POLYPLOID MUTATIONS IN CREPIS. TRIPLOID AND PENTAPLOID MUTANTS OF *CREPIS CAPILLARIS*

M. NAVASHIN

Timiriazev Federal Institute of Scientific Research, Moscow, Russia

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INTRODUCTION

Polyploidy or the increase of the number of basic haploid chromosome sets above the normal double number, is justly attracting the attention of cytologists and geneticists. Besides numerous cases of polyploidy in separate somatic cells, cases are generally known in which all cells composing the organism contain more than two haploid sets of chromosomes. This last category of cases deserves special attention, as this is the simplest form of mutation, yielding itself most easily to investigation.

However, not all described cases of polyploidy are indubitable, as the majority of authors citing them base their conclusions entirely on counting the chromosomes, although it is quite clear, that the counting in itself is insufficient, because the number of chromosomes may increase for other reasons having nothing in common with polyploidy; for instance, from the crossing of species with many chromosomes, or as a consequence of segmentation of the chromosomes, etc. (GATES 1924). Thus, I have proved (NAVASHIN 1925) that in 4- and 5-chromosome species of Crepis there are present new additional chromosomes, not peculiar to a 3-chromosome set, contrary to the established opinion that the "not x-ploid conditions," within the limits of the genus, originate from the repetition of definite chromosomes already contained in the "fundamental" 3-chromosome set of *Crepis capillaris* (L.) Wallr. (=*virens*).

The cases of polyploidy which I have mentioned here are especially interesting in that they do not permit the slightest doubt. Thanks to extremely sharp morphological differences between the chromosomes of

Legend for Plate 1

All the figures were made by means of an Abbé camera lucida, with homogeneous immersion 2 mm objective and compound ocular 18, at the level of the working table.

The ends of chromosomes, turned toward the observer, are schematically represented by white circles.

Figures 1, 2 and 3 belong to the diploid individual; 4, 5 and 6, to the triploid one; and 7 and 8, to the pentaploid one.

In reproduction the original drawings were reduced about 1.5 times.

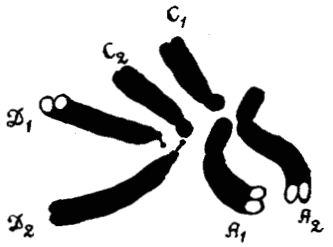

Fig. 1

Fig. 2

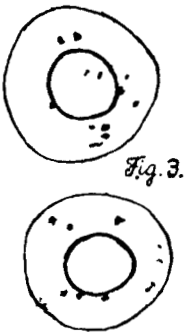

Fig. 3.

Fig. 4.

Fig. 5

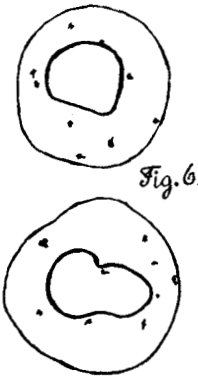

Fig. 6.

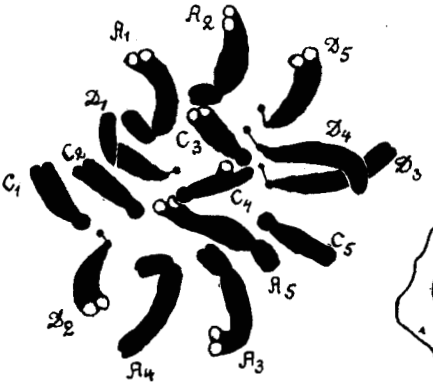

Fig. 7.

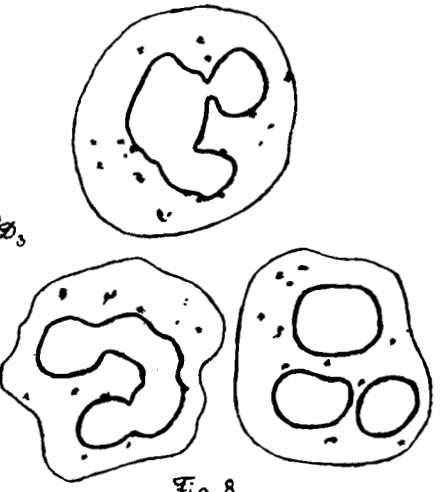

Fig 8.

Crepis capillaris, I could establish with complete certainty, that in the nuclei of triploid and pentaploid mutants there are actually present homologous chromosomes, three of each in the former and five in the latter.

MATERIAL

The material which I have used for my observations was derived from the same specimen (H_5) of *Crepis virens* the caryological study of which has been published in another article (NAVASHIN 1925). The seeds left over from this research were sown in April, 1924, in my seed-plot at Tiflis, and gave 10 individuals which bloomed on the 20th of June and bore abundant fruit. About 150 seedlings, grown from the newly collected achenes, were subjected to a careful caryological study and two polyploid plants were discovered, one triploid and the other pentaploid.

Unfortunately, I could not keep these interesting plants, as they had been destroyed during the fixation of the young roots. But a large stock of seeds, which I am reserving for sowing during the coming spring, will, I hope, give at least several such polyploid individuals. (It is quite improbable that only a single pair of mutants should have occurred accidentally in the first 150 of many thousands of seeds, obtained from one and the same source and under entirely similar conditions.) During the next season I shall sow all the seed I possess for caryologic investigation and the polyploid individuals will, of course, be kept for further study.

The morphology of the cell nucleus of *Crepis capillaris* has been examined in detail in my previous article. It will be sufficient here briefly to characterize it. The normal diploid nucleus (figure 1, plate 1) contains three sharply different haploid chromosomes: one long A-chromosome of two links, one short-capitate C-chromosome and one D-chromosome, supplied with a satellite. These morphological characters of the chromosomes are so constant and clear, that the recognition of the three chromosome forms presented no difficulty in any case.

THE TRIPLOID MUTANT

One of the 150 seedlings appeared to contain constantly nine chromosomes in the cell nuclei of the root-tips. The homologous chromosomes were in threes and, using my previous symbols for different forms of chromosomes, I am designating the nucleus of this mutant thus: 3A, 3C, 3D. In figure 4 can be seen, without further explanation, the triploid composition of the nucleus of this individual; two D-chromosomes are lying obliquely to the plane of section, it is true, but they are unmistakably

recognizable by their satellites; as for the rest, every chromosome can be identified with its homologous chromosome of the normal set, illustrated in figure 1.

In the behavior of the nucleus of this mutant I have observed no peculiarities. The division proceeds entirely normally and I was even able to establish that the satellites, present here in the number of three, arise on the nucleolus just as in the normal (diploid) nucleus, in the prophases. This stage may be seen in figure 5; for comparison the same condition of the diploid nucleus is illustrated in figure 2.

A certain peculiarity is seen in the resting nucleus in that the nucleolus tends to assume a "biscuit-like" shape (figure 6) which suggests the approaching division. In normal (diploid) individuals this has almost never been observed (figure 3).

The dimensions of the nuclei and cells of the described individual are noticeably increased; if the average dimension of the meristem cell of the root of a diploid individual be taken as 1.0, the corresponding dimension in the triploid mutant will be equal to 1.3; and the relation for nuclei (resting) is 1.0 : 1.9. This increase of the size of the root cells is reflected in the enlargement of the whole organ; thus, the root of the triploid individual is considerably thicker than that of the diploid plant.

THE PENTAPLOID MUTANT

This remarkable mutant was discovered in the same material from which the triploid individual originated. In its cell nuclei there were constantly 15 chromosomes, five each of the usual three forms, A, C and D, as shown clearly by figure 7, illustrating the metaphase of the nuclear division of this mutant. The chromosomal formula of this individual is, therefore, 5A, 5C, 5D.

The division of the cell-nuclei in this plant is also entirely normal. The lobulation of the nucleolus, as noted in the resting nucleus of the triploid individual, is here greatly increased. As may be seen from figure 8, illustrating the resting nuclei of the pentaploid mutant, there are nearly always several nucleoli present, or at least, the nucleolus appears elongated, with several interruptions, indicating an approaching breaking up into parts.

The dimensions of the cells and of the nuclei included in them, as well as the dimensions of the whole root, appear greatly enlarged. The cells of the pentaploid mutant exceed those of the diploid individual in average volume 2.5 times, and the resting nuclei appear enlarged 4.1 times.

DISCUSSION

These cases of polyploidy raise a whole line of interesting questions. First of all, a decision must be reached concerning their origin. Generally, it is considered established that polyploidy can arise in two basic ways: one of these may be called vegetative (for instance the polyploid chimera of WINKLER (1910), formed by the fusion of vegetative nuclei in wounded cells); the other may be called generative (GATES 1924). There is no doubt that my described mutants could arise only in the latter way. Two possibilities may be suggested also as to how generative polyploidy may arise: (1) as a consequence of polyspermy; and (2) as a result of a combination of gametes with increased number of chromosomes, as compared with the normal haploid condition. Polyspermy appears to me to be highly improbable, for, although dispermy might possibly occur occasionally (it has never been observed by any one), yet the simultaneous union of more than two sperms with one egg is even less probable. The possibility that four generative nuclei fused with the nucleus of the egg cell, thus giving rise to my pentaploid mutant, cannot, in my opinion, be admitted. The case becomes entirely different, if we assume that these two mutants arose from a combination of gametes containing more than one haploid chromosome set. According to the well-known idea of DE VRIES (1917), I imagine the origin of the triploid mutation to have resulted from the union of a normal, unchanged haploid gamete with a diploid one. That diploid gametes frequently occur in plants is proved by many instances. Besides the "normal" sporogenesis of tetraploid individuals such abnormal gametes may result from an abnormality of the reduction division,—even its complete omission. Such abnormalities of reduction are not rare phenomena, and may be supposed to arise oftenest under the influence of external agencies, probably the most important of which is the temperature, as is indicated especially by the work of DE MOL (1923). By increasing the temperature during the process of pollen formation DE MOL secured diploid pollen grains, and applying this pollen to the stigmas of normal individuals of the same species, he obtained seeds, which gave triploid plants. It is not improbable that in my cultures there prevailed conditions similar to those which were created artificially by DE Mol in his experiments. The Tiflis climate is distinguished by the quick approach of a dry and extremely hot summer; the development of the plants proceeds very quickly, and my plants of *Crepis capillaris*, grown under these unusual circumstances, experiencing a period of accelerated development, probably gave a certain quantity of diploid

pollen. It is perfectly clear that the meeting and combination of two diploid gametes is very much less probable, than the combination of a diploid gamete with a normal haploid one. Thus, the origin of the triploid mutant seems to me sufficiently clear.

It is more difficult to imagine the origin of a pentaploid mutation. The most probable manner of origin of such a mutant, it seems to me, is by the combination of a tetraploid gamete with a normal haploid one. No one has, so far as I know, reported the occurrence of tetraploid gametes, but it is possible to imagine them, theoretically, as a result of non-disjunction of daughter chromosomes after the homeotypic division, if the preceding reduction were omitted, (as during the formation of diploid gametes).

Particular experiments which I have undertaken in this direction will, I hope, explain this question.

However these mutants may have originated, they are of extreme interest for genetics. If they prove to be capable of forming viable gametes, their progeny, thanks to their varied nuclear composition, will give valuable material for research. Based on analogous observations (BELLING 1924), one may think that the extra three chromosomes of our mutants, not finding for themselves homologues, will have to be distributed during sporogenesis, between the daughter nuclei of the diads, in all possible combinations, according to the law of chance. Table 1 includes all kinds of chromosome combinations, which must arise during sporogenesis by triploid and pentaploid individuals.

TABLE 1

Chromosome combinations which must arise during sporogenesis.

TRIPLOID MUTANT			PENTAPLOID MUTANT		
Number of chromosomes	Formula	Extra chromosomes	Number of chromosomes	Formula	Extra chromosomes
3	1A, 1C, 1D		6	2A, 2C, 2D	1A, 1C, 1D
4	2A, 1C, 1D	1A	7	3A, 2C, 2D	2A, 1C, 1D
4	1A, 2C, 1D	1C	7	2A, 3C, 2D	1A, 2C, 1D
4	1A, 1C, 2D	1D	7	2A, 2C, 3D	1A, 1C, 2D
5	1A, 2C, 2D	1C, 1D	8	2A, 3C, 3D	1A, 2C, 2D
5	2A, 1C, 2D	1A, 1D	8	3A, 2C, 3D	2A, 1C, 2D
5	2A, 2C, 1D	1A, 1C	8	3A, 3C, 2D	2A, 2C, 1D
6	2A, 2C, 2D	1A, 1C, 1D	9	3A, 3C, 3D	2A, 2C, 2D

From this table we can see, that both of our mutants must form gametes of 8 different kinds. The triploid mutant will form gametes with different

numbers of chromosomes, ranging from normal haploid up to diploid ones, and the pentaploid from diploids up to triploids.

The progeny anticipated from these mutants would be particularly remarkable. The nuclear composition of all the different zygotes which, according to this theory, must be formed by self-pollination and by crossing with a normal individual, are listed in table 2.

TABLE 2

Chromosome combinations expected in the progeny of two mutants.

TRIPLOID MUTANT						PENTAPLOID MUTANT					
Self-pollination			Crossed with normal			Self-pollination			Crossed with normal		
Number of chromosomes	Number of zygotes	Formula	Number of chromosomes	Number of zygotes	Formula	Number of chromosomes	Number of zygotes	Formula	Number of chromosomes	Number of zygotes	Formula
6	1	2A, 2C, 2D	6	1	2A, 2C, 2D	12	1	4A, 4C, 4D	9	1	3A, 3C, 3D
7	2	3A, 2C, 2D	7	1	2A, 3C, 2D	13	2	5A, 4C, 4D	10	1	4A, 3C, 3D
7	2	2A, 3C, 2D	7	1	3A, 2C, 2D	13	2	4A, 5C, 4D	10	1	3A, 4C, 3D
7	2	2A, 2C, 3D	7	1	2A, 2C, 3D	13	2	4A, 4C, 5D	10	1	3A, 3C, 4D
8	1	4A, 2C, 2D	8	1	2A, 3C, 3D	14	1	6A, 4C, 4D	11	1	3A, 4C, 4D
8	1	2A, 4C, 2D	8	1	3A, 3C, 2D	14	4	5A, 5C, 4D	11	1	4A, 3C, 4D
8	1	2A, 2C, 4D	8	1	3A, 2C, 3D	14	4	5A, 4C, 5D	11	1	4A, 4C, 3D
8	4	2A, 3C, 3D	9	1	3A, 3C, 3D	14	4	4A, 5C, 5D	12	1	4A, 4C, 4D
8	4	3A, 3C, 2D				14	1	4A, 6C, 4D			
8	4	3A, 2C, 3D				14	1	4A, 4C, 6D			
9	2	2A, 4C, 3D				15	2	4A, 6C, 5D			
9	2	4A, 2C, 3D				15	2	4A, 5C, 6D			
9	2	2A, 3C, 4D				15	8	5A, 5C, 5D			
9	8	3A, 3C, 3D				15	2	6A, 4C, 5D			
9	2	3A, 2C, 4D				15	2	6A, 5C, 4D			
9	2	3A, 4C, 2D				15	2	5A, 4C, 6D			
9	2	4A, 3C, 2D				15	2	5A, 6C, 4D			
10	1	2A, 4C, 4D				16	1	4A, 6C, 6D			
10	1	4A, 2C, 4D				16	4	6A, 5C, 5D			
10	1	4A, 4C, 2D				16	4	5A, 5C, 6D			
10	4	3A, 3C, 4D				16	4	5A, 6C, 5D			
10	4	4A, 3C, 3D				16	1	6A, 4C, 6D			
10	4	3A, 4C, 3D				16	1	6A, 6C, 4D			
11	2	3A, 4C, 4D				17	2	5A, 6C, 6D			
11	2	4A, 3C, 4D				17	2	6A, 5C, 6D			
11	2	4A, 4C, 3D				17	2	6A, 6C, 5D			
12	1	4A, 4C, 4D				18	1	6A, 6C, 6D			

From this table it may be seen, that both mutants by self-fertilization must give, according to the usual scheme, zygotes of 27 different kinds,

and consequently, descendants of the triploid mutant will consist of individuals with all kinds of nuclear composition from the normal diploid to the tetraploid; and the descendants of the pentaploid will range from tetraploid up to hexaploid. The most frequent combination (8 in 64, that is, 12.5 percent) in the descendants of both mutants will be again of the parental type,—respectively, triploid or pentaploid. Besides, in the descendants of both mutants there are 8 different homozygotes, which are capable of continuation without segregation.

The greatest interest from the genetic view-point is found in the 7- and 8-chromosome combinations from the descendants of the triploid mutant: 3A, 2C, 2D; 2A, 3C, 2D; 2A, 2C, 3D; 4A, 2C, 2D; 2A, 4C, 2D; 2A, 2C, 4D. If they prove to be viable they should solve an extremely important question about the localization of groups of genes in this or that one of the three chromosomes of the haploid set; such combinations will be extremely rare, but they are especially suitable material for investigating the influence of any definite chromosome on the phenotype.

The next task of my research will be the thorough study of these polyploid mutants, the investigation of their sporogenesis, and the exact cytologic and hybridologic analysis of their progeny.

SUMMARY

1. Among 150 seedlings of *Crepis capillaris* (L.) Wallr. two polyploid mutants were discovered. One was triploid, the other pentaploid. Three sets of three homologous chromosomes, identifiable by their characteristic form, were found in the nuclei of the triploid individual, and three sets of five homologous chromosomes in the nuclei of the pentaploid individual.

2. The hypothesis is proposed that these mutants owe their origin to the unusual climatic conditions under which their parents grew, which influenced the reduction division in such manner that some of the spores (more probably microspores) arose without reduction, thus giving rise to pollen-grains with diploid nuclei. The pentaploid individual is believed to have arisen from the combination of a tetraploid gamete with a normal haploid one.

3. The progenies of these remarkable mutants should be exceptionally favorable objects for genetic research. Among the numerous expected combinations, several should permit a quick solution of the question as to the localization of different groups of genes in this or that one of the three chromosomes of the haploid set in *Crepis capillaris*.

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