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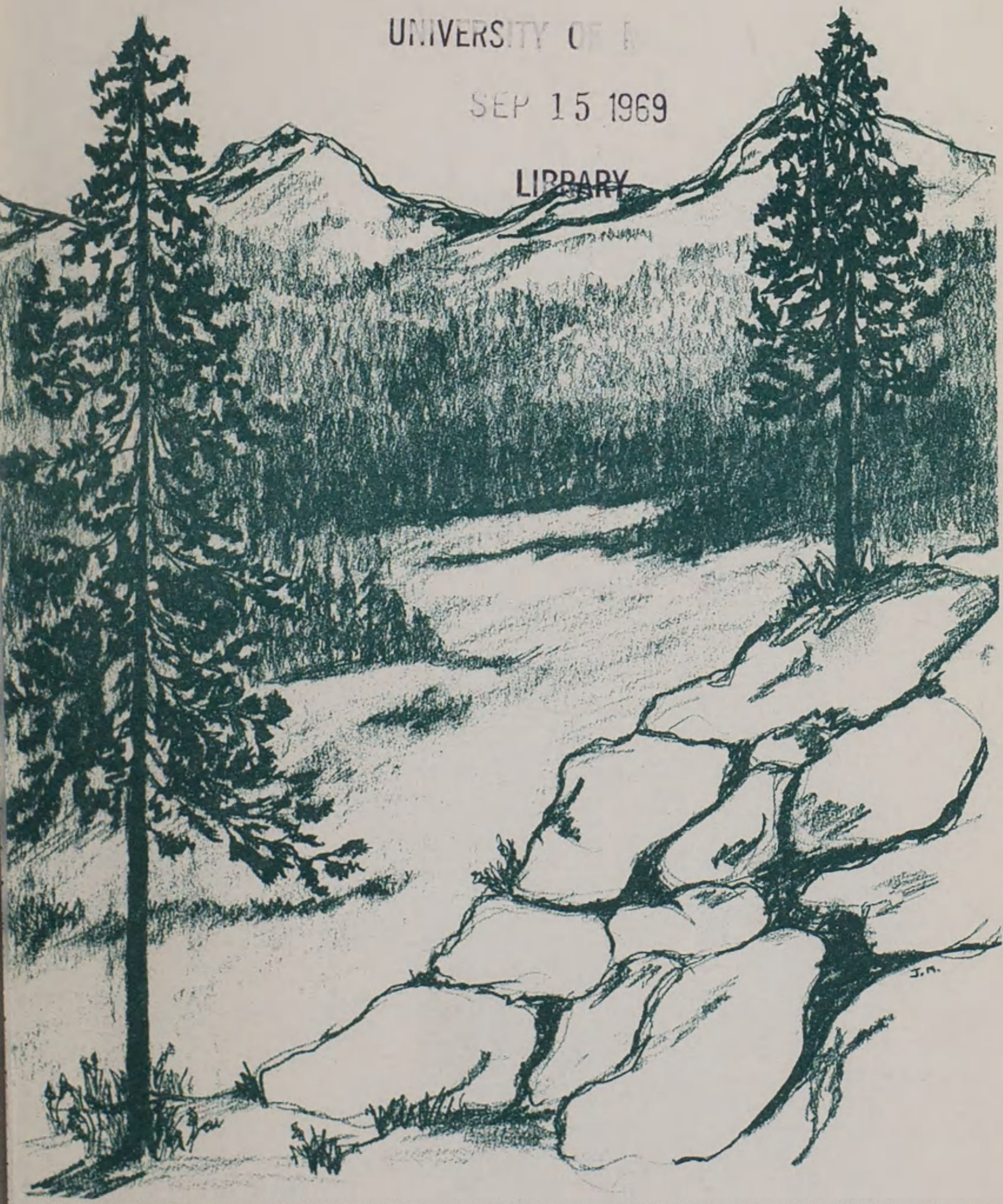

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Hybridization of Western and Subalpine Larch

By CLINTON E. CARLSON[1] and GEORGE M. BLAKE[2]

Introduction

At least four barriers can prevent hybridization between two plant species (Stebbins, 1950 and Dobzhansky, 1951). The species may be isolated reproductively, ecologically, or geographically. Protandry and protogyny may also prevent hybridization. Thus many species may live sympatrically and hybridize only intraspecifically, whereas others may hybridize both intra- and interspecifically.

Western larch (*Larix occidentalis* Nutt.) grows throughout the Columbia River drainage system at elevations of 2,000 to 6,500 feet; subalpine larch (*L. lyallii* Parl.) grows within the same area, at elevations of 7,000 to 10,000 feet. Elevation isolates the two species (Figure 1). Ostenfeld and Larsen (1930) and Schoenike (1961) noted that western larch and subalpine larch occupy overlapping geographical ranges in the northwestern United States, but these authors found no reliable evidence that the two species had hybridized.

However, we investigated the possibility of interspecific hybridization between western larch and subalpine larch.

The objectives of the study were (1) to test the hypothesis that subalpine and western larch would cross under artificial conditions; (2) to study the morphology of both species and so provide a basis for differentiating natural hybrids if such should occur; and (3) to locate putative natural hybrids between the species.

Procedures

We selected isolated populations of western and subalpine larch as the sources of breeding materials. The western larch parents grow on the Lubrecht Experimental Forest, 30 miles northeast of Missoula, Montana, and the subalpine larch on Carlton Ridge near

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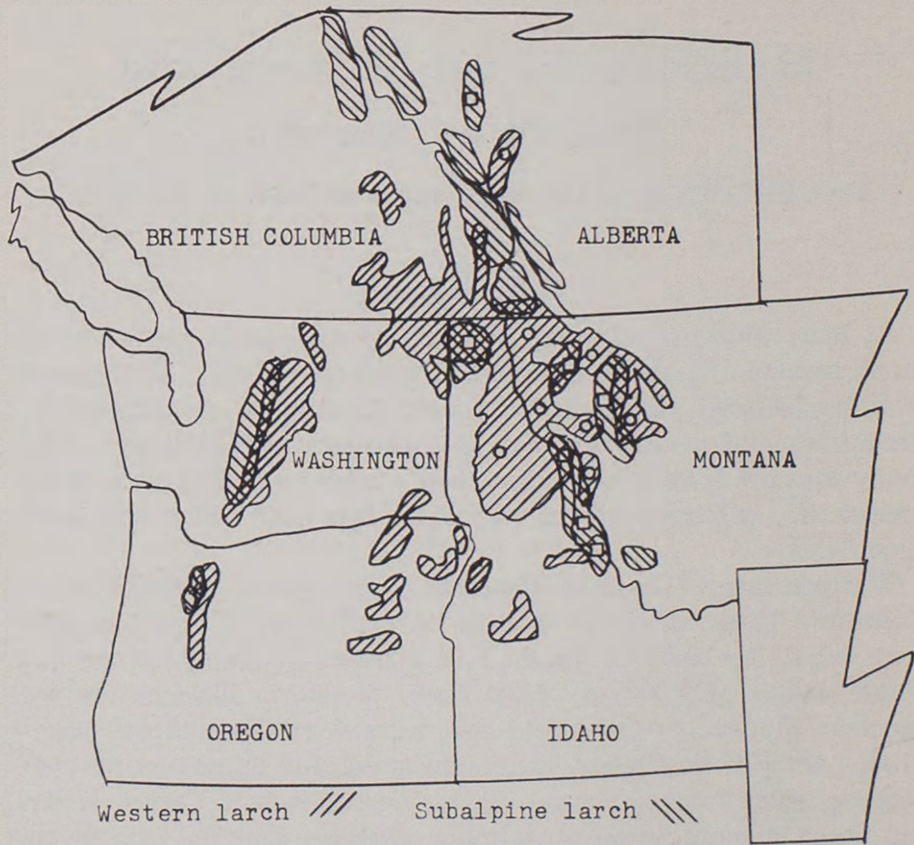


FIGURE 1.—Geographical ranges of western and subalpine larch. Although the geographic ranges appear to overlap, the species are separated altitudinally. Circles = western larch sampled; squares = subalpine.

Lolo Peak, 15 miles southwest of Missoula. Pollen was collected directly from pollinating trees or from cut branches kept in water in a greenhouse. Collected pollen was stored in plastic vials at 38° F.

To test pollen a drop of distilled water containing pollen was placed on a glass slide, covered with a cover glass which was ringed with petroleum jelly, and incubated at 80° F for 72 hours. Counts of 1,000 grains per slide indicated germination of 70 to 75 percent for western larch and 50 to 60 percent for subalpine larch.

In mid-June unreceptive pistilate strobili of subalpine larch were isolated in sausage casing bags. In late June, these pistilate strobili were artificially pollinated with western larch pollen. Three weeks later, when pollen from subalpine larch was no longer present and the pistilate strobili had closed, the isolation bags were removed. Reciprocal crosses also were made. Seeds from these crosses were extracted, stratified for 30 days at 38 degrees F in moist sand and planted in pots in a greenhouse.

Concurrent with the artificial hybridization, sampling of morphological characteristics was initiated in stands of western and subalpine larch. Descriptions of the two species differ in pubescence of twig, resin canals of needle, shape of needle, cone length and possibly bark (Table 1). However, we have not learned

TABLE 1.—A composite of the morphological characteristics which are used to distinguish western from subalpine larch.

	Cone Length	Resin Canals in Needle X-Section	Pubescence of Second Year Twigs	Young Bark	Transverse Needle Shape
Western	1-1½ inches	none	nearly glabrous	smooth	triangular
Sub-alpine	1½-3 inches	2, one in each of the smaller angles	very dense	scaley	quadrangular

[1]For a more complete description, the reader is referred to Bow, 1958, Collingwood and Brush, 1947, Green, 1933, Kirkwood, 1930, and Ostenfeld and Larsen, 1930.

whether these differences are actual and, if so, are caused by genetic dissimilarities or by environmental modifications of phenotypes produced by similar genotypes.

We selected the populations that produced the artificial hybrids for the morphological samples. Analyzed samples of each population from three different sites on two different aspects suggested the effect of determined environmental control. Six trees, one tree from each site, were sampled. Site was fixed as bottom, middle, or top of slope. Aspect was fixed as northeast or southwest. In case position in the crown might affect morphology, subsequent samples were taken from each of the cardinal directions at three crown positions in each tree. The sampling was done in such a manner that the quantitative data could be analyzed by analysis of variance. The design was a two-way classification with sub-sampling (Table 2). The following characteristics were studied:

(1) Number of needles per fascicle.

(2) Pubescence. This was measured as number of hairs on a square millimeter of twig surface selected at random on

TABLE 2.—Analysis of variance of morphological characteristics with mean square estimates.

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

[1] r = site, p = position, s = species

[2] 1% Probability

[3] NS = Non-Significant

the last 3 cm of the previous year's growth. The sample was placed on the stage of a binocular dissecting scope; a small grid was placed over the sample.

(3) General shape of twigs. (Western larch is long, slender; subalpine is shorter, blunt.)

(4) Texture of bark within four feet of the top of the tree. (Western larch is smooth; subalpine is rough.)

(5) Needle morphology. A transverse section was taken from the mid-portion of a selected needle, sectioned by the paraffin method (Johansen, 1940), and examined for the following characteristics:

(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. (Western larch triangular; subalpine quadrangular.)

Although not mentioned in previous descriptions, general twig shape, needles per fascicle, and endodermis cells were studied because observation indicated possible species differences. During 1964 and 1965, samples were collected from within the ranges of both species (Figure 1) and analyzed for the above characteristics. Collection areas included Alberta, British Columbia, Montana and Idaho. This part of the study involved samples from 41 trees.

The final phase of the study involved searching for stands of natural hybrids. This, of course, could only occur in an area in which both geographic and elevational ranges overlap. A geographic overlap is common throughout the ranges of the species, but elevational overlap has not been reported. The two species have similar ecological requirements in that both are found on the moist, north aspects, but they are separated by a "buffer zone" consisting of Engelmann spruce (*Picea engelmannii*, Parry) and subalpine fir (*Abies lasiocarpa* (Hook), Nutt.). Thus, for the species to overlap, the habitat must offer such a unique feature as a slide zone in which the larch can compete with the Engelmann spruce and the subalpine fir.

The Carlton Creek drainage again offered the best study area. It is oriented in an east-west direction. Lolo Peak rises over 9,000 feet above sea level at the upper extremity of Carlton Creek. The mouth of the creek is about 4,000 feet above sea level. Cliffs, talus slopes, and snowslide areas intersperse the north, more gentle side of the canyon. A preliminary excursion to the south side of the drainage revealed that both western larch and subalpine larch grow in a large snowslide path. Anderson (1949) presented an argument for the existence of natural hybrids which may apply in this situation. He claimed that a natural disturbance creates a multitude of microsites, to which one plant may be more suited than another. Thus, because the recurring snowslides form microsites similar to the ridge-top environment, subalpine larch can survive at elevations lower than its normal range. The possibility of locating natural hybrids in the Carlton Creek drainage was excellent for two reasons: (1) the parent species were sympatric here, and (2) the environment had been periodically disturbed, creating suitable microsites for establishing hybrids.

Western larch extends from the mouth of the drainage to three miles up the floor and one third of the way up the south side of the canyon. Subalpine larch grows on the upper ridges paralleling the canyon and in many slide areas. A five-mile transect was made diagonally from the mouth to the upper extremity of the canyon, crossing the areas occupied by western larch, a species overlap area, and subalpine larch. Samples were taken at half-mile intervals; at each stop all larch trees in a 20-foot radius were sampled. Six transects, each about one-quarter mile long, were made through the species overlap area. All larch trees—a total of 48—within 10 feet of the transect were sampled. The bark was observed and recorded in the field as smooth, medium or rough. Samples of twigs and needles were put in labeled paper bags for later analysis.

A hybrid index (Anderson, 1949) was used for analysis of all the tree samples. Table 3 shows the values assigned to variations of

TABLE 3.—Hybrid Index Design. Selected characteristics given along with the associated numeral rating.

Pubescence		Epithelial Cells		Bark	
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		

each character. By totaling the values for the characteristics, the hybrid index of a tree is obtained. The highest possible total is 10, indicating strong subalpine larch tendencies. In addition, the scatter diagram technique of Anderson (1949) was used to compare all the sample trees.

Results and Discussion

The strobili of western and subalpine larch are normally monoecious and monosporangiate. However, during the process of isolating pistilate strobili, abnormal strobili were observed on both species of larch. These strobili consisted of two distinct parts: one typically pistilate, the other typically staminate. The pistilate structure was positioned over the staminate in the subalpine larch; in western larch the situation was reversed (Figure 2). Kirkwood

Western Larch **Subalpine Larch**

FIGURE 2.—Bisporangiate strobili of western and subalpine larch. Note difference in arrangement of staminate and pistilate parts.

(1916) observed the structures in western larch. Chandler (1959) found both arrangements in Japanese larch, but Kirkwood found only the type in which the pistilate part was above the staminate.

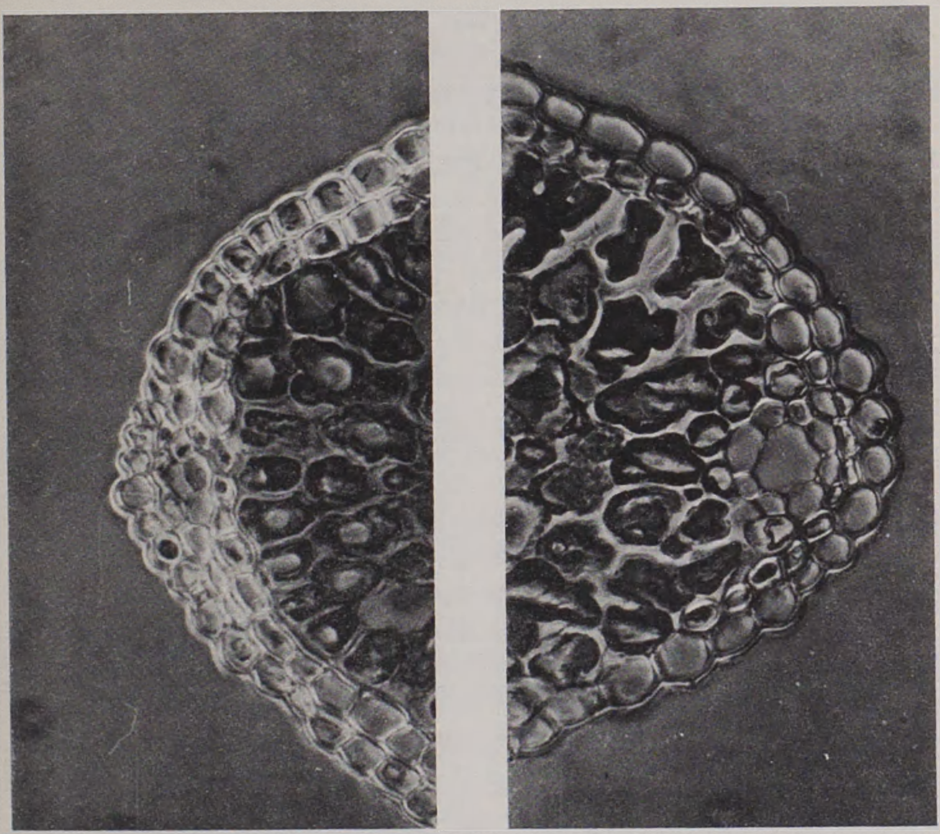
Approximately 30 percent of the putative hybrid seed from the cross western and of the open subalpine pollinated western larch seed germinated, whereas only 15 percent of the open pollinated subalpine (control) seed germinated. After six months, western larch was six inches high, putative hybrids were of intermediate height, and subalpine larch was one inch high. Seed did not germinate from the reciprocal cross. The hybrids did not exhibit abnormally rapid growth. A single albino seedling was observed during germination of the subalpine larch seed, but efforts to keep the plant alive failed. This phenomenon did not appear in western larch or hybrid seedlings.

The midportion of a cotyledon from each of the western, putative hybrid and subalpine larch was sectioned transversely. Microscopic examination revealed that internal structure differs among western, hybrid and subalpine larch. Subalpine larch has two resin canals, the perimeter of each comprising four secretory cells. The hybrid also has two resin canals, but each canal comprises six secretory cells. No resin canals occur in the cotyledons of western larch. Thirteen cells constitute the circumference of the endodermis in western larch, 16 in the hybrid, and 15 in the subalpine.

The data from needles per fascicle, pubescence, epithelial cells, and the endodermis cells were analyzed by analysis of variance. All characteristics were highly significant between species (Table 2). Of the other recognized sources of variation, site within species was significant in all cases; the interaction between species and crown position was nonsignificant for all characteristics except endodermis cells. Because of the relatively high variation of individual values and the small difference between species means for needles per fascicle and endodermis cells, these characteristics were not useful in a hybrid index analysis.

Contrary to previous descriptions, this study showed that the needles of western larch do possess resin canals, and that the species distinguishes itself from subalpine larch by the number of epithelial cells composing the circumference of the canal (Figure 3). The findings of Budkevich (1956) support the use of this characteristic as a diagnostic tool. He stated that, on the basis of wood morphology, the number of thick-walled epithelial cells lining the vertical and horizontal resin canals divide the larch species into two groups.

Casual observation suggests that species may differ in twig form. Subalpine larch seems to grow much thicker twigs than



Western Larch **Subalpine Larch**

FIGURE 3.—Difference in number of epithelial cells around resin canal between mature western and subalpine larch. Western has five cells, subalpine eight.

western larch. However, fast-growing western larches possess thick twigs similar to those of subalpine, and slow-growing subalpine larch has thin twigs similar to those of slow-growing western larch. Because of this apparent dependence on growth rate, twig form is not suitable for a hybrid index.

Bark texture within four feet of the top of the tree is consistent within species and does not appear to be altered by growth rate or site. Western larch has very smooth, greenish-gray bark, with longitudinal fissures. Subalpine larch has much coarser, darker bark. The fissures are both longitudinal and horizontal, giving the bark a scaly appearance. The bark difference in the two species is so pronounced that they can be distinguished from a considerable distance. This characteristic has not been mentioned in previous descriptions of the species.

Earlier descriptions indicate that the general transverse shape of the needle can be used to separate the species because the needles of western larch are triangular and the subalpine needles are quadrangular (Collingwood and Brush, 1947; Green, 1933; Ostenfeld and Larsen, 1930). However, most of the subalpine larch needles sectioned were more triangular than quadrangular, and in many cases were indistinguishable from western larch needles.

The morphological variation portion of the study showed that epithelial cells of the resin canals, pubescence, and bark within four feet of the growing tip would be most useful in describing natural hybrids. The samples collected, i.e. from the Lubrecht Experimental Forest and Carlton Creek populations, varied similarly in pubescence, epithelial cells, and bark, giving additional support to the usefulness of these characteristics.

The occurrence of both western larch and subalpine larch on a homogeneous site with reference to light, soil, and water indicated that genetics strongly controls the characteristics of epithelial cells, pubescence, and bark. Results of the morphological variation phase showed that these characteristics are consistent within species. The distribution of the trees on the hybrid index is given in Table 4.

TABLE 4.—The distribution of sample trees along the hybrid index.

				Hybrid Index					
0	1	2	3	4	5	6	7	8	9
				Number of Trees					
21	29	4	6	1	7	4	7	11	11
western larch				putative hybrids			subalpine larch		

The index includes all 101 sample trees in the study (48 from Carlton Creek, 12 from the morphological variation, and 41 from the geographic range). Western larch scored from 0-3, putative hybrids from 4-6, and subalpine larch from 7-9. No tree received a total of 10. All but two of the trees in the 4-6 classes were from the species overlap area in Carlton Creek; the exceptions came from an area of suspected natural hybridization in the Mission Mountains in northwestern Montana.

The scatter diagram (Figure 4) shows a distinct grouping of the sample trees in their respective species classes. Examination indicates that the bark character is the least reliable: medium bark occurs in all three groups. In some cases, differentiating medium from smooth or rough bark was very difficult; however, most of

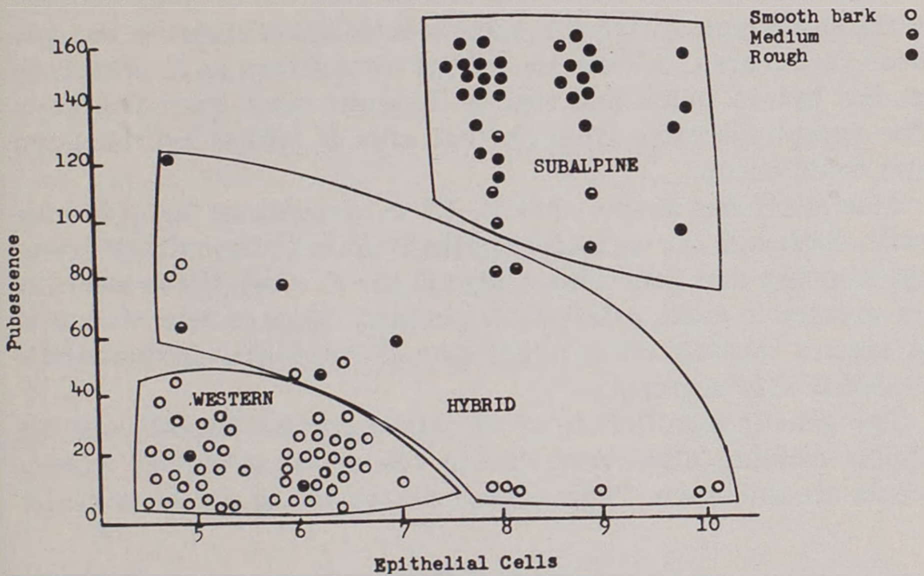


FIGURE 4.—Scatter diagram of all trees sampled in the study. Note groupings of western, subalpine, and hybrid larches. All except two of the trees shown as hybrids were from the species overlap area in Carlton Creek.

the medium-barked trees were also intermediate in the other characteristics. The trees included as hybrids at the upper left and lower right of the scatter diagram were not intermediate in any single characteristic. This may be a result of hybrids backcrossing with the parents. However, these trees appear in the intermediate range on the hybrid index.

Summary

The study investigated whether western larch and subalpine larch naturally hybridize. A cross using subalpine larch as the female parent was completed and the seedlings are now growing. Reciprocal crosses were also made. Transverse section of cotyledons revealed that the hybrid has resin canals quite similar to those of subalpine larch, and that western larch has no resin canals.

With the use of statistical analysis, three morphological characteristics proved useful in detecting putative natural hybirds between the species. The traits were pubescence of the year-old twigs, number of epithelial cells composing the circumference of the resin canals in the needles, and texture of bark within four feet of the top of the tree.

Analysis of hybrid index and scatter diagram of trees sampled in the study indicated that natural hybridization occurs in the species overlap area. All but two of the trees shown as intermediate on the hybrid index and scatter diagram came from this area. The exceptions came from another area in which hybridization also could occur.

This study has shown that F_1 (first generation) hybrids may exist. Although the variability of the trees in Carlton Creek drainage suggests that generations beyond the F_1 exist, the evidence is too meager to make a definite statement. More extensive studies in species overlap areas might provide stronger circumstantial evidence of introgression.

The genetic significance of the bisporangiate strobili and the albino seedling discovered during the cross-pollination experiments are unknown. These abnormalities, too, merit more study.

Literature Cited

Anderson, E. 1949. Introgressive hybridization. John Wiley, New York 109 p.

Budkevich, E. V. 1956. The anatomical structure of wood in the species of the genus *Larix* in relation to their systemmatical classifications. Botanicheskii Zhurnal XLI (1):64-80.

Chandler, C. 1959. Bisporangiate cones in larch. Contr. Boyce Thompson Inst. 20 (1):107-110.

Collingwood, G. H., and W. D. Brush. 1949. Knowing your trees. American Forestry Assoc., Washington. 312 p. (illus., maps)

Dobzhansky, T. 1941. Genetics and the origin of species. Columbia Univ. Press, New York. 2nd ed. 446 p.

Green, G. R. 1933. Trees of North America (exclusive of Mexico). Vol. I. Edwards Brothers, Inc. Ann Arbor, Michigan. 186 p.

Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill, Inc. New York and London. 523 p.

Kirkwood, J. E. 1916. Bisporangiate cones of *Larix*. Bot. Gazette 61: 256-257. illus.

Ostenfeld, C. H. and C. S. Larsen. 1930. The species of the genus *Larix* and their geographical distribution. Det. Kgl. Danske Videnskabernes Selskab. Biologiske Medd. IX(2). 106 p.

Schoenike, R. E. 1961. The genus *Larix* (a literature review). School of Forestry, University of Minnesota. 61 p.

Stebbins, G. L. 1950. Variation and evolution in plants. Columbia Univ. Press, New York. 643 p. illus., maps.

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