



SAMPLING STRATEGIES FOR CAPTURE OF DIVERSITY AND CONSERVATION OF RARE ALLELES

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INTRODUCTION

Rare alleles are often considered a minor element in gene conservation programs and yet they can be very important for long-term evolution or to meet new breeding objectives such as resistance to introduced insects or diseases. For example, if low frequency alleles become a target for future breeding objectives or for survival of wild populations, the possibility of relatedness among selected or surviving genotypes may not allow effective use of these low-frequency alleles due to excessive inbreeding build-up. It is therefore desirable to raise the probability of maintaining rare alleles in enough unrelated genotypes for future use in wild or breeding populations.

Strategies for collection of seed are much more flexible than the design of *in situ* reserves, and can be designed to maximize the capture of genetic diversity across the landscape, particularly rare alleles. This work focuses on sampling strategies for conservation of rare alleles through a study of the distribution of rare alleles at different spatial scales, using Sitka spruce as a model species.

OBJECTIVES

To develop a sampling strategy that allows rare alleles to be captured for *ex situ* gene conservation. The specific objectives are as follows:

- (i) What is the distribution and frequency of rare alleles in continuous and disjunct populations of Sitka spruce?
- ii) What sampling strategy (number and distribution of parent trees within a seed management or conservation captures the highest proportion of rare alleles for *ex situ* gene conservation efforts?

- (iii) How do these results compare to the distribution of rare alleles in other forest tree species?

MATERIALS AND METHODS

Experimental Design and Genetic Material

Sitka spruce distribution was classified by both ecology (core or peripheral environments relative to realized niche) and geography (continuous or disjunct distribution of populations) (Table 1; Figure 1). A total of 200 trees were sampled from each population. At each sampling site, a transect 50 m wide was established and fresh needle tissue (current year's growth) was collected from individuals 30 m apart. The number of trees sampled was expressed on a unit area basis to allow for spatial distribution analysis, which monitors changes in genetic variability and relatedness over space.

We are using co-dominant Sequence-Tagged Site (STS) markers developed by Perry and Bousquet (1998) and screened for amplification and polymorphisms in natural populations of Sitka spruce. We anticipate using 10 primer pairs in this study.

Table 1. Two-way classification of Sitka spruce sampling sites by ecological and geographical distribution within its range

	CORE POPULATIONS	PERIPHERAL POPULATIONS
CONTINUOUS	Fort McNeill, BC Prince Rupert, BC	Brookings, OR Seward, AL
DISJUNCT	Qualicum, BC Queen Charlotte Islands, BC	Fort Bragg, CA Kodiak Island, AL

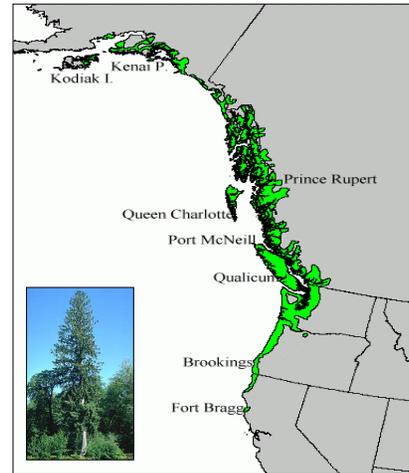


Figure 1. Natural distribution of Sitka spruce and location of collection sites

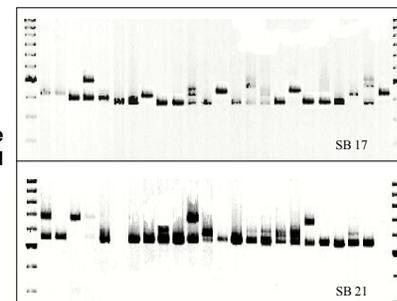


Figure 2. Panels showing amplification and allelic polymorphisms at locus SB17 & SB21 among 24 genotypes of Sitka spruce from Prince Rupert

ONGOING RESEARCH

Based on STS co-dominant marker data, allele frequencies and genetic diversities will be calculated in continuous and disjunct populations. Allelic "capturing curves" are introduced to give information on the minimum number of individuals that must be sampled in a given area to capture an expected number of rare alleles. The results will then be used to model and compare alternative sampling strategies to capture rare alleles at different spatial scales.

The overall findings of this study have implications for devising optimal sampling strategies for conservation purposes. It is expected that a large number of individuals will be necessary to capture a significant proportion of rare alleles.

REFERENCES

- Doyle J. J. and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. *Focus* 23:13-15
- Perry, D. J. and Bousquet, J. 1998. Sequence-Tagged-Site (STS) Markers of Arbitrary Genes: Development, Characterization and Analysis of Linkage in Black Spruce. *Genetics* 149: 1089-1098.

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