Cytological Studies in Oryzeae and Phalarideae

II. Further studies in Oryza

By

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Introduction

Recent cytological studies on Oryza sativa (Nandi 1936, Sakai 1935, and Ramanujam 1938) have led to the conclusion that it is a secondary allotetraploid derived from a primary basic set of five pairs of chromosomes. Hence it should have at least two pairs of satellited chromosomes, forming four nucleoli at somatic telophase. Nandi in fact has observed in several varieties of rice two pairs of SAT-chromosomes in somatic metaphase and found that two pairs of chromosomes were attached to the nucleolus at heterotypic prophase. Selim (1930) found varietal difference in the number of nucleoli at heterotypic prophase, some with mostly a single nucleolus and some with two. He presumed, however, that the second nucleolus arose as a result of budding of the single nucleolus. Hedayetullah (1933) crossed two varieties of rice, one with two large nucleoli and the other with a single large nucleolus at meiotic prophase. The F₁ had two nucleoli, one large and the other small. Ramanujam (1937) found three nucleoli at somatic telophase in an autotriploid isolated in a pure line derived from a cross between two varieties of rice with two and four nucleoli respectively. He explained the origin of such a condition on the principles of simple Mendelian segregation from the F₁ of such a cross which could give pure-breeding types with two and four nucleoli respectively, while the triploid arose from the type
with two nucleoli. Hedayetullah was evidently dealing with a cross between a variety with two and another with four nucleoli at somatic telophase, which by fusion in the premeiotic telophase would give rise to a single or two large nucleoli at meiotic prophase.

The origin of a plant with two SAT-chromosomes from one with four can thus be assumed to have resulted from the loss of function to develop a nucleolus in one of the SAT-chromosomes, through a mutation causing the disappearance of the satellite, and subsequent segregation in the progeny of this hybrid. Kato (1930) classified rice varieties into two groups: var. japonica and var. indica, according to geographical distribution, and found that while the hybrids within each group were fertile, the hybrids between varieties of the two groups were more or less infertile. Professor Gates suggested the examination of some Japanese and Indian rice varieties, as regards the number of their satellited chromosomes and the determination whether partial sterility, if it existed in the hybrids between these, could be due to such a condition.

This investigation is preliminary to further cytological investigations in India, in hybrids between varieties with two and four nucleoli.

**Technique**

Root-tips from some Japanese and Indian varieties grown at the Courtauld Genetical Laboratory, Regent’s Park, were fixed in La Cour 2 BE and blocked by the ordinary paraffin method. Sections were cut 12 μ thick and stained in Newton’s gentian violet. Flower buds from T. 24, a pure strain from an Indian variety, were fixed in Navashin’s fluid for the examination of meiosis.

All drawings were made at bench level with the aid of a camera lucida. An achromatic objective N. A. 1.3 was used in conjunction with Zeiss eye piece K. 25, giving approximate magnification of 4000 diameters. These were reduced to four-fifths in reproduction.

**Observations**

**Number of nucleoli in rice varieties.**

The number of nucleoli formed at somatic telophase was assumed to represent the number of satellited chromosomes, on the principle of Heitz’s hypothesis (Heitz 1931). As the chromosomes themselves are too small, the satellites could not be distinguished in the preparations. The following varieties in Table 1 were examined. (Figs. 1 and 2).

It is found that both in India and Japan there are varieties with two and with four SAT-chromosomes. The causes of sterility could
only be determined by hybridising the races with the same number of SAT-chromosomes as well as those with different numbers. It is probable that structural differences may exist in the geographical races which may bring about partial sterility in the crosses. That varieties with two satellited chromosomes could have evolved from varieties with four is quite possible. Examination of wild races of *O. sativa* should throw more light on the problem.

**Nucleolar fusion at meiotic prophase.**

In a strain T. 24, of Coimbatore, India, with four SAT-chromosomes, it was found at early leptotene, that almost all the P.M.C.'s contained two nucleoli. (Fig. 3). Each of these nucleoli should have resulted from the fusion of two nucleoli from a single pair of SAT-chromosomes at late prophase of the cell cycle. At diakinesis 67 cells out of 108 cells examined had a single large nucleolus (Fig. 4) while the rest had smaller double nucleoli, and in all the cells with double nucleoli, two pairs of chromosomes were found at the point of contact. (Fig. 5). Nandi (1938) has also observed such a condition at zygotene, where two threads pass between the two nucleoli. It is clear from the present observations that the intervening chromosomes prevent fusion of nucleoli.

The average measurements of the single fusion nucleolus and the double nucleoli at early diakinesis, showed that the volume of the single nucleolus was definitely more than the sum of two single nucleoli. (Table 2). Similar results were obtained by De Mol (1926) in *Hyacinthus*, Selim (1930) in rice and Bhatia (1938) in wheat. The reason for this change in nucleolar volume was attributed to loss of material during fragmentation by De Mol, while Bhatia considered that it was due to growth after fusion. By fusion of two single spherical nucleoli, the volume remaining constant, the surface area of the fusion nucleolus will be reduced to nearly three-fourths, with the result that it will offer a smaller surface for the absorption of nucleolar material by the cell. This I presume may be the cause of the greater volume of the fusion nucleolus, which loses its material much less quickly than the double nucleoli which present greater surface for cell absorption.
The fusion of nucleoli, which takes place in more than half the number of pollen mother cells, could occur in two ways—one, by the nucleoli, which are already close together, meeting by slight movement. Such movement is possible if there is attraction between the pairs of nucleolar chromosomes which are ancestrally homologous, as the second pair of nucleolar chromosomes is derived by the duplication of the basic complement. Whether there is such secondary attraction during zygotene is not known. To bring the nucleoli into contact, it has to be assumed that at least there is attraction between the chromosome segments near the attachments to the nucleolus. The fact that in early leptotene the nucleoli are always near each other leads to the inference that during the organisation of the nucleoli, these chromosomes are always near each other. The existence of somatic pairing in rice is an indication of the proximity of at least some of the homologous chromosomes in prophase.

Another way by which fusion between two nucleoli could take place is by a slight increase in the size of the nucleoli during meiotic prophase, bringing them into contact. But the average measurements of 25 nucleoli at early leptotene and early diakinesis showed that there was only a decrease in the size.

### Table 2. Volume and surface area of nucleoli in meiotic prophase

<table>
<thead>
<tr>
<th>Measurement of size</th>
<th>Early leptotene, all double</th>
<th>Early diakinesis, single 67, double 41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean diameter of nucleolus in mm.</td>
<td>8.75</td>
<td>10.72</td>
</tr>
<tr>
<td>Volume in cu. mm.</td>
<td>2×350.9 = 701.8</td>
<td>2×272.6 = 545.2</td>
</tr>
<tr>
<td>Surface area in sq. mm.</td>
<td>2×240.5 = 481.0</td>
<td>2×203.6 = 407.2</td>
</tr>
</tbody>
</table>

**Size of the nucleolus in haploid and diploid rice.**

The mean volume of a single large nucleolus at mitotic prophase was determined both for the diploid strain T. 24 as well as for the haploid mutant isolated from that strain. In each case 100 nucleoli taken from different sections were drawn at the same magnification and the mean volume was calculated.

The results (Table 3) show that the volume of the nucleolus in the diploid is more than double the volume of the nucleolus in the haploid. This is in accord with De Mol's (1927) observations in *Hyacinthus*, where the volumes of the fusion nucleolus in diploids and polyploids increased progressively with the increase in the chromosome sets. On the other hand Dermen (1933) found no significant correlation between the size of the nucleolus and the number of chromosomes in *Petunia* polyploids. He thinks that the size of the nucleolus is influenced by other factors besides the increase in the chromosome complements.
Table 3. Measurements of diameter of nucleolus in the haploid and diploid rice (Expressed in mm., see text)

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<tr>
<th>Diameter</th>
<th>5.5</th>
<th>6.0</th>
<th>6.5</th>
<th>7.0</th>
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<th>8.0</th>
<th>8.5</th>
<th>9.0</th>
<th>9.5</th>
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<td>4</td>
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<td>Diploid root-tip</td>
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<td>Total</td>
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<td>27</td>
<td>5</td>
<td>39</td>
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<td>4</td>
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Mean diam. | 7.40 | 9.78 |
Mean vol. | 212.2 | 489.9 |

Somatic pairing.

In one of the varieties of *O. sativa*, the paired arrangement of the chromosomes at somatic metaphase was very significant. The degree of somatic pairing was variable and Fig. 6 shows that nearly all except four chromosomes are in pairs, lying parallel close together though not in contact. Evidence of somatic pairing in rice was reported by Kuwada (1910) in *O. sativa* and by Ramanujam (1938) in *O. officinalis*, though Nandi (1938) did not find it in his material.

Pairing of chromosomes in somatic divisions occurs in a large number of plant and animal species. Strasburger in 1905 (quoted by Gates, 1912) first found this type of pairing in somatic tissues of plants. This was found later by various investigators in a number of plants, Gates (1912) in *Oenothera*, Huskins (1932) in *Matthiola*, Lawrence (1931) in *Dahlia*, Newton (1924) in *Galtonia*, and others. Watkins (1935) lists a number of species of plants in which this phenomenon is known to occur. Metz (1916) reports somatic pairing in many species of *Diptera*, where the small number and distinct size differences make it particularly evident. This characteristic juxtaposition of similar chromosomes in all these cases must be due to specific attraction resulting from homology between the paired chromosomes. Gates (1911) infers that such attraction may exist between parental chromosomes throughout the life cycle of the sporophyte.

The favourable conditions for somatic pairing are: (1) the number of chromosomes should be small, as otherwise the chances of all chromosomes being sorted out in pairs are small, and further the
chromosomes would interfere with each other's movement, (2) the chromosome size should be small or the volume of the nucleus should be sufficiently large to allow of free movements. It was found by Upcott (1936) in Eremurus, where there were distinct size differences in the chromosome complements, that somatic pairing was more frequent among the short chromosomes.

Darlington assumes (1937) that somatic pairing does not usually show itself at mitosis in plants with secondary pairing at meiosis, since the chromosomes in somatic tissue are widely distributed and are not brought within sufficiently close range of one another to show any association. This idea is contrary to the observations of Skovsted (1933) and Davie (1933) in cotton, and of myself in rice, where there is secondary pairing in meiosis as well as somatic pairing in mitosis. Secondary pairing and somatic pairing are similar phenomena of specific attraction, but there is a distinction. While secondary pairing manifests itself by attraction between ancestrally related bivalents in metaphase I, and between related univalents in metaphase II, somatic pairing, like meiotic pairing, expresses affinity between the parental chromosomes without coming into actual contact as in meiosis. Simple diploids can thus show somatic pairing while secondary association is not possible.

Favourable conditions for somatic pairing exist in doubled nuclei, where through the failure of the divided chromosomes to separate, the chromosomes had probably remained together at the resting stage and so were able to pair in the next metaphase, e.g. Huskins and Smith (1932) in Sorghum, and Manton (1935) in Iberis. Peto, (1935) observed paired arrangement of chromosomes in tetraploid nuclei in roots of Pisum treated with chloral hydrate, but considers that such pairing is not due to homologous attraction but to the failure of the spindle mechanism. Evidently the failure of the split chromosomes to move towards the pole retains the paired arrangement. Such a condition was also obtained by Kemp (1910) in the cells of root-tips of peas subjected to chloral hydrate treatment.

**Secondary association.**

The observations regarding secondary association in rice (Coimbatore strain T. 24) confirm the results of Sakai, Nandi and Ramanujam in establishing the maximum association as \((3(2) + 2(3))\) five. (figs. 7 and 8). The fact that in the related genera Lygeum and Zizania the chromosome numbers are in multiples of five (Ramanujam 1938) confirms the inference drawn from the results of secondary pairing. The results of the observations are summarised in Table 4.
Secondary association is an expression of ancestral homology and as such is of great value in the analysis of the constitution of polyploids when it is combined with other evidence. The evidence from secondary pairing alone as a criterion of general homology cannot be complete, for the following reasons:— (1) structural differentiation of the supposed homologous chromosomes through a longer period of evolution in many polyploids, may have occurred to such an extent that their affinity is no longer enough to cause attraction, (2) structural changes like simple translocations and reciprocal translocations, which are recognised to be important in species evolution, can on the basis of attraction by homology give rise to higher associations, so that the inference of basic number from the results of maximum associations may be misleading; and (3), it is quite probable that secondary association may also be gene controlled, similar to primary pairing, (Beadle 1930, Ramanujam and Parthasarathy 1935), and so absence of secondary pairing may not be an indication of non-homology.

**Oryza coarctata ROXB.**

This is a wild species natural to the coastal regions of Sind in India, and evidently thrives well under saline conditions. Attempts to germinate the seed under normal conditions, as well as by soaking the seed in different concentrations of sea water, were tried without any success. Professor Gates obtained plants from India by air, but they unfortunately did not survive the journey. I am indebted to Dr. S. Hedayetullah for the fixed root-tip materials of this species. Chromosome counts (Fig. 9), showed that 2n = 48. This species is thus a tetraploid on the basis of the secondary basic number of *Oryza*, which is 12. The chromosomes are small like those of rice and no satellites are visible. Four nucleoli are formed at somatic telophase, which indicates that 4 satellited chromosomes are present in the complement (Fig. 10).

The nucleolus, which usually gets reduced and disappears before the dissolution of the nuclear membrane and the onset of metaphase,
is seen in some cells however, to persist at metaphase. Sometimes it is found to divide at the equator or pass to one of the poles. (Figs. 11, 12, 13 and 14). In all these cases, it is not included in the daughter nuclei and is left out in the cytoplasm where it probably

Figs. 1-2. Somatic telophase. 1, Kuruvai, an Indian variety of rice, showing 4 nucleoli; 2, Tiyodawase, a Japanese variety of rice showing 2 nucleoli. Figs. 3-8. Type 24 (Coimbatore). 3, early leptotene, showing two nucleoli; 4, diakinesis, single nucleolus with 2 bivalents attached; 5, diakinesis, two nucleoli with a pair of bivalents attached between them; 6, metaphase plate, showing an exceptional amount of somatic pairing; 7, metaphase I, Polar view showing the maximum secondary association, i.e. 2(3)+3(2); 8, metaphase II. Polar view showing the maximum secondary association, 2(3)+3(2) in one cell. Figs. 9-15. *Oryza coarctata*. 9, somatic metaphase 2n = 48; 10, somatic telophase showing 4 nucleoli; 11-14, persistence of the nucleolus at metaphase; 11 & 12, nucleolus dividing at the equator; 13, being pulled towards one pole; 14, nucleolus dividing, at one of the poles; 15, not included in the daughter nucleus.
degenerates. (Fig. 15). Examples of such cases both in lower and higher plants are given by Frew and Bowen (1929). The division of the nucleolus on the equator and its movement to the poles suggests the presence of forces at the spindle region which are responsible for the polar migration of the nucleolus. This passage to a pole suggests convection currents in the spindle substance, since the nucleolus is not attached to the spindle in any way.

Discussion

Nucleolus in relation to changes in the chromosome complement.

The nucleolus has been the subject of numerous investigations. Its function in the economy of the cell is still a matter of speculation, and literature dealing with it is quite extensive. (Ludford 1922, Zirkle, 1928, Frew and Bowen 1929, and others). Though the exact role of the nucleolus is still not established, its regular appearance and disappearance during the life cycle of the cell signifies that it has an important part in nuclear metabolism.

The problem of the origin of the nucleolus however, has been satisfactorily solved, and Gates (1937) has recently reviewed the relevant literature. To Heitz (1931) must be given the credit of establishing that SAT-chromosomes are associated with the formation of the nucleolus. He found that the number of nucleoli formed in telophase depended upon the number of SAT-chromosomes present in the complement. While Heitz thought that the satellite stalk or thread was responsible for the organisation of the nucleolus, McClintock (1934) demonstrated the presence of a definite body on the chromosome adjacent to the satellite stalk which performs this function. Starting on the assumption that in a normal diploid, definite bodies located in a specific pair of chromosomes organise nucleoli at telophase, it is interesting to consider the corresponding changes in the number of nucleoli with changes in the chromosome complement. De Mol (1927) found 2, 3 and 4 nucleoli in the root-tips of 2n, 3n and 4n plants respectively in Hyacinthus. In rice, an autotriploid had three nucleoli while the corresponding diploid had two. (Ramanujam, 1937). These facts show that the number of nucleoli is an indication of the number of haploid complements present in the organism, unless it be that the satellited pair alone had duplicated (trisomy and tetrasomy). Cases, however, are known where the apparently simple hypothesis is subject to modifications. Navashin (1927, 1934) found in certain Crepis crosses that a satellite normally present in one of the contributing complements did not appear in the hybrid. This phenomenon, termed amphiplasty by him, may
be interpreted as either due to the inability of the nucleolar body to function in a new environment or due to competition in the nucleolar organisation by the nucleolar chromosomes, resulting in the apparent inability of one of them to function.

Such a phenomenon has a direct bearing on allopolyploidy, where an amphidiploid should have four SAT-chromosomes. All the four may be functional, but if hybridity has an amphiplastic effect in the suppression of a satellite, the organisation of the nucleolus is also suppressed and two instead of four may result. In Rice another type of change is inferred in the evolution of varieties with two from varieties with four nucleoli. A mutation leading to the inactivation of the nucleolar body or the loss of a satellite in varieties with four, will give in subsequent generations varieties with four and two nucleoli, (Ramanujam 1937), and the existence of varieties with two and four nucleoli in both the Indian and Japanese types of rice confirms that such a change is quite possible in the evolution of species. Warmke and Johanson (1935) have found in two related species of *Trillium* with the same chromosome number, that one of them has a satellite while the other lacks the same. In *Crepis tectorum*, one of the D chromosomes, which are usually satellited, lost its satellite in the parent plant. (Navashin 1932). The progeny consisted of plants having the two normal D chromosomes and plants in which one D chromosome lacked the satellite. Plants lacking both satellites were found only as embryos, as that condition is lethal to further development. Such a mutation, leading to the loss of a satellite, is thus possible, and hence even in autopolyploids the number of satellited chromosomes may be modified by mutation.

That translocations may involve the fragmentation of the nucleolar body has been demonstrated by McClintock (1934) and different numbers of nucleoli may be formed according to the number of nucleolar bodies present as a result of such fragmentation. Thus the simple rule of the correspondence of satellites and nucleoli may not be applicable to definitely known segmental interchange heterozygotes as *Oenothera, Aucuba, Anthoxanthum*, etc.

All the above instances show the limitations to the general rule that the number of nucleoli is in direct proportion to the number of haploid complements present in the genome.

**Summary**

Examination of several Japanese and Indian varieties of rice indicates that both the Indian and the Japanese types are in two groups, one with two nucleoli and the other with four in the somatic telophase, corresponding to the number of SAT-chromosomes.
The behaviour of nucleolar chromosomes in preventing the fusion of nucleoli in the prophase of meiosis is described.

The basic chromosome number for *Oryza sativa* L. is confirmed by observations of secondary association as five.

The 2n number for *Oryza coarctata* Roxb. is determined for the first time as 48.

The modifications in the nucleolar chromosomes are discussed in relation to changes in chromosome complement.

**Acknowledgments**

It is with great pleasure that I acknowledge my indebtedness to Professor R. R. Gates, for his help, guidance and criticism during the progress of this investigation.

**References**


