Negative Heterochromatin, Positive Heterochromatin, and Chromosome Condensation in *Vicia faba*

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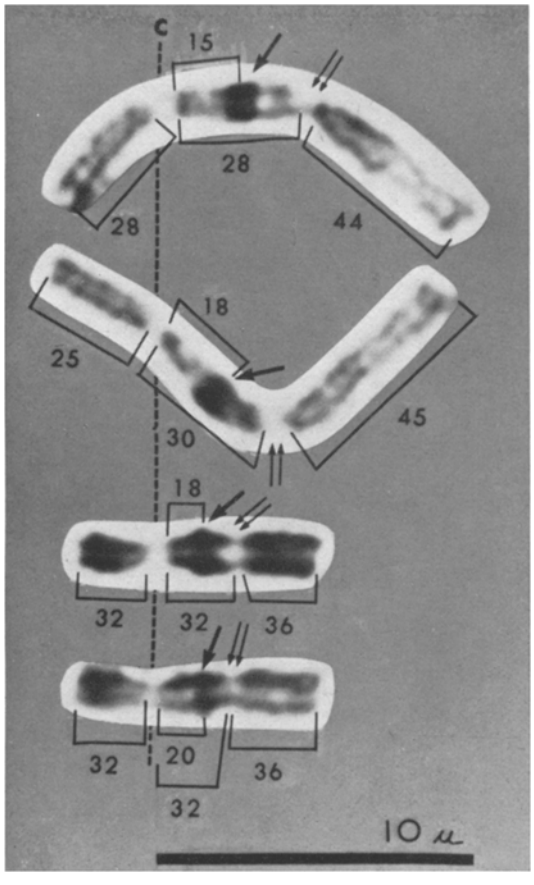
Summary. In *Vicia faba,* cold treatment disturbs more effectively the vanishing from late prophase on of the −H (negatively heterochromatic segment) than that of the +H (positively heterochromatic segment), and eventually produces the −H and +H revelation on metaphase chromosomes.

Various methods to differentiate the heterochromatic (H) segments of metaphase chromosomes, such as Q-, G-banding, have become very useful tools to probe the longitudinal differentiation of chromosome structure. Characteristics of each H segment within a karyotype are being disclosed through understanding the differentiation mechanism in each method. Among these various banding methods the cold treatment has been known for decades as the method to reveal negatively heterochromatic, i.e., faintly stained segments (negative heterochromatin, −H) on metaphase chromosomes. Differentiation of −H brought about by cold treatment is considered to be due to the differential chromosome condensation between heterochromatin and euchromatin (E)[2–5]. In *Vicia faba* the existence of cold-induced −H has been well known[6,7]. However, some[8–10] reported the simultaneous occurrence of −H and +H (positively heterochromatic, i.e., more darkly stained segments than E) in the cold treatment. Ockey[8] claimed that the tightly-coiled +H occurred at the expense of loosely-coiled −H. For better understanding of the mechanism of cold-induced H revelation, it is important to investigate whether or not the +H occurrence is −H occurrence-dependent as Ockey supposed, and to know how the differential chromosome condensation correlates with the simultaneous +H and −H occurrence.

Materials and methods. Since it has been shown that in *Vicia faba* the +H in the middle of the long arm of the 5th chromosome of the haploid complement appears clearly[8,10], the relative position and the occurrence frequency of +H and −H in the 5th chromosome was investigated at late prophase and at metaphase, respectively. In the standard karyotype of *Vicia faba,* it is difficult at prophase correctly to distinguish the 5th chromosome among the 10 S chromosomes which are very similar in size and shape. Thus the reconstructed karyotype ACB[11,12] of *Vicia faba,* which was produced by Michaelis and Rieger, was used in this experiment. In ACB the +H and −H of the 5th chromosome of the standard karyotype are in the structurally changed 5th chromosome, which is easily identifiable among the 12 chromosomes of the complement[12]. Seedlings of *Vicia faba* karyotype ACB were cold-treated at 0 ± 0.5 °C for 2 days. Main root tips were excised, pretreated with 0.05% colchicine at 0 °C for 2.5 h before fixation with La Cour 2BD at 0 °C for 30 min. After bleaching in 0.05% H_2O_2 for 5 min, roots were hydrolyzed in 1 *N* HCl at 60 °C for 20 min, stained by Feulgen's, and squashed.



Cutouts of the 5th chromosome in the complement of cold-treated *Vicia faba* karyotype ACB. Upper two, chromosomes at late prophase; lower two, at metaphase. C, centromere; single arrow, +H; double arrow, −H. The numbers indicate the share of chromosome segments in the total length of the 5th chromosome.

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Frequencies of the cold-induced +H and −H on the long arm of the 5th chromosomes in *Vicia faba* karyotype ACB

	Cells observed	% of 5th chromosomes showing			
		+H and −H	Only +H	Only −H	Neither +H nor −H
Late prophase	22	52	25	9	14
Metaphase	40	21	22	34	22

Late prophases and metaphases in which 12 chromosomes of the complement were interdistinguishable and well spread were chosen and photographed. Count and judgement of +H and of −H were made on the positive prints with magnification × 2600.

Results and discussion. The cold treatment revealed that the 5th chromosome possessed one +H and one −H on its long arm (Figure). The frequencies of the +H and those of the −H at late prophase and at metaphase are summarized in the Table. At late prophase, the +H and the −H appeared with similar frequencies (+H, 77%; −H, 61%). The chromosomes showing neither +H nor −H increased in number at metaphase more than at late prophase. This implies that both +H and −H become less recognizable when the cell cycle approaches metaphase. From late prophase to metaphase, the +H frequencies decreased more rapidly than the −H frequencies (+H, from 77% to 43%; −H, from 61% to 55%). This explains, at least in part, why many researchers who studied cold-treated *Vicia faba* have described only −H. Through chromosome condensation from late prophase to metaphase, the relative position of the +H and that of the −H along the chromosome did not change as shown in the Figure. If the tightly-coiled +H occurs at the expense of loosely-coiled −H, the chromosomes showing only +H are not to be observed. This, however, was not the case, as shown in the Table. The occurrence of the chromosomes showing only +H supports the view that the occurrences of +H and −H are mutually independent matters, but not that the occurrence of +H is −H occurrence-dependent as OCKEY suggested.

In *Vicia faba* the chromocenters, i.e., heterochromatin regions at interphase, decondense transiently to a euchromatic state twice during mitotic prophase[13]. The H disappears first at the beginning of prophase ('Zerstäubungsstadium'[13]), and reappears at the stage subsequent to the 'Zerstäubungsstadium'. As prophase proceeds, chromosomes undergoing spiralization become recognizable separately as elongated threads (spiral-prophase[13]). At an early stage of this spiral-prophase, the distinction between H and E becomes impossible again; i.e., H disintegrates to E. In this experiment, the prophases which corresponded to the early stage of spiral-prophase did not show any H differentiation. As chromosome condensation proceeds, H reappears (late prophase in this paper). It was known that without the cold treatment both +H and −H became apparent at this late prophase[10, 14]. Afterwards, in the case of non-cold-treatment, this H differentiation vanishes up to metaphase. It is considered that cold treatment disturbs the vanishing of the H differentiation from late prophase on and eventually brings about the +H and −H revelation on metaphase chromosomes.

The cold-induced −H has been shown in *Trillium*[4, 15] and in *Vicia faba*[16] to be a differentially darker stained segment by Giemsa banding methods. The present results showed that +H and −H were independent phenomena. In Chinese hamster, the darker Giemsa bands were the tightly-coiled, i.e., +H chromosome segments[17]. In *Vicia faba*, too, some of the Giemsa-positive segments revealed by Giemsa banding methods[12, 15, 16, 18, 19] may correspond to cold-induced +H segments. This, however, is at present an open question.

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Genetic Control of LDH Isozymes in the *Rana esculenta* Complex

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Summary. Studies of LDH isozymes in the European green frogs showed that the synthesis of the B subunits is controlled by 3 alleles at a single genetic locus. The genetic evidence supports the hypothesis that *Rana esculenta* is the hybrid of *R. lessonae* × *R. ridibunda*.

Hybridization experiments and biometric studies of the 3 types of European green frogs indicate that *Rana lessonae* and *Rana ridibunda* are 2 distinct species, while *Rana esculenta* represents their hybrid[2–6]. Cytological analysis of the karyotypes[7], as well as electrophoretic examination of the serum proteins[8–11], support this conclusion. In order to obtain further information about the taxonomic relationships of these 3 frog types, we carried out a detailed study of the genetic control of the lactate dehydrogenase (LDH) isozyme. The present paper is a brief report of this study. The detailed results will be published later.

Materials and methods. Adult frogs were collected from the vicinity of Zürich and kept in running water in the laboratory. The different crosses, which were partly made by artificial fertilization, are summarized in Table I.