

超高压電子顕微鏡による染色体のラセン構造の観察

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SHORT COMMUNICATION

DEMONSTRATION OF THE SPIRAL STRUCTURE OF CHROMOSOMES BY HIGH VOLTAGE ELECTRON MICROSCOPY

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It has been difficult to visualize three-dimensional structure of chromosomes by electron microscopy (Lafontaine and Lord 1969). This mainly due to the fact that conventional 100 kV or lower voltage electron microscopes can observe only thin specimens, being less than 1,000 Å in thickness. This difficulty may be overcome to a great extent with the aid of a high voltage electron microscope which is operated at accelerating voltages higher than 500 kV, because of its large penetrating power (Nagata *et al.* 1969). A preliminary study has made possible the examination of biological specimens sectioned at 0.5 to 1 micron in thickness.

The present paper deals with a preliminary observation on the fine structure of meiotic chromosomes of *Trillium kamtschaticum* by high voltage electron microscopy with the hope of demonstrating three-dimensional structure of chromosomes.

Pollen mother cells were treated for 40 seconds with a hypotonic solution, a 1:3:8 mixture of 1 N KCl, 1 N NaNO₃ and distilled water and fixed for 2 to 3 hours in ice-cold 6.25% glutaraldehyde buffered to pH 6.8 with 0.1M phosphate buffer. After the materials were washed in phosphate buffer for about 16 hours, they were postfixed in ice-cold osmium tetroxide buffered to pH 6.8 with 0.1M phosphate buffer for 2 hours. The fixed materials were dehydrated in ethanol, and embedded in methacrylate. Sections, 0.3 to 0.5 micron thick, were made with a Porter-Blum microtome, and stained with 2% uranyl acetate in 50% ethanol for 2 hours at room temperature. The specimens were examined in a Hitachi high voltage electron microscope (HU-650) operated at accelerating voltage of 650 kV, and photographed at direct magnifications of 5,000×, 10,000× and 20,000×.

In Fig. 1a taken in the comparative thick specimen, about 0.5 micron thick, major and minor spirals of the meiotic chromosome are clearly demonstrated. The major spiral is made up of several gyres of the minor spiral. The chromatid shown in Fig. 1a is presumed to be one of short arms of a B chromosome (Fig. 1b), or one of long arms of an E chromosome (Fig. 1c) named by Matsuura (1935) on account of the fact that the gyre number of the major spiral nearly corresponds to that observed by light microscopy. The chromatid in the early metaphasic cell appears to consist of two thick strands in the form of a plectonemic spiral (Fig. 1a). The gyre diameter of the strands

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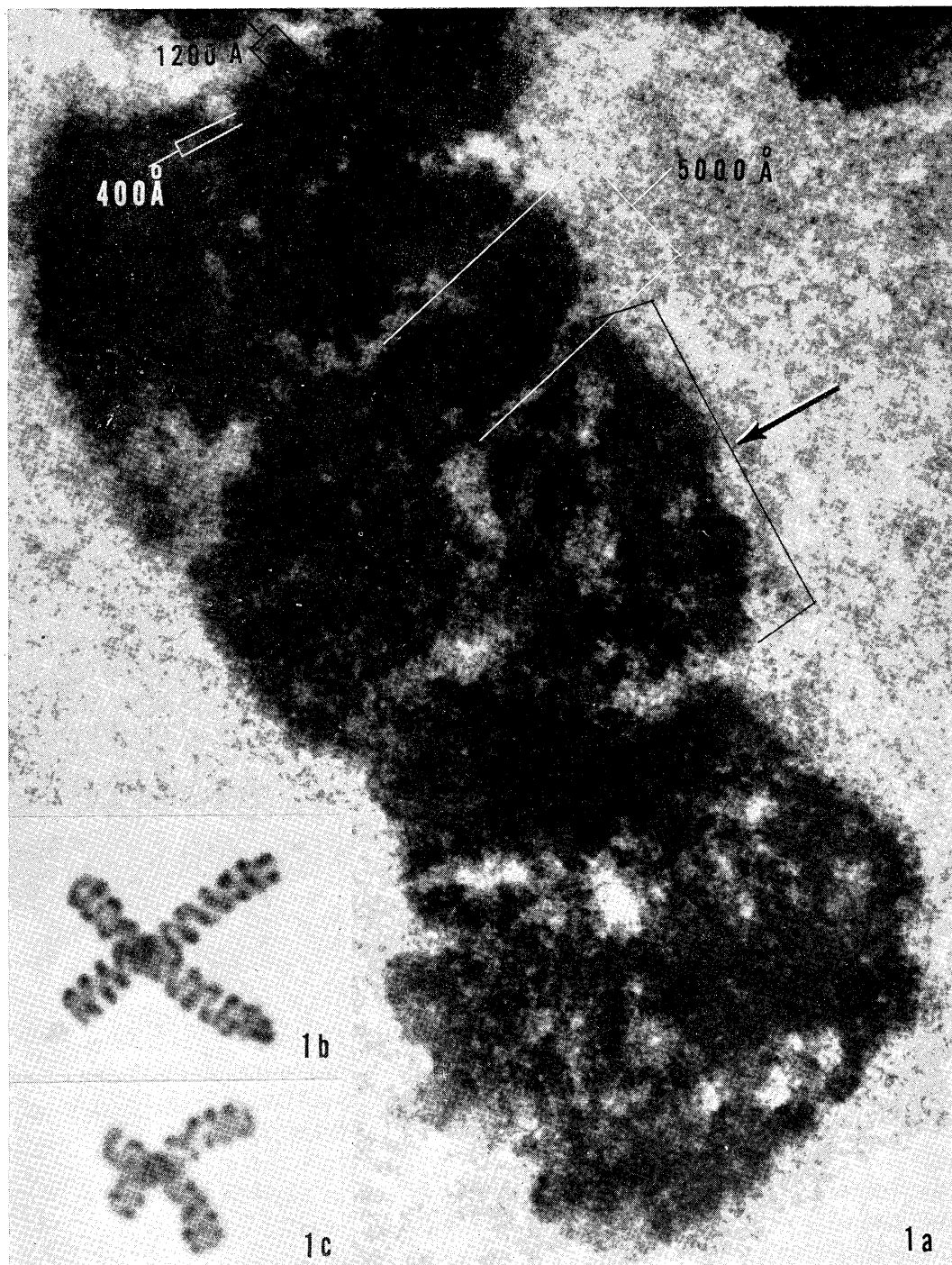


Fig. 1. Meiotic chromosomes in pollen mother cells of *Trillium kamschaticum*. a, Electron micrograph taken by high voltage electron microscopy. $\times 40,000$. b and c, Photomicrographs taken by light microscopy. b, B chromosome. c, E chromosome. $\times 2,000$,

forming the major spiral is about 5,000 Å. An area which may represent a change in direction of the major spiral is indicated by an arrow in Fig. 1a. The 5,000 Å strands appear to be formed by coiling of fibers 1,200—1,500 Å in diameter. The 1,200—1,500 Å fibers seem to be made up of fine fibrils which are less than 400 Å in diameter, showing a helical configuration. The 400 Å fibril has a periodicity indicating that a thin fibril is coiled into a helix, though its diameter is undetermined. Detailed observations are now in progress.

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