SILVICULTURAL MANAGEMENT AND THE MANIPULATION OF RARE ALLELES

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ABSTRACT

Because rare alleles provide a means for adaptation to environmental change they are often considered important to long-term forest health. Through the selective removal of trees (and genes), silvicultural management may alter the genetic structure of forests, with rare alleles perhaps being uniquely vulnerable to manipulation due to their low frequencies or associations between rare alleles and harvest selection criteria. We tested this possibility in two ways. First we evaluated the influence of long-term management on the genetics of an eastern hemlock (*Tsuga canadensis* (L.) Carr.) forest within the Penobscot Experimental Forest in Maine. Plots received one of three treatments: 1) a selection cut in which small and poor form trees were preferentially removed in 1957 and 1977, 2) a diameter limit cut in which the largest trees in each diameter class were removed in 1952, 1973 and 1994, and 3) an unmanaged control. Results from isozyme analysis indicated that the number of rare alleles decreased in the selection cut relative to the control. In contrast, rare alleles increased in frequency within diameter limit plots as poor quality trees were preferentially retained. As a separate test, we conducted simulated computer-based harvests within a genetically mapped forest that included isozyme data for 220 eastern white pines (*Pinus strobus* L.) growing in central Vermont. Most harvest scenarios had no discernable impact on stand genetics. However, due to an unequal distribution of rare alleles among tree age and crown classes, harvests that used these parameters as selection criteria altered the frequency of rare alleles relative to random harvests of equal intensity. Similar to the hemlock study, rare alleles either increased or decreased in frequency depending on the selection criteria used. The loss of rare alleles could diminish the potential of populations to successfully adapt to and survive ongoing environmental change, whereas an increase in rare alleles could reduce current stand fitness.

INTRODUCTION

Genetic diversity is essential to the long-term health and survival of biological populations because this diversity is critical for the physiological plasticity and adaptation needed to survive natural and man-made environmental change. This is particularly true for forest trees, which have limited mobility and are likely to encounter environmental change throughout their long life spans. Considering the fundamental importance of genetic diversity to the continued adaptation, health, and long-term productivity of tree populations, a loss of genetic diversity (whatever the cause) can be a serious threat to forest ecosystems and the many products and services they provide. This generalized threat is particularly serious today as a result of escalating human activity which has not only initiated pervasive and sometimes dramatic changes in the earth's environment but has also led to measurable declines in the genetic diversity of at least some tree populations. Although many human influences may work to reduce the genetic diversity within forests stands (e.g., land conversion and forest fragmentation, pollution exposure, climate change, etc.), another factor that could alter the gene pools of woodland ecosystems is intensive forest management.

Forest management represents an anthropogenic force in which sometimes large numbers of trees (and the genes they contain) can be removed from a natural system within a relatively short period of

time. Although probably not consequential to species that vegetatively reproduce, such removals could be important to species reliant upon sexual reproduction. Certainly, tree harvests are critical to supply human resource needs. Indeed, an important element of silvicultural selection is the belief that through the prudent removal of select trees, favorable commercial attributes (e.g., fast growth, good form, etc.) can be fostered in both residual trees (through an improvement of growing conditions) and their progeny (through a manipulation of the gene pool). Still, the long-term impact of tree removal on the genetic base and ecological resiliency of forests is largely unknown.

Can timber harvesting alter forest gene pools and, as a result, the long-term health and productivity of forest communities? Do various types or intensities of harvest differentially impact the gene pool? How might these potential impacts differ among species? The few studies that have examined these questions have provided a mixed indication of the influence of timber harvesting on genetic resources. For example, Neale (1985) and Neale and Adams (1985) found that shelterwood harvesting had little impact on the genetic structure of Douglas-fir (*Pseudotsuga menziesii* Carr.) forests. However, Cheliak et al. (1988) reported that phenotypically selected white spruce (*Picea glauca* (Moench) Voss) possessed on average only 75% of the genes found among randomly selected trees from the same population, indicating that phenotypic selection resulted in gene loss. Indeed, recent data indicates that rare alleles may be particularly prone to loss following silvicultural selection (Buchert et al. 1997; Adams et al. 1998).

We took two approaches toward evaluating the influence of silvicultural selection on the presence and frequency of rare alleles within forest stands. First we assessed the effect of long-term management on the genetics of an eastern hemlock (*Tsuga canadensis* (L.) Carr.) forest within the Penobscot Experimental Forest in Maine. As an additional test, we conducted simulated computer-based harvests within a genetically mapped forest that included isozyme data for 220 eastern white pines (*Pinus strobus* L.) growing in central Vermont. Here we discuss the results of these studies and highlight potential implications of results for short- and long-term forest management and health.

METHODS

Sites

Assessments of the genetics of eastern hemlock were conducted in a long-term silvicultural experiment at the Penobscot Experimental Forest in east-central Maine, USA. This experiment was established in 1952 and included a no-harvest control and two silvicultural treatments: 1) a fixed diameter limit treatment, and 2) a selection treatment. The fixed diameter limit treatment involved a ten-hectare stand that had three harvests: June 1952, May 1973, and January 1994. At each harvest, all merchantable hemlocks with DBH $\geq$ 24 cm were cut, resulting in approximately one-fifth to one-half of all hemlock trees being removed per harvest. The selection treatment was conducted on an eight-hectare stand that had two harvests: May 1957 and January 1977. Harvesting criteria were effectively opposite those for the diameter limit cut: per diameter class, phenotypically superior trees were retained, while smaller, unmerchantable, and poor-risk trees were preferentially removed. Nearly one-third of the eastern hemlock trees of poor merchantability were removed in 1957 and an additional 75% were removed in 1977.

For the simulated harvesting study, the study site was an approximately ten-hectare stand in Jericho, Vermont, USA, dominated by mature eastern white pine. The genetically mapped stand was constructed by first gathering inventory data, including size, age, growth, vigor, crown class, and spatial location for all 232 eastern white pine trees in the forest. These data were combined with genetic data determined from starch gel electrophoresis in a GIS framework to produce a virtual reconstruction of the actual stand's spatial, physical, and genetic components (Nijensohn et al. in review).

Genetic assessments

Eastern hemlock: Seeds were collected in the fall of 1994 from 40 to 50 randomly selected residual eastern hemlock trees from each treatment stand. Starch-gel electrophoresis of megagametophyte tissue was used to assess the genetics of individual trees. A minimum of seven haploid megagametophytes from each tree were used in genetic analyses. A total of 106 trees were examined at 27 gene loci representing 19 enzyme systems. Electrophoretic gel buffers and enzyme recipes were prepared according to procedures described by Jech and Wheeler (1984) and Cheliak and Pitel (1984), including appropriate modifications made in our laboratory. Seeds from individuals of known genotype that have been shown to be highly homozygous were included as standards in each electrophoretic run.

Eastern white pine: Cones were collected from individual trees in the fall of 2000, when almost all trees (220 of 232 trees) produced seeds, allowing for a nearly complete assay of stand genetics. Using the methods described above, starch gel electrophoresis was used to resolve 46 loci (26 of which exhibited polymorphisms) from 19 enzyme systems.

Statistical analyses

Multi-locus genotypes were determined for each tree. Although numerous genetic parameters were assessed, the focus of this manuscript is the effect of actual or simulated harvests on the presence of rare (frequency $P < 0.05$) alleles. Uncommon (frequency $0.25 > P > 0.05$) alleles were also assessed for the study involving eastern hemlock. Here, the lack of replication limited data analysis to assessments of trends among treatment means. For the simulated harvest of eastern white pine, analyses of variance and Tukey-Kramer HSDs were used to test for significant differences in the number of rare alleles among age and crown classes. Harvesting scenarios were developed using phenotypic characteristics (e.g., tree age, DBH, and crown class) as silvicultural selection criteria at various harvesting intensities (e.g., simulating the removal of different proportions of the original stand). Monte Carlo simulations were used to compare the genetics of residual stands following harvest simulations to repeated random harvests of equal intensities (1000 resampling iterations) (Simon 2002).

RESULTS

Hemlock silvicultural trial

Compared to the control stand, the repeated removal of small, poor-formed trees in the selection cut was associated with fewer rare alleles, five alleles no longer detected, and a reduction in the estimated number of the genotypes that could arise from the stand given random mating (i.e., genetic multiplicity; Bergman et al. 1990).

In the diameter limit cut, an apparent loss of rare alleles actually resulted from an increase in the frequency of some alleles that were rare (occurred in < 5% of individuals) in the control, but now occurred at a higher frequency in the treated stand (i.e., were now low frequency alleles that occurred in up to 25% of individuals; Table 2). Even though a fewer total number of alleles were considered rare, the percentage of trees with rare alleles was about double for the diameter limit cut relative to the control. Indeed, compared to the control stand where no trees were homozygous for rare alleles, a remarkable 42% of trees in the diameter limit were homozygous for some rare allele. The concentration of unusual gene forms (rare or low frequency alleles, often in a homozygous form) resulted in a ten-fold increase in the estimated number of genotypes potentially generated by the diameter limit stand compared to the control.

Table 1. The influence of silvicultural selection on the presence and frequency of rare alleles in mature eastern hemlock stands in eastern Maine. Data based on Hawley et al. (in review).

Stand parameter	Treatments		
	Control	Diameter limit	Selection
# sample trees	35	31	40
# rare alleles	9	5	4
# low frequency alleles	2	8	4
% trees with rare alleles	29	68	34
% trees homozygous for rare alleles	0	42	5
Lost alleles relative to control	---	2	5
Genetic multiplicity (millions)	1.26	11.34	0.1

Genetically mapped eastern white pine stand

An important objective in creating this genetically mapped white pine stand was to assess patterns of subpopulation structuring. Indeed, multiple levels of structuring were detected. For example, spatial structuring was evident: trees within 5 m of one another were highly related, and levels of relatedness generally decreased with increasing distance between trees (Nijensohn et al. in review). Temporal structuring (a generation gap) was also evident: trees of similar age showed significant positive relatedness, as did trees 30-40 years apart (Nijensohn et al. in review). In addition, trees in suppressed crown classes exhibited a high concentration of rare alleles, a trend also evident for older age classes.

To test the potential influence of tree removal on stand genetics, a series of computer-based simulated harvests were conducted on this genetically mapped stand. Most harvest scenarios did not significantly alter genetic parameters relative to random harvests of equal intensity. However, due to their association with rare allele frequency, harvests that targeted tree age and crown classes were an exception to this trend (Table 2). For example, when 50% of the stand's oldest trees were preferentially removed, there was a significant reduction in the number of rare alleles relative to random harvests of equal intensity. If wolf trees (large, knot-ridden trees usually avoided in commercial harvests) were excluded from harvest, a significant reduction in the number of rare alleles occurred at even lower harvest intensities (e.g., removal of 43% of the oldest trees). In contrast, selective retention of suppressed trees had the opposite effect, i.e. a significant increase in the number of rare alleles relative to random harvests of equal intensity (Table 2). Similar to results from actual harvests in the eastern hemlock stands (Table 1), results from simulated harvests indicate that silvicultural selection can alter the frequency of rare alleles within forests, and that depending on the criteria used, tree removals can either increase or decrease the presence of rare alleles within a stand.

DISCUSSION

Our results are consistent with recent findings showing that the presence and frequency of rare alleles can be altered by silvicultural selection (Buchert et al. 1997; Adams et al. 1998). Because they already occur at low frequencies, rare alleles are particularly vulnerable to loss. But what are the implications of these alterations to forest productivity and health?

It is possible that rare alleles may represent localized adaptations beneficial to specific locations in space and time. However, evidence suggests that rare alleles are uncommon at the population level for a reason: they have been selected against because they impart some generalized competitive disadvantage. Numerous studies have shown that rare alleles, in both heterozygous and homozygous forms, are associated with weaker phenotypes (e.g., Bergman and Sholz 1987; Cheliak et al. 1988; Bush and Smouse 1991; Adams et al. 1998). As a recent example of this, Zhong et al. (2001) found that Douglas-firs that were susceptible to defoliation by the western spruce budworm (*Choristoneura occidentalis* Freeman) had a higher proportion of uncommon and rare alleles than resistant trees. It was postulated that these alleles remained rare because they were associated with reduced fitness (greater defoliation) (Zhong et al. 2001). In another study, Bongarten et al. (1985) reported a negative correlation between growth rate and heterozygosity for rare alleles.

Table 2. Changes in the number of rare alleles following simulated harvests in the genetically mapped eastern white pine forest in Jericho, Vermont. Monte Carlo simulations were used to detect significant changes (* denotes $0.05 \leq P \leq 0.10$; **denotes $P \leq 0.05$) in the number of rare alleles compared to 1000 random harvests of equal intensity. Data based on Nijensohn et al. (in review).

Trees removed in simulated harvest	Residual n	Mean # rare alleles per tree	Significant Δ rare alleles
oldest 25%	165	0.99	
oldest 50%	110	0.81	decrease*
oldest 75%	55	0.80	
youngest 25%	165	1.10	
youngest 50%	110	1.14	
youngest 75%	55	0.93	
oldest but leave:			
18% wolfs	181	0.96	
43% wolfs	126	0.79	decrease**
68% wolfs	71	0.76	decrease*
understory (U)	218	0.97	
all but Dominant (D)	62	0.82	
all but Codominant (C)	115	0.94	
all but Intermediate (I)	36	1.17	
all but Suppressed (S)	5	1.80	increase*
D & C	43	1.28	
D, C & I	7	1.86	increase*
all but D & C	177	0.90	
Original population	220	0.97	

Because of an apparent association between rare alleles and the negative phenotypes (small size, poor form, etc.) preferentially retained, the diameter limit treatment resulted in a residual stand in which rare alleles become unusually common (Table 1). By repeatedly removing larger competitors, gene forms that were rare (presumably because they imparted reduced fitness) following natural selection in the control plot were allowed to accumulate in the diameter limit stand. This concentration of marginally

fit individuals was associated with reduced woody biomass accumulation relative to other treatments (Hawley et al. in review), which may reflect a reduction in stand fitness under prevailing conditions.

Although they likely impart a fitness disadvantage under current conditions, rare alleles are also assumed to be an important reservoir of adaptive potential for populations. Rare alleles have been important to the survival of a range of biological populations due to the development of resistance following exposure to a variety of selective forces (Table 3). The development of antibiotic, insecticide, and herbicide resistance is a headline-grabbing example of this. Yet, evidence of the importance of rare alleles for the long-term survival of forest trees presented with novel selection pressures also exists. Dutch elm disease (caused by the fungus *Ophiostoma ulmi* (Buisman) Nannfeldt) provides a prominent case in point. Through one outbreak in North America and two in Europe, the majority of elms (e.g., *Ulmus americana* L. and *Ulmus glabra* Huds.) were killed within only a few decades following the accidental introduction of this pathogen native to Asia (Tainter and Baker 1996). Similarly, chestnut blight (caused by the fungus *Cryphonectria parasitica* (Murrill) Barr.) virtually eliminated the American chestnut (*Castanea dentate* (Marsh.) Borkh.) throughout its range within 50 years (Tainter and Baker 1996). Even though disease-induced population (and presumably gene) losses were extreme, rare alleles may yet form the basis of species survival. Rare genotypes resistant to both chestnut blight and Dutch elm disease have been identified and are considered critical to species recovery efforts (Cheng et al. 1997; Griffin 2000).

Examples of dramatic population declines following exotic disease or other novel selection pressures are not merely historic curiosities. The number, intensity, and frequency of stresses (e.g., exotic pests and pathogens, climate change, pollutant exposures, etc.) that tree populations are subjected to seem only to be escalating. Considering the growing burden of anthropogenic stresses that individually or in combination may tax the physiological limits of individual trees, the rich genetic base needed to support population adaptation and survival may be more important now than ever.

Table 3. Examples of organisms for which rare alleles provided the basis for stress resistance that supported population survival despite strong selection pressure.

Organism	Novel selective force	Reference
Bacteria	Antibiotics	Abramson and Sexton (1999)
Insects	Pesticides	Georghiou (1991)
Insects	*Bacillus thuringiensis* toxin in transgenic crops	McGaughey and Whalon (1992)
Herbaceous plants	Herbicides	Heap (1997)
Populus tremuloides Michx.	Air pollution	Berrang et al. (1989)
Ulmus americana L.	Invasive pathogen	Cheng et al. (1997)

Given the likely importance of rare alleles to population adaptive potential, what could mean the legacies of the harvest-induced changes in rare allele frequency we found? The decline in rare alleles (i.e. in the hemlock selection cut for hemlock and preferential removal of the oldest white pine via simulated harvests) could reduce the genetic diversity needed by populations to successfully adapt to and survive ongoing environmental change. Conversely, for the hemlock diameter limit cut and when suppressed trees were preferentially retained in simulated harvests of white pine, residual stands

showed an increase in the frequency of (previously) rare alleles. Although the preservation of rare alleles is of theoretical adaptive benefit, it is unknown whether this hyper-abundance of typically rare alleles imparts any advantage beyond that afforded by the continued presence, but lower frequency, of these same alleles.

Although natural selection tends to remove phenotypes that are generally inferior, the interplay of environmental heterogeneity and stochastic events also allows some gene forms to persist at low frequencies despite apparent fitness costs. Thus, perhaps because it is variable and "incomplete", natural selection preserves rare alleles at levels that balance stand fitness under current environmental conditions with long-term benefits for potential adaptation when conditions ultimately change.

Our data from both actual silvicultural treatments and computer-simulated harvests, as well as studies with different coniferous species (e.g., Buchert et al. 1997; Adams et al. 1998), indicate that silvicultural selection can lead to shifts in the frequencies of rare alleles. But is this potential for alteration meaningful? One factor that could have a substantial influence on the importance of silvicultural manipulations of allele frequencies is the extent to which gene migration ameliorates harvest-induced alterations. In particular, seed and pollen migration from surrounding stands could offset allelic losses provided transport distances were not too great. Although gene flow for species such as eastern white pine are reported to be very high (Beaulieu and Simon 1994), evidence for the same species from our genetically mapped forest indicates that the average distance of genetic influence can actually be quite short, i.e. about 35 m (Nijensohn et al. in review). Furthermore, patterns of species distribution, land use and forest fragmentation, as well as the scale of silvicultural application (e.g., stand vs. forest vs. region), would all likely influence the functional limits of gene migration.

In addition to uncertainties about the influence of gene migration, many basic questions exist concerning the influence of silvicultural treatments on genetic resources. For example, how much can managers increase or decrease the frequency of traditionally rare alleles before the balance between short-term (productivity and fitness) and long-term (enhanced resilience to environmental change) benefits is compromised? Which silvicultural practices most alter genetic reserves? Are some species more vulnerable to genetic manipulation than others? What is the combined effect of management with other anthropogenic factors (e.g., pollution-induced mortality, population losses following exotic pest and pathogen introductions, shifts resulting from climate change, etc.) that may also reshape forest gene pools? These and other questions will need to be addressed to fully evaluate the true vulnerability of forest stands to genetic alteration following silvicultural treatment.

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