THE RELATIONSHIP BETWEEN GENETIC DIVERSITY AND STAND VIABILITY: A CASE STUDY WITH JACK PINE

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Abstract.-- Observed heterozygosity and allelic frequencies were compared for four distinct, but sympatric, even-aged jack pine populations of different ages to examine possible genetic changes associated with age and natural thinning. Populations resulted from four separate fires between 1919 (age 68) and 1965 (age 22) and are growing on the same uniform site. Genic variation of 36 trees per population was examined at 25 loci coding for 19 enzymes. Possible genetic mechanisms responsible for individual tree selection, and the impact of selective thinning of certain individuals on the genetics of the resulting stand are discussed. Stand density estimates revealed a 75% reduction in jack pines due to natural thinning between ages 22 and 68. The 68 year-old population had a significantly higher observed heterozygosity estimate than the 22 year-old population, despite only minimal differences in allelic frequencies. Thus, it appears that individuals with high heterozygosities were favored, while individuals with low heterozygosities generally succumbed to natural thinning. Large heterozygosity differences between the oldest and youngest jack pine population can be explained by either a survival advantage for individuals with a high proportion of heterozygous loci or a selection against individuals that are highly homozygous as a result of consanguineous mating.

Additional keywords: Electrophoresis, loci, alleles, natural selection, heterozygosity, Pinus banksiana.

INTRODUCTION

A great deal is known about changes in the physical structure and ecology of natural forest stands as they develop from seedlings to pole-sized stands and eventually to mature forest stands. During this development, natural thinning occurs and fast-growing dominant individuals out compete slow-growing trees. This is the natural process by which stand densities are reduced from perhaps tens of thousands of seedlings per hectare to several thousand, or even several hundred, trees per hectare at maturity. In contrast to the relative wealth of knowledge about ecological changes in forest stands, very little is known about genetic changes in forest stand dynamics. For instance, the importance of genetic characteristics in the selection of individuals for natural thinning and the possible impact of individual selection on the genetic structure of stands is virtually unknown. In this study, we discuss the possible impact of differential survival of individuals as a result of the natural thinning process on the genetic variability of the resulting population and possible genetic mechanisms responsible for individual tree selection. In a related paper to be submitted elsewhere, further discussion of heterozygosity-fitness relationships and additional data from this study will be presented.

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Selection Mechanisms During Thinning

There are several possible mechanisms by which certain individuals may survive the natural thinning process. These include: selection favoring individuals with a high proportion of heterozygous loci, selection for or against individuals with specific allelic combinations, and stochastic selection.

Heterozygosity and fitness. Several theories discuss the relationship between genetic diversity and fitness. For outbreeding plants, Ledig (1986) briefly discussed the balance and neutralist theories of genetic diversity and their relationship to fitness. Neutralists generally believe that there is no short term relationship, while the balance theory maintains that genetic diversity is favored by natural selection that promotes heterozygosity. However, Ledig (1986) pointed out that both theories maintain that genetic variation is a prerequisite to long term survival by adaptation and evolutionary change. Individuals heterozygous at a particular locus produce two forms (two alleles) of an enzyme and each form may have a different optimum for important environmental factors, such as pH and temperature. As a result, such an enzyme may function normally in a wide range of environmental conditions and individuals possessing a large proportion of heterozygous loci may be buffered against fluctuating environmental conditions. In contrast, individuals that are highly homozygous produces a single form of most enzymes which may be desirable in extreme, but stable environments. However, because of the longevity and immobility of trees, temporal and spatial variability is a reality and one might expect that highly heterozygous individuals would be resilient and able to cope with such diverse conditions.

In a review of the animal literature, Allendorf and Leary (1986) found there is generally, but not always, a positive relationship between heterozygosity and survival. Heterozygosity relationships with other fitness measures such as growth rate, dominance, morphological variation and various measures of symmetry have also been identified in animals (Soule 1967, Baker and Fox 1978, Singh and Zourus 1978, Zourus et al. 1980, Kat 1982, Fleischer et al. 1983, Leary et al. 1984 and 1987).

In a review of heterozygosity-fitness relationships in outbreeding plants, Ledig (1986) discussed results from greenhouse experiments that examined fitness values such as size, precociousness, and number of flowers. In these experiments, individuals with a high proportion of heterozygous loci generally exhibited greater fitness than less genetically variable individuals. In forest trees, Ledig et al. (1983) reported a strong positive relationship between heterozygosity and mean annual growth rate in old stands of pitch pine (Pinus rigida Mill.), but not in young stands. Positive associations between levels of heterozygosity and growth rate or variability have been identified in ponderosa pine (Pinus ponderosa Laws; Knowles and Grant 1981) and quaking aspen (Populus tremuloides Michx.; Mitton et al. 1979), while this relationship was not found for lodgepole pine (Pinus contorta Dougl.; Mitton et al. 1979). Based on an increase in heterozygosity between embryos and seedlings following some early seedling mortality, ponderosa pine seedlings that were relatively high in heterozygosity appear to have a survival advantage over less genetically variable seedlings (Farris and Mitton 1984)

Allelic combinations. Another possible mechanism that may be responsible for the differential survival of individuals is that they may possess specific alleles or combinations of alleles that convey increased or decreased fitness over individuals that do not possess those alleles. With the relatively limited number of loci that are examined using electrophoresis, the probability of detecting loci with specific allelic combinations that are related to differences in fitness is extremely small and unlikely. Furthermore, if gene frequency differences are detected among populations, it is difficult to know if they are a function of selection or an association with population mating system. Despite the difficulty in finding an association between specific alleles and fitness, these relationships have been identified. For example, Bongarten et al. (1985) found a significant reduction in growth to be associated with the presence of rare alleles in Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) trees growing in full-sib progeny tests. Greaves and Ayers (1967 and 1969) reported on several studies where resistance to a commonly used rodenticide was found to be due to a specific allele at a single locus in some Norway rat (Rattus rorvegious) populations. In another example, the frequency of specific ADH alleles in fruit flies (Drosophila melanogaster) and LDH alleles in teleost fish (Fundulus heteroclitus) appear to be maintained by selective environmental gradients associated with latitude of population origin (Oakeshott et al. 1982, Powers and Place 1978).

Stochastic selection. A stochastic model for individual selection would suggest that individual survival is purely random with respect to genetic characteristics. Survival may be a result of an advantageous microsite or some other nongenetic advantage over other individuals.

Approaches to Examine Genetics and Survival

Several approaches can be utilized to examine the relationship between genetic characteristics and survival. Probably the most reliable relationship would be obtained by examining the genetics of the exact same stand at different stages of stand development and natural thinning. Even though this approach might yield reliable results, it is often impractical due to the long time period that is usually necessary to examine different stages of stand development. Another possible approach would be to examine different stands at different stages of development. These stands, however, are likely to be of different genetic composition, have developed under different environments, and are probably subjected to different selection pressures. All of these factors and others are confounded with stand development stage and make it virtually impossible to interpret heterozygosity differences in terms of fitness. One could also examine the genetic variability of individuals of various ages in the same stand. With this approach, however, stand density information associated with age is difficult to obtain, and overstory trees are probably exposed to different environmental conditions than young understory trees. As a result, heterozygosity-age relationships are confounded with these factors and are difficult to interpret.

An approach that would minimize some of the problems mentioned, would be to examine the genetics of several distinct even-aged populations of different age growing on the same uniform site. If these populations developed under similar environments, experienced little or no gene immigration, and had similar genetic structures at the time of establishment, significant changes in genetic structure associated with increasing stand age could be validly interpreted as genetic changes resulting from the natural thinning process. Obviously, this approach requires a very unique population. A population of jack pine (Pinus banksiana Lamb.) located in northern New York, consisting of four discrete even-aged stands at different stages of development, was utilized in this case study to determine the extent of genetic changes associated with increasing stand age and decreasing stand density.

MATERIALS AND METHODS

Stand Description

Jack pine populations examined in this study are located at the Flat Rock Pine Barrens in Chazy, New York. This area is owned primarily by the Miner Institute, with small portions of the area under state, town, and private ownership. There are approximately 2,000 hectares in which jack pine is the major tree species.

There are several attributes of this area that are unique, making it a valuable ecosystem for studies of changes in genetic structure with population maturity. This is a large apparently panmictic population of jack pine with no obvious barriers to internal gene flow that might promote genetically distinct groups within the population. Jack pine needles found near the bottom of a bog within the area are estimated to be about 12,000 years old (personal communication, Janet Coles, Botany Dept., UVM) indicating this population existed shortly after glaciation. The environment within the Flat Rock Pine Barrens is very uniform. Potsdam sandstone soil is found throughout nearly the entire area, with soil depths ranging from bedrock to only about 4 cm. The elevational range is only about 50 meters in those areas inhabited primarily by jack pine. This area is located a minimum of 160 km from the contiguous range of jack pine, thereby minimizing gene immigration.

Perhaps the most important attribute of these pine barrens is the fire history. Four fires occurring between 1919 and 1965 have resulted in four distinct even-aged populations of jack pine that were 22, 30, 47, and 68 years old at the time of seed collection. These even-aged populations range in size from about 10 to 230 hectares. Jack pine within this area are almost exclusively closed cone. As a result, a regenerated population resulting from fire is almost entirely even-aged and is comprised of trees resulting from seed that was pollinated and matured in several different years. Thus, one would not expect gene frequency or genic diversity differences among populations due simply to a unique pollen or seed year.

The combination of stand and species attributes along with the fire history clearly illustrates the value of this ecosystem for studies of genetic structure changes with population age. Based on these attributes, we have made a necessary assumption that at any particular age all four of the stands were very similar in their genetic and physical characteristics.

Stand Density Assessments

Estimates of the number of stems per hectare for all woody tree species were obtained from several fixed radius plots per population to characterize changes in stand density of the four even-aged jack pine populations. In the

22 and 30 year-old stands, where stand densities were high, eight and thirteen 182 m^2 randomly-located plots were examined, respectively. Eighteen randomly-located 720 m plots were examined in each of the populations resulting from the 1919 and 1940 fires. In each plot, all trees taller than 1 m height were counted and the total number per plot were recorded by species. Number of trees per plot per species was converted to estimates of number of trees per hectare. Analysis of variance was used to determine the significance of jack pine density differences among the four populations of different ages.

Electrophoresis

In fall 1987, cones were collected from 36 randomly selected jack pine trees in each of the four populations. A comparison of several genetic parameters among the four jack pine populations provides an estimate of genetic changes associated with age. Data used for generating these estimates were obtained by examining the genetic variability of 25 gene loci representing 19 enzyme systems using starch gel electrophoresis (Table 1.). Because 8 of the 25 loci exhibited no evidence of genetic variability, these monomorphic loci were not used and all subsequent data analysis and interpretations were completed using only the 17 polymorphic loci listed in Table 1. Electrophoretic procedures used in this study were similar to those described by Jech and Wheeler (1984).

Following germination, seven haploid megagametophytes per tree for each of 36 trees per population were examined using electrophoresis. By examining seven megagametophytes per tree, there is a 987 chance of detecting a heterozygous locus if the alternative allele is present. Because genotyping each tree identifies the maternal and paternal allelic contribution, a total of 72 gametes per locus were examined for each population. By examining 72 gametes per locus per population, there is a 97% probability of identifying alleles in each population that occur at a frequency of 0.05 or greater.

The percentage of heterozygous loci (observed heterozygosity) was determined for each of the 36 trees per population. For each population, the following estimates of genetic variability were obtained: total number of alleles per locus, effective number of alleles per locus, population mean observed heterozygosity, and allelic frequencies. The effective number of alleles per locus is computed using weighted inputs for each allele at a locus with alleles of higher frequency contributing more (Crow and Kimura, 1970). The effective number of alleles per locus will always be less than the actual number of alleles per locus unless all alleles are of equal frequency.

Analysis of variance, using individual tree estimates as observations, was used to determine the significance of differences among the four populations in observed heterozygosity. For total number of alleles per locus and effective number of alleles per locus, analysis of variance was used to assess the significance of population differences using individual locus measures as observations. Contingency Chi-square tests of heterogeneity were conducted to determine the significance of population differences in allelic frequencies.

Table 1.-- Enzyme systems, electrophoresis buffers utilized, and number of gene loci examined for jack pine megagametophytes

Enzyme system variability	Enzyme system abbreviation	Buffer system[1]	Number loci examined
Polymorphic	ACP	6MC	1
	ACO	LB	1
	AAT	TC	1
	G2DH	TC	1
	DIA	LB	2
	FUM	8MC	1
	GDH	TC	1
	IDH	6MC	1
	LAP	LB	1
	MDH	8MC	2
	6PGD	6MC	2
	PGI	LB	1
	PGM	LB	1
	UGPP	6MC	1
	No. polymorphic loci		17
Monomorphic	CAT	TC	1
	DIA	LB	1
	F-EST	6MC	1
	B-GLU	8MC	1
	GPT	LB	1
	F-HEX	6MC	1
	ME	8MC	1
	PGI	8MC	1
	No. monomorphic loci		8
Total number of loci			25

[1] TC=Tris citrate, 8.7 pH; LB=Lithium borate, 8.3 pH; 6MC=Morpholine citrate, 6.1 pH; 8MC=Morphiline citrate, 8.1 pH.

Results and Discussion

Population Differences in Density

There were eleven woody tree species counted in the fifty-seven randomly located plots in the four populations of different ages. However, jack pine was by far the most prevalent. The four populations were comprised of 91.5, 97.6, 99.5, and 99.9% jack pine for the populations resulting from burns in 1919, 1940, 1957, and 1965, respectively (Table 2.). As expected, there was a significant reduction in stand density as the stand increased in age and the natural thinning process eliminated trees that were less competitive (Table 2.). Jack pine densities ranged from an estimated 2,379 trees per hectare in the oldest population (68 years old) to 8,642 trees per hectare in the youngest (22 years old) population (Table 2). Assuming all populations were similar in density at the same stage of stand development, nearly three-fourths of the less competitive trees were eliminated by natural thinning between ages 22 and 68. The elimination of nearly 75% of the trees during natural thinning provides an excellent opportunity to assess genetic changes associated with this rather extensive natural thinning process.

Table 2.-- Information on jack pine stands resulting from four natural fires at the Flat Rock Pine Barrens, Miner Institute, Chazy, NY

	Stand				Reduction in density with age
Year of fire	Age (yrs.)	Size (ha)	Composition (% jack pine)	Density (# stems/ha)	(%)
1965	22	22	99.9	8,642a [1]	--
1957	30	212	99.5	7,565a	12
1940	47	202	97.6	3,225b	63
1919	68	227	91.5	2,379b	73

[1] Means followed by the same letter are not significantly different, Duncan's ($P \leq 0.05$).

Population Differences in Genetic Parameters

An examination of observed heterozygosity estimates for each population revealed significant population differences. The oldest population had an observed heterozygosity of 26.9%, which was the highest of all populations and over thirty percent higher than the youngest population. The youngest population had an observed heterozygosity of 20.5%, which was the lowest observed heterozygosity of all populations (Table 3). Populations that were intermediate in age were also intermediate in observed heterozygosity estimates. Analysis of estimates of the actual and effective number of alleles per locus revealed no significant differences among jack pine populations and no apparent trend associated with population age (Table 3.). Plessas and Strauss (1986) found similar significant increases in observed heterozygosity from embryos to saplings (3-6 years old) to adolescent trees (17-20 years old) in three disjunct populations of Monterey pine (Pinus radiates D. Don). Ponderosa pine embryos were also found to have a lower proportion of heterozygous loci than seedlings, after some early seedling mortality (Farris and Mitton 1984). For old trees, Tigerstedt (1983) (as cited by Ledig, 1986) found 300 to 350 year old Scots pine (Pinus sylvestris L.) overstory trees to have higher average heterozygosity than understory trees that were 80-100 years old.

There are two possible explanations for the large heterozygosity differences between the oldest and youngest jack pine stands. There may be a heterozygote advantage; that is, trees that possess a higher proportion of heterozygous loci may be more likely to survive the natural thinning process than trees with low observed heterozygosities. Trees with relatively high observed heterozygosities possess a greater proportion of enzymes with two forms than trees with low observed heterozygosities. Thus, these trees might be expected to have an advantage of possessing enzymes that function normally in a wide range of environmental conditions. This explanation, where the heterozygote is superior to either homozygote, is referred to as overdominance (Ledig 1986, Smouse 1986, Bush et al. 1987), and could be responsible for the survival of highly heterozygous trees that was apparent in the oldest jack pine population.

Table 3.-- Estimates of genic diversity for four jack pine populations of different ages growing in the Flat Rock Jack Pine Barrens

Population #	age	# of trees genotyped/ population	Genetic parameter # alleles per locus	 Effective # alleles/locus	 Observed Heterozygosity (%)
1513	68	36	2.24a [1/]	1.43a	26.90a
1514	47	36	2.18a	1.43a	22.36b
1515	30	36	2.29a	1.42a	23.79ab
1516	22	36	2.12a	1.40a	20.53b

[1/] Means within a column that are followed by the same letter are not significantly different, Duncan's, ($P \leq 0.05$)

Rather than a true advantage for individuals that possess a high proportion of heterozygous loci, it is possible that the inability to survive the natural thinning process of individuals with relatively low heterozygosity may be due to inbreeding depression (Ledig et al. 1983, Ledig 1986, Smouse 1986, Bush et al. 1987). With the inbreeding depression or dominance explanation, individuals that posses deleterious alleles in the homozygous form as a result of consanguineous mating would be less competitive than heterozygous individuals that are the progeny of parents that are more distantly related. This mechanism could be used to explain the high proportion of individual trees with low observed heterozygosities in the youngest compared to the oldest jack pine population. With this explanation, individuals with a high proportion of homozygous loci would be less competitive and therefore more likely to be eliminated as the population matured. Such mild inbreeding may be normal and result from natural family structuring or microhabitat selection. Either a heterozygote advantage or inbreeding depression could conceivably explain the higher observed heterozygosity in the oldest compared to the youngest jack pine population that was evident in this study.

<u>Gene frequency changes</u>. It is conceivable that individuals surviving the natural thinning process may do so because they possess specific alleles or combinations of alleles conferring increased fitness over individuals that do not possess these alleles. Chi-square tests of heterogeneity revealed no significant differences among populations in allelic frequencies at most of the 17 polymorphic loci. For three loci, these jack pine populations did differ significantly in allele frequency. However, these differences appear to be due to slight absolute, but relatively large, differences in the frequency of rare alleles (Table 4.). Because these differences were quite

Table 4.-- Allele frequency differences among four sympatric jack pine populations of different ages

		Allele frequency (%) of population # (age):				Significance of χ^2 heterogeneity test
Enzyme system and locus	Allele #	1513 (68)	1514 (47)	1515 (30)	1516 (22)	
AAT-2	1	64.3	62.5	62.5	73.6	NS 1/
	2	35.7	37.5	37.5	26.4	
GDH-1	1	98.6	98.6	91.7	100.0	*
	2	1.4	1.4	8.3	0.0	
G2DH-1	1	53.7	50.0	52.6	37.2	NS
	2	38.9	42.9	44.7	52.3	
	3	7.4	7.1	2.7	10.5	
LAP-1	1	91.7	94.4	91.7	95.8	*
	2	8.3	0.0	6.9	4.2	
	3	0.0	5.6	1.4	0.0	
ACO-1	1	48.6	59.7	62.5	54.2	NS
	2	41.4	20.8	31.9	29.2	
	3	5.7	13.9	4.2	11.1	
	4	0.0	0.0	0.0	2.8	
	5	4.3	4.2	0.0	2.7	
	6	0.0	1.4	1.4	0.0	
PGM-1	1	100.0	100.0	100.0	98.6	NS
	2	0.0	0.0	0.0	1.4	
DIA-1	1	90.0	98.6	94.4	98.6	*
	2	10.0	1.4	5.6	1.4	
DIA-2	1	62.9	58.3	55.6	69.4	NS
	2	37.1	41.7	44.4	30.6	
6PDG-1	1	65.7	75.0	72.2	63.9	NS
	2	34.3	25.0	25.0	36.1	
	3	0.0	0.0	2.8	0.0	
6PGD-2	1	88.6	79.2	88.9	82.0	NS
	2	7.1	9.7	4.2	8.3	
	3	4.3	11.1	6.9	9.7	
IDH-1	1	100.0	100.0	98.6	100.0	NS
	2	0.0	0.0	1.4	0.0	
UGPP-2	1	95.7	97.2	98.0	100.0	NS
	2	0.0	0.0	2.0	0.0	
	3	4.3	2.8	0.0	0.0	
ACP-1	1	72.1	80.6	69.4	72.2	NS
	2	20.6	15.3	23.6	9.7	
	3	5.9	4.1	7.0	18.1	
	4	1.4	0.0	0.0	0.0	
PGI-1	1	90.0	91.7	75.0	88.9	NS
	2	10.0	8.3	25.0	11.1	
FUM-1	1	91.4	93.1	94.4	97.2	NS
	2	8.6	6.9	5.6	2.8	
MDH-2	1	92.9	93.1	88.9	81.9	NS
	2	7.1	6.9	11.1	18.1	
MDH-3	1	98.6	100.0	100.0	100.0	NS
	2	1.4	0.0	0.0	0.0	

1/ χ^2 test for heterogeneity significant ($P \leq 0.05$), NS non-significant ($P > 0.05$)

subtle and there is a no obvious trend in allele frequency associated with increasing population age, there appears to be little evidence indicating a relationship between specific allele frequencies and fitness, or survival, in these populations of jack pine.

CONCLUSIONS

Stochastic events alone are not responsible for the selection of trees for removal during the natural thinning that eliminated nearly 75% of the jack pine between ages 22 and 68. In fact, the selection process is decidedly non-random as evidenced by the fact that a greater proportion of individuals with high heterozygosities than those with relatively low heterozygosities survived thinning. These data generally support the balanced theory of enhanced fitness, as measured by long-term survival, with increased heterozygosity. Whether this increase in heterozygosity with increasing age is actually a result of a heterozygote advantage, or a disadvantage of homozygosity resulting from inbreeding is not discernible from data presented in this paper. This issue, along with additional data from this study, will be examined in a paper to be submitted elsewhere.

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