Cytological Investigations of Rice Varieties*

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Introduction

Rice is a tropical plant, but its cultivation has spread far beyond the limits of the Tropics. It is now grown as far North as Spain, Italy, Japan, and in South America, while it grows in all parts of India where it is the most important staple food of the populations.

There are many races and varieties of rice with different local names, but botanically all the races belong to the single species *Oryza sativa*, L., an annual grass maturing very rapidly. Recently Kato and his colleagues (1930) have distinguished two types of rice which they call variety *Japonica* and variety *Indica*. The former are indigenous to Japan, Korea and N. China; the latter to India, S. China and Java, etc. They found that both "varieties" as well as the hybrids within each are highly self-fertile, but the hybrids between members of the different varieties are much less fertile, owing to the formation of many abnormal and imperfect pollen grains.

Very little work has been done on the cytology of the different races of the rice plant. Kuwada (1910) was the first to make a cytological examination of a few races of rice and he found the haploid number of chromosomes to be 12. This result has been confirmed by several later workers, specially by Nakatomi (1923), who found a varietal difference in size of chromosomes. Rau (1929) reported the diploid number to be 24. Morinaga and Fukushima (1930) made extensive observations on the chromosome number of numerous varieties and found no variation in chromosome number. Selim (1930), working with five races of rice belonging to both Kato's varieties, found that although these five races have the same size of nuclei and the same number of chromosomes, yet they showed characteristic differences in the absolute size of the nucleolus in different races. Thus up to the year 1930 no chromosomal mutants with less or more chromosomes than the typical number were reported

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and it was thought that *Oryza sativa* is a species composed of a single karyological type.

In the year 1931, Morinaga and Fukushima first discovered a haploid plant of rice among the F_1 individuals of a variety hybrid Dekiyama $\times$ Bunketu-to. After this a large number of haploid rice plants have been found by these authors and also by Nakamura (1933), and Ramiah, Parthasarathy and Ramanujam (1934).

Nakamori (1932) obtained an abnormal plant among certain varietal lines belonging to the eighth generation of a cross between *Seniti* $\times$ *Takenari*, which on cytological examination revealed 36 chromosomes in the root-tip cells. Morinaga and Fukushima (1933) found in one season more than 150 triploid individuals in common rice fields under cultivation. They say "It is certainly a remarkable fact to find such a great number of auto-triploid plants in a species reproduced sexually. How the auto-triploid individuals come to exist so often in *Oryza sativa* is an interesting problem still to be solved."

Nakamori (1933) first reported tetraploid individuals of the rice plant in the F_1 progeny of an intervarietal cross. More recently Ramiah, Parthasarathy and Ramanujam (1935) report a tetraploid plant in wild rice.

Thus we find that the recent discoveries of chromosomal mutants, viz. haploid, triploid, and tetraploid individuals of *Oryza sativa* L., have brought a turning point in the field of cyto-genetical studies of rice, a plant which is of so great economic importance to the tropical countries, particularly India.

The present paper deals with the meiotic divisions of the pollen mother cells of a large number of rice varieties, with special regard to the origin, development and behaviour of the nucleolus. It also reports the cytological features of the origin of polyploid gametes and hence the formative causes of the triploid and tetraploid mutants which are found so frequently in rice fields.

The writer is continuing his cyto-genetical investigations of a large number of Indian and Japanese rice varieties and other species of *Oryza*, the results of which will be published later.

Material and Methods

A large number of Indian and Japanese varieties of *Oryza sativa*, L., obtained through several sources are under investigation. The cytological studies embodied in the present paper belong to the following races:—

1. 'Basanta-Bahar', received from Assam.
2. 'Garikkasan-navari', obtained from Coimbatore.

3. 'Thekkas-chesa', obtained from Coimbatore.
4. 'Bonsari-to', obtained from Japan.
5. 'Murasaki-ine', „ „ „
6. 'Tanko-hoira', grown in Formosa.
7. 'Konan-sen', grown in South China.

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The plants were grown in pots during the years 1934 and 1935 in greenhouses at the Genetical Laboratory, Regent's Park, London and at Chelsea Physic Garden. The iron-acetocarmine method was used for examining the different stages of division in the pollen mother cells. The flower buds were collected between 11 a.m. and 3 p.m., on bright sunny days. The glumes of the buds were cut off at the tip just above the level of the anthers before fixing. The buds were then dipped into Carnoy's solution for a few seconds and fixed in Navashin's fluid (Maeda 1930), for 5 to 6 hours. An exhaust pump was always used. Although several other fixatives were used the above method gave the most satisfactory results.

Paraffin sections were cut from 10–14 microns in thickness according to the requirements. The gentian-violet-iodine stain was exclusively used.

Description

Observations on the somatic cells. The chromosomes were counted in seven varieties of both *Indica* and *Japonica* types. The counts confirmed that the diploid number 24 represents the typical chromosome number. The chromosomes in the somatic cells of rice do not make paired arrangements as described by Kuwada (1910). Some of the chromosomes show differences in size and shape, but owing to the extreme smallness of the chromosomes morphological peculiarities are not distinct. At somatic prophase in diploid plants two chromosomes with terminal knob-like bodies remain attached to the single nucleolus (Fig. 1). Sometimes there are two nucleoli of smaller size each attached to a particular separate chromosome by a slight terminal enlargement or knob (Fig. 2). It is suggested that the two chromosomes concerned in forming the nucleolus here lie far apart in the nucleus and the two nucleoli therefore remain separate; whereas when the two chromosomes lie very near each other the two nucleoli as they grow come into contact and fuse result-

ing in a single nucleolus. Hence some of the nuclei in diploid somatic cells are characterised by two small nucleoli while others show only one big nucleolus. When there are two nucleoli in a nucleus the size of each of them is nearly half the size of the single nucleolus in other nuclei. It will be shown in the following studies of meiosis that the terminal chromatic knob-like bodies possessed only by the two chromosomes of the diploid complement are responsible for the orderly organization of the nucleoli in telophase.

Microsporogenesis. The pollen development of *Oryza sativa* L., is very similar in all the races examined and consequently the following account is applicable to all. A general description of the Japanese race 'Murasaki-ine', will be given and the other races will be compared when the differences arise.

The resting nucleus of the pollen mother-cell. The pollen mother-cells at the resting stage are polygonal in outline (Fig. 3), and closely packed in two rows within the loculus against the tapetum. The tapetal cells are smaller than the pollen mother-cells and always binucleate (Fig. 4). The two daughter nuclei here show one large and two smaller nucleoli in each respectively, the large nucleolus in one daughter nucleus no doubt resulting by the fusion of the two smaller ones in the previous telophase. The resting pollen mother-cell nucleus is always spherical with a thin nuclear membrane. Within the membrane the nuclear cavity is filled with a faintly staining material of granular appearance, a large deep-staining spherical nucleolus occupying the centre of the nucleus (Fig. 3). Sometimes in the race 'Basanta Bahar', two separate nucleoli each nearly half the size of the more commonly occurring single nucleolus were observed in the resting nucleus (Fig. 5). At the resting as well as later stages the nucleolus was destined to examine the nucleolar contents. The nucleolus appears to be homogeneous without any crystal-like body but sometimes vacuoles were observed in it. There was never found a clear area around the nucleolus as described by Latter (1926) in *Lathyrus*, Zirkle (1928) in *Zea*, and many others. McClung (1929) and Dermen (1933) are of opinion that the clear area around the nucleolus is due to fixation and therefore is an artefact.

The meiotic prophase. The heterotypic prophase begins with an increase in the size of the nucleus. The leptotene threads become conspicuous, but they are so very delicate in rice that their relationship to each other cannot be made out (Fig. 6). At this stage two of the leptotene threads are always attached to the nucleolus by their

terminal knobs. Occasionally these knobs may be seen to be double (Fig. 6a), indicating that the leptotene thread is already split. These two deeply-stained bodies presumably represent the nucleolar body first described by Latter (1926) in *Lathyrus* and recently described as the 'nucleolar-organizing body' in the satellited chromosome of *Zea* by McClintock (1934). In *Oryza*, these two bodies having terminal position as deeply-stained chromatic elements with their attached threads represent the ends of the two particular chromosomes of the diploid complement responsible for the orderly organization of the two nucleoli in the telophase. The doubleness of each nucleolar body and the twisted aspect of each separate leptotene thread represented in Fig. 6a, suggests that the threads are probably double being composed of two chromonemata, closely approximated, but they are so near the limits of resolution of the microscope that their double nature is not made clear.

The nature of the leptotene thread, whether single or double has been a matter of great controversy among cytologists. Darlington (1931) in his 'precocity theory' assumes that in mitosis chromosomes divide during the interphase so that they are double in the prophase. In meiosis, however, the leptotene thread is supposed to be single, the attraction of the leptotene threads being satisfied by the pairing of similar homologues. Meiosis is therefore assumed by him to be brought about by a premature contraction of the chromosomes and a delayed splitting. The recent observations of McClung (1928), Robertson (1931) in *Orthoptera*, and Koshy (1934) in *Allium*, have demonstrated duality of the chromosomes in the last premeiotic telophase.

Darlington (1932) still maintains that duality of the anaphase and telophase chromosomes is due to optical illusion. The works of Sharp (1929), Kaufmann (1926, 1931), Hedayetullah (1931), Perry (1932), Koshy (1933), and Hoare (1934), have confirmed the split and twisted nature of the chromosomes at anaphase and telophase. This would mean that the early meiotic prophase chromosomes are double.

Evidence has since been obtained by Kaufmann (1931) in *Tradescantia*, Smith (1932) in *Galtonia*, Koshy (1934) in *Allium*, Hoare (1934) in *Scilla*, Gates and Nandi (1935) in *Oenothera*, that the leptotene threads, like the somatic early prophase chromosomes, are double, presenting a beaded appearance under the microscope, owing to the intertwining of two chromonemata. Naturally this disposes of Darlington's hypothesis that the difference between mitosis and meiosis lies in the postponement of a split and in the singleness of the leptotene thread.

The leptotene threads now aggregate towards one side of the nucleus, the two particular threads (representing the two chromosomes) with their deeply-stained nucleolar bodies still remaining in contact with the apparently single nucleolus (Fig. 7). This condition has been described before by Gates and Nandi (1935) in trisomic *Oenothera*, where a loop of thread passing through these two bodies or one body resulting from the fusion of the two could be clearly traced over the surface of the nucleolus. This stage is obviously that of synzesis. The contracted threads never take the form of a knot as described by various authors. Professor Gates is of opinion that synzesis represents a sensitive condition of the nucleus, when the delicate threads are easily compacted together by the entrance of the fixing fluid. During this contraction stage the nucleolus in some of the nuclei produces a little bud (Fig. 8), which with the growth of the nucleus gradually increases in size and ultimately reaches the size of the original nucleolus at early diakinesis. Figs. 8-12 show such successive stages of synzesis, zygotene, pachytene, diplotene and early diakinesis respectively where only the nucleoli are represented. The presence of a small bud on the nucleolus was also reported by Gates and Latter (1927), Maeda (1930), and Koshy (1934). Selim (1930), working with five varieties of rice, finds in three of them a single large nucleolus in the pollen mother nucleus, budding off a "secondary nucleolus" which becomes as big as the original "primary" nucleolus, by the time the synaptic contraction is maximum. He also finds that these two nucleoli remain attached to each other by a short bridge and the two nucleoli show a constant difference in staining properties. The 'secondary nucleolus' disappearing at early diakinesis was believed to contribute material to the formation of the chromosomes while the 'primary' nucleolus, which disappears at late diakinesis, contributed material to the formation of the spindle. Two other varieties which Selim studied were characterised by a single large nucleolus in each. His comparison of the five varieties shows characteristic differences in the nucleolar content, which are correlated with the absolute and relative size of the nucleoli and also with the time of division of the 'primary nucleolus' into a 'secondary nucleolus'.

As shown in Figs. 8-12, it is quite evident that as the 'secondary nucleolus' becomes larger, the 'primary nucleolus' becomes gradually smaller until the two nucleoli attain the same size at early diakinesis. In most of the pollen mother nuclei there is no budding, the single nucleolus which was formed by the fusion of the original two nucleoli at the last premeiotic telophase, being present as a single body from the resting stage to the late diakinesis, when it gradually disappears

at metaphase. The single nucleolus is always about twice the size of either of the two nucleoli.

Numerous measurements of a large number of nuclei and nucleoli at the resting stage and early diakinesis were taken, to compare the absolute and relative sizes of the nucleus and its nucleolus or two nucleoli in six varieties of rice.

The measurements were taken from figures drawn by camera lucida magnified about 2800 times.

Table 1. Each of the figures represents here an average of 25 measurements, in m.m.

Race	1 nucleolus. Nuclear diameter at resting stage.	1 nucleolus. Nuclear diameter at diakinesis.	2 nucleoli. Nuclear diameter at diakinesis.	1 nucleolus. Nucleolar diameter at resting stage.	1 nucleolus. Nucleolar diameter at diakinesis.	2 nucleoli. Nucleolar diameter at diakinesis.
'Basanta Bahar'	16.3	24.2	28.1	6.8	7.1	8.6
'Garikkasan- navari'	15.8	24.1	28	6.5	7	8.5
'Bonsari-to'	16.3	24.2	28.2	6.7	7	8.6
'Murasaki-ine'	16.2	24.1	28.1	6.7	7.1	8.6
'Tanko-hoira'	15.8	24	28	6.5	7	8.5
'Konan-sen'	16.2	24	28	6.7	7	8.5

From Table 1 it is quite evident that there is no difference in the size of the nuclei and the nucleolar contents in these six varieties of rice. But it shows a significant difference in the size of the nuclear diameter in those cells with one nucleolus at diakinesis (column 2) and with two (column 3). This is correlated with the difference in the size of the nucleolar diameter when one nucleolus (column 5) or two (column 6) are present at diakinesis. This shows that the size of the nucleolus depends upon the size of the nucleus, or in other words the larger the nucleus the larger the total nucleolar diameter. It is possible, therefore, as Selim (1930) suggested, that the division of the nucleolus may be due to the relative increase of the nucleolar material within the mother-nucleus.

From Table 1 it may be concluded that only the larger nucleoli bud, and that these occur in the larger nuclei. The budding moreover takes place after the nucleolar growth and means simply a transfer of material from the larger nucleolus to the smaller, not an increase in the total amount of nucleolar material.

In a single preparation of the variety 'Thekkas-chesa' three or more nucleoli of different sizes have been noticed, some of them connected with the 'primary nucleolus' and others present as small separate bodies (Figs. 13, 14). The stage probably represents the synzinesis stage. The nuclear threads were not clear and the later stages were not available in this variety. In the resting stage there

was always a single nucleolus. These smaller nucleoli no doubt correspond with the several 'dwarf' nucleoli, observed by Kuwada (1910) in rice varieties. The same phenomena were noticed by Mazumdar and Datta (1935) in the pollen mother nucleus of *Hibiscus mutabilis*. They supported the view that the nucleolus by such fragmentation contributed chromatin to the developing spireme. It seems to the writer that such a fragmentation of the nucleolus is due to the action of the fixing fluid when the fixation is not critical. There is no advantage gained by such fragmentation and from other evidence it appears that the nucleolus does not contribute chromatin to the chromosomes directly.

Small dark-stained bodies were never found on the nuclear threads at any stage as observed by Selim (1930), or aggregations of chromatin suggested as prochromosomes by Kuwada (1910). At the termination of synizesis the locule increases in size, a cavity appears in the central part of the loculus and the two rows of pollen mother-cells get separated from each other. Then the compact pollen mother-cells increase in size and become spherical, separate from one another and cling to the tapetum (Fig. 15).

The two particular chromosomes of the diploid complement of rice with their deeply-stained terminal nucleolar bodies become clearly recognizable when the separate leptotene threads begin to pair side by side at zygotene. Fig. 16 shows the stage of early zygonema, where only the two particular nucleolar organizing chromosomes are represented. The two threads with terminal deeply-stained nucleolar bodies are seen free from each other while remaining in contact with the surface of the single nucleolus. When there are two nucleoli the two bodies with their chromatic threads remain situated near the point of attachment between the two nucleoli (Figs. 17, 18, 19). Similarly, Smith (1933) found in the pollen mother nucleus of *Galtonia* both the satellites in contact with the single nucleolus at the early prophase stage, but when there was a bud on the nucleolus the satellites appeared in the construction at the base of the bud either encircling or lying at one side of the bud. McClintock (1934) clearly demonstrated the structure of the 'nucleolar-organizing body' present only in the satellited pair of chromosomes in *Zea*, at the mid-prophase of meiosis where they remain attached to the surface of the single nucleolus. In *Zea*, this body is subterminal and adjacent to the secondary constriction and is farther away from the satellite proper. In rice the satellite and the secondary-constriction are not present and the nucleolar body in each of the two particular chromosomes of the diploid plants is terminal.

In Figs. 16, 17, it is seen that the unpaired leptotene threads with their free ends are beginning to run parallel to one another, pairing closely with each other here and there. In some cases the threads seem to twist round each other as they pair. By this time there is considerable growth of the nucleus, the chromatic threads become more prominent and the double nature of the thread which Kuwada (1910) referred to is quite evident. The 12 pairs of zygotene threads representing the 24 diploid chromosomes may now be easily counted. Fig. 18 is an illustration of this stage where at regions "a" the leptotene threads are still apart although on both sides of these regions they have completely paired. Delay in pairing in these regions does not mean that there is lack of pairing. Darlington (1935) finds in all the species of *Fritillaria* with or without localized pairing, inhibition of pairing. He further finds the nucleolar chromosomes showing incomplete pairing at zygotene. There was never found at this stage the two particular chromosomes attached to the nucleolus or nucleoli showing any incomplete pairing. According to him delay in pairing means failure of pairing. As is shown here, lack of pairing at certain regions means delay in pairing, i.e., the chromosome threads are still in the process of pairing.

In *Oryza*, similar chromosomes usually associate in the zygotene stage to produce side by side pairing, i.e. the mode of pairing is parasynaptic. The pairing commences at the ends of the chromosomes as observed by Belling (1931) in Liliaceous plants, Huskins and Hearne (1933) in Oats, Hedayetullah (1933) in *O. missouriensis*, and Koshy (1934) in *Allium*. This confirms the suggestion of Catchside (1931), that synapsis begins at the ends of the chromosomes and not at the spindle-fibre attachment regions, as assumed by Darlington (1935).

In the meantime the zygotene threads undergo thickening and shortening by axial contraction. The beaded appearance representing the so-called 'chromomeres' becomes clear. The beaded character of each pairing thread is believed to be due to the fact that it is composed of two chromonemata twisted about each other and thus forming nodes and internodes (Gates and Nandi, 1935). The two by two association of homologous chromosomes present in the diploid complement is maintained by the forces of specific attraction. This question has been the subject of much discussion owing to its great bearing on genetical problems. Gates (1932) in his Presidential Address to the Royal Microscopical Society, states that "We are quite without any basis for an explanation of this phenomenon at the present time, and even a knowledge of how genes divide would not appear to help us."

Fig. 19, represents pachytene stage where pairing of the two chromosomes is complete. The appearance of loops described in the so-called second contraction in *Oenothera* (Gates and Nandi, 1935), and the brochonema stage of Latter (1926) in *Lathyrus*, do not occur in the present material.

Further contraction of the chromosome threads leads to the formation of diplotene loops (Fig. 20). The paired bivalent chromosomes begin to separate, remaining attached at some points. At this stage it is difficult to distinguish a twist in two chromosomes from an actual chiasma, where there is an interchange of two chromatids. Three to four nodes were occasionally seen at this stage, some of which might represent the actual exchange of partners. Such an interchange does not necessarily result in genetical crossing-over.

Early diakinesis:—As the shortening and thickening of the threads progresses, the 12 bivalents lie scattered at the periphery of the nucleus (Fig. 21). During this stage the quadruple nature of each bivalent chromosome can be easily traced. The differences are evident in sizes and shapes of the twelve bivalents. The members of one bivalent may be in the form of straight rods or spirally intertwined or twisted over each other forming a figure of eight, and those of other bivalents may be ring-shaped, v-shaped or x-shaped. Generally one pair (Fig. 21, marked "a") is recognizably large and spirally inter-twined. Since the chromosome pairs differ constantly in length and shape it is apparent that the pairing is between homologous chromosomes of similar length and shape. The volume of the nucleus and the nucleolar material reach a maximum at this stage, one pair of bivalent chromosomes always remaining attached to the single nucleolus. The 'secondary nucleolus' by this time reaches the same size as that of the 'primary nucleolus' and in that case the particular bivalent chromosome concerned in the organization of the nucleoli at telophase remains attached at the junction point of the two nucleoli (Fig. 22). Sometimes two nucleoli of similar size which are separate but very near each other were found. In this case the two particular chromosomes are attached separately to the two nucleoli (Fig. 22a). Probably the attraction developed between the homologous chromosomes keeps the nucleoli attached to them in contact. These two nucleoli never fuse. At the diakinesis stage they represent the two original nucleoli which were produced at the last telophase of the archesporial division and remained separate as such throughout all the intervening stages. Whether two nucleoli or one, their total volume at this stage is always greater than at the resting stage. This indicates that the size of the nucleolus depends

upon the size of the nucleus and consequently upon the stage of development of the nucleus.

Late diakinesis:—As the nucleus passes to the late diakinesis stage the bivalents assume a more peripheral position in the nucleus. This was first pointed out by Gates (1909) in *Oenothera*, who concluded that the chromosome bivalents are mutually repelled due to the superficial electric charges. The chromosomes become shorter and thicker, undergo condensation and stain more deeply. The quadruple nature of each bivalent chromosome becomes less evident. In Fig. 23 it is clear that some of the bivalents are more condensed than others. Apparently the condensation of the chromosomes does not take place equally in all the 12 pairs of bivalents. Frequently they exhibit a rough and ragged appearance which may be due to the accumulation of nucleolar material which is now being incorporated in them. The members of the bivalents are now more intimately associated and the difference in size and form is less marked. They show a wide range of sizes from small round chromosomes to elongated ones (Fig. 24). The chromosome pairs still undergo progressive condensation and become very compacted together (Fig. 29). They still show an indication of their bivalent nature, but the dual nature of each univalent is no longer evident.

From late diakinesis there is a gradual decrease in the size of the nucleolus or the two nucleoli contemporaneous with the gradual condensation of the chromosomes, until at the termination of diakinesis they have completely disappeared. Figs. 25–28 show a series of successive stages where the nucleolus gradually decreases in size with the gradual increasing condensation of the chromosomes. These figures also show a gradual decrease in size of the nucleus. Such a decrease of nuclear volume from diakinesis to metaphase was described by Lawson in *Smilacina* (1912), where he supposed that the decrease of nuclear volume is due to osmotic diffusion of the karyolymph.

It is also evident here that one pair of chromosomes always remain attached to the nucleolus, or more properly stated the nucleolus remains attached to this pair of chromosomes until the time of its disappearance at metaphase. The nucleolus always keeps its spherical form and gets paler and paler staining gradually. When there were two nucleoli, both disappeared simultaneously and by similar stages, indicating their similarity.

There was no evidence of nucleolar fragmentation or any indication of extrusion of nucleolar material into the cytoplasm at this stage of its disappearance. The disappearance of the nucleolus or the nucleoli is not so sudden as has been assumed by other workers.

As has been shown here, it is seen that they do not disappear by a process of fragmentation (Wilson, 1925, p. 95), but rather by a progressive reduction in size.

The increased stainability of the chromosomes at this stage (Fig. 29), with the disappearance of the nucleolus suggests that all the nucleolar substance is now incorporated into the chromosomes.

Generally the pollen mother nucleus at diakinesis has the 12 normal bivalent chromosomes, but sometimes in the Japanese race 'Tanko-hoira', a trivalent and a univalent (Fig. 23), or two trivalents and two univalents (Fig. 24), were found.

In the Indian race 'Basanta Bahar' (Fig. 30), 3 pairs of chromosomes show non-conjugation (Belling, 1925), that is, there are 9 bivalents and 6 univalents present in the nucleus. Fisk (1925) noted such early separation of the members of one bivalent in *Zea* and suggested that there might be less intimate conjugation between the members of this pair. Randolph (1928) in certain inbred strains of maize showed such phenomena in many microsporocytes. Beadle (1930) in asynaptic maize showed the presence of 20 univalents at diakinesis. Ramanujam and Parthasarathy (1935) report asynaptic rice where they show 24 univalents at diakinesis completely separate.

It is difficult to say whether the dissociation of the 6 univalents at the diakinesis stage in the race here reported is due to non-homology of the chromosomes or whether there was an earlier association of these chromosomes.

Pro-metaphase:—At this stage the nuclear membrane and the nucleoli have completely disappeared and the spindle is formed. The condensation of the chromosomes is complete and the bivalents show the greatest degree of contraction. The specific identity of the chromosome pairs is lost and the bivalents appear as single subglobular bodies. By this time the widely separated bivalents move towards each other, which results in a very close assemblage in the centre of the nuclear area. This is prometaphase (Fig. 31).

Metaphase:—In a polar view of this stage the 12 bivalent chromosomes assume an arrangement similar to that of floating magnets. In rice, the chromosomes in most of the cases are arranged in two rings, an outer one with nine and an inner one with three (Fig. 32). This arrangement is quite in agreement with the theory of floating magnets of Cannon (1923) and Kuwada (1929). But sometimes this arrangement is disturbed, which may be due to the fact that at this stage the chromosomes are in oscillating movement and the effect of fixative at that particular moment may result in such distortions.

In a side view at this stage the bi-polar spindle is quite evident; the counting of the chromosomes is easy in some cases and the biva-

lents are arranged in one plane on the equator of the spindle. The forms of the bivalent chromosomes have altered by this time becoming elongated and taper-pointed in the direction of the axis of the spindle (Fig. 33). This is no doubt due to the forces applied at the spindle-fibre attachment to the middle of each chromosome, which pull them apart to the opposite poles.

Anaphase:—At the approach of anaphase each member of a chromosome bivalent is attached to a spindle fibre which appears median in every case, and a side-view at this stage shows each chromosome on one side having a recognizable homologue on the other side (Fig. 34). In the early anaphase which is represented in Fig. 35 (Plate 17), the univalents are in the process of separation. Figs. 36, 37, represent mid-anaphases. The univalent chromosomes do not always pass to the poles simultaneously. Fig. 37 shows a case where 11 univalents are passing to each pole, and the other two univalent chromosomes are being stretched out by forces apparently pulling in opposite directions. Such a condition is similar to that described by Gates and Nandi (1935) in a trisomic *Oenothera*. Sometimes lagging chromosomes are found on the spindle. Fig. 36 shows a case where a chain of three chromosomes are lagging. Morinaga and Fukushima (1935) have shown the separation of such trivalents at anaphase which may disjoin to three univalents or to one bivalent and one univalent. At the late anaphase several of the chromosomes show a constriction which is generally sub-median (Fig. 38). This condition has been described before in the heterotypic anaphase chromosomes of *Oenothera paralamareckiana* by Gates and Nandi (1935), where a full explanation of such appearances was given.

In a polar view of the anaphase stage 12 univalent chromosomes in each group can be counted easily (Fig. 39).

As the chromosomes reach the poles they become compacted together corresponding to the "tassement polaire." No evidence of anastomosing matrices connecting all the chromosomes and carrying dispersed chromatin, as described by McClintock (1934) in *Zea*, was found. The individual chromosomes are still visible although they are in closely compact groups (Fig. 40).

Telophase:—With the outset of heterotypic telophase nuclear membranes are formed round the tightly pressed chromosome groups and the two daughter nuclei then continue to increase in size. During this stage the two daughter nuclei of the heterotypic telophase show a number of small nucleolus-like globules of different sizes, no nuclear threads being visible (Fig. 41). Fig. 42, shows a later stage than in Fig. 41, where the nucleolus-like globules are undergoing

fusion and with the decrease in their number the size of the nucleolus is increasing.

Van Camp (1924) in *Clivia* and Dermen (1933) in *Callisia* and *Paeonia*, applying Ehrlich-Biondi mixture which stains nucleoli red and chromosomes pale blue, were able to show that the nucleoli originate from the chromosomes in telophase in the form of small nucleolus-like globules which later, by fusion form one large nucleolus. Latter (1926) figures such small nucleolus-like globules in contact with the more faintly staining chromosomes in an interkinetic daughter nucleus of *Lathyrus*. From her (Fig. 42) it is quite evident that the number of these nucleolus-like globules exactly corresponds with the number of univalent chromosomes, which is 7. Similar stages were described by her in the grand-daughter nuclei, where she stated, "These nucleoli are first formed in contact with the chromosomes and gradually become liberated into the nuclear sap. They then coalesce to form the one large nucleolus of the resting microspore nucleus." From all this evidence it appears therefore probable that the nucleolus-like globules at the telophase represent the original nucleolar material of the mother nucleus, which passed into the metaphase chromosomes and was carried as such to the daughter nuclei in the anaphase chromosomes. In the heterotypic telophase the nucleolar material present in each chromosome gets liberated as small globules which under the influence of the nucleolar body present in one chromosome of the haploid complement is organised into a definite nucleolus that remains associated with one particular chromosome (Fig. 43).

By this time the spindle fibres disappear rapidly, the daughter cells are completely formed and become separated.

Homotypic division mitosis:—As the prophase II progresses the size of the nucleolus, which is always single, gradually decreases with the gradual contraction and condensation of the chromosomes, and as soon as the metaphase is reached the nucleolus and the nuclear membrane completely disappear and the chromosomes stain more deeply (Fig. 44).

The second division proceeds as a rule simultaneously in the two sister cells. Fig. 45 shows the two homotypic plates, one in side-view where the 12 pairs of univalent chromosomes are grouped in the equatorial plane of the spindle, the other plate in polar view, 9 chromosomes being in the periphery and 3 in the centre. The univalent chromosomes, which are half the size of the bivalent chromosomes, assume the same arrangement of floating magnets as described in metaphase I. Here also this arrangement is disturbed as shown in Fig. 46, where at metaphase II, 8 chromosomes are in the periphery

and 4 in the centre. The paired arrangement of certain chromosomes at this stage observed by Kuwada (1910) in *Oryza sativa* L., and Ramiah, Parthasarathy and Ramanujam (1935) in *Oryza longistaminata* was not found in the present material.

Fig. 47 represents an early anaphase of division II where the chromosomes are just separating. An exactly similar stage to this has been described by Ramiah, Parthasarathy and Ramanujam (1935, cf. figs. 5 and 6) as the metaphase stage in a supposed tetraploid plant of wild rice. A careful study of this stage and the other stages figured by them yields no evidence of its tetraploid nature. Moreover they were unable to produce any evidence of its tetraploid nature from chromosome numbers in somatic nuclei.

In the late anaphase II, (Fig. 48), 12 chromosomes are seen passing to each pole in the two daughter sporocytes. The disjunction of the chromosomes at anaphase II seems to be simultaneous, no lagging chromosomes being observed. As the late anaphase chromosomes reach the poles the granddaughter-nuclei are organised. A nucleolus is developed in each granddaughter-nucleus by the same process as described in heterotypic telophase. Here it is also clear that one chromosome remains attached to the single nucleolus which it produced (Fig. 49). The homotypic division is completed in the ordinary way and is followed by the formation of 4 haploid pollen grains.

Irregularities in the reduction divisions. *Cytomixis*:—The phenomenon of cytomixis was described by Gates (1911) in *Oenothera gigas*, where he showed the transference of chromatin between two contiguous cells taking place through gaps in the cell-walls by means of protoplasmic connections. This process of transference of chromatin material from one pollen mother-cell nucleus into the cytoplasm of an adjacent pollen mother-cell has been described in a large number of plants by several authors. Katterman (1933) gives a reference to these works and shows the various stages of cytomixis in the pollen mother-cells of *Triticum* $\times$ *Secale* hybrids.

The process of nuclear migration in various stages of development was noticed in a variety of rice 'Tanko-hoira'. Fig. 50 shows such a stage at early prophase where the two contiguous pollen mother-cells are in contact with each other and the whole of the nuclear contents from one cell are being transferred into the cytoplasm of the adjacent pollen mother-cell. It is probable that at a later stage this pollen mother-cell will become bi-nucleate by this process. Fig. 51 shows a bi-nucleate pollen mother-cell at diakinesis stage in the same variety, where one of the nuclei contains the normal 12 bivalents and the other nucleus, a portion of which is cut by the

microtome knife, still shows 10 bivalents.

Kihara and Lilienfeld (1934) have made a study of cytomyxis in the pollen mother-cells of certain hybrids between *Triticum* × *Aegilops*. They figured cases where pollen mother-cells have become binucleate in this way. In the present case in rice there is no doubt that the binucleate condition of the pollen mother cell is the result of cytomyxis.

Binucleate pollen mother-cells have been described by Gates and Rees (1921) in *Lactuca*, Randolph and McClintock (1926) in *Zea*, and Karpechenko (1927) in the hybrids of *Raphanus* × *Brassica*. Binucleate pollen mother-cells described in this paper, when followed by normal cytokinesis in both the divisions, would result in the formation of diploid pollen grains.

In the variety 'Tanko-hoira', Fig. 52 shows a case where not only the two nuclei but the whole of the two adjacent pollen mother-cells are in the process of fusion, the intervening cell walls having completely disappeared. That such a process takes place is supported by the case found in the same variety and represented in Fig. 53 in metaphase polar view, where not only is the number of chromosomes doubled, but the volume of the cytoplasm is also doubled. These 24 chromosomes, are as large as the normal bivalents and therefore cannot be unpaired univalent chromosomes. Such a condition would result in the production of diploid pollen grains. In both the above cases, if the nucleus undergo a 'semi-heterotypic division', (Rosenberg (1927), in *Hieracium*, and Karpechenko (1927) in the hybrids of *Raphanus* × *Brassica*), resulting in a 'Restitution-nucleus', where the homotypic division proceeds normally, then there would arise dyads with tetraploid pollen grains. It is not yet known whether such a process takes place in rice.

Failure of cytokinesis:—This has been shown to be due to various treatments by several investigators. Sharp (1934) has given a full list of literature on the subject and discussed the question fully. Beadle (1932) described a gene in *Zea* for failure of cytokinesis during the meiotic divisions of some of the microsporocytes. In a variety of rice 'Garikkasan-navari', many microsporocytes were found where cytokinesis failed both in the first and second divisions. Fig. 54 shows two homotypic spindles at anaphase stage where the first cytokinesis failed. Here it is quite possible that the two spindles might fuse and result in a tetraploid pollen grain. A later stage is represented in Fig. 55, where due to the failure of cell wall formation in both the divisions the microsporocyte has resulted in a 4-nucleate pollen grain. A measurement of this cell showed that the 4n pollen grain thus produced is four times the volume of the haploid

pollen grain. Fig. 56 represents a case where cytokinesis had taken place during the first division but had failed during the second division, which resulted in the production of two binucleate pollen grains. A measurement of this cell showed that the $2n$ pollen grain thus produced is twice the volume of the haploid pollen grain.

There is thus direct cytological evidence of the production of unreduced microspores through various abnormalities in the meiotic divisions of certain rice varieties. It is also quite possible that such irregularities happen in the megasporogenesis of rice varieties, resulting in diploid megaspores. The union of a diploid male gamete thus produced with the normal haploid female gamete will result in triploid rice plants, and the union of diploid male gamete with the diploid female gamete would result in the production of tetraploid rice plants. This will account for the frequent occurrence of triploid and tetraploid plants of *Oryza sativa* L., in the common rice fields.

It may be pointed out here that De Mol (1923) was able to induce the formation of diploid pollen grains experimentally and demonstrated their viability by the occurrence of triploids in the offspring from the treated bulbs of *Hyacinthus*. Gates (1909) suggested that polyploidy might arise (1) by a split of the chromosomes in the fertilized egg and their subsequent failure to separate owing to a suspended mitosis, (2) by the production of polyploid gametes. Jørgensen (1928), working with the genus *Solanum*, expressed the opinion that the majority of polyploid forms of plants owe their origin to a doubling process (endo-duplication) in the somatic tissue.

Tschermak and Bleier (1926) observed "Die beiden fertilen *Aegilotriticum*-Bastarde, *A. ovata* $\times$ *T. dicoccoides* und *A. ovata* $\times$ *T. durum* zeigen 28 Chromosomen als haploide Chromosomenzahl in den untersuchten F_5 und F_6 Pflanzen." The authors could not show any evidence of the mode and time of origin of this doubling of the chromosomes.

Kagawa (1928) observed that giant pollen grains were produced in meiosis of the pollen mother-cells of the hybrid between *T. durum* $\times$ *A. ovata*.

Percival (1930) repeating the cross of Tschermak and Bleier's fertile *Aegilotriticum* hybrids, actually found the suspension of the first division in the pollen mother cells of the hybrids which leads to the formation of diploid pollen grains. So, it is most probable that the case reported by Tschermak and Bleier with the double number of chromosomes resulted from the union of diploid germ cells thus formed in the meiosis of F_1 hybrids.

Considering the above cases and the origin of polyploid gametes in rice through various abnormalities in the reduction division, it is

natural to conclude that such a process must be an important factor in the formation of other polyploid plants.

The recent discoveries of haploid, triploid and tetraploid mutants of rice on a large scale raise some of the fundamental problems of polyploidy, which will be dealt with in further studies on the cytogenetics of rice which are in progress.

Discussion

The nucleolus. The problem of the nucleolus has been the subject of numerous investigations,* various theories having been put forward as to its origin, composition and function. The extensive literature concerning the nucleolus has been well summarized by Ludford (1922), De Mol (1927), and Sharp (1934). Wilson (1925) has provisionally classified the nucleoli into two groups:—(1) plasmosomes, or true nucleoli, and (2) karyosomes, or chromatin nucleoli. Gates (1932) has pointed out that “there has been no clear evidence of two types of nucleoli in the cells of higher plants.”

The fact that the nucleolus is probably present in all types of cells throughout the plant and the animal kingdom raises Wilson's question (1925) “whether the nucleolus may not play a more active and important part in cell-metabolism than most writers have hitherto assumed. . . and it now seems probable that the nucleoli are not merely accumulations of waste products but contain materials that play some definite part.”

Although many earlier workers noted a connection between the nucleolus and the spireme, it is only comparatively recently that any light has been thrown on the significance of this relationship.

So long as the continuous spireme theory held sway, it was usually held that the nucleolus functioned as a repository of chromatin material; it stored this material during telophase and resting stage and used it up during prophase. Fikry (1930) and Dermen (1933) pointed out the difficulties in explaining the passage of nucleolar material to or from the chromosomes when, as appeared to be the case, the spireme was not continuous.

We have seen that in rice the nucleoli, one or two in number, are closely connected with a particular pair of chromosomes at a definite position on them. A connection of a “loop of the spireme” with one or two bodies as a darker staining area on the nucleolus in the pollen mother-cells of *Lathyrus* was first discovered by Latter (1926). The same condition has been described in the pollen mother-cells of *Lathraea* (Gates and Latter, 1927), *Oenothera* (Sheffield, 1927), *Malva* (Latter, 1932), and *Oenothera* (Gates and Nandi, 1935). From present knowledge it is evident that these two nucleolar

bodies with their attached threads represent the particular pair of chromosomes which is responsible for the orderly organisation of the nucleolus in telophase.

A relationship between the nucleolus and a particular pair of chromosomes was also noted by several authors. S. Navashin (1912) in *Galtonia*, first observed a pair of satellites attached to the nucleolus. Wenrich (1916) established the relationship between the nucleolus and a particular pair of chromosomes in the meiotic nuclei of certain grasshoppers. Sorokine (1924, 1929) reported a pair of satellite chromosomes in *Ranunculus acris* and *R. chius* respectively, attached to the nucleolus. Senjaninova (1926), studying *Ranunculus acer*, found the two satellite chromosomes attached to the nucleolus in the resting nucleus. Heitz (1913, a, b) in *Vicia* observed a pair of satellite chromosomes connected with the nucleolus. Sax (1932) and Dermen (1933) find the nucleolus in the pollen mother-cells of *Callisia* constantly attached to a satellite chromosome pair. Smith (1933) in *Galtonia* observes one pair of satellite chromosomes regularly in contact with the nucleolus from the leptotene stage until the time of disappearance of the nucleolus at metaphase. Kaufmann (1933) finds the X- and Y-chromosomes of *Drosophila* connected with the two nucleoli present in the spermatocytes, and Dearing (1934) clearly demonstrates in *Amblystoma tigrinum* the association of the two nucleoli at a localized segment of a particular pair of satellite chromosomes. The relation of a particular pair of chromosomes to the nucleolus is thus well established.

Previous to the discovery of the 'nucleolar-organizing region' by McClintock (1934) in *Zea mays*, most of the workers associated the origin of the nucleolus with the secondary constriction of a satellited chromosome.

Heitz (1931a, 1931b) developed a theory concerning the origin of the nucleolus at telophase. According to him, the nucleolus originates from the achromatic thread of the satellited chromosome, their number at telophase corresponding with the number of satellites and secondary constrictions present. Darlington (1935) finds in every species of *Fritillaria* two or more pairs of chromosomes bearing nucleoli and supports Heitz's theory of the origin of nucleoli at the secondary constrictions.

Heitz (1931b), however, reports in *Vicia faba* and *Vicia monanthus* which possess only two satellited chromosomes in the diploid complement, a small extra nucleus was occasionally found with a small nucleolus formed by a lagging chromosome; besides the two main sister nuclei each with two normal nucleoli produced by the two satellited chromosomes.

It has been shown by the writer that in the diploid somatic nucleus of rice two nucleoli remain attached at the position of the nucleolar body of one pair of chromosomes. The development of the nucleolus in somatic telophase of rice is very rapid and therefore not favourable for studies. But we have clearly demonstrated in the sporocytes of rice one pair of chromosomes regularly in contact with the one or two nucleoli from the leptotene stage until the time of disappearance of the nucleoli at metaphase. In *Oryza*, the nucleolar body in the two chromosomes occupies the end of the chromosomes, as terminal knobs which always stain deeply. There is no evidence of satellites or secondary constrictions in the chromosomes of rice. In the heterotypic telophase the nucleolar substance present in each chromosome appears in the form of small globules, which are organised into a definite body, the nucleolus, through the activity of the nucleolar body present in one chromosome of the haploid complement, to which it remains attached.

The origin of the nucleolus at telophase by the flowing together of small nucleolus-like globules has been noticed by several authors. Such a method of origin of the nucleolus was first described by Van Camp (1924) in the somatic telophase nuclei of *Clivia*, where by using Ehrlich-Biondi differential mixture (which stains chromosomes pale blue and nucleolus globules bright red) he was able to show that the nucleolus originates from the chromosomes at telophase in the form of small globules which later fuse and collect into one large nucleolus.

Dermen (1933), using the same stain, finds in the somatic and meiotic telophase nuclei of *Callisia* and *Paeonia* that the nucleolus originates in the form of small globules on the surface of every chromosome, which later collect by fusion into two or one large nucleolus.

Manton (1935) in *Biscutella* finds the nucleolus present as a single body in the telophase and resting stage and supports the view that all the chromosomes contribute nucleolar substance to form a single structure by the flowing together of all the nucleolar material.

McClintock (1934) has shown that:—(1) The nucleolus is organised in the telophase through the activity of a distinct deep-staining body (nucleolar-organizing body) immediately adjacent to an achromatic region delimiting a satellite in one chromosome (chromosome VI), of the haploid complement of *Zea mays*, the material however being contributed by all the chromosomes. (2) In diploid somatic tissue with two such chromosomes the telophase nucleus shows two nucleoli of similar size which sometimes fuse to form one. (3) In an interchange this 'nucleolar-organizing body' is

broken into two unequal parts, each interchange chromosome possessing a segment. Plants heterozygous for the interchange developed three nucleoli and plants homozygous for the interchange developed four nucleoli in their somatic telophases. (4) In plants heterozygous for the interchange two kinds of spore quartets are sometimes produced as the result of non-disjunction, the satellited chromosome with the normal 'nucleolar-organizing body' and the other two interchanged chromosomes, each possessing part of the 'nucleolar-organizing element', passing into the same nuclei. Two spores of a quartet thus lack this 'nucleolar-organizing element' and therefore show numerous nucleolus-like globules in contact with all the chromosomes, the other two spores having normal nucleoli. Moreover, it has been shown by Miss Creighton (quoted in Sharp, 1934) in a strain of *Zea mays* resulting from the functioning of X-rayed pollen in which the 'nucleolar-organizing region' of the satellited chromosome was deficient and the other satellited chromosome contributed by the female was normal; that the somatic nucleus of this plant was characterised by the presence of one nucleolus. In the microspore quartets, two spores with the normal 'nucleolar-organizing' chromosome produced one definite nucleolus in each, while the other two spores with the 'nucleolar-organizing body' absent were characterised by small nucleolus-like globules attached to each chromosome.

All this evidence shows that the nucleolus is organised in the telophase by the nucleolar body present in one chromosome of the haploid complement having different positions in different plants. In *Oryza* this body is terminal, in *Zea* it is situated near the secondary constriction delimiting a satellite, the nucleolar body near the secondary constriction in *Vicia* not being recognized by Heitz.

It may therefore be concluded that nucleolar substance present in each chromosome collects as nucleolus-like globules on the surface of each chromosome at telophase and is therefore organised into a definite nucleolus by the agency of the nucleolar body seen in *Oryza* as a terminal knob at one end of a chromosome of the haploid complement. The nucleolus remains closely attached to this terminal knob.

The question arises what is the material that the nucleolar body uses to organise a definite nucleolus at telophase? Several workers have concluded that the nucleolar substance of each cell-generation enters into the chromosomes at prophase and goes out of them again in the telophase. Zirkle (1928), using a fixative "nickel-chromacetate", which dissolves all chromatin but preserves nucleolar material (which he calls plastin) has been able to demonstrate the "plastin" entering into the spireme in prophase and passing back

into a nucleolus in telophase. Marshak (1931), with materials fixed in "chrom-valeric-formalin" and stained in haematoxylin, obtained blue reticulum and red nucleoli at the resting stage. With the progress of the prophase the spireme became purple and then red, and in metaphase the chromosomes stained the same colour as the nucleolus. In the telophase the reticulum again appears blue and nucleoli red. He never observed any extrusion of nucleolar matter into the cytoplasm. This indicates that all the nucleolar material has entered the chromosomes at metaphase. Marshak obtained evidence for the identity of the nucleolar substance with the matrix of the chromosomes. Zirkle (1931) finds the last traces of nucleolar material passing into the chromosomes before metaphase. No evidence of any granules of nucleolar substance was noticed by him in the cytoplasm. All the nucleolar substance is carried to the daughter nuclei in the chromosomes and the "plastin is thus continuous from one cell generation to another".

McClintock (1934) obtains evidence that the nucleolar substance which enters the chromosomes at late prophase constitutes the visible matrix material of the anaphase and early telophase chromosomes, and a definite nucleolus is organized from this substance through the activity of the nucleolar-organizing element of the satellite chromosome. The present work shows that during the meiotic prophase of rice there is a marked increase in the size of the nucleoli with the growth of the nucleus. They remain undiminished in size, with no apparent change in their staining capacity till early diakinesis when the chromosomes are fully formed. From late diakinesis onwards there is a gradual decrease in the size of the nucleoli with the gradual condensation of the chromosomes until the nucleolus completely disappears at the metaphase when the chromosomes reach their complete condensation and full development. Thus all the nucleolar substance appears to be passed into the metaphase chromosomes by this time. No nucleolar fragments were found in the metaphase and there was no indication of extrusion of any nucleolar material into the cytoplasm. It appears probable that all the nucleolar substance is incorporated into the metaphase and anaphase chromosomes, the telophase nucleoli being derived from the pre-existing material which is continuous from cell-generation to cell-generation like the chromosomes. According to this view the chromosomes contain a combination of chromatin and nucleolar material at metaphase and anaphase and the latter is again separated at telophase from each chromosome to form a definite nucleolus under the activity of the nucleolar body present in one chromosome of the haploid complement.

Micro-chemical tests carried out by Zacharias (1882), Yamaha

and Sinotô (1925) and Zirkle (1928, 1931) indicate that the nucleolar substance is not composed chemically of "nucleic acid", "chromatin" or "karyotin."

Recent work thus shows two substances present in the chromosomes, one of which is of nucleolar origin. The evidence obtained in this study and those of Zirkle (1928, '31), Marshak (1931), Dermen (1933), and McClintock (1934) suggests that the nucleolar substance contributes to the matrix of the chromosomes.

Thus the nucleolus may be regarded as a compound product consisting of substances from all the chromosomes and hence perhaps from all the genes, and since it is present almost universally in all organisms and is perpetuated from cell-generation to cell-generation it may have a certain rôle in inheritance.

Since there is no physical connection between the nucleolus in diakinesis and the various chromosomes, it appears that transference of material must take place by its solution from the nucleolus followed by its reconstitution on the matrix of the chromosomes. Similarly in telophase, the globules of material derived from the matrix of the chromosomes do not appear to be transferred bodily to the young nucleolus at the nucleolar locus of one pair of chromosomes, but rather to be dissolved or chemically changed and afterwards reconstituted to contribute to the growth of the nucleolus—no doubt under the influence of the nucleolar body.

Summary

1. Seven varieties of rice plants have been cytologically examined. The chromosome number in each is determined as 12 haploid, 24 diploid. Satellites and secondary constrictions are not found in the chromosomes.

2. In the somatic prophase a pair of chromosomes with terminal knob-like bodies remain attached by the knob to two small nucleoli or one large nucleolus which results by the fusion of the two. These knobs must be concerned in the growth of the telophase nucleoli.

3. The resting nucleus of the pollen mother-cells generally has a single nucleolus with no crystalline bodies.

4. The leptotene thread is split like the somatic early prophase chromosomes, the two chromonemata being closely twisted about each other.

5. During the heterotypic prophases the nucleoli, one or two in number, remain closely connected with a particular pair of chromosomes at one end (referred to as the nucleolar body) from the leptotene stage until the nucleolus disappears completely at metaphase.

6. During synizesis the nucleolus in some of the nuclei of the

varieties studied produces a bud, which grows until the two nucleoli attain the same size at early diakinesis by transfer of material from the larger to the smaller.

7. Comparison of six varieties of rice shows no difference in the size of the nuclei and nucleolar contents. But it shows a significant difference in the size of the nuclear diameter in those cells with one nucleolus at diakinesis and with two (Table 1). This is correlated with the differences in the size of the nucleolar diameter when one nucleolus or two are present at diakinesis. Only the larger nucleoli (which are present in the larger nuclei) bud, and the "budding" takes place after the maximum size of the nucleolus is reached.

8. Budding of the nucleolus may be due to the relative increase of the nucleolar material within the mother nucleolus or it may be an artefact produced by the treatment. When budding takes place it is always at the point of attachment of the terminal chromosome knob.

9. The method of chromosome pairing is parasynaptic, pairing commencing at the ends of the chromosomes. Variation in size and shape of the 12 bivalent chromosomes is found in early diakinesis, one larger twisted pair being particularly noticeable.

10. From resting stage until early diakinesis there is an increase in the size of the nucleolus, followed by a gradual decrease from late diakinesis with the gradual condensation of the chromosomes until the time of its complete dissolution at metaphase.

11. It is concluded that the nucleolus contributes matrix substances (not chromatin) indirectly to all the chromosomes, which is carried over by metaphase and anaphase chromosomes, and a definite nucleolus is organized at telophase from this substance under the influence of the nucleolar body present on the end of one chromosome of the haploid complement. Trivalent and univalent chromosomes are sometimes found at diakinesis in certain Japanese varieties.

12. The phenomena of cytomyxis, pollen mother-cell fusion, binucleate and tetraploid pollen mother-cells and failure of cytokinesis during meiotic divisions of some of the microsporocytes, are described. As the result of these aberrations, diploid gametes are formed, which will account for the origin of triploid and tetraploid plants of rice.

13. The origin of the nucleolus is discussed and its relation to the nucleolar body; also the possible rôle of the nucleolus in inheritance.

14. It is emphasized that further studies of a biochemical nature are required before definite conclusions may be drawn as to the rela-

tionship between the matrix substance of the chromosomes and the nucleolus.

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Explanation of Plates

All figures were sketched at table level with the aid of a camera lucida. A 2 mm. Zeiss Apochromatic, 1.3 aperture, and Zeiss oc. $\times 15$ were employed for all drawings. Magnification $\times 2800$. Fig. 15 is magnified $\times 1500$.

The figures are from pollen mother-cells of *Oryza sativa*, L., a Japanese race 'Murasaki-ine', except those mentioned below.

Plate 16

- Fig. 1. Somatic prophase nucleus showing 24 chromosomes of different size and shapes. Two chromosomes with terminal knob-like bodies are attached to the single nucleolus.
- Fig. 2. The same stage, but the two small nucleoli are each attached to a separate chromosome by a terminal knob.
- Fig. 3. A polygonal resting pollen mother-cell with a single nucleolus.
- Fig. 4. A binucleate tapetal cell.
- Fig. 5. 'Basanta-Bahar', a resting nucleus with two nucleoli.
- Fig. 6. Leptotene stage showing the beaded appearance of the threads. Two threads are attached to the nucleolus.
- Fig. 6a. Two leptotene threads attached to the nucleolus by terminal nucleolar bodies which are double in each.
- Fig. 7. Synizesis. Two threads attached to nucleolar body.
- Figs. 8-12. Successive stages of synizesis, zygotene, pachytene, diplotene and early diakinesis, showing the budding of the nucleolus. Note that as the 'secondary' nucleolus becomes larger the primary nucleolus becomes smaller.
- Figs. 13-14. 'Thekkas-chesa', synizesis with several nucleoli of different sizes.
- Fig. 15. Section of whole anther showing binucleate tapetum and four pollen mother cells at zygotene clinging to the tapetal layer.
- Fig. 16. Zygonema, a pair of threads attached to the nucleolus, by terminal knobs.
- Fig. 17. The two threads situated at the point of attachment between the two nucleoli. This condition is invariable when two nucleoli are present.
- Fig. 18. A pollen mother-nucleus with 12 pairs of zygotene threads. At regions "a" the two threads are still unpaired.
- Fig. 19. Pachytene stage.
- Fig. 20. Diplonema, showing a few twisted bivalents, one attached to the nucleolus.
- Fig. 21. Early diakinesis with 12 bivalents, one pair attached, as always, to the nucleolus.
- Fig. 22. The same stage, one pair of chromosomes attached at the junction of the 'primary' and 'secondary nucleolus'.
- Fig. 22a. Same stage, the particular pair of chromosomes attached separately to the two nucleoli.
- Fig. 23. 'Tanko-hoira' diakinesis, showing differential condensation of the bivalents, one trivalent, and one univalent. Only the bivalent on the left is attached to the nucleolus.
- Fig. 24. Diakinesis showing two trivalents and two univalents.
- Figs. 25-28. Late diakinesis, showing gradual condensation of chromosomes with the gradual decrease in size of the nucleolus, one bivalent remaining attached.
- Fig. 29. Later diakinesis, where the nucleolus has completely disappeared.

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- Fig. 30. 'Basanta-Bahar', diakinesis showing six univalent and nine bivalent chromosomes.
 Fig. 31. Prometaphase.
 Fig. 32. Metaphase in polar view showing the chromosome arrangement 9, 3.
 Fig. 33. Metaphase in side view with 12 bivalent chromosomes, and bipolar spindle.
 Fig. 34. Early anaphase.

Plate 17

- Fig. 35. Early anaphase.
 Fig. 36. Mid-anaphase with a chain of 3 chromosomes lagging.
 Fig. 37. Same stage, showing two univalent chromosomes stretched out.
 Fig. 38. Late anaphase, several of the chromosomes showing a constriction.
 Fig. 39. Polar view of anaphase with 12 chromosomes in each group.
 Fig. 40. "Tassement polaire"
 Fig. 41. Telophase of first meiotic division, showing many nucleolus-like globules of different sizes. No threads visible.
 Fig. 42. A later stage where the nucleolus-like globules are undergoing fusion and with the decrease in their number the size of the nucleolus is increasing.
 Fig. 43. Prophase II, one chromosome attached to a nucleolus in each.
 Fig. 44. Metaphase II. The nucleolus and the nuclear membrane have completely disappeared.
 Fig. 45. Two homotypic plates, one in side view and the other in polar view.
 Fig. 46. Metaphase II, showing 8 chromosomes in the periphery and 4 in the centre of each group.
 Fig. 47. Early anaphase II.

Plate 18

- Fig. 48. Late anaphase II.
 Fig. 49. A microspore, one of the 12 chromosomes attached to the nucleolus.
 Fig. 50. 'Tanko-hoira' cytomyxis at early prophase I.
 Fig. 51. " " a binucleate pollen mother-cell at diakinesis. Left nucleus cut, in right nucleus one bivalent attached to nucleolus.
 Fig. 52. 'Tanko-hoira', fusion of two pollen mother-cells at leptotene stage.
 Fig. 53. " " a gigantic pollen mother-cell at metaphase I, in polar view with 24 bivalents.
 Fig. 54. 'Garikkasan-navari', second meiotic division after failure of cytokinesis.
 Fig. 55. " " a four-nucleate pollen grain.
 Fig. 56. " " a bi-nucleate pollen grain.
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