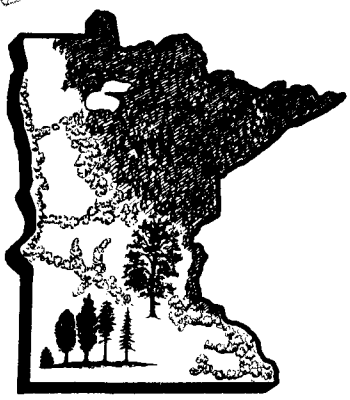

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CYTOTECHNIQUES FOR SPRUCE CHROMOSOMES[1/]

Lawson L. Winton[2/]

In a study of natural polyploidy in juvenile white and black spruce (Winton, 1963, 1964a), the first reliable chromosome counts were obtained in 1960, using a squash technique devised by combining parts of the methods by Mikaelsen (1952) and Johnsson (1953). White spruce vegetative buds were collected when the bud scales were just opening. More division figures were found in meristems collected at night, just as Mikaelsen (1952) found in Norway spruce. In 1960, needle meristems were pretreated in 0.002 M oxyquinoline for 6-12 hours at 24° C., then at 3° C. for 3 hours. Fixation in 1:3 acetic-ethanol for 24 hours was followed by storage in 70% ethanol at 20° C. for 1-5 days.

Individual slides were prepared by placing a single needle meristem in a watchglass with 3 drops of 9:1 acetolacmoid-1N HCl. After heating 3-4 times, the 9:1 solution was replaced with 1% acetolacmoid and the watchglass was again heated. The addition of stain and heating continued until the material turned dark and sank to the bottom of the solution.[3/] This method did not completely hydrolyse the pectin, but good cell separation was obtained by adding one drop of 1N HCl to the 9:1 solution after the initial heating. The increased acid also decreased the stainability of the chromosomes by acetolacmoid. Consequently, in 1961 and 1962, the 1% acetolacmoid was replaced in all solutions by 2% acetocarmine, which was not affected by additional acid.

Several tests failed to reveal any advantage of saturated peradichlorobenzene over oxyquinoline as a pretreatment solution. However, 0.2% colchicine used in both light and darkness gave better results than oxyquinoline, paradichlorobenzene, or non-pretreated but fixed needle meristems. Also, the substitutions of propionic acid for acetic acid and absolute ethanol for 95% ethanol appeared to increase the quality of slide preparations.

On the basis of this study, it is recommended that growing needle meristems of spruce be collected during mid-morning and shaken vigorously a few times in 0.5 ml of 0.2% colchicine in a 2 dram vial. After 3 hours in sunlight, replace the colchicine with 1:3 propionic-ethanol for 12-24 hours. Store in 70% ethanol at 3° C. After at least 12 hours of storage, remove all needles from the meristem and hydrolyse the latter in 9:1 acetocarmine-1N HCl. After initial heating, add one drop of 1N HCl and reheat. Replace the 9:1 solution with acetocarmine and gently reheat. Dissect out the promeristem, and under a dissecting scope tease it apart on a clean slide in a drop of stain, cover, squash; and heat. The slide may be temporarily sealed with a 1:1 mixture of gum mastic-Parawax, or made permanent by the dry ice method (Conger and Fairchild, 1953), using balsam in ethanol as the mounting medium. The Figure shows one tetraploid cell complement of 48 chromosomes from a juvenile white spruce prepared by this method.

1/ This report is based on a portion of the author's M.S. thesis (Winton, 1963). Support of this research was provided by the Charles K. Blandin Foundation, Grand Rapids, Minnesota.

2/ Research Assistant, School of Forestry, University of Minnesota.

3/ This method was later found to give good results for unfixed somatic chromosomes of white and black spruce, as well as their hybrid, in microsporangiate tapetal cells (Winton, 1964b).

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The cell cytoplasm of stored material tends to stain lightly with acetocarmine. Thus, meristems stored longer than one or two months may give better results when propionocarmine stain is substituted, even though chromosomes will not stain as darkly as with acetocarmine.

Using methods just described, diploid (2n) chromosome counts of 24, 36, 48, and 96 were observed among juvenile white spruce. Counts of 2n = 24 and 48 have also been found for juvenile black spruce. For both species care must be exercised during squashing not to break the long chromosomes.

In addition to somatic counts, 12 haploid (n) chromosomes were counted for both species in female gametophyte cells. For these preparations, immature cones were collected in mid-June, 1961, from mature trees, and split longitudinally before fixing in 1:3 acetic-ethanol for 10 hours. Individual female gametophytes were dissected out and hydrolysed in cold Warmke's (1935) solution (1:1 conc. HCl-absolute ethanol) for 10 minutes, then squashed in acetolacmoid.

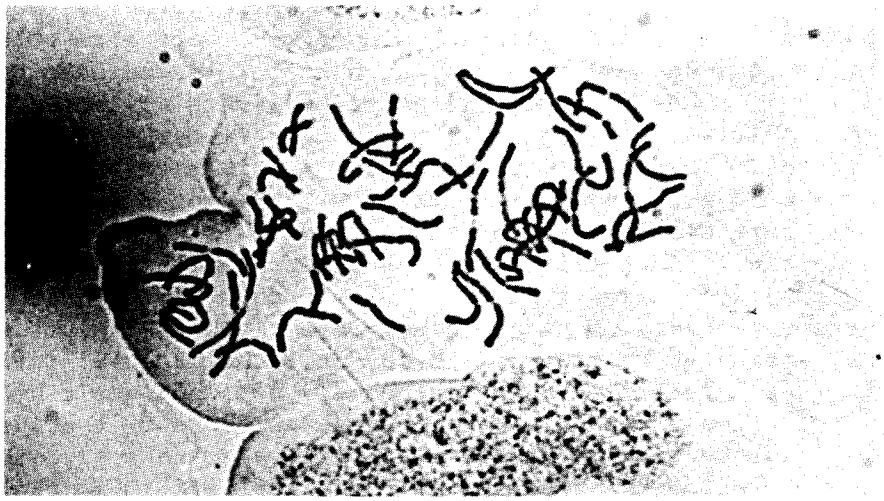

Figure. A tetraploid (4X = 48 chromosomes) cell complement prepared from a needle meristem of a juvenile white spruce by the colchicine-acetocarmine method.

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