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Genetic Structure of *Pinus rigida* Mill. in an Expanding Population Originating from a Few Founder Trees[1]

Min Sup Chung[2]

數本의 兩親樹에 의해 傳播增殖중에 있는 리기다소나무 集團의 遺傳的 構造[1]

鄭 珉 燮[2]

ABSTRACT

Allozyme study on a small pitch pine stand originating and expanded rather rapidly from a few founder trees indicated that the colonization of the pitch pine population was made progressively from the place where the founder trees located to another by moving in cohorts of seeds from a limited number of family or genetically closely related family groups in line with the succeeding generations. This pattern of migration and colonization resulted marked differences in allelic and genotypic frequencies at many of the allozyme loci between the initially colonized subpopulation on the south-facing slope and the lately colonized subpopulation on north-facing slope of a hill.

It appeared that gene fixation due to inbreeding and genetic drift occurred at some loci in the pitch pine population or subpopulations. However, even in the inbreeding small pitch pine population or subpopulations, a comparatively large amount of genetic diversity or heterozygosity was maintained due to the high levels of gene recombination at many of the gene loci and natural selections favoring for heterozygotes.

Key words ; Allozyme variation ; inbreeding ; genetic drift ; Pinus rigida *Mill.*

要 約

처음 8본의 리기다소나무 양친수로부터 전파 증식된 리기다소나무 집단에 대한 유전변이를 AAT, GDH, LAP 등의 Allozyme에 의해 조사한 결과 다음과 같은 사실을 밝혀냈다.

표본으로 선정된 리기다소나무 집단은 산의 남쪽 낮은 지대에 처음 식재되었던 8본의 양친수로부터 종자가 무더기 무더기로 군데군데 colony를 형성하면서 동쪽, 서쪽, 북쪽으로 전파 증식되어 산지의 각 부분 부분마다 유전적으로 서로 밀접하게 관련된 소수의 家系群을 형성하였다. 이러한 형태의 移植와 colony 형성 과정에서 부분적으로 Inbreeding과 Genetic Drift 현상이 심하게 진행되고 있는 것으로 추정되었으며, 그 결과 처음 리기다소나무 colony가 형성되었던 산의 남쪽지역과 나중에 colony가 형성되었던 북쪽 지역의 소집단 사이에 상당량의 유전자 빈도 차이가 확인되었다.

[1] 接受 12月 14日 Received on December 14, 1985.
[2] 경북대학교 농과대학 College of Agriculture, Kyungpook National University, Daegu, Korea.

Inbreeding과 Genetic Drift 현상에 의해 소수 유전자座에 유전자 고정 현상이 나타났으나 기타의 유전자座에서는 유전자 Recombination이 일어났다. Gene Recombination에 의한 異型接合體의 형성과 이들의 자연도태 현상에 의해 유전적으로 서로 밀접하게 관련된 소수의 리기다소나무 집단에 있어서도 상당량의 유전적 다양성과 異型接合性이 유지되고 있었다.

INTRODUCTION

Living organisms, particularly those of outcrossing species, generally show a vast array of genetic variation. Recurrent mutations and involvement of mutated genes to the gene pool and the genetic systems that enhance genetic variability of a species usually increase and/or maintain genetic variation of the species.

In natural populations, the function of genetic system is to bring about a workable compromise between the contradictory demands for fitness and flexibility in population variability. Genetic uniformity of a population, accompanied by an effective physiological function of the genes involved, may enhance competitive function of the population,at the expense of genetic variability that endows population stability, in the given environment of a certain specific site. On the other hand, genetic diversity of a population with diversified physiological functions that shared by various individuals within the population may hamper physiological functions of the population as a whole in a way but enhance the population stability on the other. Natural population of many forest tree species are expected to have an innate genetic flexibility that enables them to live and evolve successfully under various genetic and environmental conditions. Genetic structures of such natural populations are also expected to change at various stages of their life cycle in response to various factors that affect their genetic systems.

Genetic drift is a random process that involves chance fluctuations of gene frequencies in reproductively isolated populations. It is generally believed that genetic drift occurs particularly in small populations established by a few founders provided that gene flow from neighbouring population is effectively restricted. In this process there is a tendency towards gene fixation and thus reduced hereditary variation of the population in question.

Small populations with an extremely reduced genetic variation in consequence of genetic drift may become extinct unless the individuals consisting of the populations contain some combinations of fixed genes that have become specifically adapted to their natural habitats.

Pitch pine (*Pinus rigida* Mill.) has been introduced to Korea in the beginning of this centruy(the earliest introduction was in 1907) from the U. S. A. and has been used extensively for reforestation of poor and/or erroded forest sites. Most of pitch pine populations at present are descended from a few of these originally introduced populations. In most cases, foresters in Korea care nothing either for the genetic bases or genecological background of the pitch pines they have used in producing seedlings for reforestation. Large quantities of pitch pine seedlings are usually produced with seed lots originated from a limited number of young maternal trees, and small portions of these seedlings are sent to many local places for reforestation. Pitch pine seedlings may be produced again in the same way from the local places and sent to other places from generation to generation. This kind of reproduction and population expansion process in many localities may lead to genetic drift of pitch pines in various sites.

The aim of this study was to investigate genetic structure of pitch pines in relation to genetic differentiation in an expanding population originating from a few founder trees.

MATERIALS AND METHODS

1. Seed collection

Open-pollinated seeds (from eight cones at four

different aspects of a tree crown) were collected from a pitch pine stand (of about 600-700 population size) spread over about one hectare area at elevations of 100-200 m above sea level. The pitch pine stand is located in Shinpyung-Myun, Euseong-Gun, Kyungsangbuk-Do (36° 29′ N latitude, 128° 28′ E longitude).

The forest owner established the pitch pine stand by natural reproduction originated from eigh founder trees. The founder trees were planted in early part of 1950s and cut down in early 1970s. Therefore, most of the trees in that pitch pine stand are expected to be genetically related to one another. The pitch pine stand consists mainly of young trees that are mostly less than fifteen years old and are about three to five(largest ones are about 8 m) meters in height. Initially the founder trees planted at a low part of south-facing slope of the hill. Later progenies of the founder trees appeared to colonize gradually towards the northern, eastern, and western slopes by invading exposed sites. In sample stand pitch pines are mixed with red pines (*Pinus densiflora* S. et Z.), oaks (*Quercus* spp.) and alder (*Alnus hirsuta var. tinctoria*) trees. Cones were collected from 101 and 100 pitch pine trees respectively for N- and S-facing slopes in 1982 for allozyme analysis.

2. Electrophoresis

Seeds were germinated on moist sands in petri dishes at 22°-26°C. Each macrogametophytes and developing embryos of eight seeds per tree were separated and homogenized for enzyme assay in buffer solution consisting of 0.09 M. trisaminomethane, 0.27 M. borate and 0.004 M. EDTA (pH 7.4). The solutions of crude homogenates were absorbed onto filter paper wicks (7 X 3 mm). These paper wicks that absorbed crude extracts of enzymes from macrogametophytes and the corresponding embryos of the same seeds were then inserted, side by side, into a starch gel at 2 cm distance from the cathodal end. The insertion of the paper wicks were aided by a narrow razor blade especially designed for this purpose.

Electrophoresis was carried out at constant current of 2.6 mA per one square centimeter of gel surface (cross-section) for six hours at temperatures of 3°-5°C. The dimension of a starch gel for electrophoresis was 12 cm (length, from cathodal to anodal ends) X 22 cm (width) X 7 mm (thickness).

Buffer Systems and the gel preparations are as follows;

(1) Electrode buffers.

for cathode ; H_3BO_3 0.2 M., LiOH 0.04 M. pH 8.1

for anode ; H_3BO_3 0.2 M., LiOH 0.05 M. pH 8.4

(2) Gel buffer.

Trisaminomethane	0.047 M.
Citrate	0.007 M.

(3) Gel preparation

Gel buffer	540 ml
Electrode buffer (cathodal)	16 ml
Starch (hydrolized)	64 g
Saccharose	0.5 g
Urea	0.5 g

Glutamate dehydrogenase(GDH) and leucine aminopeptidase(LAP) enzymes were stained according to the methods described by Shaw and Prassad (15) and of aspartate aminotransferase(AAT or GOT) by Siciliano and Shaw(16).

RESULTS AND DISCUSSIONS

1. Allele frequencies

Number of alleles and loci including zymograms for aspartate aminotransferase(AAT or GOT), glutamate dehydrogenase(GDH) and leucine aminopeptidase(LAP) on pitch pine has been reported in a study carried out by the same author(4). At present study additional alleles B_2 for AAT and A_3 for LAP that could not be found in the previous study were detected. Alleles B_2 for AAT and A_3 for LAP migrate slightly slower than the alleles B_1 and A_2 of the respective enzyme loci. The allele B_2 for AAT is very rare and most likely to be originated from pollen pool (paternal origin).

The alleles A_1 for GDH and A_3 for LAP are

obviously from pollen pool. The parental population(s) either of the allele A_1 for GDH or of A_3 for LAP is not known yet. It may be one or some of the following pitch pine and/or pitch-loblolly hybrid pine populations; (1) the same pitch pine population studied at present, (2) neighbouring pitch pine population(s) and neighbouring pitch-loblolly hybrid pine population(s). The allele A_1 for GDH most likely come from the nearest pitch-loblolly hybrid pine population. There is a small pitch pine stand approximately 1 Km north of the sample stand. There are some other pitch pine stands approximately 8 Km south east of the sample stand. There are some other pitch pine stands approximately 8 Km south east of the sample stand. These stands are not examined for their species identity by the author.

Expected allele frequencies at locus B for AAT in maternal group were estimated from those of the progeny group because, in many cases, the alleles at locus B for AAT in macrogametophytes were silent. Allele frequencies for AAT, GDH and LAP loci of the sample stand are presented in Table 1. Allele frequencies of the same enzyme systems for pitch pines in a pitch-loblolly hybrid seed orchard (4) are also included in Table 1 (last two rows) for comparison.

As it was found in the previous work by the same author(4), all the investigated allozyme patterns except GDH showed polymorphisms in pitch pine. Polymorphic genes usually exist in more than two allelic forms. However, the definition of polymorphism is arbitrary (7, 10). A locus is polymorphic if the frequency of commonest allele is equal to or less than 1-q. The value for q may either be 0.01 or 0.05 (2, 19, 20). In the present work 0.01 is adopted for q.

Test of allele frequencies for their differences by Chi-square contingency Tables indicated that gametic segregations in parental population were normal, with a few exceptions, at most of the loci examined. Segregation of alleles at locus A for AAT in macrogametophytes of pitch pine subpopulation on south-facing slope deviated significantly from the expected Mendelian segregation ratio. Lower level of allele A_1 for AAT was observed than that of the expected one ($\chi^2 = 4.74 > p_{0.05} = 3.84$, df = 1). When the allele frequencies of the subpopulations on N-facing and S-facing slopes combined into one, the same tendency was found for the allele

Table 1. Allele frequencies for AAT, GDH and LAP in the maternal (macrogametophytes), paternal (pollen) and the progeny (embryo) populations or subpopulations of the north-facing(N) and south-facing (S) slopes. Allele frequencies of the same enzyme systems for pitch pines in an F_1-hybrid seed orchard are presented in the last two rows.

Source		Allele frequency																			
		AAT										GDH			LAP						
		A_1	A_2	A_3	B_1	B_2	B_3	B_4	C_1	C_2		A_1	A_2		A_0	A_1	A_2	A_3	B_1	B_2	B_3
Maternal	N	0.08	0.92	–	0.36	–	0.64	0.00	0.04	0.96		–	1.00		0.14	0.83	0.03	–	0.32	0.64	0.04
	S	0.30	0.70	–	0.23	–	0.77	–	0.01	0.99		–	1.00		0.23	0.77	0.00	–	0.52	0.48	–
	Total	0.19	0.81	–	0.29	–	0.71	0.00	0.02	0.98		–	1.00		0.19	0.79	0.02	–	0.42	0.56	0.02
Paternal	N	0.08	0.91	0.01	*				0.03	0.97		0.00	1.00		0.03	0.93	0.04	–	0.28	0.66	0.06
	S	0.17	0.83	0.00					0.02	0.98		0.00	1.00		0.03	0.95	0.02	0.00	0.50	0.50	–
	Total	0.12	0.87	0.01					0.02	0.98		0.00	1.00		0.03	0.94	0.03	0.00	0.39	0.58	0.03
Progeny	N	0.07	0.93	0.00	0.36	0.00	0.64	0.00	0.03	0.97		0.00	1.00		0.09	0.88	0.03	–	0.29	0.66	0.05
	S	0.21	0.79	0.00	0.23	–	0.77	0.00	0.01	0.99		0.00	1.00		0.12	0.86	0.01	0.01	0.53	0.47	–
	Total	0.14	0.86	0.00	0.29	0.00	0.71	0.00	0.02	0.98		0.00	1.00		0.11	0.87	0.02	0.00	0.41	0.57	0.02
Pitch loblolly F_1-hybrid seed orchard																					
Maternal		0.02	0.96	0.02	0.49	–	0.51	–	0.04	0.96		–	1.00		0.09	0.87	0.04	–	0.46	0.52	0.02
Progeny		0.02	0.96	0.02	0.49	–	0.50	0.01	0.06	0.94		0.00	1.00		0.06	0.88	0.06	–	0.45	0.53	0.02

* Alleles originating from pollen could not be identified

A_1 at AAT locus ($\chi^2 = 5.63 > p_{0.05} = 3.84$, df = 1). No such tendency as described above was found in the subpopulation of N-facing slope. Thus it appears that the rare allele A_1 for AAT was selected against during gametogenesis in the subpopulation on S-facing slope. Rudin(12), Rudin and Ekeberg(13) reported differential segregation of gametes at some loci of AAT allozyme in *Pinus sylvestris* L.

There were substantial differences in allele frequencies at many loci between tree groups or allele sources(Tables 2, 3, 4, and 5). In spite of gametic selection against the allele A_1 for AAT in the subpopulation on S-facing slope, frequency of the same allele was much higher in the subpopulation on S-facing slope than in the subpopulation on N-facing slope in parental population (Tables 1 and 2). Frequency of the same allele A_1 for AAT reduced drastically from maternal (0.30) to progeny (0.21) populations (Table 1). From these results, it is inferred that the initial frequency of the allele A_1 for AAT in the founder trees which had been planted on the S-facing slope might have been much higher than that of the present level. As the population expands rather rapidly, frequency of the same allele decreased in line with the population increase probably due to gametic selection in S-facing slope. Selections during pollination-fertilization and embryogenesis (including competitive ability of the allele during polyembryo development in a seed) may also play an important role in changing allele frequencies(14). Data on zygotic selection for this sample stand was not available at present work. It is also suspected that only a small portion of the seeds that contain allele A_1 for AAT migrated to the north-facing slope of the sample stand.

Trees of the upper S-facing slope and N-facing slope are migrants originated from the initial founders. The migrants may consist of the first, second and/or even third generations of the founder trees. Pitch pines can produce a considerable amount of seeds at the age of seven to eight under open canopy condition, particularly on the exposed sites where incident solar radiation is abundant. From the allozyme study on the pitch pine sample stand, it appeared that colonization of pitch pines on the upper S-facing slope and N-facing slope was made progressively from the south to the north by moving in cohorts of seeds originated from a limited number of family or genetically closely related family groups and by occupying small spots of exposed sites. Such pioneer tree family or families are expected to be characterized by fecundity of seeds and/or by early incipiency of reproduction. The frequent occurrence of rare alleles in groups of trees at certain spots along transects from the south to the northern direction obviously supports this pattern of migration.

This kind of discriminative gene flows from the south to the north and the following regenerations of the related trees in the small patches might have resulted marked differences in allele frequencies at many of the investigated loci between the two subpopulations located on N-facing and S-facing slopes (cf. Tables 2 and 3). Under such pattern of migration and colonization change of genic and genotypic frequencies in the colonizing small population is indispensable. Tigerstedt and his coworkers (18) suggested that seeds in natural Scots pine stands move in and land in cohorts and thus mixing up the population structure. Significant variation of gene

Table 2. Differences in allele frequencies between subpopulations on the north-facing(N) and south-facing(S) slopes of maternal population

Enzymes	AAT						LAP					
Alleles	A_1	A_2	B_1	B_3	C_1	C_2	A_0	A_1	A_2	B_1	B_2	B_3
Difference	*** N<S	*** N>S	*** N>S	*** N<S	*** N>S	*** N<S	*** N<S	*** N>S	*** N>S	*** N<S	*** N>S	*** N>S

Calculated by 2 x 2 or 2 x n contingency tables.
Significance at probability of *: 0.05, **: 0.01, ***: 0.005

Table 3. Differences in allele frequencies between subpopulations on the N-facing(N) and S-facing(S) slopes of progeny(embryo) population

Enzymes	AAT						LAP				
Alleles	A_1	A_2	B_1	B_3	C_1	C_2	A_0	A_1	A_2	B_1	B_2
Difference	*** N<S	*** N>S	*** N>S	*** N<S	*** N>S	*** N<S	*** N<S	*** N>S	*** N>S	*** N<S	*** N>S

Designation the same as in Table 2

frequencies that associated with slopes of different aspects was reported on ponderosa pines spread over distances of several hundred meters on a mountain (8).

When the allele frequencies of subpopulations on the N-facing and S-facing slopes are combined into one, the frequencies of alleles A_1 for AAT and A_0 for LAP were much lower in progeny(embryo) than in maternal populations (Table 4).

Table 4. Differences in allele frequencies between parental(M) and progeny(F) populations (subpopulations on the N- and S-facing slopes combined).

Enzymes	AAT		LAP	
Alleles	A_1	A_2	A_0	A_1
Difference	*** M>F	*** M<F	*** M>F	*** M<F

Designation the same as in Table 2.

Also in parental population there were differential segregation of male gametes at some loci. The observed allele frequencies for male gametes (pollen) of A_1 ($\chi^2 = 43.18 > p_{0.005} = 10.60$, df = 2), C_2 ($\chi^2 = 4.82 > p_{0.05} = 3.84$, df = 1) alleles for AAT, A_0 ($\chi^2 = 154.74 > p_{0.005} = 12.84$, df = 3) for LAP in subpopulation on S-facing slope and allele A_0 ($\chi^2 = 62.57 > p_{0.005} = 10.60$, df = 2) for LAP in subpopulation on N-facing slope were significantly lower than the expected Mendelian segregation ratio of the corresponding maternal ones (Table 5). When all alleles of the two subpopulations on N-facing and S-facing slopes were combined together into one, the observed allele frequencies of A_1 ($\chi^2 = 34.24 > p_{0.005} = 10.60$, df = 2) for AAT and of A_0 ($\chi^2 = 214.34 > p_{0.005} = 12.84$, df = 3) for LAP were much lower than the expected segregation ratios (Table 5). The allele A_1 of male gametes for AAT in subpopulation on S-facing slope was selected against during the stages of male gametogenesis, pollination-fertilization and embryogenesis. On the other hand, the allele C_1 of male gametes for AAT in subpopulation on S-facing slope was selected for during the stages of development described above. An influent gene flow of alien allele C_1 for AAT to this subpopulation may have changed the allele frequency at this locus. However, considering distant locations of the same and/or reproductively related species, the extent of gene influx may not have been so great as to change the allele frequency at the locus significantly.

Deviation of observed paternal allele frequencies from those of maternal ones for LAP locus A(Table 5) appeared to be caused by the silent(null) allele A_0 which was usually masked in zymograms under heterozygous state by functional alleles. Many of the silent A_0 alleles that were masked under heterozygous states might have not been detected as an allele A_0 and thus it might have given a lower frequency for the allele than that of the actual one in embryos. In embryos the silent allele A_0 for LAP originating from male gametes can surely be detected only when the allele is homozygous state for the embryo. Detailed analysis of the allelic (maternal and paternal) and genotypic (expected and observed) frequencies for this locus indicated that gametic segregation of the allele A_0 for LAP did not deviate significantly from that of the Mendelian segregation ratio in subpopulation on N-facing slope and also in the combined group of the two subpopulations. However, gametic segregation of the same allele in subpopulation on

S-facing slope deviated significantly from the expected ratio ($\chi^2 = 5.34 > p_{0.05} = 3.84$, df = 1). This fact indicates that there was a selection against this allele during one or some of the stages of gametogenesis, pollination-fertilization and embryogenesis in subpopulation on S-facing slope. It is not yet known whether the silent allele A_0 for LAP has a selective disadvantage on a relatively (compared to N-facing slope) xeric environments of the S-facing slope. Sexually asymmetric fertility selection discussed by Müller-Starck(9) may also play an important role for the change of allele frequencies in a small tree population.

Comparison of the allele frequencies between pitch pine populations investigated at present (subpopulations on N- and S-facing slopes combined) with the previous work(pitch pines in an F_1-hybrid seed orchard) shows significant differences in allele frequencies at one half of the total investigated loci ($\chi^2 = 98.80 > p_{0.005} = 10.60$, df = 2, $\chi^2 = 57.00 > p_{0.005} = 10.60$, df = 2, and $\chi^2 = 26.64 > p_{0.005} = 10.60$, df = 2) for respective loci of AAT A, B and LAP A. (refer to Table 1).

Table 5. Differences in allele frequencies between Maternal (macrogametophyte, M) and Paternal (pollen, P) groups.

Sources	S-facing slope							N-facing slope		N- and S-subpopulations combined				
Enzymes	AAT				LAP			LAP		AAT		LAP		
Alleles	A_1	A_2	C_1	C_2	A_0	A_1	A_2	A_0	A_1	A_1	A_2	A_0	A_1	A_2
Difference	*** M > P	*** M < P	*** M < P	*** M > P	*** M > P	*** M < P	*** M < P	*** M > P	*** M < P	*** M > P	*** M < P	*** M > P	*** M < P	*** M < P

Designation the same as in Table 2.

Considering the planted years of the pitch pine populations, it is quite certain that the two investigated pitch pine populations are derived from an initially introduced pitch pine population in early 1900s. In this case the two pitch pine populations appeared to have differentiated genetically and have formed different population structures at two different localities within a relatively short period of time span. If this is true, the rapid change of gene frequencies in the tree populations derived from the same ancestral population should not be overlooked in silvicultural and forest genetics point of views.

Further comparative study on relatively large pitch pine population is required to elucidate the extent of genetic differentiation in different pitch pine populations because the sample size at present work, particularly the initial effective population size of the founders was very small.

2. Genotype frequencies.

The genotype frequencies of the parent(maternal) and progeny groups are presented in Table 6. There were statistical differences in genotype frequencies between the tree groups at some loci for AAT and LAP allozymes(Tables 7 and 8). The difference in genotype frequency between the two parental subpopulations on N- and S-facing slopes appeared due partly to changes in allele frequencies during the migration and colonization the pattern of which was described in the previous section. There were substantial differences in genotype frequencies between the parental and progeny (embryo) groups. In general, much more homozygous genotypes were found in progeny groups than those of the parental counterparts where natural selections encountered by external environmental factors were much less than those of the parental groups (Table 8). This fact indicates that viability selection against homozygotes at early stages of stand development was considerable.

A detailed investigation on individual tree level also showed that less than 5% and 3% of trees in the respective parental subpopulations on N- and S-facing slopes were homozygous all over the six investigated loci. On the other hand, 31% and 21% of the respective progeny subpopulations (embryos) from N- and S-facing slopes were homozygous all

over the six enzyme loci. The proportions of homozygotes between the progeny subpopulations from N- and S-facing slopes ($\chi^2 = 11.02 > p_{0.005} = 7.88$, df = 1) and between the parent and progeny ($\chi^2 = 34.76 > p_{0.005} = 7.88$, df = 1) groups were different significantly.

Chi-square contingency analysis for Hardy-Weinberg equilibrium indicated that genotype frequencies at locus A for AAT in parental subpopulation on S-facing slope were not under equilibrium. This locus has a higher number of heterozygote A_1A_2 than that of the expected under Hardy-Weinberg equilibrium ($\chi^2 = 6.99 > p_{0.05} = 5.99$, df = 2). All the other loci in parental population (subpopulations on N- and S-facing slopes combined) or in subpopulations on N- and S-facing slo-

Table 6. Genotype frequencies in the subpopulations on the N-facing(N) and S-facing(S) slopes of the parental(M) and progeny(F) groups.

Enzyme		n	AAT														GDH
Genotype			A_1A_1	A_1A_2	A_2A_2	A_2A_3	B_1B_1	B_1B_2	B_1B_3	B_1B_4	B_2B_4	B_3B_3	B_3B_4	C_1C_1	C_1C_2	C_2C_2	A_1A_2
M	N	101	–	0.16	0.84	–	0.13		0.46	–	–	0.41	–	–	0.09	0.91	–
	S	100	0.02	0.56	0.42		0.05	–	0.35	–	–	0.60	–	–	0.01	0.99	–
F	N	806	0.00	0.14	0.85	0.01	0.17	0.00	0.38	0.00	0.00	0.45	0.00	0.00	0.06	0.94	0.00
	S	803	0.03	0.35	0.61	0.01	0.06	–	0.33	0.00	–	0.61	0.00	–	0.02	0.98	0.00
Total	M	201	0.01	0.36	0.63	–	0.09	–	0.41	–	–	0.50	–	–	0.05	0.95	–
	F	1609	0.02	0.24	0.73	0.01	0.11	0.00	0.36	0.00	0.00	0.53	0.00	0.00	0.04	0.96	0.00

Enzyme		GDH	LAP														
Genotype		A_2A_2	A_0A_0	A_0A_1	A_0A_2	A_0A_3	A_1A_1	A_1A_2	A_1A_3	A_2A_2	A_3A_3	B_1B_1	B_1B_2	B_1B_3	B_2B_2	B_2B_3	B_3B_3
M	N	1.00	0.02	0.28	–	–	0.64	0.06	–	–	–	0.13	0.38	0.01	0.41	0.07	–
	S	1.00	0.02	0.42	–	–	0.55	0.01	–	–	–	0.21	0.61	–	0.18	–	–
F	N	1.00	0.03	0.11	0.00	0.00	0.82	0.02	–	0.02	–	0.23	0.13	0.01	0.55	0.08	0.00
	S	1.00	0.03	0.19	–	0.00	0.76	0.02	0.00	–	0.00	0.39	0.27	–	0.34	–	–
Total	M	1.00	0.02	0.35	–	–	0.60	0.03	–	–	–	0.17	0.49	0.01	0.30	0.03	–
	F	1.00	0.03	0.15	0.00	0.00	0.79	0.02	0.00	0.01	0.00	0.31	0.20	0.01	0.44	0.04	0.00

Table 7. Differences in genotype frequencies between subpopulations on the N-facing(N) and S-facing(S) slopes.

Enzyme	AAT							LAP				
Genotype	A_1A_2	A_2A_2	B_1B_1	B_1B_3	B_3B_3	C_1C_2	C_2C_2	A_0A_1	B_1B_1	B_1B_2	B_2B_2	B_2B_3
Difference	*** N < S	*** N > S	* N > S	* N > S	* N < S	** N > S	** N < S	* N < S	*** N < S	*** N < S	*** N > S	*** N > S

Designation the same as in Table 2.

Table 8. Differences in genotype frequencies between the parental(M) and progeny(F) groups.

Source	N-Slope subpopulation					S-slope subpopulation			
Enzyme	LAP					AAT		LAP	
Genotype	A_0A_1	A_1A_1	B_1B_1	B_1B_2	B_2B_2	A_1A_2	A_2A_2	A_0A_1	A_1A_1
Difference	*** M > F	*** M < F	*** M < F	*** M > F	*** M < F	*** M > F	*** M < F	*** M < F	*** M > F

Source	S-slope subpopulation			N and S combined						
Enzyme	LAP			AAT		LAP				
Genotype	B_1B_1	B_1B_2	B_2B_2	A_1A_2	A_2A_2	A_0A_1	A_1A_1	B_1B_1	B_1B_2	B_2B_2
Difference	*** M < F	*** M > F	*** M < F	*** M > F	*** M < F	*** M > F	*** M < F	*** M < F	*** M > F	*** M < F

Designation the same as in Table 2

pes were under Hardy-Weinberg equilibrium. However, this fact does not necessarily imply that the allelic and genotypic frequencies at most of the loci reached equilibrium by panmictic matings in proportion to the initial allelic frequencies of parental population or of the subpopulations.

Genotypic frequencies at many loci of the progeny population (subpopulations on N- and S-facing slopes combined) or subpopulations deviated significantly from the expected frequencies under Hardy- Weinberg equilibrium states (Table 9). From this observation it is obvious that an excess amount of homozygotes was produced during the reproductive phases up to seed stage in this small pitch pine stand. However, a large part of the excessive homozygotes was eliminated during the early stages of stand development by the selective forces against these homozygotes. Yazdani and his coworkers(20) found a similar result that the author obtained at the present work in their isozyme study on mature, young and embryo populations of Scots pines in a seed tree stand. Gene flow and natural selection favoring for heterozygotes was discussed by various authors(3, 17).

It is not yet clear how the two counteracting forces, one for producing excessive amounts of homozygotes at early stages of reproductive phase up to seed stage and the other for eliminating the excessive homozygotes at early stages of stand development, render the population or subpopula-

Table 9. Deviations of observed(O) genotype frequencies from the expected(E) under Hardy-Weinberg equilibrium.

Source	N-slope subpopulation										S-slope
Enzyme	AAT		LAP								LAP
Genotype	B_1B_1	B_1B_3	A_0A_0	A_0A_1	A_1A_2	A_2A_2	B_1B_1	B_1B_2	B_1B_3	B_2B_2	B_1B_1
Difference	* E < O	* E > O	*** E < O	*** E > O	*** E > O	*** E < O	*** E < O	*** E > O	*** E > O	*** E < O	*** E < O

Source	S-slope subpopulation		Subpopulations N- and S-slope combined								
Enzyme	LAP		AAT		LAP						
Genotype	B_1B_2	B_2B_2	B_1B_1	B_1B_3	A_0A_0	A_0A_1	A_2A_2	B_1B_1	B_1B_2	B_1B_3	B_2B_2
Difference	*** E > O	*** E < O	* E < O	* E > O	*** E < O	*** E > O	*** E < O	*** E < O	*** E > O	*** E > O	*** E < O

Designation the same as in Table 2.

tions to reach allelic and genotypic equilibrium states at most of the investigated loci in this pitch pine population.

3. Heterozygosity and fixation index

Average number of alleles examined over six loci were 2.33 for maternal and 3.00 for the progeny (embryo) populations. Values for average heterozygosity(10, 11) and genetic diversity(6) on different tree groups are presented in Table 10. The same parameters on pitch pines studied by the same author in the previous work(4) were also presented in Table 10 for comparison.

There were no statistical differences among those parameters that stand for average heterozygosities of different tree groups. It appears that average heterozygosity does not change drastically, as it is expected, even in small inbreeding pitch pine popu-

Table 10. Average heterozygosity(H) and genetic diversity(V_p) of the parental(M) and progeny(F) groups computed over six enzyme loci.

Source		n	H	$V_{(p)}$	Remarks
M	N	101	0.247	5,624	
	S	100	0.273	7,362	at present work
N & S Combined		201	0.269	6,913	
F	N	806	0.227	4,661	
	S	803	0.233	5,349	at present work
N & S Combined		1609	0.241	5,330	
M		49	0.235	5,409	in F_1-hybrid
F		784	0.238	5,569	seed orchard

N; Subpopulation on the N-facing slope.
S; Subpopulation on the S-facing slope.

Table 11. Fixation indices for subpopulations on N-facing(N) and S-facing(S) slopes of the parental(M) and progeny(F) groups.

Enzyme		AAT			GDH	LAP	
Locus		A	B	C	A	A	B
M	N	-0.067	--	0.000	0.000	-0.172	0.080
	S	-0.333	--	0.000	0.000	-0.194	-0.220
	Total	-0.161	--	0.000	0.000	-0.167	-0.039
F	N	-0.009	0.163	-0.154	0.000	0.353	0.543
	S	-0.055	0.053	0.000	0.000	0.119	0.462
	Total	-0.003	-0.133	0.047	0.000	0.226	0.524

lations or subpopulations within a few generations. It also appears that gene fixation does occur in small inbreeding populations not at most of the loci but at a limited number of loci. Fixation indices(5) in Table 11 explain a relatively strong gene fixation at loci A and B for LAP in progeny subpopulations. Fixation indices for many of the loci in maternal and for a few loci of the progeny groups were negative. This fact indicates that gene fixation at some loci and gene recombination at the other loci occurs simultaneously at population level even in small inbreeding populations. Viability selection favoring for heterozygous individuals at some or many of the loci during the early stages of stand development gradually augments the level of heterozygosity. In this way tree populations appear to maintain their genetic diversity from generation to generation. This fact may give a partial answer for "heterozygosity paradox" discussed by Brown(1).

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