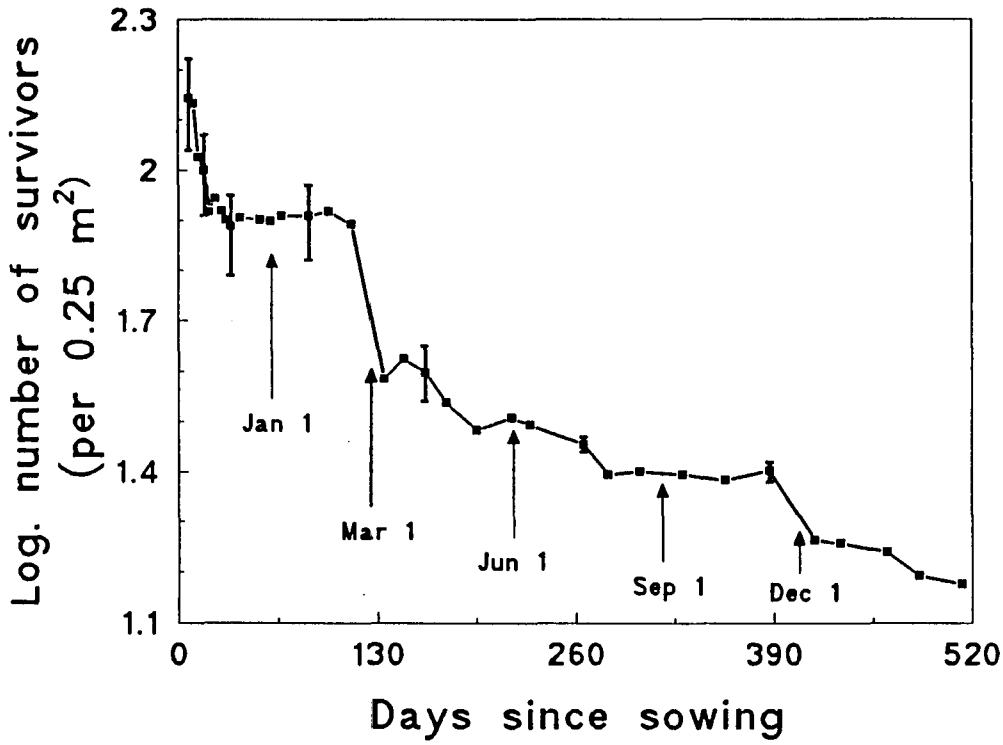

Fig 1. Survivorship curve of the population AZ-88NDC during the first 515 d following sowing; values represent means of 16 quadrats for the first 195 d, and 12 quadrats thereafter.

Table 1. A cohort life table for AZ-88NDC, beginning with the number of seeds sown.

Age interval (days)	No.plts surviving 0.25 m^{-2}	Surviving rate (%)	Mean mortality rate per day†	SD	Duncan grouping (5%)
0	484.5†				
7	139.3	100	49.32	47.1	A
10	137.	98.3	0.70	43.2	A
10-13	106.6	76.5	10.17	36.5	B
13-17	99.6	71.5	1.73	36.9	B C
17-20	82.6	59.3	5.66	28.2	C D
20-24	87.7	62.9	-1.2	32.2	C D
24-28	82.9	59.5	1.20	28.5	C D
28-31	79.7	57.2	1.08	28.7	D
31-34	76.1	54.6	1.2	25.1	D
34-40	80.3	57.6	-0.63	28.8	D
40-53	79.6	57.1	0.04	27.1	D
53-60	79.1	56.8	0.08	29.1	D
60-67	80.9	58.1	-0.26	28.7	D
67-85	81.1	58.2	-0.01	29.6	D
85-98	82.6	59.3	0.12	28.1	C D
98-113	77.9	55.9	0.31	22.1	D
113-134	38.4	27.5	1.88	8.7	E
134-147	42.1	30.2	-0.73	13.3	E
147-161	39.5	28.4	0.18	8.6	E
161-175	34.5	24.8	0.35	8.8	E F
175-195	30.5	21.9	0.20	4.4	E F G
195-218	32.2	23.1	-0.07	7.3	E F G
218-230	31.2	22.4	0.08	5.5	E F G
230-265	28.5	20.4	0.07	5.3	E F G
265-281	24.8	17.8	0.22	5.2	E F G
281-302	25.2	18.0	-0.01	5.0	E F G
302-330	24.8	17.8	0.01	5.5	E F G
330-358	24.2	17.3	0.02	4.7	E F G
358-387	25.3	18.2	-0.03	4.4	E F G
387-416	18.3	13.1	0.23	4.0	E F G
616-433	18.1	13.0	0.01	4.4	F G
433-464	17.3	12.4	0.01	4.3	F G
464-485	15.5	11.1	0.11	3.5	G
485-513	15.0	10.8	0.02	3.2	G

† Total number of seeds sown.
‡ Plants 0.25 m^{-2}.

First stand counts conducted 7 d after planting showed that only 28.3% of the seeds sown had germinated and emerged (Table 1). The emergence rate of a sample of seeds from the same lot as used in the field was tested in the greenhouse and germination rate was tested in the laboratory at same time seeds were planted in the field experiment. Germination rate of the seeds in the laboratory was 99.5% and emergence rate in greenhouse was 85%.

Apparently, most of the seeds that were planted in the field were viable and this suggests the poor emergence rate observed in the field might be due to environmental factors. Many factors can influence the germination and emergence of alfalfa seeds. Among them, seed age, daily temperature, amount of available water and soil salinity dominate (Fick, 1988). However, no obvious environmental factors could be found to explain the low emergence observed in the field. Townseed and McGinnies (1978) showed that the rate of alfalfa germination depended on temperature. Generally, alfalfa seeds germinates between 2 and 40 ° C with an optimum about 19 to 25 ° C. Bula (1972) reported that most rapid and vigorous seedling emergence occurred when daily mean temperatures were near 25 ° C and seedling emergence would be minimal at temperatures below 10 and above 35 ° C. In this study, during the first 7 DAP, daily mean, minimum and maximum temperature averaged 22, 14 and 30 ° C respectively (Table 2). This would seem to greatly reduce the possibility of temperature as a possible causes of the poor stand establishment. Salinity can affect both germination and emergence of alfalfa seeds (Fick,

Table 2. Climatological data for the year Nov. 29 - Mar.1989 at Tucson, AZ.

Time interval (DAP)	Mean daily temperature (°C)			Light†
	Minimum	Average	Maximum	
0-7	13.9	22.0	30.1	98.8
7-10	11.9	21.5	31.1	98.8
10-13	13.1	21.1	29.1	92.0
13-17	9.0	18.0	27.0	96.8
17-20	4.8‡	13.1	21.4	100
20-24	2.2	10.3	18.4	95.7
24-28	3.1	13.3	23.5	79.7
28-31	3.9	8.7	13.5	36.0
31-34	2.0	11.5	21.0	100
34-40	8.5	16.1	23.7	97.5
40-53	4.7	14.0	23.3	68.3
53-60	6.1	13.2	20.3	65.0
60-67	1.5	7.0	12.5	76.4
67-85	4.0	7.9	11.8	83.8
85-98	4.0	11.7	19.4	72.1
98-113	4.5	12.5	20.5	69.6
113-134	6.4	15.5	24.6	84.2
134-147	10.6	21.2	31.8	99.3
147-161	8.9	18.3	27.7	90.4
161-175	13.6	24.0	34.4	99.0
175-195	13.4	23.5	33.6	96.5
195-218	14.2	23.2	32.2	96.1
218-230	16.8	27.1	37.4	99.5
230-265	22.3	31.9	41.5	92.5
265-281	24.2	31.8	39.4	65.1
281-302	23.5	30.8	38.1	71.3
302-330	21.9	29.8	37.7	86.4
330-358	18.2	26.5	34.8	81.9
358-387	10.2	19.1	28.0	88.6
387-416	7.1	14.8	22.5	85.3
416-433	3.0	11.4	19.8	67.6
433-464	3.3	10.9	18.6	76.8
464-485	2.5	10.3	18.1	66.3
485-513	5.2	13.9	22.7	69.5
513-556	11.2	20.2	29.2	78.8

† Percent of total possible sunshine.
‡ Minimum temperature was below or equal to 0 ° C in at least one day.

1988). But the low emergence rate can not be contributed to high salt concentrationsin the soil. Soil and water sample from the same site had been tested for salinity one year before and data demonstrated that it was not a potentially saline site (Smith et al., 1989).

While the poor emergence rate can not be easily explained by seed quality or environmental factors such as temperature, and salinity, seedbed preparation and predation may provide an explaination. Tesar and Jackobs (1972) showed that compaction of the seedbed before planting usually resulted in a much higher emergence rate than simply harrowing. In this study, the experiment field was not compacted before sowing. Predation can also greatly reduce the potential emergence rate. Rodent and rabbit activities were observed in and around the field and may have lead to seedling mortality.

From 10 to 13 DAP, a significant decline in plant population occurred, with average daily mortality being approximately 40 plants m^{-2} (Fig. 1; Table 1). By day 13, 24.4% of the population that had emerged had died. A second significant decline in the population density occurred between 13 and 20 DAP, during which the population lost an additional 23% of its members, with average mortality per day of 16 plants m^{-2}. By 20 DAP, only 60% of the original seedlings were still alive.

Low temperatures during seedling growth may have resulted in some seedling death during the first 20 DAP. The optimum temperature for alfalfa seedling growth is between 21 and 27 ° C, 6 d after gemination (Fick et al.,

1988). However, during the period 13 to 20 DAP, average daily temperature was 13 to 18 ° C while average minimum temperature was only 4.8° C. On day 19 and 20, minimum temperatures were 0 ° C (Table 2). Some of the seedlings might have failed to endure these low temperatures.

As Fig. 1 reveals, after the heavy mortality in the first 20 DAP, the population appeared to stabilize. From 20 to 113 DAP (mid-November, 1988 to the end of January, 1989), average mortality per day was approximately 0.5 plants m^{-2} and the population lost only an additional 5% of its members. By day 113, 55.3% of the original seedling population was still in the field.

This short stable period may be best explained by the principles of microevolution and natural selection. Table 2 shows that there were not great differences in average daily temperature between 10 to 20 DAP during which heavy mortality occurred and 20 to 113 DAP during which the population density was stable. Most seedlings that were susceptible to low temperatures might have died during the first 10 to 20 DAP. Survivors might be those that were adapted to the cold mid-winter environment.

The population experienced another period of heavy mortality following the initial stable period (Fig.1, Table 1). During period from day 113 to 134 DAP (12 Feb. to 5 mar., 1989), average mortality increased from approximately 0.5 to 20 plants m^{-2} and an additional 29% of the population was lost. By day 134, only 27.7% of original seedlings were still represented in the field. A sudden increase in density stress among the plants might have resulted in this

heavy population mortality. As shown in Table 2, from 113 to 134 DAP, daily mean temperatures began to rise. With higher temperatures, plant growth rates would be increased. On the other hand, at 113 DAP, the population density was still about 300 m^{-2} (Table 1), which was higher than what is considered an optimum alfalfa population density (60-150 plants m^{-2}). High density and increased growth rate would make the strong density stress in the population inevitable and relatively weak plants could die under this stress. During the rest of the stand establishment year, the population did not experience heavy mortality. Instead, mortality became relatively low and stable (Fig. 1). The low but constant mortality pattern has been found in many different plant populations after seedling establishment (Harper, 1977). However, few consistant reasons can be found to explain this phenomenon. Yoda et al. (1968) did observed a relationship between mortality and plant weight and proposed the self-thinning law which states that when the log. of plant weight is plotted against the log. of number of survivors in a population, the line has a slope of -3/2. Unfortunately, this law can not be verified in this study because plant weight was not measured over time.

Genetic Changes among Populations and its relationship with Demographic Variation

Differences in Genetic Structure of the Populations

Highly significant differences in allele frequencies were found between the unselected (UNS) and 300-d progeny (300-P) populations at all the loci except locus PRX-B (Table 3). Allele frequency differences between UNS and 60-d survivor (60-S) populations appeared to be larger than that between 60-d and 300-d populations; G values of UNS - 60-S comparisons were larger at all loci, except APS-1, than those of 60-S - 300-P comparisons. This implies the population experienced more changes in allele frequencies during the first 60 DAP than it did between 60 to 300 DAP. Systematic changes in allele frequencies (either increases or decreases) occurred at loci LAP-1, LAP-2, APS-2 and EST. This suggests that frequency changes of some alleles or chromosome segments that carry these allelels during establishment were not the result of a random process. Alleles that increased in frequency systematically may be those favored by natural selection while those alleles that systematically decreased in frequency may be ones disfavored by natural selection.

H values observed in the three populations were about 90% (Table 4). The major proportion of average heterozygosity of the three alfalfa populations were di- and triallelic heterozygosity. Tetraallelic heterozygosity was relatively less common. There were no significant differences in H among the three

Table. 3 Observed allele frequencies of three populations and results of G-test among the populations

Locus	Pop.	N	Alleles				G		
			A	B	C	D	a-b	a-c	b-c
PRX-B†	(a)Unsel.†	66	0.127	0.224	0.325	0.325	1.6	1.1	0.9
	(b)60-S.	102	0.152	0.213	0.318	0.318			
	(c)300-P.	59	0.149	0.246	0.310	0.296			
LAP-1	(a)Unsel.	48	0.309	0.105	0.330	0.225	105.8**	97.0**	4.8
	(b)60-S.	95	0.487	0.036	0.467	0.005			
	(c)300-P.	60	0.563	0.031	0.406	0			
LAP-2	(a)Unsel.	48	0.295	0.288	0.295	0.123	52.6**	68.1**	35.2**
	(b)60-S.	95	0.332	0.304	0.332	0			
	(c)300-P.	60	0.435	0.111	0.442	0			
APS-1	(a)Unsel.	52	0.041	0.422	0.423	0.114	5.6	12.4**	25.6**
	(b)60-S.	48	0.009	0.449	0.449	0.094			
	(c)300-P.	60	0.053	0.355	0.355	0.237			
APS-2	(a)Unsel.	52	0.625	0.375	0	0	5.5*	22.8**	5.0*
	(b)60-S.	48	0.737	0.263	0	0			
	(c)300-P.	60	0.825	0.175	0	0			
EST.	(a)unsel.	65	0.189	0.305	0.323	0.097	6.8	23.2**	6.6
	(b)60-S.	60	0.200	0.242	0.300	0.168			
	(c)300-P.	53	0.288	0.184	0.2756	0.214			

* ** Significant at P=0.05 P=0.01, respectively.

† Unsel.= unselected (greenhouse-grown); 60-S = surviving plants dug 60 d after sowing; 300-P = progeny produced from intermating plants surviving 300 d after sowing.

PRX-B: peroxidase locus B. LAP-1 LAP-2: leucine amino pepetidase locus 1 and 2, respectively. APS-1 APS-2: acid phosphotase locus 1 and 2, respectively.

Table 4. Average heterozygosities and its partitioning for unselected, 60-day survivors and 300-day progeny populations.

Pop.	No. loci	$H^{\dagger}$	SE	$P_{di}^{\dagger}$	$P_{tri}^{\dagger}$	$P_{tetra}^{\dagger}$
Unselected	6	0.945	0.008	0.510	0.424	0.049
60-d survivors	6	0.908	0.016	0.611	0.339	0.024
300-d progeny	6	0.877	0.029	0.6195	0.338	0.033

$\dagger$ H= expected average heterozygosity; P_{di} =expected diaallelic heterozygosity/H;

P_{tri} = expected triallelic heterozygosity/H; P_{tetra}= expected tetraallelic heterozygosity/H;

populations. However, partitioning average heterozygosity showed that the proportions of diallelic heterozygosity of the 60-S and 300-P survivor populations were 20% higher than that of unselected population. Correspondingly, the proportions of triallelic heterozygosity of 60-S and 300-P populations were 20% lower than that of unselected populations.

At the four of the six loci (PRX-B, LAP-1, APS-1, EST), there was a trend toward increasing values of diallelic genotype fixation indices (F_{di}) in the 60-S population compared to that of the unselected population (Table 5). The change was greater for LAP-1, APS-1 and EST than at locus PRX-B. For loci LAP-1, APS-1 and EST, an excess of diallelic genotypes were present in UNS. But after 60 d of growth, the frequencies of these genotypes became much less than what would be expected at equilibrium. A chi-square test of independence indicated a highly significant difference between the F_{di} values of these two populations. Meanwhile, out of the five loci that had triallelic genotypes, there was an apparent decrease in the triallelic genotype fixation indices (F_{tri}) values. An excess of triallelic genotypes appeared in the 60-S population at loci PRX-B, LAP-2 and APS-1. At loci EST and APS-1, a large excess of tetraallelic genotypes were found in the 60-S population. When tri- and tetraallelic genotypes were combined, out of the five loci which had available data, four of them had decreased F values. A chi-square test again showed that the differences in F_{tri} and $F_{t\text{-}tet}$ between UNS and 60-S populations were highly significant.

Table 5. Comparison of fixation indices between unselected and 60-d survivor populations

Locus	Pop.	F_{di} **	F_{tri} **	F_{tetra}	F_{ttet} **
PRX-B	Unselected	0.459	0.093	-3.255	-0.314
	60-d survivor	0.542	-0.024	-2.535	-0.347
LAP-1	Unselected	-1.213	0.551	1.000	0.602
	60-d survivor	0.238	0.159	1.000	0.166
LAP-2	Unselected	0.946	-0.691	0.169	-0.582
	60-d survivor	0.818	-1.084	1.000	-1.324
APS-1	Unselected	-0.241	0.372	1.000	0.4075
	60-d survivor	0.264	-0.447	-4.059	-0.505
APS-2	Unselected	0.910	1.000	--	1.000
	60-d survivor	0.738	1.000	--	1.000
EST	Unselected	-0.345	0.300	-0.737	0.232
	60-d survivor	-0.160	0.604	-4.584	0.165

F_{di} = 1-(observed diaallelic heterozygosity/expected diaallelic heterozygosity);
F_{tri} = 1-(observed triallelic heterozygosity/expected triallelic heterozygosity);
F_{tetra} = 1-(observed tetraallelic heterozygosity/expected tetraallelic heterozygosity);
$F_{t\text{-}tet}$ = 1-(observed tri and tetraallelic heterozygosity/expected tri and tetraallelic heterozygosity).
** significant difference (P=0.01) in F between the two populations by chi-square test.

Table 6. Comparison of fixation indices between 60-d and 300-d survivors populations.

Locus	Population	F_{di}	F_{tri}	F_{tetra}	$F_{t\text{-}tet}$
PRX-B	60-d survivor	0.542	-0.024	-2.535	-0.374
	300-d progeny	0.678	-0.021	-2.733	-0.373
LAP-1	60-d survivor	0.238	0.159	1	0.166
	300-d progeny	0.250	1	1	1
LAP-2	60-d survivor	0.818	-1.084	1	-1.324
	300-d progeny	0.161	-0.431	1	-0.756
APS-1	60-d survivor	0.264	-0.447	-4.06	-0.505
	300-d progeny	0.486	-0.396	-1.577	-0.485
APS-2	60-d survivor	0.738	1	--	1
	300-d progeny	0.908	1	--	1
EST	60-d survivor	-0.160	0.604	-4.582	0.165
	300-d progeny	0.495	0.219	-3.199	-0.156

F_{di} = 1-(observed diaallelic heterozygosity/expected diaallelic heterozygosity);
F_{tri} = 1-(observed triallelic heterozygosity/expected triallelic heterozygosity);
F_{tetra} = 1-(observed tetraallelic heterozygosity/expected tetraallelic heterozygosity);
$F_{t\text{-}tet}$ = 1-(observed tri and tetraallelic heterozygosity/expected tri and tetraallelic heterozygosity).

In contrast to what was observed in Table 2, there were not any significant differences in F_{di}, F_{tri} and $F_{t\text{-}tet}$ values between these two populations (Table 6); a chi-square test indicated that the differences in F values between 60-S and 300-P populations were nonsignificant and therefore could be assumed to be the result of random processes.

Data in Table 5 suggests that at most loci, the genetic structure of the populations was moving towards the higher degree of heterozygosity (i.e. fewer diallelic and more triallelic and tetraallelic structures) during the first 60 days of stand establishment. However, the opposite conclusion could be drawn from Table 4. The apparent disagreement can be explained by recognizing that of the six loci, no triallelic and tetraallelic genotypes were found for one and two loci, respectively, in all three populations. Since data from only six loci were used to measure average heterozygosity of the populations, either the absence of data or a large sampling error at even one locus may have seriously affected the precision of the measurement. Nei (1975) stated that to calculate average heterozygosity, a large number of loci are needed so that a precise estimation can be achieved. In this study, information from Table 5 (fixation indeces) may therefore be more dependable than that from Table 4 (average heterozygosity).

Fixation indices in Table 6 indicate that during stand establishment changes in population genetic structure occurred primarily in the early stage of growth. No significant genetic changes were observed in the population

between 60 and 300 DAP.

Decreases in F_{di} at loci LAP-2 and APS-1 and increases in F_{tri} at locus EST in 60-S survivor population could be the result of unknown Mendelian genetics of marker loci, null alleles or dominant alleles at modifier loci (Brown, 1980). They could also because alleles at those loci are neutral in terms of fitness of the populations.

Relationships between Genetic Change and Demographic Variation of the Population

Because of the differences in genetic structure between unselected and 60-d selected populations and the fact that the 60-d population may have been affected by natural selection, one may wonder what the relationship is between genetic and demographic change during the establishment of alfalfa seedlings.

Few studies have been done to link demographic variation of alfalfa populations with genetic structure. However, according to Neo-Darwinist theory, genetic change will happen in a population as long as there is sufficient genetic variation and strong selection pressure including competition and environmental variation both in time and space. Both conditions may exist in an alfalfa population during establishment and in subsequent years.

Although alfalfa is a largely cross-fertilized in nature, Wilsie (1958) showed that under field conditions, selfing can be as high as 11%. It is therefore possible that in alfalfa populations, a certain proportion of plants

could be partially inbred. The detrimental effects of inbreeding in alfalfa have been well documented (Hanson, 1974). Tremendous reductions in vegetative vigor and reproduction are associated with selfing of alfalfa. For example, Wilsie (1958) compared the forage yields of eight parental clones with those of their S_1 and S_2 progenies. S_1 and S_2 populations had a yield of only 80 and 50 % of that of parental clones, respectively. The agronomic traits of S_0 and S_1 populations were compared by Aycock and Wilsie (1968). The S_0 population was superior to the S_1 in many characteristics such as spring vigor, yield per plant, seed production, summer and fall plant height, and summer and fall plant width. The S_0 population also had a lower percentage of winter injury than the S_1 population. Kirk (1927) also observed a pronounced and progressive reduction in important vegetative morphological and physiological characters with increased inbreeding. Kiffman and Wilsie (1961) observed that inbred progenies exhibited a more erect growth habit, a higher incidence of leaf disease, and darker green foliage color than the parental clones. These finding suggest that in an alfalfa population, the smallest and weakest plants may be partially inbred and therefore least likely to survive, especially under density dependent selection pressures.

Under strong natural selection pressure, smallest and weakest would be eliminated most frequently. Veronesie and Lorenzetti (1983) studied intra-specific competition in an alfalfa population. They found that selective elimination of S_1 plants began during the first year. Selection against S_1

plants increased over time and they suggested that one year after seeding, few S_1 plants would remain regardless of their percentage in the initial seed lot.

Two possible genetic consequences of the elimination of inbred plants from an alfalfa population can be imagined. One is a change in allele frequency. In order to achieve lower mean level of inbreeding depression in particularly self-fertilizing species, selfing acts to purge populations of deleterious alleles (Charlesworth and Charlesworth, 1987). Deleterious alleles carried by less competitive plants that do not survive will be automatically eliminated and the frequency of these alleles in the population lowered. The second possible genetic consequence of the elimination of partially inbred plants is a change in the overall level of heterozygosity. Two major hypotheses, overdominance and partial dominance, have been proposed to explain the general superiority of heterozygosity over homozygosity. Even though disagreement as to the relative importance on these two hypotheses continues, it is unanimous that heterozygosity is generally associated with improved adaptation, especially in variable environments (Charlesworth and Charlesworth, 1987).

In this study, the population was reduced by 58% after 60 d growth. Possible genetic changes associated with the elimination of partially inbred plants were observed during this period. We may conclude that the eliminated seedlings may largely be partially inbred and the elimination of

these inbred plants resulted in observed increase in heterozygosity of the population. Since no significant changes were found in the overall population heterozygosity level between 60 and 300 d growth, we may also conclude that the mortality in the later stage of stand establishment may have been due to reasons other than the elimination of partially inbred plants.

Biochemical Mechanisms for Observed Heterosis

Three similar hypotheses that explain enzyme polymorphism heterosis have been proposed. Schaal and Levin (1977) stated that the basis for heterosis may lie in the greater biochemical versatility or efficiency conferred to the presence of different alleles. Genotypes with different level of heterozygosity may achieve a superficially standard phenotype but still differ markedly in the extend to which their metabolism was affected in the process. Under less favorable conditions those with weaker or less well controlled enzymic "equipment" could have reduced chance of survival.

Finchan (1972) proposed two ways in which the heterozygous combination of co-dominant alleles could confer a selective advantage. Fist the new allele could, in combination with the old, produce a quantity of enzyme activity closer to the optimum in the prevailing environment than had been given by the progenitor homozygote. Another is the possibility that interaction between an old allele with a new allele could give a qualitatively distinct enzyme with uniquely advantageous properties.

The heterozygous advantage can also be explained as that enzyme

polymorphism may have a metabolic regulatory function (Johnson, 1974). Johnson's hypotheses is that enzyme polymorphism increases fitness by providing a means of metabolically compensating for a varable environment. In other words, an individual that is heterozygous at an enzyme locus has two or more available different forms of the enzyme. However, because substrate concentrations are usually very low under physiological conditions, the allozyme form having the lowest K_m (greatest affinity for substrate) will determine the reaction rate. Because K_m is a sensitive function of temperature and other variables, a small change in reaction condition may significantly raise the K_m of the rate-determining form, lowering its activity. If this was the only enzyme available to catalyze the reaction, a marked change in reaction rate could result. The same change in reaction condition, however, may simultaneously lower the k_m of an alternative form, increasing its activity and thus minimizing the change in reaction rate. Thus individuals with multiple molecular forms of an enzyme may be capable of minimizing the effect of change reaction conditions.

Selection vs Neutral Theory

Many population geneticists and ecologists have adopted a selectionist's point of view that states that allelic diversity allows for increasing population fitness in heterogeneous habitats. This hypothesis, known as the niche-variation hypothesis, predicts a positive correlation between protein polymorphism and morphological variation in a population and the relative

heterogeneity of the most relevant abiotic and biotic variables of the habitat. The basic assumption underlying this hypothesis is that the presence of more than one variant of a given protein broadens the tolerance limits or the optimal functioning range of the structure or reactions associated with the protein. For example the presence of multiple forms of an enzyme, each with a different thermal optimum, may broaden the thermal tolerance range of an organism (Nevo and Beiles, 1989).

Even though selectionists have collected enormous data to support their hypothesis, they are seriously challenged by data favoring the neutral theory of molecular evolution. The neutral theory which is more precisely called the neutral-mutational/random drift hypothesis, was first proposed by Kimura in 1968. Kimura (1968) found that on average the evolutionary rate was about approximately one substitution in 28×10^9 yr for a polypeptide chain consisting of 100 amino acids. Though this evolutionary rate appeared to be very low for each polypeptide chain of small size, the average time taken for one base pair replacement within a genome was very high (roughly 1.8 years). This means that in evolutionary history, nucleotide substitution had been so fast that on average, one nucleotide pair has been substituted in the population roughly every two years (Kimura, 1968). Substitutional load calculated based on this rate was so large that no species could tolerate it, which is to say mortality of population would be too high for evolution to proceed. Kimura claimed that the very high rate of nucleotide substitution could be reconciled with the limit

set by the substitutional load by assuming that most mutations produced by nucleotide replacement were almost neutral in selection. He further developed two formulae showing that (i) for a neutral mutation the substitutional load could be very low and there would be no limit to the rate of gene substitution in evolution, and (ii) for a mutant allele, the probability of fixation was roughly equal to its initial frequency. He then related his discovery that neutral mutations are occurring at high rates with the high frequency of heterozygous loci that had been observed by studying protein polymorphism and concluded that most polymorphism observed at the molecular level were selectively neutral. Mutations that were selectively neutral produce such small effects on the ability of their carriers to survive and reproduce that they were completely equivalent in terms of natural selection. Therefore, the ultimate fate of neutral alleles in a population was determined largely by the process of random genetic drift.

To solve this important theoretic problem, the relation of marker enzymes and fitness of an organism must be fully understood. This study was far from being able to answer this question. But the results we observed strongly support selectionists' theory since higher levels of heterozygosity at most loci investigated were found to be associated with survival in the plant population.

CONCLUSIONS

High mortality occurred in the alfalfa population investigated during the first year after planting. However, the mortality rate was not consistent over time. Seeds were planted in late Oct. 1988 and heavy mortality took place in the newly emerged seedling population during the period of 10 to 20 DAP. Low minimum temperatures may have been one factor resulting in the death of these seedlings. Population density remained relatively stable until the middle of Feb. 1989, when the population experienced another heavy loss of its members due to perhaps increased temperature and a high population density that lead to increased density stress. Thereafter, population mortality was low and constant over time.

Based on the protein polymorphism data from six loci, genetic changes could be related to changes in plant population. During the first 60 DAP, an increase in level of population heterozygosity based on table 5 was associated with the 48% seedling mortality. Since the elimination of less heterozygous individuals in a population would result in the increase in the overall level of population heterozygosity, I conclude that the increased level of heterozygosity was due to the elimination of partially inbred plants. However, the elimination of the less fit inbred plants apparently occurred only in the early growing stage. This is consistent with those data presented by Muona (1987) and others in comparable long-term perennial populations.

APPENDIX 1. NUMBER OF PLANTS IN EACH 0.25 m^2 QUARDRAT AT EACH CENSUS TIMES

Days after planting	Quadrat No.	Replication			
		I	II	III	VI
7	1	82	167	221	208
7	2	144	181	165	98
7	3	116	116	121	191
7	4	63	81	122	152
10	1	77	177	206	177
10	2	136	190	149	82
10	3	112	132	111	163
10	4	50	157	122	152
13	1	52	156	163	169
13	2	105	131	120	61
13	3	101	91	78	86
13	4	59	130	90	113
17	1	48	157	144	142
17	2	121	138	112	40
17	3	111	82	76	76
17	4	49	116	75	107
20	1	42	128	124	109

Appendix 1. (continued)

Days after planting	Quadrat No.	Replication			
		I	II	III	VI
20	2	104	104	89	36
20	3	96	66	70	62
20	4	48	93	64	87
24	1	38	119	122	112
24	2	97	92	96	38
24	3	97	70	71	61
24	4	48	102	155	86
28	1	39	119	121	120
28	2	99	102	93	37
28	3	97	74	62	67
28	4	39	96	71	91
31	1	33	117	125	124
31	2	90	94	86	44
31	3	87	62	63	59
31	4	40	98	69	84
34	1	42	118	110	100
34	2	95	107	86	43

Appendix 1. (continued)

Days after planting	Quadrat No.	Replication			
		I	II	III	VI
34	3	81	62	62	57
34	4	44	99	70	77
40	1	42	125	120	117
40	2	92	90	92	40
53	1	0	123	122	110
53	2	90	98	88	44
53	3	79	64	67	59
53	4	41	102	65	82
60	1	34	121	126	111
60	2	91	93	97	41
60	3	79	53	69	58
60	4	41	104	64	83
67	1	38	115	127	126
67	2	86	92	99	43
67	3	87	55	67	61
67	4	44	101	67	86
85	1	38	109	129	130

Appendix 1. (continued)

Days after planting	Quadrat No.	Replication			
		I	II	III	VI
85	2	90	83	100	32
85	3	75	57	79	62
85	4	48	110	72	83
98	1	41	112	101	136
98	2	99	92	110	40
98	3	71	57	76	78
98	4	43	108	77	81
113	1	37	107	94	100
113	2	87	68	91	37
113	3	79	62	97	71
113	4	51	67	75	94
134	1	25	46	41	60
134	2	42	36	45	25
134	3	32	42	45	30
134	4	33	39	38	39

Appendix 1. (continued)

Days after planting	Quadrat No.	Replication			
		I	II	III	IV
147	1	26	64	53	50
147	2	59	51	51	23
147	3	53	33	40	30
147	4	26	46	25	44
161	1	27	50	51	51
161	2	44	33	48	31
161	3	42	33	47	33
161	4	25	41	40	36
175	1	20	50	47	43
175	2	36	29	38	21
175	3	45	28	36	33
175	4	27	39	30	34
195	1	24	32	37	
195	2	34	25	32	
195	3	36	30	30	

Appendix 1. (continued)

Days after planting	Quadrat No.	Replication		
		I	II	III
195	4	25	34	27
218	1	22	33	38
218	2	43	22	41
218	3	38	31	33
218	4	24	35	26
230	1	22	31	26
230	2	36	27	40
230	3	36	35	30
230	4	27	37	27
265	1	21	29	25
265	2	36	23	36
265	3	32	32	25
265	4	24	34	25
281	1	18	23	22
281	2	33	24	30

Appendix 1. (continued)

Days after planting	Quadrat No.	Replication		
		I	II	III
281	3	24	33	20
281	4	20	29	22
302	1	16	20	24
302	2	30	26	31
302	3	27	29	24
302	4	18	31	26
330	1	17	22	25
330	2	29	24	32
330	3	25	29	17
330	4	19	34	25
358	1	19	18	20
358	2	28	25	29
385	3	27	31	22
358	4	21	30	20
387	1	20	18	22
387	2	28	25	28

Appendix 1. (continued)

Days after planting	Qudrat No.	Replication		
		I	II	III
387	3	28	32	28
387	4	23	30	21
416	1	17	15	23
416	2	20	10	22
416	3	17	16	21
416	4	15	21	23
433	1	15	16	22
433	2	18	19	24
433	3	17	17	18
433	4	15	21	25
464	1	14	15	21
464	2	18	19	24
464	3	17	18	18
464	4	15	21	24
485	1	11	12	18

Appendix 1. (continued)

Days after planting	Qudrat No.	Replication		
		I	II	III
485	2	14	19	24
485	3	16	15	15
485	4	14	17	22
513	1	10	13	18
513	2	15	11	19
513	3	12	14	17
513	4	14	15	22
556	1	12	12	17
556	2	15	12	20
556	3	14	13	19
556	4	12	16	20

LITERATURE CITED

Allard, R. W., A. L. Kahler, and B. S. Weir. 1972. The effect of selection on esterase allozymes in a barley population. Genetics 72: 489-503.

Allard, R. W. 1988. Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. J. Heredity 79: 225-238.

Aycock, M. K., and C. P. Wilsie. 1968. Inbreeding Medicago sativa L. by sib-mating. II Agronomic traits. Crop Sci. 8: 481-485.

Barnes, D. K., T. Bingham., J. D. Axtell, and W. H. Davis. 1972. The flower, sterility mechanisms, and pollination control. p. 123-140. In A.A. Hanson (ed.) Alfalfa Science and Technology. ASA. Madison, WI.

Barta, A. L. 1980. Regrowth and alcohol dehydrogenase activity in waterlogged alfalfa and birdsfoot trefoil. Agron. J. 72: 1017-1020.

Black, J. N. 1958. Competition between plants of different initial seed sizes in swards of subterranean clover (Trifolium subterraneum L.) with particular reference to leaf area and the light microclimate. Aust. J. Agric. Res. 9:299-318.

Bula, R. J., and G. O. Mott. 1957. Some effects of light intensity, temperatures, and soil moisture on the growth of alfalfa, red clover, and birdsfoot trefoil seedlings. Agron. J. 49: 33-36.

Canfield, R. M. 1957. Reproduction and life span of some perennial grasses of

southern Arizona. J. Range Manag. 10: 199-203.

Chapman, F. D. 1987. Natural re-seeding and Trifolium repens demography in grazed hill pastures. II. Seedling appearance and survival. J. Appl. Ecol. 24: 1037-1043.

Charles, A. H. 1961. Differential survival of cultivars of Lolium, Dactylis and Phleum. J. Brit. Grassl. Soc. 16: 69-75.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Ann. Rev. Ecol. Syst. 18: 237-268.

Clarke, B. 1975. The contribution of ecological genetics to evolutionary theory: detecting the direct effects of natural selection on particular polymorphic loci. Genetics 79:101-113.

Cowett, E. R., and M. A. Sprague. 1962. Factors affecting tillering in alfalfa. Agron. J. 54:294-297.

Deevey, E. S. 1947. Life tables for natural populations of animals. Quart. Rev. Biol. 22: 283-314.

Fick, G. W., D. A. Holt, and D. G. Lugg. 1988. Environmental physiology and crop growth. P. 163-165. In A. A. Hanson, D. K. Barnes, and R. R. Hill, Jr.(ed.) Alfalfa and Alfalfa improvement. ASA. Madison, WI.

Finchans J. R. S. 1972. Heterozygous advantage as a likely general basis for enzyme polymorphisms. Heredity 28: 387-391.

Gallais, A. 1968. Interactions between alleles and their variability in autotetraploid cross-fertilized plants; consequences for selection. 5th

Congress of Eucarpia, Milan.

Garza, R. T., R. F. Barnes, G. O. Mott, and C. L. Rhykerd. 1965. Inference of light intensity, temperature and growing period on the growth and chemical composition and digestibility of Culver and Tanverde alfalfa seedlings. Agron. J. 57:417-420.

Gist, G. R., and G. O. Mott. 1957. Some effects of light intensity, temperature, and soil moisture on the growth of alfalfa, red clover, and birdsfoot trefoil seedlings. Agron. J. 49: 33-36.

Gorman, G. C., and J. Renzi, Jr. 1979. genetic distance and heterozygosity estimates in electrophoretic studies: effect of sample size. Copeia 2: 242-249.

Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111:1169-1194.

Guries, P. R. and Ledig T. F. 1982. Genetic diversity and population structure in pitch pine (Pinus rigida M.). Evol. 36:387-402.

Harper, J. L. 1977. Population biology of plants. Academic Press. London.

Harper, J. L. and J. White. 1974. The demography of plants. Ann. Rev. Ecol. Syst.5:419-463.

Harris, H., D. A. Hopkinson and Y. H. Edwards, 1977. Polymorphism and the subunit structure of enzymes: A contribution to the neutralist-selectionist controversy. Proc. Natl. Acad. Sci. USA. 74:698-701.

Hartle, D., 1988. A primer of population genetics. Sinauer Associates, Sunderland, MA.

Hayward, M. D. 1978. Survival of allozyme variants in swards of Lolium perenne L. Z. Pflanzenzuchtg. 81:228-234.

Hesterman, O. B., L. R. Teuber, and A. L. Livingston. 1981. Effect of environment and genotype on alfalfa sprout production. Crop Sci. 21:720-726.

Janson, C, G. 1975. Irrigation of lucerne in its establishment season. New Zeal. J.Exp. Agric. 3:223-228.

Johnson, M. S., 1971. Adaptive lactate dehydrogenase variation in the crested blenny, Anoplarchus. Heredity 27:205-226.

Jones, R. M. and E. D. Carter. 1989. Demography of pasture legumes. In G. C.Marter, et al.(ed.) Persistence of forage legumes. ASA. Madison, WI.

Kimura, M., 1968. Evolutionary rate at the molecular level. Nature 217:624-626.

Kimura, M., 1977. Preponderance of synonymous changes as evidence for the neutral theory of molecular evolution. Nature 267:275-276.

Kirk, L. E. 1927. Self-fertilization in relation to forage crop improvement. Sci. Agr. 8:1-40.

Koehn, R. and W. F. Eanes. 1977 Subunit size and genetic variation of enzymes in natural populations of Drosophila. Theor. Pop. Biol. 11:330-340.

Koffman, A. J., and C. P. Wilsie. 1961. Effects of inbreeding on various

agronomic traits in DuPuits alfalfa. Crop Sci. 1: 239-240.

Kojima, K. I. and M. Yarbrough. 1967. Frequency-dependent selection at the esterase 6 locus in Drosophila melanogaster. Proc. Nat. Acad. Sci. 57:645-649.

Leach, G. J. 1979. Lucerne survival in southeast Queensland in relation to grazing management systems. Aust. J. Exp. Agric. Anim. Husb. 19: 208-215.

Lehman, W. F., and R. A. Flock. 1970. Two microscopic mites, Aceria medicaginis and Tarsonemus setifer, found on alfalfa in the desert area of the southwestern United States. J. Econ. Entomol. 63: 293-294.

Leath, K. T., D. C. Erwin, and G. D. Griffin. 1988. Diseases and nematodes. p.523-526. In A. A. Hanson et. al. (ed.) Alfalfa and alfalfa improvement. ASA. Madison, WI.

Lesins, K. 1950. Investigations into seed setting of lucerne at Ultuna, Sweden, 1945-1949. Ann. Roy. Agr. Col. of Sweden 17:441-483.

Lodge, G. M., and A. C. Gleeson. 1984. A comparison of methods of estimating lucerne population for monitoring persistence. Aust. J. Exp. Agric. Anim. Husb. 24: 174-177.

Matches, A. G., G. O. Mott, and R. J. Bula. 1962. Vegetative development of alfalfa seedlings under varying levels of shading and potassium fertilization. Agron. J. 54:541-543.

Mather, K. 1968. Selection through competition. Heredity. 23:529-540.

McElgunn, J. D. 1973. Germination response of forage legumes to constant and alternating temperatures. Can. J. Plant Sci. 53:797-800.

Milkman, R. 1973. Electrophoretic variation in Escherichia coli from natural sources. Science 182:1024-1026.

Mukai, T., H. Tachida, and M. Icinose. 1980. Selection for viability at loci controlling protein polymorphism in Drosophila melanogaster is very weak at most. Proc. Natl. Acad. Sci. USA 77:4857-4860.

Mukai, T., O. Yamaguchi and S-I. Kusakabe. 1982. Lack of balancing selection for protein polymorphism. p 81-120. In M. Kimura (ed.) Molecular evolution, protein polymorphism and the neutral theory. Japan Sci. Soc. Press, Tokyo.

Muona. O., R. Yazdani, and D. Rudin. 1987. Genetic change between life stages in Pinus sylvestris: allozyme variation in seeds and planted seedlings. Silvae Genetica 36:39-42.

Nei, M. 1975. Molecular population genetics and evolution. Elsevier. New York.

Nei, M., P. A. Fuerst, and R. Chakraborty. 1976. Testing the neutral mutation hypothesis by distribution of single locus heterozygosity. Nature 262:491-493.

Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populaitons. Ann. Human Genet. London 41: 225-233.

Nevo, E. and B. Avigdor. 1988. Genetic parallelism of protein polymorphism in nature: ecological test of the neutral theory of molecular evolution. Biol.

J.Lin. Soc. 35:229-245.

Pipkin, S. B., C. Rhodes, and N. Williams. 1973. The frequency of the Drosophila alcohol dehydrogenase allele, Adh. Genetics 74:s213.

Rossiter, R. C. 1966. The success or failure of strains of Trifolium subterranenm L. in a mediterranean environment. Aust. J. Agri. Res. 17: 425-443.

Rowe, D. E. 1988. Alfalfa persistence and yield in high density stands. Crop Sci. 28:491-494.

Schaal, B. A., and D. A. Levin. 1976. The demographic genetics of Liatris cylindracea Michx. (Compositae). Amer. Nat. 110:191-206.

Silvertown, W. 1982. Introduction to plant population ecology. p 40-70. Longman, London.

Shaw, D. V., and R. W. Allard. 1982. Isozyme heterozygosity in adult and open-pollinated embryo samples of douglas-fir. Silva Fenica 16:115-121.

Smith, S. E., and D. J. Fairbanks. 1989. Registration of AZ-88 MS and AZ-88 NDC, nondormant male sterile and male fertile maintainer alfalfa germplasms. Crop Sci. 29: 1095.

Smith, S. E., D. C. Poteet, D. C. Robinson, and A. K. Dobrenz. 1989. Stability of salt tolerance at germination in lucerne following seed multiplication at a non-saline site. Seed Sci. Tec. 17: 555-561.

Somero, G. H. 1974. Genetic variation in marine fishes as a test of the niche-variation hypothesis. Nature 249:670-671.

Tesar, M. B., and V. L. Marble. 1988. Alfalfa establishment. p.235-236. In A. A. Hanson et al.(ed.) Alfalfa and alfalfa improvement. ASA. Madison, WI.

Veronesi, F., and F. Lorenzetti. 1983. Productivity and survival of alfalfa hybrid and inbred plants under competitive conditions. Crop Sci. 23: 577-580.

Williams, O. B. 1970. Population dynamics of two perennial grasses in Australian semi-arid grassland. J. Ecol. 58:869-875.

Williams, O. B., and R. Roe. 1975. Management of arid grassland for sheep: plant demography of six grasses in relation to climate and grazing. Proc. Ecol. Soc. Aust. 9:142-156.

Wilsie, C. P. 1958. Effect of inbreeding on fertility and vigor of alfalfa. Agron. J. 50:182-185.

Wright, S. 1951. The genetical structure of populations. Ann. Eugenics 15: 323-354.

Yamazaki, T. 1982. Genetic variability in natural populations of the haploid plant, C.donicum. p.123-134. In M. Kirmura (ed) molecular evolution, protein polymorphism and the neutral theory. Japan Sci. Soc. Press. Tokyo.

Yazdani, R., O. Muona, D. Rudin and A. Szmidt. 1985. Genetic structure of a Pinus sylvestris L. seed-tree stand and naturally regenerated understory. Forest Sci. 31:430-436.

Yoda, K., T. Kira, H. Ogawa, and K. Hozumi. 1963. Intraspecific competition

among higher plants; XI self-thinning in overcrowded pure stands under cultivated and natural conditions. J. Biol. Osaka City Univ. 14: 107-129.