

Positively Heterochromatic Segment in Mitotic Metaphase Chromosomes of *Vicia faba*

It is well established that heterochromatic segments in the metaphase chromosomes of *Vicia faba* are revealed by cold treatment or other methods. But all the reports¹ available so far concerned the understained, i.e. negatively heterochromatic (-H) segments except the one by OCKEY² who recognized the existence of more densely stained, i.e. positively heterochromatic (+H) segments in addition to the -H segments. The present report deals with the +H segments of metaphase chromosomes which are disclosed by a new method.

Primary root-tips of *Vicia faba* (Nagasaya Soramame) were treated according to the procedure shown in the Table, and squashed after Feulgen stain. +H segments were clearly observed as the segments which remained dense in stainability in the metaphase chromosomes which swelled slightly and were more weakly stained than usual (Figure 1).

It is well known that DNA and/or histones are selectively extracted by TCA^{3,4}, HCl⁵ or acetic acid⁶. Therefore, it is supposed that the present procedure caused the preferential extraction of chromosomal constituents and consequently the differential staining along chromosomes. This implies that the +H segment revealed by the present method is a representation of heterogeneity in the chemical make-up of chromosomes. The +H segment was not detected when the sequence of TCA and HCl-acetic acid treatments was reversed, or when the HCl-acetic acid treatment was omitted. Relevant to the in situ selective extraction of chromosomal constituents, it was already suggested by HOLTZMAN⁷ that the sequence or combination of the treatments was also important.

The number and position of the +H segment in the present experiment are different from those plotted by OCKEY². The site of +H was confined only to a very tiny segment at the satellite of the M chromosome directly adjacent to the satellite stalk where the nucleolus is

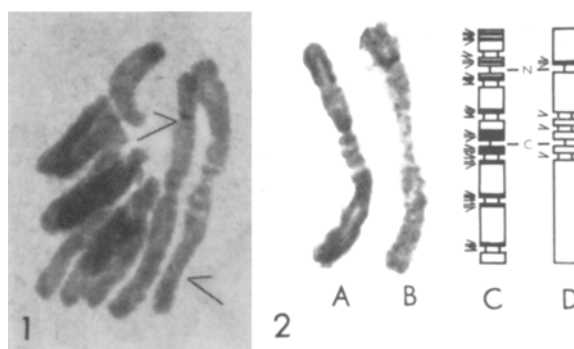


Fig. 1. The metaphase chromosome complement of *Vicia faba* after the application of the procedure shown in the Table. Arrows indicate +H segments in the single pair of M chromosomes.

Fig. 2. M chromosomes at metaphase showing -H and +H segments after various treatments. A, treatment with HCl-acetic acid⁹; B, present method; C and D, schematic representation of M chromosome. The position of the centromere (C), nucleolus organizing region (N), -H (gaps indicated by single arrows) and +H segments (black parts indicated by double arrows) as described by OCKEY² (C) and myself (D).

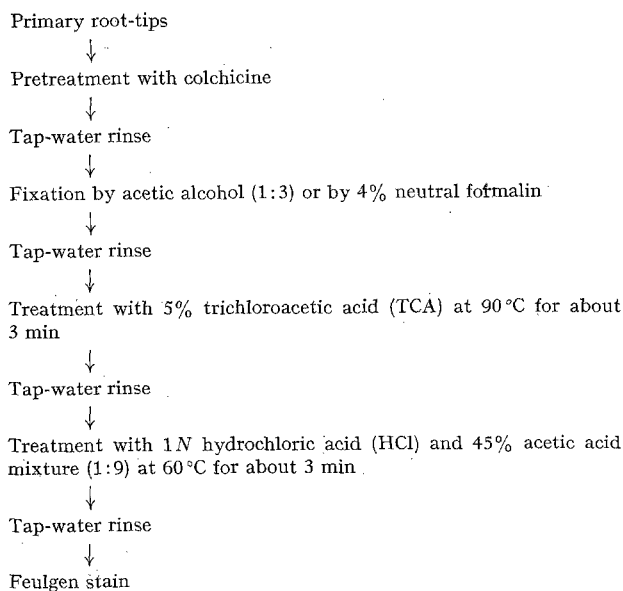
formed (Figures 1 and 2). McCLINTOCK⁸ reported that in *Zea mays* the part of the SAT chromosome immediately proximal to the satellite stalk is responsible for the organization of the nucleolus and this part shows positively heterochromatic, deep staining characteristic at pachytene. Therefore, the +H segment in the present case may be a nucleolus organizing body in *Vicia faba*¹⁰.

Zusammenfassung. Nach sukzessiver Behandlung der Wurzelspitze von *Vicia faba* mit 5%iger Trichlorsäure und dem Gemisch von einem Teil 1 N HCl und 9 Teilen 45%iger Essigsäure treten bei Feulgenfärbung die schmalen, tief gefärbten +H-Segmente der Trabanten beider metaphasischen M-Chromosomen direkt an der Astbasis hervor. Das +H-Segment ist als «nucleolus organizing body» nach McCLINTOCK⁸ anzusehen.

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Scheme outlining the procedure for demonstration of +H segment



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