A Cytological Study of *Oryza sativa* L.*

By

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(With Plate VIII).

Recent studies have brought to light several interesting phenomena relating to the difference of chromosomes and their behavior in certain plant species of very close affinities. Thus the comparative studies of closely allied plant species or varieties became a subject of certain interest. Inasmuch as some closely allied plants show differences of chromosomes, while others present no such tendency it is desirable to know what groups of plants have generally such tendency. For the study of the real significance of this chromosome difference, investigations of cultivated plants rich of races were suggested by Professor Fujii. Such plants may be considered as plants which are liable to certain changes, and it is likely that this changeable nature is correlated in certain manner with the internal cellular structure, especially chromosomes and their behavior. *Oryza sativa* L. or the rice-plant, which I have chosen for the purpose of investigation is the most important of the cultivated plants in Japan; and of this plant there are so many races as they are counted by thousands. Among them, I have studied chiefly the race 'Shiriki,' partly the races 'Kishūwase,' 'Usuaka,' 'Kurobo' (all these races belonging to subsp. *Oryza utilissima* Kcke.), 'Kuromochi' and 'Akashimochi', (these two races belonging to subsp. *Oryza glutinosa* Lour.). So far as these races are concerned, however, I could ascertain in the main only a little difference of sizes of chromosomes in the maototic phase, but an

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abnormal case with a considerable difference was found, and a variable number of chromosomes was also observed in the somatic cell. Besides these points, some peculiarities in the development of the gametophytes, inclusive the formation of the endosperm in ‘Shinriki’ will be described in this paper.

The material was collected in a rice field in the vicinity of the city of Osaka, and was fixed either with the chrom-acetic mixture or chrom-osmium-acetic mixture. The microtome-sections were made generally 5–10 µ thick. For staining, Heidenhain’s iron-alum-haematoxylin was used in general, but sometimes Flemming’s safranin-gentian-violet-orange was used. In the former case I have often used congo-red as after-stains for the differential staining of chromatin and limin-substance.

I. Mioticis in the Development of Pollen-Grains.

In the young stage of the pollen-mother-cell-nucleus, there are several masses or aggregations of chromatin which Overton (9) has designated as prochromosomes. They are used to lie in pairs (Figs. 1, 2). The number of pairs of these chromatin-masses was found to be nearly equal to the haploid number, though I could not determine it with certainty. In the next stage they begin to grow, take thread-form, and run parallel in pairs (Fig. 3). Then they enter the synapsis stage, in which they conglobate themselves very closely (Fig. 5). In the section cut 2 µ thick, however, the double nature of the threads can be still distinctly observed in this stage (Fig. 6), as the fact itself was also pointed out in other plants by Cardiff (1).

Whether the conglobation of the nuclear substance at the synapsis is a natural process or not has been a subject of question. Some authors took it for an artifact chiefly caused during the process of fixation, while the others thought it to be a natural phenomenon. Since it was observed by Sargant and other investigators in the living state, however, the matter stands in favour of the latter view; and at the same time authors generally tend to regard the synapsis
characterized by the contraction phenomenon or synizesis of McClung as an important stage in the maiosis, as has been first maintained by Moore (8). In 1905 Strasburger (12) expressed a view, that at the synapsis the pangens undergo a definite orientation through the mutual action of the paired gamosomes, so that at the subsequent elongation of the gamosomes, their pangens will get successive positions in such a way as the homologous ones come close to each other. In my case with Oryza sativa the elongation of the gamosomes takes place before the synaptic contraction begins. Consequently we may say, that the conglobation of the nuclear substance does not always occur in a definite moment of the maiotic phase with respect to other behaviors of the chromatin-threads.

Moreover the fact that the synaptic contraction is a natural process, does not necessarily imply that it has an important biological significance.

It is often mentioned that at the synapsis the nucleolus makes its appearance in a lens shape. It seems to be probable that a considerable amount of nutrition is needed during the mitotic preparation partly as the source of energy for the division, and partly as the building material. As the consequence, there must be a strong metabolic interchange between the nucleus and the cytoplasm. That such a strong metabolic interchange or some other special chemical process involved in the maiotic prophase is the cause of this change of shape of the nucleolus, is not improbable. In the living nuclei in the marginal cells of the leaf of Elodea canadensis and in those of the leaf-hair of Tradescantia virginica, Kohl (7) has observed the change of shape of the nucleus, and the shifting of the nucleolus through an increasing substance-interchange between the nucleus and the cytoplasm as a consequence of the addition of asparagin-solution. The similar process may well cause the conglobation of the chromatin-threads at the synapsis, especially because they are very slender at this stage of prophase, and have likely much smaller power of resistance for pressure than in the later stages, where they are much thicker. In this connection we may note that in some cases, as has been clearly described by
Farmer and Moore (2), Grégoire (3) &c., contractions of nuclear substance occur more than once in the meiotic phase. This fact too strengthens the possibility that the contraction phenomenon or synizesis is not biologically an essential process, but a result of strong metabolic activities.

The nucleus in the prophase is often found provided with several dwarf-nucleoli beside the ordinary large one. They are placed close to the large nucleolus or scattered about between the chromatin-threads (Figs. 8, 10), a phenomenon, probably related to the metabolic activity in the nucleus and to the nutrition of chromosomes.

In the next stage the chromatin-threads increase in length and thickness, and the synaptic ball begins to loosen (Fig. 7). and the chromatin-threads stretch out over the nuclear cavity. The paired threads become closer and closer, till finally they unite themselves into one (Fig. 8). As has been pointed out by Overton (9), the moment of actual union seems to vary in different cases: a case of pre-synaptic fusion was also observed by Stomps (10), and my case is one of the post-synaptic union. Thus the true synopsis in the meaning of the word and the chief contraction take place separately. The united threads grow more and more, and stretch themselves in the entire nuclear cavity. Then they reappear as separated threads (Fig. 9), and their segmentation follows. Thus twelve gemini of twisted threads make their appearance. They become shorter and thicker, and take a ring- or X-shape (Figs. 10, 11). At the later stages of the diakinesis, the chromosomes get still shorter and there are found no more ring- or X-shaped gemini. The paired chromosomes lie close to each other and form dumb-bell-shaped gemini (Figs. 12, 13). The further change of gemini does not proceed in equal pace among themselves, so that the chromosomes or gemini are found in different shapes. One may present a dumb-bell-shape, while the other assumes a more or less square shape (Figs. 14, 15, 20). The formation of the spindle begins around the nucleus (Fig. 12), and as soon as the nuclear membrane and the nucleolus disappear, it assumes at first the tripolar (Fig. 14),
and later the bipolar structure (Fig. 15). The chromosomes, being caught by the spindle-fibres, are arranged at the equatorial plate. In the polar view at this stage the number of gemini was found always to be 12 (Text-fig. A. a). Geminial chromo-

![Text-fig. A. Polar view of chromosomes at the equatorial plate. x 2360. a, Heterotype division. b, Abnormal case of the same, 2 or 3 out of 12 chromosomes are especially small. The side-view of the same is shown in Fig. 16. Pl. VIII. c, d, Divisions of somatic nuclei. e, f, Homotype divisions. g, Sister chromosomes at anaphase of the same.]

somes soon separate and move toward the poles. As soon as they reach the poles the new daughter-nuclei are organized, which soon become ready for the second division (Fig. 17). No resting condition of nucleus was found in the interkinesis. The partition wall is formed and the daughter-cells are separated
from one another (Text-fig. A. e, f), as is commonly the case with Monocotyledons.

In the polar view of the second division, we find 12 chromosomes as is natural, but their behavior is very remarkable in presenting a paired arrangement (Text-fig. A. e, f, g) as we may expect it in a diploid cell, but not in an ordinary homotype division, or in a haploid cell. This peculiar behavior of chromosomes in homotype division was first discovered by my friend Dr. M. Takara in Morus (14), and the present case with Oryza sativa is the second example. In Oryza sativa not all of the 12 chromosomes form pairs, and some of them remain single, while there is also a tendency among them to form a group of more than two or three. Besides, it seems to me that the arrangement of chromosomes, though not in all cases, is somewhat similar in both sister nuclei of the homotype division. This pairing of chromosomes in the second division can not be looked upon as an artifact. But its biological significance is left undecided for the present.

The homotype division passes over otherwise in the ordinary way and the four young pollen-grains are organized.

The size of chromosomes observed in the meiotic phase is by no means the same, but their individual difference in size is not large enough to be recognized throughout the phases.

**Abnormal case of heterotype division.** In the course of this study I got a preparation, in which all the gemini were somewhat smaller than the ordinary ones, and two or three of them took a rod-shape (Fig. 16) which were quite small in the polar view, when compared with the other gemini (Text-fig. A. b). The material of this preparation was collected in a 'Shinriki' rice-field, but the very plant to which this material belonged was not specially noted. It is likely, however, that plants of a different race grew mixed with 'Shinriki.' At the same time there is no proof that this plant did not represent a mutant of 'Shinriki.' In fact I have observed a few plants, while collecting the materials, which had, unlike usual 'Shinriki,' the dark violet stigma. To know whether the latter plant has such chromosomes as above stated, I have specially collected the material
from individuals with the dark violet stigmas or with the colored glumes or leaves. The investigations of such materials have shown, however, that in all these cases the plants are provided with 12 gemini which are as large as those of 'Shinriki.' This abnormal case is certainly of an interest, but the impossibility of the identification of the plant itself makes it difficult to carry further investigations on this point for the present.

II. Behavior of Chromosomes in the Somatic Cell.

Here a brief account of the diploid chromosomes may be given. The number of chromosomes in the nucellar tissue is 24 (Text-fig. A. c). The paired arrangement of chromosomes which was noted at first by Strasburger (12, P. 19) and afterwards by several authors is clearly seen. They are sometimes closely attached end to end, and present an appearance of a single bent chromosome, and the differences of their size and shape are well shown. Some of the paired chromosomes are smaller than others as found in the abnormal case of the heterotype division above stated. Very often, however, I have met with cases where there were a greater number of paired chromosomes; in one case even more than 16 pairs were observed, some of them being smaller than the others (Text-fig. A. d).

III. Embryosac and the Endosperm-Formation.

The embryosac-mother-cell is formed subepidermally (Text-fig. B. a). The meiosis takes place in the same way as in the pollen-mother-cells. The chromatin-masses in paired arrangement are also here observed in the pre-synaptic stage (Fig. 18), and the actual union of the spirem-threads takes place in the same stage as in the case of pollen-mother-cells (Fig. 19). The four macrospores are formed in a vertical row, and as usual only the lowest one develops to be the embryosac. The upper sister-cells are gradually disorganized and remain for a time as deeply staining masses which finally disappear altogether. The embryosac contains at first as usual an egg-cell, two synergidæ,
two polar nuclei, and three antipodal cells. The antipodals divide further, as is the case with other Gramineae. The nuclear division of these antipodals is either direct or indirect, and the former seems to be more usual than the latter.* I have observed

Text-fig. B. a, Abnormal formation of two embryosae-mother-cells. The left hand one is about to disintegrate. ×1280. b, c, Telophase of the free nuclear division in endosperm. ×2360. d, metaphase of the same in the polar view. ×2360. e, Antipodal cells. The lower one contains 14 nuclei. ×1280.

the mitosis only in a few cases; and the chromosomes were found making a compact mass, so that the individual chromosomes were not well distinguished. The nuclear division is not always accompanied with the cell-wall formation. Thus a large cell is formed, which may contain as many nuclei as 14, so far as I have counted (Text-fig. B. e). The number of the cells varies from 6 to 20, or sometimes much more.

*KOERNICKE (6) found no mitotic figure in the antipodals of Triticum. So he is inclined to believe that only the amitosis takes place here.
The disintegration of the antipodal cells seems to occur in different stages, sometimes already before the fertilization, and sometimes after one or two cell-layers of the endosperm tissue have been formed. As soon as the nuclear membrane is dissolved, as the disintegration proceeds, bodies like 'Mitokondrin' make their appearance in the cytoplasm, which are well stained with Heidenhain's iron-alum-hæmatoxylin or safranin of Flemming's triple staining.

The young antipodal cells are filled up with many colourless granules in the cytoplasm which turn reddish brown when treated with the chloriodide of zinc. The similar granules may be seen in the egg-cell, synergidæ, and in the cytoplasm around the polar nuclei. Their occurrence here seems to be transient; but in the antipodals they remain pretty longer. These granules probably represent a mixture of amyrodextrin and amylose.

The synergidæ disintegrate before the fertilization.

The fertilized egg-nucleus migrates downward and prepares for division. The first division of the fertilized egg takes place just after many free endosperm-nuclei have been formed.

Before the fertilization both polar nuclei, each containing a large nucleolus, come in contact with each other, but do not fuse together. They migrate toward the micropylar end of the embryosac, where they are to receive a male nucleus. The 'double fertilization' is indicated here by the existence of two nucleoli in one of the polar nuclei, one of the two nucleoli probably representing the one derived from the sperm-nucleus, although I was not able to find the male nucleus itself in contact with the polar nuclei. The male nucleus seems to unite at first with the upper polar nucleus. The Figs. 21 and 22 show stages just after the union of the male nucleus. The polar nuclei have already well developed the chromatin-threads before

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† HUSS (5).

*STRASBURGER (11, P. 299), in examining the passage of the male nucleus to the secondary embryosac-nucleus in living materials of Monotropa, describes: "So ist der betreffende Spermakern auch sehr schwer zu unterscheiden, nachdem er mit dem sekundären Embryosackkern in Contact trat,...Deutlicher wird der Spermakern hier erst, nachdem seine innere Differenzierung begonnen und, wie meist, auch ein Kern körperchen in ihm sich zeigte."
the fusion of the three. The large nucleus formed by their union (Fig. 23) divides as a rule without formation of the cell-wall. In the telophase of the division the chromosomes crowd in a lump, and then the vacuoles are used to appear between them, so as to form three lumps (though not always three) of the chromosomes. With the growth of the vacuoles, the lumps of chromosomes are scattered about the surface of the nuclear cavity; and by this time three large nucleoli, as found before the complete formation of the primary endosperm nucleus, always make their appearance (Text-fig. B. b). Among them, two nucleoli unite at first to form one (Text-fig. B. c), and then the third comes to the union with the latter. The course of event during the free nuclear division is very regular. In the later stages it is not so well marked. A large nucleolus is here usually to be found, and the appearing of three nucleoli is rather rare. These processes seem to indicate the autonomy of the nuclear elements belonging to those three nuclei which form the initial of the endosperm, during the early stages of development of the endosperm.

It reminds us of a paper by HACKER (4), in which the view of autonomy of the paternal and the maternal portions in the nucleus of the 'Keimbahn' is expressed, and the regular appearing of two nucleoli in the symmetrical positions at the end of each cell division is pointed out as an evidence. I have not observed the actual separation of the nuclei or the spindles, as it is the case with Copepoda &c; but I have once met with the figure as shown in the Text-fig B. d. The relative positions of the chromosomes are not distinct, but they seem to have been divided, so as to make three groups as the marks of straight lines in the figure will indicate. STRASBURGER (13) has observed clear figures of chromosomes in the endosperm-nuclei of Galtonia candidans, in which paired and unpaired arrangements of chromosomes were found, a fact which indicates the actual fusion of 3 nuclei of endosperm-initials. I am inclined to believe that in the case of Oryza sativa this fusion of three nuclei is postponed until the free nuclear division stage has passed over.

The endosperm-formation in Oryza sativa proceeds as
usual with the free nuclear divisions, and after the embryosac has been lined with free nuclei, the wall-formation begins. In the later stage of development the nuclear division is sometimes not accompanied by the wall-formation. Thus there may be found two nucleated cells (Fig. 24). The both nuclei finally come to fuse, and make a syntriploid nucleus. I have once observed the mitotic figure of these double nuclei, in which the two spindles were visible (Fig. 25).

Summary.

Maiosis in the development of pollen-grains. In the young stage of the pollen-mother-cell, the chromatin-masses, nearly as many as the diploid number of chromosomes, are scattered in the nuclear cavity, each two forming a pair.

The chromatin-masses stretch out into the double threads somewhat with an appearance of pearl-strings.

They enter the chief contraction stage of synapsis. But the double nature of the threads can be still clearly made out in thin sections cut 2 μ thick.

The conglobed threads begin to become loose and at last stretch themselves over the nuclear cavity, where the double threads unite together to form simple threads. Thus the conjugation of chromatin-threads does not take place in the contraction stage itself, but after this stage has passed over.

After a while the separation of the united threads takes place. Then the segmentations occur, and 12 double segments or gemini make their appearance.

Thus through all stages of prophase, the paired arrangement or union of chromatin-threads or chromosomes is formed by parallel association, not by an end-to-end association, viz. the process is a parasynodesis, not a metasyndesis.

They become shorter and thicker, and assume a dumb-bell shape or a somewhat square shape.

In the prophase of the division the nucleus is usually provided with several dwarf-nucleoli beside the ordinary large one.

The behavior of chromosomes in the homotype division is
very remarkable. Some of them present paired arrangements and form pseudo-gemini and they show even a tendency to form a group of more than two.

An abnormal case in the heterotype division has been observed, the chromosomes are smaller than the ordinary ones and two or three of them, which are especially small, take a rod-shape.

**Chromosomes in the somatic cell.** The somatic number of chromosomes is 24, but very often we find a larger number. Chromosomes always present paired arrangements. The chromosomes show here the difference of size and shape clearly, while it was not so clear in the maiotic phase.

**Embryosac and endosperm-formation.** The development of the embryosac is normal. In the antipodals, which consist of 3 cells at first, further cell- or nuclear divisions take place, as is the case with other Gramineae.

The ‘double fertilization’ takes place in this plant, and the sperm-nucleus reaches the upper polar nucleus.

The regular appearance of the three nucleoli in the young daughter-nuclei of the endosperm-tissue is looked upon as an indication of the autonomy of the three different nuclei, which have given rise to the endosperm-initial, up to a certain stage of endosperm development.

The endosperm-formation proceeds at first as usual with free nuclear divisions, and is followed later by the simultaneous wall-formation. In the further development of the tissue the cell-wall formation is often suppressed, and the fusion of the daughter-nuclei occurs, which results in the formation of syntriploid nuclei.

The present work was carried out under the guidance of Professor Fujii during the academic year of 1907-1908. I wish to express my obligation to him for his kind advice and suggestions throughout the work.

Botanical Institute, College of Science,

November, 1910. Imperial University,

Tokyo.
LITERATURE.


3. **Grégoire**, V., La formation des gemini hétérotypiques dans les végétaux. La Cellule. t. XXIV. 2d fasc. 1907.


EXPLANATION OF PLATE.

All figures are drawn with the aid of Abbe's camera, and with a Leitz's achromatic \( \frac{1}{4} \) objective and a comps. ocular 12 or an ordinary ocular 4. Except where otherwise indicated all the figures refer to the race 'Shinriki.'

Figs. 1–17, Consecutive stages in the development of pollen-mother-cells.

Figs. 1–3, Consecutive stages in pre-synapsis. \( \times 2360 \).
Fig. 4, The beginning of the conglobation of chromatin-threads. \( \times 2360 \).
Fig. 5, Synapsis. \( \times 2360 \).
Fig. 6, The same cut \( \frac{2}{\mu} \) thick. \( \times 2360 \).
Fig. 7, Conjugation of chromatin-threads after synapsis (synizesis). \( \times 2360 \).

Figs. 8–9, Spirem. Spirem-threads cut by the knife, showing many sectional ends. \( \times 2360 \). In Fig. 8 a nucleolus with two dwarf-nucleoli and a very small one lying close to a thread. Fig. 9 shows the separation of threads.

Fig. 10, Shortening of the threads after segmentation. \( \times 2360 \).
Fig. 11, An advanced stage, showing X- or O-shaped chromosomes. \( \times 2360 \).
Fig. 12, The more advanced stage. Spindle-fibres are found around the nuclear membrane. \( \times 2360 \).
Fig. 13, Somewhat similar stage in pollen-mother-cell of the race 'Kishūwase.' The nucleolus and a gemini were a little dislocated by the knife in cutting. \( \times 2360 \).
Fig. 14, Tripolar spindle. \( \times 2360 \).
Fig. 15, Bipolar spindle. \( \times 2360 \).
Fig. 16, An abnormal form of chromosomes. The two gemini are found very elongated. \( \times 2360 \).
Fig. 17, Nucleus in interkinesis. Longitudinal splits are visible. \( \times 2360 \).
Fig. 18, Pre-synaptic stage of embryosac-mother-cell-nucleus. \( \times 2360 \).
Fig. 19, Spirem of the same. Double nature of spirem-threads can be recognized at one of the ends of threads cut off by the knife. \( \times 2360 \).
Fig. 20, Heterotype division of the same. \( \times 2360 \).
Figs. 21–22, Polar nuclei before the fusion. The upper one provided with two nucleoli. \( \times 1280 \).
Fig. 23, Fusion accomplished. A large nucleolus is formed. \( \times 1280 \).
Fig. 24, A two-nucleated cell of the endosperm-tissue. The lower nucleus has 3 nucleoli, while upper nucleus 2, of which the larger one has been probably formed by the fusion of 2 nucleoli. ×2360.

Fig. 25, Double spindles in the endosperm-tissue. Two small extra-nuclear nucleoli are found in symmetrical positions. ×2360.

Ueber die Kernteilung bei Morus.

von

Masato Tahara.

(Hierzu Tafel IX.)


*) Im vorigen Jahre erschienen die vorläufige Mitteilung dieser Arbeit auf japanisch in dieser Zeitschrift, Bd. XXIII.