SEX AND CHROMOSOMES IN *SPINACIA OLERACEA* L.

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INTRODUCTION

Since *Spinacia oleracea*, a dioecious plant, was cytologically studied for the first time by Stomps (1911), several cytologists have dealt with this plant. Among them Winge (1917, '23), Sinotô (1929), and Tuschnjakowa (1929) attempted to find any unequal chromosome pair or sex chromosome at the meiotic divisions in pollen mother-cells, but they failed to find it and concluded that there is no sign of unequal chromosome pair in the male side of this plant. They, therefore, pointed out the importance of the cytological studies of the female plant as to the problem of sex determination in this plant. The study on this line was undertaken only by Stomps (1911) long before, however, paying any attention to the question here concerned.

To the writer's deep regret, their conclusion that there is no unequal chromosome pair in the male plant was based on figures not absolutely convincing. In this connection the present work was undertaken to prepare more exact figures with which the previous conclusion would be well sustained, and on the other hand, to ascertain whether the unequal pair is present or not in the female plant.

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METHODS

The male flower buds were fixed in Carnoy-2BE and the female ones in Bouin's solution. The both materials were sectioned in paraffin at a thickness of 10 microns and stained with Newton's gentian-violet-iodine methods. Drawings were made with a Leitz Abbe camera lucida using a Leitz 1/12 oil immersion and an eye piece Zeiss K.30 $\times$, giving a magnification of 3,300 diameters. They were reduced for the reproduction to the scale indicated.

OBSERVATIONS

1. *Somatic chromosomes.* The somatic chromosomes were observed only in the male plant. Figure 1 represents a somatic metaphase in an

anther-wall cell, showing 6 chromosome pairs which were indicated by the letter *m* and the numerals 1–5. The *m*-chromosome is the longest having a quite median constriction, and is easily distinguishable from the other five by these two characteristics even at the meiotic divisions. The 1-chromosome is furnished with a very small trabant which was already observed by Tuschnjakowa (1929). The other four pairs 2–5 are distinguishable from one another by their relative lengths and the positions of constrictions. Thus the unequal pair was not discernible in the male somatic cells.

Fig. 1. Somatic metaphase in an anther-wall cell, showing six chromosome pairs. 2000×

2. *Meiotic chromosomes in pollen mother-cells.* The prophasic process is entirely normal as well as those of the later phases. The chromosome behaviours from diaphase up to second anaphase were shown in Figures 2–9. Generally at mid-diaphase the chromosomes take the shape of rings (Fig. 2). With the exception of the *m*-chromosome, they take the

Figs. 2–9. Meiotic divisions in PCMs. 2000×; 2, mid-diaphase; 3, metaphase in polar view; 4, full metaphase in side view *m*-chromosome situating in centre; 5, pro-metaphase; 6, first anaphase in polar view, *m*-chromosome situating in centre at both groups; 7, first anaphase in side view; 8, two second metaphases in a P.M.C. in side and polar view respectively; 9, two second anaphases in a PMC in polar view.

shape of V's from late diaphase to pro-metaphase as the transitional form towards metaphase by the breaking off of the terminal connection at the proximal ends (Fig. 5). At full metaphase they straighten their arms of V's between the two poles, whereas even at this stage the *m*-chromosome remains its ring shaped connections (Fig. 4). The *m*-chromosome was easily identified through all the stages of the meiotic divisions (Figs. 2–9).

Now in order to give the full confirmation for the previously accepted conclusion that there is no unequal pair in the male plant, four complete sets

of the first meta-anaphasic chromosomes in side view, in which the two partners in the bivalent chromosome can be compared with most certainty, were presented in Figures 10-13. And in view of testing the conclusion, the second meta-anaphasic chromosomes in side view from the two complete plates in a pollen mother-cell were drawn out in Figure 14 *a, b*. Thus as will be seen clearly in these figures, no evidence of the size difference between two partners in the bivalent chromosome was obtained at the meiotic divisions in the male plant.

Figs. 10-13, Four complete sets of the first meta-anaphasic chromosomes in side view drawn out from a complete PMC each, showing *m*-chromosomes at the left hand. 2000×

Figs. 14 a, b. Two complete sets of the second meta-anaphasic chromosomes in side view drawn out from a complete PMC, showing *m*-chromosomes at the left hand.

Fig. 15, Diaphase in an EMC, showing the complete set of chromosomes. Nucleolus is budding. 2000×

3. *Meiotic chromosomes in embryosac mother-cells.* The prophasic process was regular as in pollen mother-cells. In several cases the first meta-anaphasic chromosomes in side view were observed, although the complete set of them was missed, to be equal in their paired partners. Fortunately at diaphase a complete nucleus very clear in this respect was observed, from

which Figure 15 was drawn. This figure will be enough to show how the chromosome partners are equal in their sizes. Here it is interesting to note that the chromosomes in embryo-sac mother-cells are much larger than those in pollen mother-cells at the same stage of diaphase, though the fixing agents are different in these two cases (cf. Figs. 2 and 15).

DISCUSSION

As the present observations indicate, no unequal chromosome pair exist in both sides of the sexes of this plant. It may be possible, however, to give certain inference on the mechanism of sex determination in this plant on the consideration of some genetic results hitherto reported for this plant and also on the comparison of them with similar cases in other plants.

Nohara (1923) studying genetic behaviours of some characters of *Spinacia oleracea* found the sex ratio to be 495 females : 496 males, closely approaching the ratio 1 : 1. In this connection Rosa (1925) obtained very important results from his extensive breeding experiments with this plant. It was shown that nutritive conditions, intensity of light, space between plants in the row, date of planting, and mutilation do not affect the sex ratio, showing as a whole 50.5% male, 49.3% female, and 0.2% intersexes among 5,198 plants. Thus it must be considered, as he pointed out, that the sex expression in this plant is due to genetic factors and not to environmental or physiological conditions. The evidence suggests the existence of a pair of genetic factors, in other word, a pair of chromosomes responsible for the sex determination in this plant. Furthermore Hirata and Yamamoto (1931) selfing the female intersexes of this plant obtained only females and female intersexes, namely 16 and 92 plants respectively among 108 offsprings. If one compares this with the breeding results of uni- and intersexual plants of *Cannabis sativa* (Hirata 1927, '29, '31), it will be inferred that the female side of *Spinacia oleracea* is homozygous for the sex determining factor or factors. Consequently, although the cytological demonstration fails, the male side of this plant would be heterozygous for the factor or factors. Thus the present case seems to be interesting in that the genetic factors differentiate into two sexes, but the morphological differentiation of the chromosomes does not occur or is too minute to observe readily, namely, the X- and Y-chromosomes in the male side are morphologically indistinguishable from each other and behave as the autosomes.

The following plan of investigations will solve the problem conclusively : 1) cytological studies on both sexes in different species and races, 2) breeding experiments of the intersexual plants, especially of the male intersexes, and

3) genic analysis searching for the sex-linked factors. The writer wishes to extend his investigations along these lines in near future.

SUMMARY

1. The gametic chromosome number 6 (in both male and female) and the somatic number 12 (in male) of *Spinacia oleracea* were counted in agreement with the previous observations.
2. Cytologically no unequal pair of the chromosomes were observed in both sides of the sexes.
3. An inference was made from the genetic results hitherto reported that the female is homozygous and the male heterozygous for the sex determining factor or factors, namely, the present case suggests the X-Y type of sex determination where X- and Y-chromosomes are morphologically indistinguishable from each other and behave as the autosomes.

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