An Occurrence of Restitution-Nuclei in the Formation of the Embryosacs in Balanophora japonica, Mak.

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By

Y. Kuwada

In his book "Parthenogenesis und Apogamie im Pflanzenreiche" (1908) WINKLER states: "Für alle die erwähnten apogamen Balanophoraceen muss übrigens noch untersucht werden, ob bei ihnen im Verlaufe der Makrosporenentwicklung eine Reduktionsteilung durchgeführt wird oder nicht. Die bisherigen Untersucher äußern sich darüber nicht, doch muss es auf Grund der von ihnen gegebenen Abbildungen, wie schon JUEL (1900, p. 41) bemerkt, als sehr wahrscheinlich gelten, dass die Reduktionsteilung unterbleibt, so dass also alle Kerne des Embryosacks die diploide Chromosomenzahl führen" (p. 67).

In 1912, Prof. FUJI kindly furnished the writer with material of Balanophora japonica, collected in the Province of Higo, suggesting that he shall carry out an investigation on the embryosac formation of this plant to make clear the question at issue. The work was immediately taken up under his direction and certain interesting results were obtained, but they looked apparently so manifold that a simple explanation would not suffice for the whole solution of the results, and further investigation was discontinued. Recently ROSENBERG has published a paper in which he has analyzed a puzzling process taking place during the heterotype division in pollen mother cells of some Euhieracium species. He has applied a term "Restitutionskerne" to those nuclei formed through this process, and suggested that it is a mechanism for maintaining the somatic number of chromosomes in the embryosac of apogamic or somato-parthenogenetic plants. In the light of this phenomenon of the restitution-nuclei the results obtained in Balanophora japonica appears very easily comprehensible. Now it seems to the writer that some benefit may accrue from the publication of the results so far obtained, though they are somewhat fragmentary.
as yet, because they may have an important bearing on the question raised by Winkler.

In some Euhieracium species Rosenberg has found two types of peculiarities of nuclear behaviour in the formation of microspores that cause the latter to be furnished with the somatic number of chromosomes. 1) In the heterotype metaphase or anaphase, the process of division stops, but the chromosomes proceed to reconstruct the nuclei—the formation of the restitution-nuclei—and enter directly the phase of interkinesis without their normal segregation to poles. The homotype division follows then, and thus pollen cells with an unreduced number of chromosomes will be produced. 2) The homotype division is intercalated into the course of the heterotype division earlier than in the first type. As the first sign of peculiarities of this second type contraction of the nucleus together with the cytoplasm about it takes place in the prophase of the heterotype division, so that the former appears darker. Soon it is restored from the contraction and becomes larger and transparent again. The restored nucleus now shows the appearance of an interkinesis nucleus, its chromosomes being longitudinally split, some taking an X-shape. The nucleus is divided into two through the homotype division without passing through the heterotype phase. The number of chromosomes is, therefore, unreduced. In both these cases only one division—the homotype division—takes place instead of the two, the heterotype segregation being omitted from the normal course of the maturation divisions. So, “Das Endresultat sind Dyadenzellen” (p. 324). Both these types of peculiarities can occur in the same flower heads in Hieracium. “Es besteht also eigentlich kein principieller Unterschied zwischen diesen beiden Teilungsvariationen. Es ist nur der Zeitpunkt für das Einsetzen der homöotypischen Teilung, der die verschiedene Art der Teilung bestimmt” (Rosenberg, 1926, p. 330). In these cases the failure of pairing in some of the chromosomes is a characteristic feature in the heterotype prophase or metaphase. This leads to certain irregularities, especially in the first type. The chromosomes do not migrate toward the poles all at once, but some go further than others, while others may lag in the region of the equatorial plate. If the formation of the restitution-nuclei takes place in this state of division the nucleus produced may, therefore, cause one who has not observed the foregoing stages of the process, to look upon it as being in the process of amitosis. There may be also a case in which the abnormality gives rise to the formation of two or more nuclei of different sizes, when some of the chromosome groups are distributed so far apart from one another that they can not be reconstructed into a single
nucleus.

In *Balanophora japonica* the contraction of the nucleus found in the second type of the peculiarities in *Hieracium* was not noticed, though it is probable that this may occur too in this plant. The first type was on the other hand a usual occurrence in the present case. Material was fixed with the Bonn modification of Flemming's solution and sections were stained with Heidenhain's iron-haematoxylin.

Observation.

In an early prophase of the embryosac mother cell there were seen many fine chromatin threads in the nucleus, some of which presented a certain indication of parallel arrangement (Fig. 7). As to the nature of these parallel threads, whether they represent both parental chromosomes, or longitudinal halves of each chromosome, or anastomoses of old chromosomes in which new ones appear in a spiral or zig-zag form, we can not say anything. No synizesis was found, just as reported by Ernst (1914) in other species of *Balanophora*. In the stage of the nuclear plate of the first division there were found many small round chromosomes which were not arranged evenly on one plane, but some of which were distributed more or less considerably above or below the rest of the chromosomes (Figs. 1, 2, 8). These irregular arrangements were more clearly observed in the side view of the division (Fig. 9). These irregularities that commonly take place in the semiheterotype division lead us to the idea that these chromosomes are those that have failed to pair. The number of chromosomes in this species is by far larger than that of *Balanophora elongata* which was counted by Ernst, but a precise counting was very difficult. The largest number counted in the heterotype nuclear plate was 112 (Fig. 2) and the smallest 94 (Fig. 1). In these two extreme cases there are recognizable size differences in the chromosomes. In the former case where 112 chromosomes were counted, there are more small chromosomes than in the latter where 94 chromosomes were found. This correlation between the size of the chromosomes and their difference in number probably shows that the number of chromosome pairs may be variable, so that counts gave us different numbers of chromosomal elements in different nuclear plates. Such is the case, according to Rosenberg, with the boreale-type of *Hieracium* (1926–27 p. 312). Somatic chromosomes were longer than the heterotype chromosomes, and thus an accurate counting was hardly possible. But their number seems to be not far from the largest number counted on the heterotype nuclear plate (comp.
Figs. 2 and 6). This approximation in the number of chromosomes or chromosomal elements found in both somatic and heterotype nuclear divisions may indicate the unpaired condition of a large number of chromosomes found in the first division of the macrospore mother cells. The formation of the restitution-nucleus takes place at the heterotype metaphase or at slightly later stages. When it occurs at the metaphase, the restitution-nuclei will be spherical. In Fig. 10 a large nucleus in a mother cell is shown. At the upper side of the nucleus a cluster of spindle fibers is found. At this side the restitution is interfered with by the presence of the spindle fibers, so that the surface of the nucleus is not smooth, but very irregular showing deep narrow depressions in places. Somewhat the same situation has been represented by ROSENBERG (1926) in his Fig. 2, E. This nucleus is understood to have been formed through the restitution process at the metaphase or a slightly later stage of the first division. Restitution-nuclei formed at later stages of the division (anaphase) were of irregular shapes, some being of the form which recalls amitosis (Fig. 13), others being of much elongated forms (Fig. 11), and still others being divided into two nuclei of unequal sizes (Fig. 12). In some cases the rest of the spindle fibers were seen near the nuclei (Figs. 14, 15, 16) as is also shown in ROSENBERG'S Fig. 2, F (1926). Fig. 17 shows probably the beginning of the formation of the restitution-nucleus; the rest of the spindle fiber is found near the periphery of the cell (at the left hand side of the figure). This figure is very much like ROSENBERG'S Fig. 13, M, which he explains thus: "M und N zeigen eine Art Restitutionskerne, jedoch ziemlich stark konstrahiert" (1926-27 p. 328). In Fig. 18 a very peculiar nucleus is shown having a ray structure surrounding it. A somewhat similar feature is seen in SAKAMURA'S Fig. 145, which was drawn from chloralized pollen mother cells in *Vicia Faba*. His Fig. 129 may explain the origin of these radiating figures. Fig. 14 is very much like Fig. 1, F and G of ROSENBERG'S (1926).

In some other mother cells which were uninucleate, nuclei were found containing very characteristic double chromosomes of X-shape in it (Fig. 5, a, b, c). In some cases the X-shape was conspicuous in only a few of the chromosomes, although the double nature was very clear in all of them. These nuclei are very much like those shown in ROSENBERG'S Figs. 9, D, F, G; 10, A, D; 13, H, &c (1926-27), and are to be held as those formed through a process belonging to the first or the second type of the peculiarity found in *Hieracium* species. The contraction of the nucleus was not noticed here, but it may occur, and the second type of the peculiarity may take place also in the present plant.
Explanation of figures.

Figs. 1–6 are camera drawings made using Zeiss' apochrom. imm. 2 mm. and comp. oc. 12, all being magnified to the same scale. Figs. 7–23 are microphotographs, all taken with the same magnification.

Figs. 1–2. Chromosomes of the heterotype nuclear plates in macrospore mother cells.

Figs. 3–4. Homotype division. Fig. 3. Metaphase in side view. Fig. 4. Anaphase in polar view.

Fig. 5 a, b, c. Interkinesis nucleus in a macrospore mother cell in three successive sections. 5a is reduced to 13/15 from the original drawing.

Fig. 6. Chromosomes on the equatorial plate in a somatic cell.

Fig. 7. Nucleus of a macrospore mother cell.

Figs. 8–9. Chromosomes in the heterotype metaphase. Fig. 8. Polar view. Fig. 9. Side view.

Figs. 10–11. Restitution-nuclei. In Fig. 11 rudimentary cell plate is seen.

Figs. 12–17. Restitution-nuclei. In Fig. 12 two nuclei of unequal sizes are seen. In Figs. 14–16 rudimentary cell plate is seen. Fig. 17 presents somewhat the same aspect as those drawn in Rosner's Fig. 13, M and N (1926–27).

Fig. 18. Probably a restitution-nucleus having cell plate surrounding it.

Fig. 19. Two daughter cells or macrospores, the lower one containing two nuclei in it.

Figs. 20–23. Produced diads or macrospores, the lower ones being very much smaller than the upper ones. Signs of degeneration of the smaller cell are seen in Fig. 23.
The interkinesis-nuclei formed are divided into two, and two cells of unequal sizes are formed (Figs. 20–23). No mitosis convincingly representing the second (homotype) division was found owing to the fact that in this case the homotype division occupies the position of the heterotype division, but it is highly probable that Fig. 3 and 4 show such mitosis, because in these figures the chromosomes are long and especially in Fig. 3 parallel arrangement of the chromosomes in pairs, which may suggest the longitudinal split, is clear. According to Rosenberg (1926–27), in Hiearcium the chromosomes may be short and thick in this second division. He states:—“Nicht immer haben die Chromosomen diese lang ausgezogene Gestalt, sondern sind mehr kurz und dick” (p. 321). The daughter cells may contain more than one nucleus in them (Fig. 19). This irregularity must have been due to an irregular distribution of chromosomes in the first division which had proceeded to a certain extent before the restitution-nucleus was formed. The further development was not followed, but it is highly probable that these daughter cells are each a macrospore. The upper one is much larger than the lower one. The former is functional and the latter will degenerate, the size of the cell being here so small that it is just a little larger than its nucleus. Signs of degeneration of the nucleus in this lower cell are seen in Fig. 23. The multinucleated macrospores should not have legitimate combinations of chromosomes, so that such cells probably will not develop further.

In his paper (1899) on Balanophora globosa Jungh, Lotsy states: “Diese subepidermale Zelle wird entweder direct zum Embryosack, oder sie ist die Mutterzelle des Letzteren; bei B. globosa theilt sie sich oftens einmal” (Fig. 15, Taf. XXVII), und es werden sehr häufig beide Tochterzellen zu Embryosäcken, die sich ganz normal entwickeln und es beide zur Bildung eines Embryos bringen (Fig. 39, Taf. XXIX)” (p. 180). This description appears to substantiate the writer’s view that the end result in the formation of macrospores is not tetrads, but diads. According to Ernst (1914, p. 134–5), when the division takes place, the daughter cells are only exceptionally of the same size, but as a rule they are of different sizes, of which only the upper, larger one develops further as an embryo sac. This behaviour in B. globosa is quite in agreement with that found in B. japonica. Such an unequal division of the mother cells seen also to occur in B. elongata in Fig. 3, Pl. IV of Treub’s paper (1898). In this figure, which Treub explains, taking it together with Figs. 1 and 2, as showing “des noyaux de sacs.

1) Italics inserted by the present writer.
embryonnaires dans différents stades de division," the beginning of the formation of the cell plate is clearly drawn. It is not drawn in his Fig. 2, which was probably assumed by him as a stage a little earlier than Fig. 3, as is suggested by its being given the lesser number, nor is it drawn in similar figures with higher number, Figs. 4 and 5. About these figures various questions may be raised: 1) Why only in Fig. 3 was the cell plate so conspicuous, while in others no trace of it was to be observed? 2) If the cell (Fig. 3) is an embryosac, the first division must give rise to two free daughter-nuclei which take their position in both polar regions of the cell symmetrically as shown in his Figs. 4 and 5. Fig. 3 suggests, however, that the result should not be free nuclei, but two cells of unequal sizes, the lower one being very much smaller than the upper, because in this figure the cell plate is shown situated nearer the lower nucleus than the upper. 3) Rutgers (1923) is probably right to take vacuolization in the cell as a criterion for distinguishing two phases of the formation and the development of the embryosac in angiosperms. In Fig. 3 no vacuolization is recognizable, while in Fig. 4 and 5, it is very distinctly drawn. These three questions lead the writer to the idea that Fig. 3, Pl. IV of Treub's may be looked upon as corresponding to Fig. 20 of the present paper.

Conclusion and discussion.

The peculiarities found in the formation of embryosacs in Baranophora japonica can be most reasonably explained by regarding them as an occurrence of restitution-nuclei in this plant. If this is the right explanation, the processes taking place in the formation of embryosacs in Baranophora japonica may be summarized as follows:—In the macrospore formation the first division is completely suppressed at a certain stage of the division. Instead of the normal reducing division, the restitution-nuclei are formed and consequently there takes place no reduction in the chromosome number. If the restitution-nuclei are formed in the anaphase, the nuclei produced may have a variable number of chromosomes in illegitimate combinations. Nuclei composed of such an illegitimate combination of chromosomes should degenerate sooner or later. The second (homotype) division follows then and takes place completely, so that only two macrospores are formed. They are of unequal sizes. The upper one is by far larger than the lower and is functionable, while the latter degenerates. The chromosome number in somatic cells is not haploid in the present plant, but diploid, because there is evidence that pairing may take place
at least in some of chromosomes in the prophase of the first division. 

Explained in this way, Baranophora japonica is a plant of somatic apogamy or somatic parthenogenesis. ROSENBERG suggests that in Taraxacum and perhaps also in Antennaria a similar mechanism for maintaining the somatic number of chromosomes in the embryosacs will possibly be found. It must be noted here, however, that the writer is not in a position to insist that such a mechanism as mentioned above normally exist in Balanophora japonica for maintaining the somatic number of chromosomes throughout its whole life cycle. Similar phenomena to what is called "Restitutionskerne" by ROSENBERG are inducible artificially by means of certain anaesthetics or some physical means. It must, therefore, be considered that the formation of the restitution-nuclei might have been due to external conditions rather than internal factors alone. What seems to corroborate this view, is that in Hieracium boreale there are two types of division in the formation of pollen cells—the semiheterotype division and the formation of the restitution-nucleus. It must, of course, be admitted here that there is an internal tendency to respond to external or environmental conditions. This tendency may be strong, in one plant and weak in another. In plants where it is very strong, such peculiarities as what is called the restitution-nuclei would be almost of normal occurrence, because in such plants a slight change in temperature &c will cause the peculiarities. Hieracium pseudoillyricum may be taken as an example of such a kind of plant. Both the plants H. boreale and H. pseudoillyricum and other 27-chromosome species belonging to Archieracium are shown by ROSENBERG to be triploid hybrids (ROSENBERG, 1917. p. 178). When a hybrid consists of two germ cells of plants in a remote relationship from each other, the behaviour of chromosomes in the maturation division may resemble that taking place in these Hieracium species, even if the chromosome number is the same in both parent plants. KARPECHENKO (1927) has recently found a beautiful example for such a case as in the Hieracium species in the Raphanus-Brassica hybrid (9 x 9), and has pointed out the importance of the influence of the external conditions in inducing such a deviation from the normal maturation divisions. He has also called attention to the point that owing to a feeble development of the spindle there is a certain lack of regularity in the distribution of the chromosomes in division, which may give rise to the doubling of the chromosome number. It is a well known fact that a feeble development or non-development of the spindle fiber is inducible by high or low temperature, ether, or certain other physical means (HERTWIG, WILSON,
Sakamura, Heilbrunn, and others). Hybrid plants consist of two different genotypical complexes, and hence, if the difference between the two complexes is sufficiently large, there may be expected a certain disturbance in the normal physiological equilibrium that has been maintained in the homozygous state. In such a disturbed condition in the protoplast, the plants are in a less consistent state and may have more tendency to respond to an abnormal environmental condition than the normal plants, and thus it does not seem improbable that they may show a certain abnormal behaviour even in a slight change in the environmental conditions to which normal plants do not respond at all. If any plants had such a tendency in a strong measure, they might produce gametes provided with the somatic number of chromosomes. Though it remains yet undecided, whether or not the formation of the restitution-nuclei is the only process that takes place in the formation of the embryosacs in Balanophora japonica, this plant seems to have a certain remarkable tendency to respond to changes in the environmental conditions.

On the number of chromosomes in Balanophora species we have hitherto only one report given by Ernst in Balanophora elongata (Tischler, 1927). It is said to be about 16 (Ernst 19). In striking contrast to this, in Balanophora japonica there are certainly more than 112 chromosomes. It has been beautifully demonstrated by Kihara and Ono (1926) that there is an extraordinarily wide range in the chromosome numbers in Eulapathum species of Rumex, extending from 10 to 100 in the haploid number. In Rumex about 100 species are known (Engler-Gilg, 1912), and the known chromosome numbers are 8, 10, 20, 30, 40, 50, 60 and 100. Kihara and Ono expect that it is possible to fill in the gap between 60 and 100. In Balanophora there are known only 12 species (Engler-Gilg) and the two known chromosome numbers are 16 and 112 + ë, numbers which show that there is another wide range in this genus too. It should be, therefore, interesting to know, if there can be found all possible derivations from the base number in other species of Balanophora, or whether there are only a few derivatives to be found in the living species of Balanophora. If the latter is the case, then we want to know what numerical relation there is to be seen between the base number and the surviving derivatives. These may throw some light on the reason why Balanophora japonica has the tendency to produce the restitution-nuclei in the natural environmental condition. Ernst states in his book “Bastardierung als Ursache der Apogamie im Pflanzenreich” (1918 p. 240): “Auch bei den Angiospermen kommt Apogamie recht häufig in poly-
morphismen Verwandtschaftskreisen vor, von welchen wir meistens viel besser als bei den Kryptogamen wissen, dass sie zahlreiche natürliche Bastarde aufweisen und experimentell besonders leicht Arthbastarde ergeben... Für die Verwandtschaft einiger anderer Fälle von Apogamie, wie z. B. Thalictrum purpurascens, stehen Angaben über Polymorphismus noch aus. Auch für Burmannia coelestis, die apogamen Vertreter saprophytischer Gentianaceen und parasitischer Balanophoraceen liegen spezielle Angaben über Polymorphismus nicht vor. Damit ist natürlich nicht gesagt, dass er, namentlich bei den letzteren, nicht doch vorhanden sein kann.

**Summary.**

In this paper an occurrence of restitution-nuclei in the formation of the embryosacs in *Balanophora japonica* has been reported.

The number of chromosomal elements found on the equatorial plate of the heterotype division varies from 94 to 112 so far as the present investigations are concerned. There is a correlation between the number of these elements and their sizes. These facts seem to show that at least in some of the chromosomes there is a tendency to form gemini, but the number of such chromosomes varies in different embryosac mother cells. The chromosome number in somatic cells seems to be not far from the largest number counted in the heterotype division, 112.

The present investigations were undertaken at Prof. Fujii’s suggestion and under his supervision more than ten years ago. In publishing this little note, the writer wishes to express his sincerest thanks to Prof. Fujii for his advice and criticism throughout the work, and for his kindness in giving the writer permission to use his material.

**Literature**


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Revisio Graminum Japoniae XV.
auctore

M. Honda.

186) **Calamagrostis kirishimensis**, Honda sp. nov.

Culmi caespitosi, erecti, 20-25 cm alti, binodi, glaberrimi. Vaginae internodiis breviore, glaberrimae, nodis laevibus. Ligula brevissina, 1 mm longa, truncata, glabra. Folia lineari-lanceolata v. linearia, tenuiter acuminatissima, 8–15 cm longa, 3–6 mm lata, utrinque glaberrima, supra glauca, subtus viridia. Panicula lanceolata v. lanceolato-oblonga, 6–8 cm longa, 1–2 cm lata, strictula, glaberrima, densiuscula, ramis binis cum ramulis glabris. Spiculæ lanceolatæ, 4 mm longæ, acutæ, glabrae, violaceæ. Glumæ steriles æquales : I a lanceolata, acuminata, 4 mm longa, 1-nervis, violacea, glabra sed nervo superne ciliolato; IIa² conformis sed obscure 3-nervis, nervis lateralibus brevissimis, laevæ v. vix carina scaberula. Gluma fertilis 3.5 mm longa, hyalino-membranacea, minutissime scaberula, oblongo-lanceolata, apice denticulata, inferne 5- superne 4-nervis, infra medium dorsum aristata, arista tenui, 2–3 mm longa, callo pilis 2/3 glumae fere aequantibus densiusculæ obsito. Paleæ 3 mm longæ, hyalina, laevæ, apice mutica, binervis, nervis in setulas brevissimas excurrentibus. Caryopsis oblongo-lanceolata, 2 mm longa. Processus rhachillae vix 1 mm longus, pilis longis densiusculæ vestitus.

Nom. Jap. Kirishima-nogariyasu (nov.)

Kiusiu : in monte Kirishima, prov. Hiuga (V. Doi, no. 71, anno 1927)