

# HYBRIDIZATION OF LARIX OCCIDENTALIS AND LARIX LYALLII

Clinton E. Carlson and George M. Blake<sup>1</sup>

## INTRODUCTION

Hybridization between species in the genus Larix is widely recognized. Probably the best known example is the Dunkeld hybrid larch (Larix x eurolepis Henry), a cross between European larch (L. europaea De Candolle) and Japanese larch (L. leptolepis Murray). Natural hybridization has been verified between other species in the genus, and the success of crossing experiments suggests hybridization is possible between species that have not yet been studied from this perspective. Ostenfeld and Larsen in 1930 and Schoenike in 1961 reported that natural hybridization may be occurring between western larch (L. occidentalis Nuttall) and subalpine larch (L. lyallii Parlatore), but that no reliable evidence had yet been presented. These two species occupy overlapping geographical ranges in the northwestern United States, although they are usually separated in terms of specific habitat. Subalpine larch normally occurs above 7,500 feet M.S.L. (mean sea level) and western larch below 7,000. Generally, there is almost no possibility of contamination of one population by pollen from the other. However, ecological phenomena have created certain niches where hybridization could occur.

Considerable variation has been observed in the wood quality of western larch (Krier, unpublished), and it is possible that hybridization with subalpine larch could be a contributing factor to this variation.

## OBJECTIVES

The present study is the first phase of a long-term project initiated by the Forestry School of the University of Montana, the goal of which is to study the genetics of western larch in terms of wood production and quality potential. The objectives of the present study are: (1) to produce, by artificial means, hybrids between western larch and subalpine larch; (2) to study the morphology of western larch and subalpine larch; and (3) to locate natural hybrids that may exist in an area of species overlap.

## PROCEDURE

Pollen was collected from an isolated population of western larch during April of 1964. In mid-June, as the buds opened, female strobili of subalpine larch were isolated. Male strobili were stripped from the branch, and females enclosed in an isolation bag. In late June, as they became receptive, the female strobili were artificially pollinated. Three weeks later, when pollen from subalpine larch was no longer present and the female strobili had closed, the isolation bags were removed. During September the mature cones were collected and the seed extracted, stratified for 30 days at 38 degrees F., and then planted. Because of the high elevation at which subalpine larch is found, pollen matured too late to make the reciprocal cross in 1964. Pollen of subalpine larch was collected and stored at 38 degrees F., and reciprocal crosses were made in the spring of 1965.

Seed from naturally pollinated western larch and subalpine larch was collected in September of 1964, stratified, and planted for comparison with the hybrid seedlings.

<sup>1</sup> Graduate student and Assistant Professor of Forestry, respectively, University of Montana, Missoula, Montana.

The cross was successful: approximately 30 percent of the western larch and hybrid seed germinated, but only 15 percent of the subalpine larch. After six months, western larch is four inches high, subalpine larch one inch, and the hybrids are intermediate. There is currently no evidence of heterosis. To obtain a good morphological comparison, the mid-portion of a cotyledon from each of western, subalpine, and hybrid larch was sectioned transversely by the paraffin method and stained with safranin 0 and fast green. Microscopic examination revealed that internal structure of the cotyledons differs among the three types. Subalpine larch has two resin canals; the perimeter of each is composed of five secretory cells. Resin canals were lacking in western larch, but two were present in the hybrid and were composed of six secretory cells each. The number of cells composing the circumference of the endodermis was counted for each specimen; subalpine larch had 15 cells, western larch 13, and the hybrid larch 16 (fig. 1).

A thorough knowledge of some of the morphological characteristics of western larch and subalpine larch was necessary before attempting to identify natural hybrids. A western larch population 30 miles northeast and a subalpine larch population 30 miles south of Missoula, Montana, were selected for intensive study. Because of the absence of the other species for a considerable distance, there is no possibility that hybrids could occur in these areas. Three different sites were selected on each of two aspects similar in each area to determine if a change in environment would alter the structure of a character. One tree from each of the six sites was sampled. Twelve positions were sampled in the crown of each tree to determine whether position affected morphology. Position and site were fixed, not selected at random. The following characteristics were studied:

1. Number of needles per fascicle.
2. Pubescence, or hairiness of twigs. This feature was measured as number of hairs on a square mm. of twig surface selected at random on the last inch of the growth of the previous year. A binocular dissecting scope was used for observation.
3. General shape of twigs. (Subjective, i.e., western larch long, slender, subalpine larch shorter, blunt).
4. Smoothness of bark within four feet of the top of the tree. (Subjective, i.e., western larch smooth; subalpine larch rough, stringy).
5. Needle morphology. A transverse section was taken from the mid-portion of the selected needle, and the following were determined:
  - a. Resin canals, if any. Number of secretory cells composing the circumference of the canal,
  - b. Number of cells composing the circumference of the endodermis:
  - c. General transverse shape of the needle. (Subjective, i.e., western larch triangular; subalpine larch quadrangular).

The data from needles/fascicle, pubescence, epithelial cells, and endodermis cells were analyzed by analysis of variance. The design was a combination one-way and two-way classification, with subsampling. The characteristic of needle morphology provided a comparison to the needle morphology of the artificial hybrids

Variation within the ranges of the species was examined. Samples from throughout the geographic range of each species were obtained and analyzed for the characteristics shown to be reliable by the preceding observations:

A summary of the analysis of variance is given in table 1. In all cases the characteristics were highly significant (1% probability) between species. The relatively small difference between species means and the relatively large variation

WESTERN LARCH

HYBRID

SUBALPINE LARCH

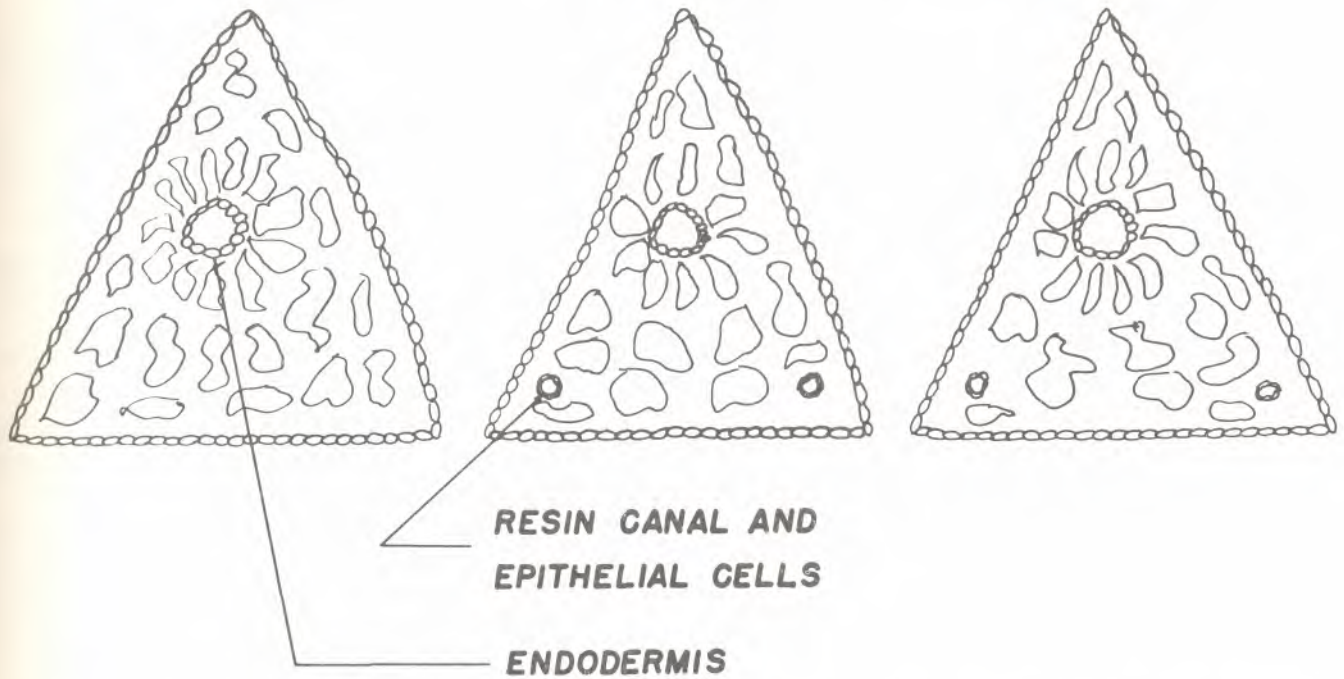


Figure 1.--Morphology of cotyledons of western larch, subalpine larch, and hybrid

Table 1

Source of Variation	Degrees of Freedom	Mean Square is 1/ an Estimate of:	Characteristic							
			Needles Per Fascicle		Pubescence		Endodermis Cells		Epithelial Cells	
			F.	Sig.	F.	Sig.	F.	Sig.	F.	Sig.
Species	(2-1)	$\sigma^2_{rps} + pr\sigma^2_s$	23.46	** <sup>2/</sup>	2711.20	**	160.17	**	241.43	**
Site Within Species	2(6-1)	$\sigma^2_{rps} + p\sigma^2_{rs}$	3.62	**	13.82	**	3.89	**	1.63	NS
Position	(12-1)	$\sigma^2_{rps} + r\sigma^2_p$	2.27	NS <sup>3/</sup>	< 1	NS	< 1	NS	1.36	NS
Position X Species	(2-1)(12-1)	$\sigma^2_{rps} + r\sigma^2_{ps}$	1.61	NS	2.21	NS	2.76	**	1.93	**
Error	2(6-1)(12-1)	$\sigma^2_{rps}$								

Mean Values for Species

Species <sup>4/</sup>	Needles/Fascicle		Pubescence		Endodermis Cells		Epithelial Cells	
	WL	SAL	WL	SAL	WL	SAL	WL	SAL
Mean	30.75	26.90	27.36	129.17	16.11	18.67	5.61	7.58

1/ r = site, p = position, s = species (From Steel and Torrie, 1960)  
 2/ \*\* = 1% probability  
 3/ NS = Non-Significant  
 4/ WL = western larch, SAL = subalpine larch

of individual values for needles/fascicle and endodermis cells indicated these characteristics would not suffice for practical description of hybrids. The data and analysis showed that epithelial cells and pubescence would be reliable characteristics for subsequent use in a hybrid index.

Among the subjective characters of twig shape, bark smoothness, and transverse needle shape, only bark smoothness was determined to be a reliable characteristic. The bark of subalpine larch was blackish, rough, stringy that of western larch was gray-green, smooth. The difference was so pronounced that it could easily be seen from a considerable distance.

The reliable characteristics in the population sample varied similarly throughout the geographic ranges of the species, giving additional support to the usefulness of those characters.

The knowledge that hybrids between western larch and subalpine larch can be produced artificially gave impetus to the idea that natural hybrids may exist. As previously stated, geographic range overlap occurs in several places over the ranges of the species. Both are frequently found in the same mountain range, generally separated by elevation, Western larch usually occurs to 7,000 feet M.S.L. Engelmann spruce (*Picea engelmannii* Parry) and subalpine fir (*Abies lasiocarpa* (Hook) Nuttall) occupy from 7,000 to 7,500 or 8,000 feet, and subalpine larch occurs above this zone. The spruce-fir complex serves as a "buffer zone" to separate western larch from subalpine larch.

In Carlton Creek, a small drainage five miles long and 20 miles south of Missoula, Montana, in the rugged Bitterroot Mountains, this buffer zone is lacking. Subalpine larch is abundant at the upper extremities of the ridges paralleling the canyon, Western larch occurs at the lower extremity and has progressed three miles up the floor of the canyon, with scattered individuals one-third the way up the south side. There are several snowslide areas on the south side of the drainage, and subalpine larch occurs from the top of the ridge (8,000 feet M.S.L.) to the bottom of the canyon (6,000 feet M.S.L.). In the lower one-third of these slide areas, both species of larch are found together.

Six transects were made through the species-overlap area on the largest slide. Each was about one-fourth mile long. All of the larch trees within 10 feet of each transect were sampled. One transect was made the entire length of the canyon, intercepting western larch, the species-overlap area, and subalpine larch. Sampling of this transect was done at half-mile intervals. At each stop, all larch trees in a 20-foot radius were sampled. The characters of epithelial cells, pubescence, and bark were observed.

The occurrence of both western larch and subalpine larch on a homogeneous site with reference to light, soil, and water, indicates that the characters studied are strongly genetically controlled. Of the 101 trees sampled, 48 were from Carlton Creek. A hybrid index (Anderson, 1930) was constructed for analysis of the trees. All 101 trees sampled in the study were analyzed on the hybrid index. Numerical ratings from 0-3 were given for pubescence, 0-2 for bark, and 0-5 for epithelial cells (table 2). The highest possible total was 10, the lowest 0. The highest value for each characteristic is associated with subalpine larch, the lowest with western larch. All characteristics combined, western larch occurred from 0-3, subalpine larch from 6-9, and possible hybrids from 3-6. All individuals occurring from 3-6 were from the overlap area in Carlton Creek, indicating that natural hybridization may be occurring.

Table 2.--Hybrid index design.

<u>Pubescence</u>		<u>Epithelial cells</u>		<u>Bark</u>	
No. of hairs	Rating	No. of cells	Rating	Type	Rating
0-60	0	5	0	smooth	0
60-110	1	6	1	medium	1
110-150	2	7	2	rough	2
150+	3	8	3		
		9	4		
		10	5		

<u>Hybrid index</u>										
	0	1	2	3	4	5	6	7	8	9
No. of trees	21	29	4	6	2	6	4	7	11	11
	western larch				:	hybrids		:	subalpine larch	

SUMMARY AND DISCUSSION

A study designed to locate natural hybrids between western larch and subalpine larch was completed in three parts. Part 1, by producing artificial hybrids, established that hybridization is possible. Part 2 studied the morphological characteristics of each species and their variation. Part 3 established the strong possibility that natural hybrids are occurring.

On the basis of physical characteristics, the study has shown that hybridization occurs between western larch and subalpine larch. The chemical and cytological properties of each species should next be investigated.

It is possible that introgression has been and is now occurring in many commercial logging areas. This may account for part of the large variation in wood quality and volume production of western larch.

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