

## The Frequency of Polyembryony and Chlorophyll Deficiency in Rye

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THE studies by Kappert<sup>1</sup> in *Linum* and those by Ramiah, Parthasarathi and Ramanujam<sup>2</sup> in *Oryza* showed that some plants, developing from twin seedlings are haploids. Namikawa and Kawakami<sup>3</sup> raised adult plants from wheat twins and found haploid, triploid and tetraploid plants among

somatic chromosome number, namely  $2n = 14$ . We also grew 39,606 seedlings in sowing 53,780 grains of *Secale cereale*—like derivatives from the interspecific cross *S. cereale* × *S. montanum*. Among these seedlings we found 32 twins and 7 albinos (Table I). Sixty plants of the twins had

TABLE I

| Plants   | Seeds sown | Seedlings grown | Pairs of twins found |           | Triplets | Albinos |           |
|--|------------|-----------------|----------------------|-----------|----------|---------|-----------|
|  |            |                 | Number               | Per cent. |          | Number  | Per cent. |
| <i>Secale cereale</i> var. <i>viatka</i> ..  | 27,020     | 10,393          | 5                    | 0.029     | 1        | 27      | 0.132     |
| <i>Secale cereale</i> -like derivatives from <i>S. cereale</i> × <i>S. montanum</i> .. | 53,780     | 39,606          | 32                   | 0.08      | ..       | 7       | 0.0176    |
| 2411 <i>Triticum vulgare</i> ..  | 23,220     | 18,275          | 4                    | 0.021     | ..       | ..      | ..        |

those with normal chromosome numbers. Studies in this field were carried out more recently on a large scale by a series of investigators. I shall recall here those by Harland,<sup>4</sup> Müntzing,<sup>5</sup> etc., which also showed that polyembryony leads to euploid chromosome alterations.

At the present time we have good methods for producing polyploid plants (colchicine, acenaphthene, bromnaphthalene, bromacenaphthene, etc.), but no reliable methods for producing haploids.<sup>6</sup> X-rays, abnormal temperatures and interspecific hybridizations seem to be less effective in this respect than polyembryony.

Haploids were reported in a large number of plants, when the latter are viable in highly inbred (homozygous) condition. *Secale cereale* is a cross fertilizer, which "degenerates" when inbred, therefore haploids from this plant would usually be lethal or semi-lethal. I attempted to verify this deduction. In doing this 27,020 grains of *Secale cereale* var. *viatka* were sown in boxes with sand. The surface was divided into 1 cm. × 1 cm. squares and then single grains were placed in each square half covered in sand. The seeds were watered regularly. Thus we raised 20,393 seedlings from 27,020 grains. Among these seedlings we found 5 twins, one triplet and 27 albinos. All ten plants from the twins and two of the triplet, that were studied cytologically had the normal

14 somatic chromosomes, and one of them was a triploid (N 38) having 21 somatic chromosomes. Six plants of the twins died in an early stage of development, three of which were not studied cytologically. One of the diploid twins was a structural hybrid forming: (1)  $5^{II} + 1^{III} + 1^I$ , (2)  $5^{II} + 1^{IV}$  and (3)  $6^{II} + 2^I$ ; its twin plant being normal. Triploid plant formed a large percentage of abortive pollen, on the average 72 per cent. (July), 68 per cent. (September), 84 per cent. (October). Most of the pollen grains appeared usually in two when matured and studied in aceto-carminic preparations, one or both of the twin pollen being usually abortive. When only one of the twin pollen is abortive, the viable twin pollen was much larger than the abortive one (Figs. 1 and 2).

During the first meiosis we found 0 to 5 trivalents, most frequently, however, 2, 3 and 4 trivalents were formed. The plant formed eight spikes, six of them had altogether 216 spikelets, i.e., 432 flowers. These six spikes set 8 grains from free pollination, two of the grains being small, shrunken with bad embryos. They did not germinate. Two spikes were bagged. No seeds were set under the bags.

The studies upon polyembryony in rye were followed up with similar studies in soft wheat—a plant that can be highly homozygotized without "degeneration" symptoms.

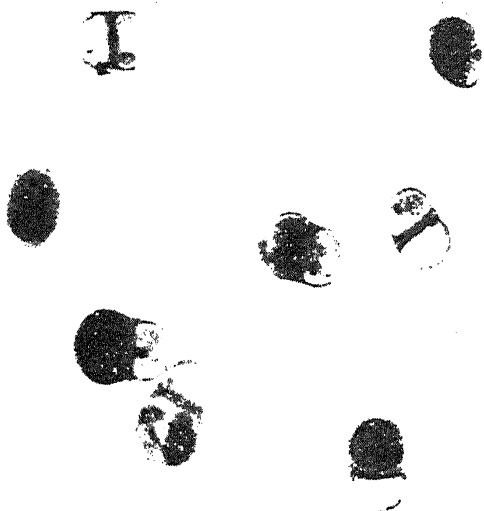


FIG. 1

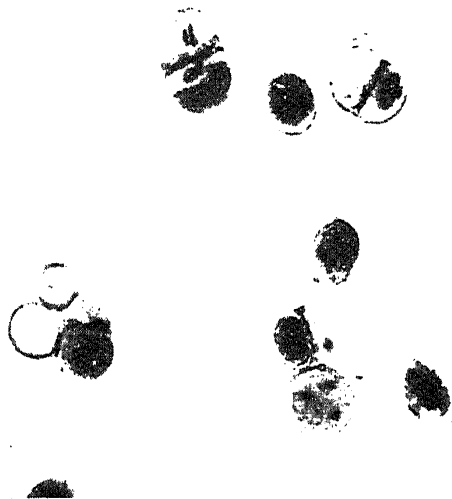


FIG. 2

Pollen from the triploid rye

We raised 18,275 seedlings from 23,220 grains of *Tr. vulgare* No. 2411. Among these seedlings we found four pairs of twins (Table I). Two seedlings of two different pairs were haploid ( $n = 21$ ) while the other six seedlings developed into normal diploid ( $(2n = 42)$ ) plants. Haploid plants were smaller, than the normal ones. Their spikes were smaller. During the first meiosis we usually found in aceto-carmin preparations from one of the haploids 21 univalents or one bivalent and 19 univalents. In a few cells two bivalents were found. In a single pollen-mother cell three bivalents were observed.

Out of 73 plants raised from rye twin pairs and studied cytologically only one triploid and one structural hybrid was found but no haploids, while two haploids were raised out of eight wheat twin pairs, i.e., 25 per cent. of haploids.

Until the present time adult haploids of *Secale cereale* have not yet been raised.

Müntzing<sup>7</sup> obtained a semi-lethal haploid rye among the material treated by abnormal temperatures. Absence of haploids among the twin pairs that we studied is due most probably to the dying off of the haploids in an early stage of development; the diploid twin pairs being only able to survive and grow further.

There is a good deal of speculation about the mode of origin of more than one embryo in a single ovule. When polyembryony is a sequence of nucellar embryony, the embryos, the seedlings, and the plants that develop from them should be diploid with maternal genetic constitution. Twin embryos should also be diploid when they originate from two cells derived from the first cleavage products of the fertilized egg. When one embryo develops from the fertilized egg and the other from an endosperm all diploid + triploid twins may originate. When one embryo develops from the fertilized egg and the other from a haploid embryo sac cell (antipodal, for example) diploid + haploid twins will be formed. In the latter case it might happen that diploid + diploid twins develop if chromosome doubling takes place in the haploid embryo at an early developmental stage. Diploid + diploid twins or diploid + diploid + diploid triplets might also originate if two pollen-tubes penetrate the micropyle, the sperm of the one fertilizing the egg nucleus, while the other sperms fertilizing the polar nucleus and one or more than one antipodal nuclei. We do not know yet the mode of origin of tetraploid plants from twins and polyploids of a higher order. Fusion of antipodal cells and then polyspermic fertilization seem to be more probable processes, than chromosome doubling in one of the pairs of diploid + diploid twins at an early stage of development, but the latter alternative is not excluded.

The twins of *Secale* that we studied do not seem to result from nucellar embryony because they differed morphologically.

The normal procedure of the meiosis and normal fertility of the twin plant of the structural twin hybrid also supports this assumption. Structural hybrid formed about 50 per cent. of abortive pollen (Fig. 3) and was partially fertile.

Diploid + diploid *Secale* twins do not seem to result from diploid + haploid twins after a chromosome doubling in the haploid

one at an early developmental stage, because a diploid, derived from a haploid rye

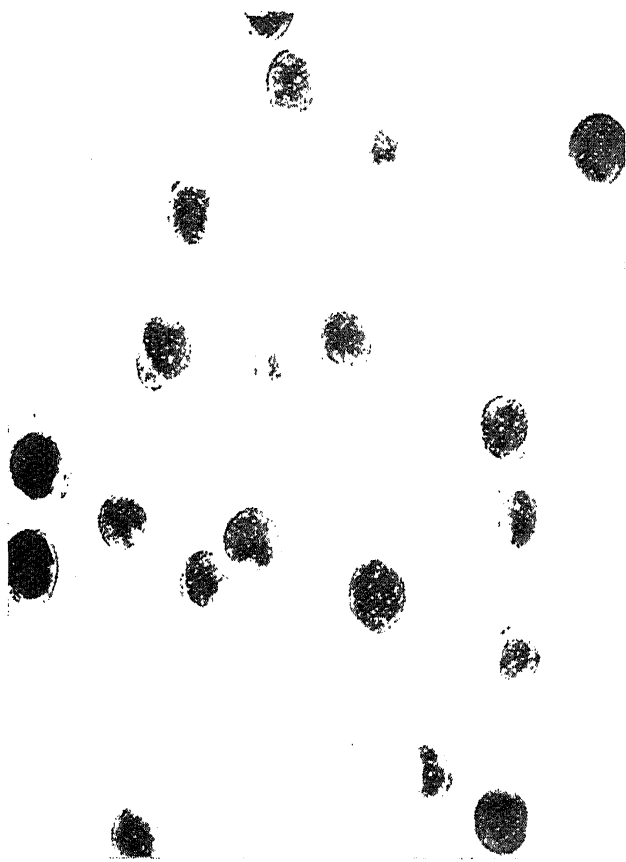


FIG. 3

Pollen from the structural hybrid raised from a twin pair

by somatic doubling will be nearly absolute homozygous form, the latter being usually semi-lethal or lethal. The most probable origin of the twin seedlings in rye that I studied seems to be: one from the fertilized egg and the other from a fertilized antipodal

cell, the triploid resulting, most probably, from an endosperm cell. It seems very probable that usually single plants develop from *Secale* twin diploid + haploid embryos, the haploid usually dying at an early developmental stage.

Polyembryony in angiosperm plants is a very common phenomenon. They are chiefly due, as a rule, to certain deviations in the developmental and fertilization processes in the embryo-sac. They lead to euploid chromosome alterations, which have great evolutionary significance, especially the tetraploid and the haploid with partial auto-syndesis during the meiosis.<sup>6</sup> The frequency of the polyembryony in our experiments is very high. In the rye *Viatka* it is about one per 3,500 and in *Secale cereale* derivatives—one per 1,200. In wheat it was about one per 5,000. The frequency of chlorophyll deficiency (albino seedlings) in *Viatka*—rye was about one per 800 and in *Secale cereale* derivatives it was about one per 6,000.

These numbers show an exceedingly high frequency of hereditary changes.

<sup>1</sup> Kappert, H., *Biol. Zentral.*, 1933, 53, 276.

<sup>2</sup> Ramiah *et al.*, *Curr. Sci.*, 1933, 1, 277.

<sup>3</sup> Namikawa and Kawakami, *Proc. Imper. Acad.*, Japan, 1934, 10, 668.

<sup>4</sup> Harland, S. G., *Jour. Heredity*, 1936, 27, 229.

<sup>5</sup> Müntzing, A., *Cytologia*, Fujii Jub. Vol., 1937, 211.

<sup>6</sup> Kostoff, D., *Bibliographia Genetica*, 14 (in the press).

<sup>7</sup> Müntzing, A., *Hereditas*, 1937, 23, 401.

## Thyroxine from Casein

LUDWIG and Mutzenbecher (*Z. Physiol. Chem.*, 1939, 258, 195) have prepared thyroxine from casein by treating it with iodine under carefully defined conditions in the presence of sodium bicarbonate and subsequent hydrolysis with barium hydroxide. The product, before hydrolysis contains 6.8 per cent. of organic iodine and has a thyroid

activity of 200–500 guinea-pig units per gm. One gm. of this material yields on hydrolysis 50–100 mg. of crystalline thyroxine.

This work has now been confirmed by Harington *et al.* (*Nature*, 1939, 144, 205) who also broadly discuss the mechanism of the synthesis.