

CYTOLOGICAL STUDY IN *PASPALUM* *CONJUGATUM* BERG⁽¹⁾

J. S. FANG and H. W. LI⁽²⁾

(Received Jan., 1965)

Introduction

According to Magoon and Manchanda (1961), the genus *Paspalum* embraces about 200 species widely distributed in the tropics and subtropics of both hemispheres. However, only 9 species have been found in Taiwan. Many species of this genus have some considerable economic importance as forage or pasture grass. Although only a few species were studied cytologically, these observations, however showed that polyploidy and irregular meiotic division were very common in this genus. The frequent occurrence of univalents would suggest hybridity and apomixis. With *P. conjugatum*, it was found that the chromosomes were invariably unpaired throughout.

In the past, studies of microsporogenesis on several species of *Paspalum* were made by Snyder (1961), Magoon and Manchand (1961), Bashaw and Forbes (1958), Chen and Hsu (1961), Chen (in press) and others. These studies showed extensive meiotic irregularity of this genus. The most striking features were complete asynapsis and meiotic non-reduction of the chromosomes in microsporogenesis and the predominant production of dyads instead of tetrads after two successive divisions. The nature and pattern of the formation of these irregularities in meiosis were differently explained by different hypotheses by different authors.

The present study concerns with the species endemic in Taiwan, particularly with those having asynapsis and non-reduction in meiosis. One of the endemic species (*P. conjugatum* Berg.) was studied by Chen (in press). However, the present authors found that these irregular meiotic divisions would be explained differently in addition to those reported by Chen. Moreover, according to Snyder (1961), Walters (1958) and Chen (in press), the formation of restitution nucleus was attributed by a return movement of chromosomes from the poles to the equatorial region and a pronounced shortening of the spindles which

(1) Paper no. 38 of the Scientific Journal Series, Institute of Botany, Academia Sinica.

(2) Research Assistant and Research Fellow of Institute of Botany, Academia Sinica respectively.

were readily apparent when the chromosomes occupied in position at the equator. By and large, it is expected that this study would lead us to further investigations of the genus *Paspalum* along this and other lines of research, especially in regard to the mechanisms involved in the irregular meiotic division in many of the species found in this genus. Furthermore, this may lead us to the study of apomixis in general.

Material and Methods

The materials used in the present study of meiosis of *P. conjugatum* were collected from the field of Northern Taiwan, and some from transplanted plants in the greenhouse. Flowering buds (spikes) were fixed in the standard 3:1 mixture of 96% ethanol-acetic acid solution for 20 minutes at 60°C. Then these were washed with 70% alcohol and were smeared and stained with propino-carmin. Ferricion was added to facilitate the staining of the spindles.

Observation

Snyder (1961) and Chen (in press) described the almost complete asynapsis at diakinesis and metaphase 1, the meiotic non-reduction and the predominant production of dyads of microspores in some species of *Paspalum*. However, in the present study in *P. conjugatum* there are other irregular meiotic divisions. The observation are given as follows:

1. *Pollen fertility*: Of more than five thousand grains examined, an average of 42% of these grains were found to be well stained. (Fig. 1). These pollen grains were presumed to be of un-reduced chromosome constitution.

2. *Meiosis in Microsporogesis*:

- A. *Normal division*: Due to the presence of a large number of chromosomes, $2n=40$, it was very difficult to identify the individual chromosome at pachynema. However, the chromosomes seemed to be unpaired showing asynapsis of this species. Snyder (1961) and Chen (in press) found the same condition in some species of the genus. In diakinesis the 40 chromosomes were found unpaired (Fig. 2). At first metaphase when the chromosomes were still univalents, they were randomly and loosely arranged in the equatorial region (Fig. 3). The spindles became well-defined at this stage, then the univalents moved randomly toward the poles (Fig. 4). Laggarads appeared quite frequently from anaphase to telophase. In the normal division, the nucleus was found to be large-sized and the spindles formed subsequently was also found to be of normal length. After two successive divisions these led to the formation of a normal tetrad. (Fig. 6). It must be mentioned here in passing that this kind of division occurred only about 1% of all the cells

examined. (diagram 1, 17-21). Since these gametes were unbalanced in number or/and in constitution, the gametes formed would be sterile.

B. *Formation of restitution nucleus*: It can be found from diagram 28-32 that the pollen mother cells with small nucleus would lead to form subsequently a compact spindle which would pave the way to the formation of restitution nucleus and finally the formation of a dyad (Fig. 7-11). The second division was usually regular. So after two successive divisions the gametes formed would be diploid in nature. However, in some cases the formation of restitution nucleus was not quite so successful. The cross wall formed at the end of first division would bisect this restitution nucleus into two parts leading to the formation of a tetrad after the end of second division. (diagram 33-36, 13-16, Fig. 12-15).

C. *The formation of a dyad after two successive divisions when the nucleus was rather normal sized with almost normal spindle*: In our observation it was found that about 98% of the pollen mother cells would evolve to become dyads after two successive divisions rather than the formation of tetrad (about 1% observed). As mentioned before a small nucleus and a compact spindle invariably would lead to the formation of a restitution nucleus. But the number of this kind of pollen mother cells was too small to explain the predominant presence of pollen mother cells with apparent normal sized nucleus and normal sized spindle. Snyder (1961) and Chen (in press) offered the explanation that the formation of restitution nucleus in this kind of pollen mother cell was accomplished by the return of the chromosomes from the poles to the equator and subsequently the shorting of the spindle at the end of first division. Our observations so far could not find any indication of the univalent chromosomes to migrate reversely to the equator again after they migrated to the poles at the start of anaphase. We found in the anaphase figures, some univalents would migrate toward the poles and others would come to the equator perhaps in the process of congression even through somewhat delayed (Fig. 16). But never in a time did we observe all the univalents would migrate backward to the equator *en bloc*. This would indicate seemingly the non-existence of reverse migration of the univalents in *P. conjugatum*, the material we used. Since most of pollen mother cells were of apparently normal sized nucleus and normal sized spindle, but after second division there were only 1% tetrad formed, it was assumed, therefore, that this type of pollen mother cells would form restitution nucleus at the end of first division irrespective of their apparently normal sized nucleus and spindle. The mechanism for the formation of restitution nucleus is unknown. (diagram 1-8, Fig. 17-19).

D. *Formation of restitution nucleus with the exclusion of one or a few chromosomes*: If our observation was correct, about 98% of the pollen mother cells

would form dyads after two successive divisions, then the fertile pollen would be 98% as the top limit. In fact, we found only 42% of the pollens to be well-stained. This showed about half of these pollen mother cells would give rise to unbalanced gametes formation. In many cases observed when the restitution nucleus was formed, one or a few chromosomes would not be included in the restitution nucleus. Rather they would stay at one pole or between the pole and the equator (Fig. 20). So the gametes formed would be unbalanced as far as number was concerned. Frequently enough ostracized chromosomes from the restitution nucleus would subsequently form a minute spindle and they would divide in second division. (diagram 9-12, Fig. 20-22).

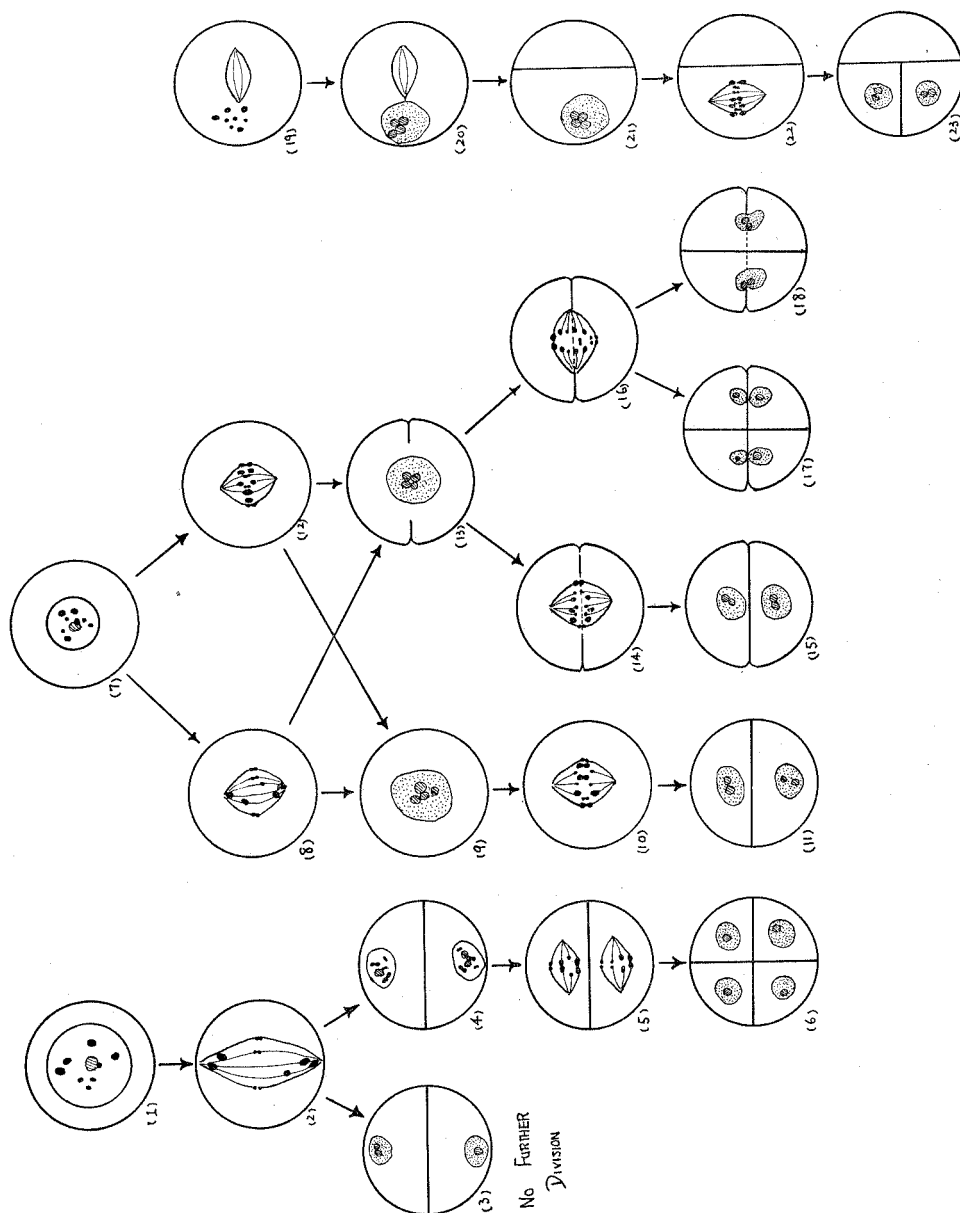
E. *Non-synchronization of nuclear division and spindle formation*: Li *et al* (1964) and Ho *et al* (in press) found that this type of non-synchronized nuclear division and cytokinesis in meiotic division in microsporogenesis was rather common in the interspecific hybrid of *Oryza sativa* × *O. officinalis*, *O. sativa* × *O. brachyantha* and *O. sativa* × *O. australiensis*. Since it was described in detail, so no further description was necessary here. At the end of second division a triad was formed with two unreduced gametes. (diagram 37-42, Fig. 23-26).

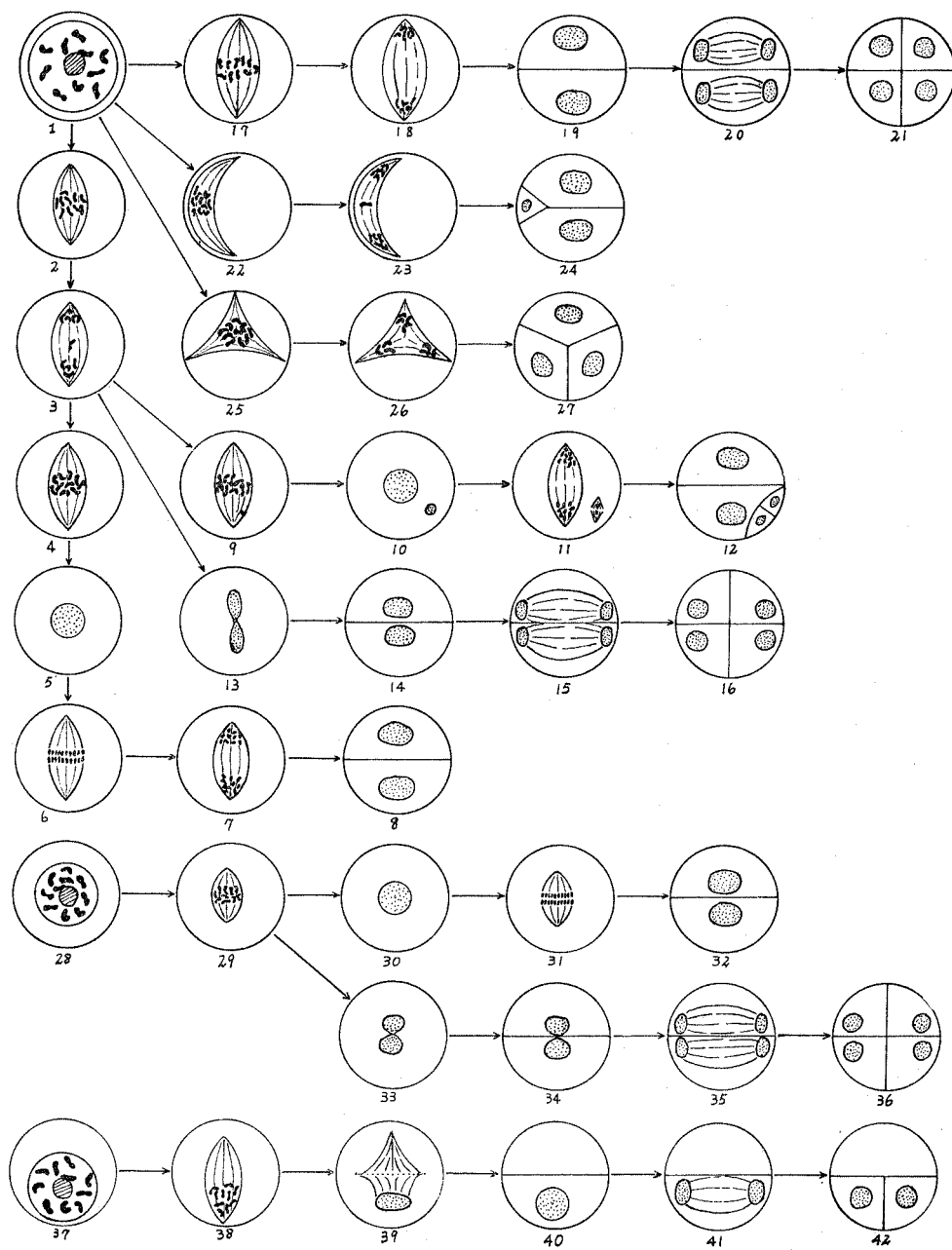
F. *Other abnormal division*: There were found in *P. conjugatum* some curved spindles (Fig. 27) and triangular spindles (Fig. 29) that would give rise to unequal triad at the end of first division (Fig. 28, 30). However, no hexads were observed. (diagram 22-24, 25-27).

Discussion

There are 9 species of *Paspalum* endemic in Taiwan and was studied by Chen (in press). According to his report, *P. Vaginatatum* is a diploid ($2n=20$) with all chromosomes well paired. *P. Urvillei* is a tetraploid ($2n=40$) with all chromosomes well paired. *P. conjugatum* is also a tetraploid as being mentioned. *P. dilatatum* is a pentaploid with $2n=50$. In microsporogenesis there are 20 bivalents and 10 univalents at diakinesis and metaphase I. *P. orbiculare* and *P. thunbergii* are hexaploid with $2n=60$. The meiotic division is irregular. *P. distichum*, *P. longifolium* and *P. scrobiculatum* (*P. commersonii* Lamk) are either tetraploid or hexaploid. The chromosome number is found to be either $2n=40$ or $2n=60$ in different collections collected at different locations. The meiotic division of the tetraploid or *P. distichum* and *P. scrobiculatum* are found to be regular. However, the meiotic division of these three species are found to be very irregular.

The pseudogamous apomictic plants that have been studied cytologically include a number of species in which irregularities occur in the microsporogenesis. The irregularities consist of multivalent formation or the occurrence





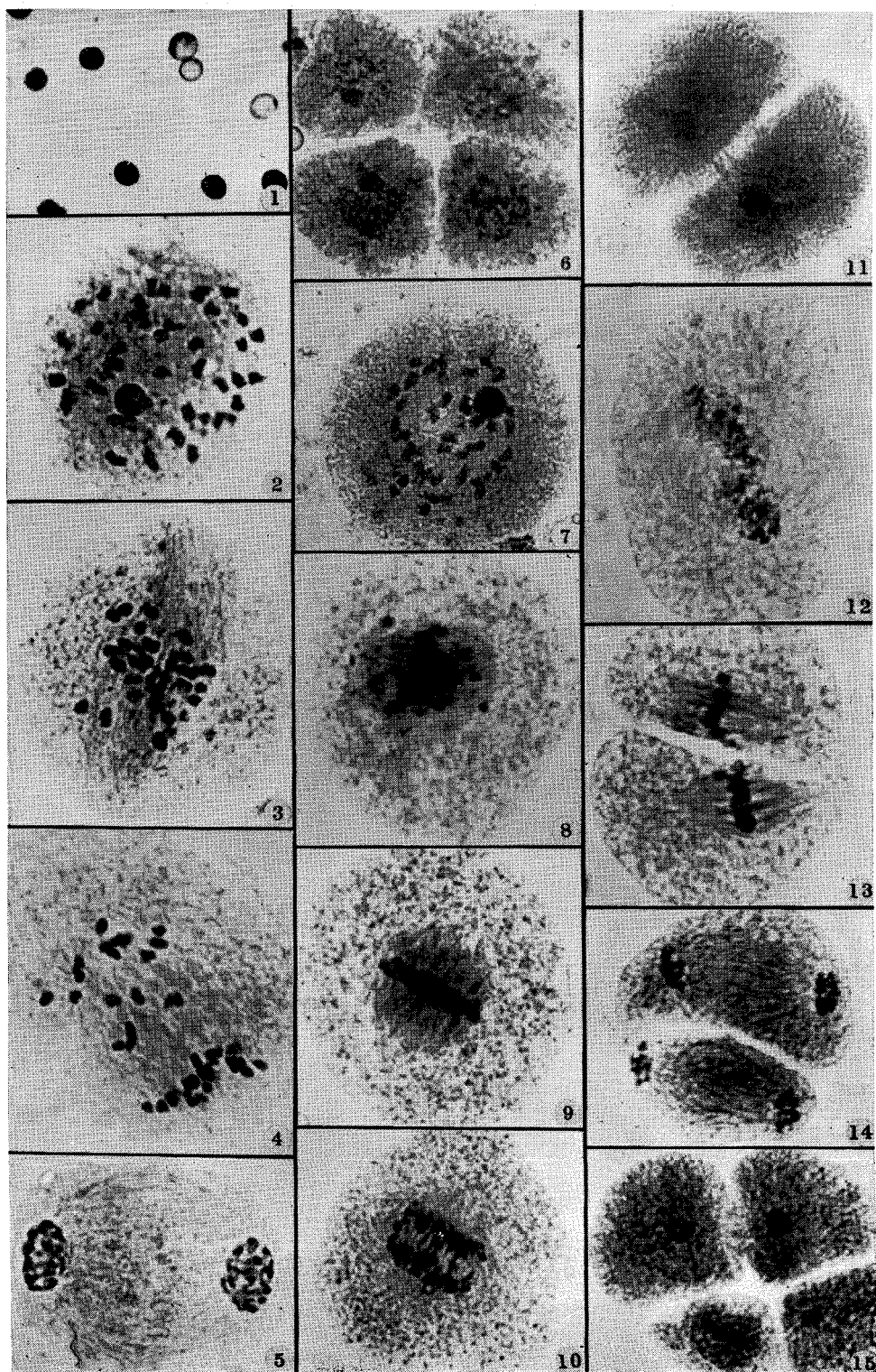
of univalents in high frequencies that would suggest hybridity. Burton (1942) examined the chromosome number in several species of *Paspalum* and concluded that 10 would be the basic number for this genus. He also demonstrated the existence of a new basic number of either 6 or 12 in the same genus. Smith (1948) found that polyploidy, meiotic irregularity and probably apomixis are frequent in the predominantly tropical genus *Paspalum*. Biotypes were also found in some species of this genus by Bashow (1958). The fact that each type maintains a constant chromosome number in the presence of extreme meiotic irregularity and the relative uniformity of progeny appearance suggests some form of apomictic reproduction. Chen and Hsu (1961) reported that *P. conjugatum* has an irregular meiosis, possibly synapsis does not take place at the zygonema. At later stages of meiosis 40 chromosomes are observed to be completely unpaired at diakinesis and metaphase (univalents) are capable of going to the equatorial plate where they clump together. The occurrence of complete asynapsis, formation of restitution nucleus, meiotic non-reduction and predominant production of dyads are observed as had been reported by these investigators.

In *P. conjugatum*, there were 42% of pollen grains to be well-stained. Possibly they are diploid microspores. These gametes are formed as result of the formation of restitution nucleus at the end of first division of a pollen mother cell with supposedly normal sized nucleus and more or less normal spindle. In pollen mother cells with small sized nucleus and rather compact spindle, dyads are formed under two successive divisions. The other possibilities for the formation of diploid gametes would be the nonsynchronization of nuclear division and spindle formation in some of the pollen mother cells leading to the formation of triad after two successive divisions.

P. conjugatum has 40 unpaired chromosomes in meiotic division. From the study of microsporogenesis it shows that the species is an enforced hybrid. Perhaps it would be similar in nature as *P. secans* described in detail by Snyder (1957) in megasporogenesis.

Summary

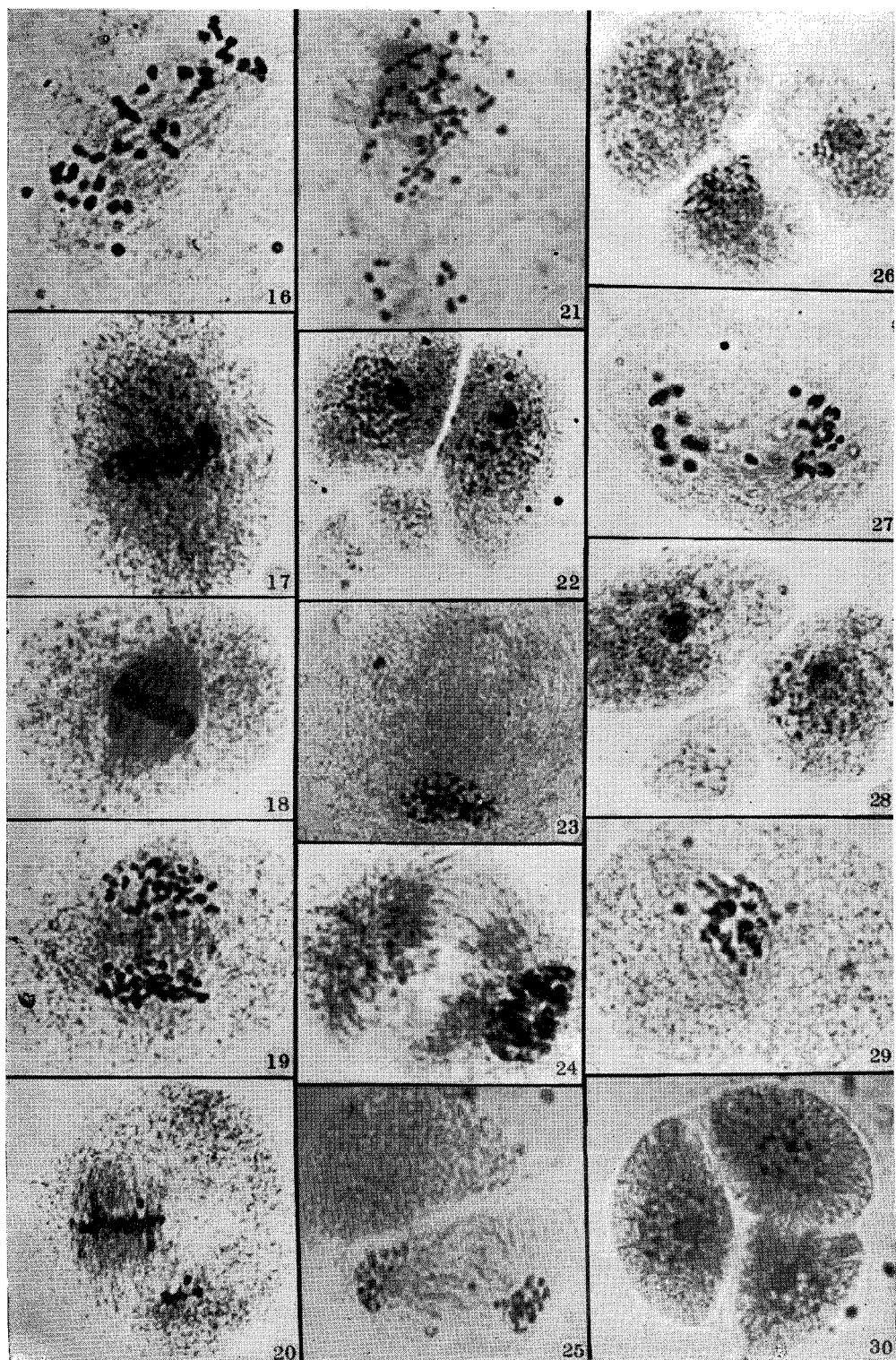
There were 9 species of *Paspalum* found to be endemic in Taiwan. *P. conjugatum* had 40 chromosomes. No pairing was found at pachynema, diakinesis and metaphase I. 42% of the pollen grains examined were found to be well-stained, presumably they were diploid pollen grains. In microsporogenesis pollen mother cells with normal sized nucleus and normal spindle would divide normally to form tetrads after two successive division. However, only about 1% of pollen mother cells were of this type. About 1% of pollen mother cells were of small nucleus and very compact spindle. This type of pollen mother



cell would give rise to the formation of restitution nucleus leading to the formation of dyads at the end of second division. In general, most of the pollen mother cells (about 98%) would be of normal sized nucleus and almost normal sized spindle. Nevertheless, restitution nucleus was formed in this kind of pollen mother cell leading also to the formation of dyads after two successive divisions. From our observation, the univalents did not make the reverse migration from the pole to the equator *en bloc* to form the restitution nucleus. Rather, they migrated in both ways which might explain the possibility that some univalents were coming to the equator to congress and others would migrate to the poles after congression took place.

Explanation of plates

- Fig. 1. Showing about half well-stained pollen grains with unreduced number of chromosomes.
- Fig. 2. Diakinesis, showing 40 univalents widely scattered in the nucleus.
- Fig. 3. MI with 40 univalents randomly and loosely arranged in the equatorial region. Notice the length of spindle.
- Fig. 4. MI-AI, 40 univalents migrated randomly toward the poles.
- Fig. 5. TI, the cross wall was formed.
- Fig. 6. Tetrad.
- Fig. 7. Diakinesis with a compact and small nucleus.
- Fig. 8. Restitution nucleus was formed with short and compact spindle.
- Fig. 9. Metaphase of post-restitution nucleus division with short spindle.
- Fig. 10. Early Anaphase of post-restitution nucleus division with short spindle.
- Fig. 11. Dyad.
- Fig. 12. The restitution nucleus was bisected by the cross wall formed at the end of first division.
- Fig. 13. MII, the chromosomes are very close between both cells.
- Fig. 14. AII, two halves not of equal size.
- Fig. 15. Tetrad with unequal sized sporads.
- Fig. 16. Anaphase of pre-restitution nucleus, showing some univalents migrate toward the poles and others would come to the equator.
- Fig. 17. Restitution nucleus formed by normal sized spindle.
- Fig. 18. Metaphase of post-restitution nucleus.
- Fig. 19. Anaphase of post-rest-restitution nucleus, showing non-reduced meiotic division.
- Fig. 20. A few chromosomes were excluded from the restitution nucleus.
- Fig. 21. Post-restitution anaphase division. The sticky nature of the chromosomes was still remaining. Five ostracized chromosomes were dividing as the restitution nucleus.
- Fig. 22. Tetrad, with two small sporads.
- Fig. 23. MI-AI, with all chromosomes located in one pole.
- Fig. 24. Telophase I, the cross wall was formed.
- Fig. 25. Early TII, all chromosomes in one of the halves only.
- Fig. 26. Triad, with the chromosomes in 2 sporads.
- Fig. 27. AI, with curved spindle and one laggard chromosome.
- Fig. 28. Triad, with one small microspore.
- Fig. 29. MI-AI, with triangular spindles.
- Fig. 30. Triad, with unbalanced chromosomal number.



In the formation of the restitution nucleus, one or a few chromosomes sometimes were ostracized naturally. These gametes formed from this type of division subsequently would be the unbalanced sterile ones. The pollen mother cells with nonsynchronization of nuclear division and spindle formation were also observed leading to the formation of triads with two unreduced gametes. Other abnormal types in meiotic division were also described.

Paspalum conjugatum 之細胞學的研究

方 菊 雄 李 先 聞

在臺灣, *Paspalum* 屬植物共有九種, 其中 *Paspalum conjugatum* 的染色體數為 $2n=40$ 。觀察其細胞分裂情形, 得知染色體在粗絲期 (pachynema), 肥厚期 (diakinesis) 乃至第一中期 (metaphase I) 均無配對現象。花粉經碘液 (I_2-KI) 染色, 只有42%是好的, 這些花粉具有40條染色體在 microsporogenesis 過程中, 細胞大小及紡錘體均正常的花粉母細胞, 經過減數分裂兩次連續的分裂, 遂形成四分子, 但是這種情形只佔 1%。另外大約有 1% 的花粉母細胞, 其細胞核較小, 紡錘體緊縮, 第一次分裂的結果, 會形成 restitution nucleus, 以致在第二次分裂後 (post restitution nucleus division), 產生二分子。一般而言, 大多數 (約98%) 的花粉母細胞, 其核的大小及紡錘體均正常, 却仍然會形成 restitution nucleus, 進而經第二次分裂而產生二分子。依 Synder (1961) 及陳其昌 (付印中) 的解釋: 當減數分裂的第一次分裂完畢, 分別移到二極的單價 (未配對) 染色體全都回到赤道板上, 同時, 紡錘體縮短, 因而形成 restitution nucleus。但由本實驗觀察的結果, 並沒有發現所有的單價染色體分向兩極後, 再由兩極回到赤道板上的例子, 而是一部的單價染色體回到赤道板來行聚合的作用, 其他的移到兩極去。所以, 到目前為止, 這類 restitution nucleus 形成的機構 (mechanism) 仍不清楚。

在形成 restitution nucleus 時, 往往有一條或數條染色體被遺落在 restitution nucleus 的外面, 經過這樣分裂而形成的配偶子, 因染色體的數目或構成分不均衡, 而為不孕者。又假如細胞核分裂與形成紡錘體的時間不一致, 也會導致三分子的形成, 其中一個為空的, 另二個為染色體未減數的配偶子。

此外, 在 *Paspalum conjugatum* 也發現了其他的不正常減數分裂的情形。

Literature Cited

- BASHAW, E. C., and I. FORBES. Chromosome number and microsporogenesis in *Dallisgrass*, *Paspalum dilatatum*. Agron. Jour. **50**: 441-445. 1958.
BURTON, G. 2. A cytological study of some species in the tribe Paniceae. Amer. Jour. Bot. **29**: 355-359. 1942.
CHEN, C. C., and C. C. HSU. Cytological studies on Taiwan grasses. 1. Tribe Paniceae. Bot. Bull. Acad. Sinica. **2**: 101-110. 1961.
CHEN, C. C. A cytological study of Taiwan *Paspalum*. Bot. Bull. Acad. Sinica. (in press).

- LI, H. W., KATHERINE K. S. YANG, and KWEI-CHI HO. Cytogenetical Studies of *Oryza sativa* L. and Its Related Species, 7. Non-synchronization of Mitosis and Cytokinesis in Relation to the Formation of Diploid Gametes in the Hybrid of *Oryza sativa* L. and *O. officinalis* Wall. Bot Bull. Acad. Sinica. **5**: 142-153. 1964.
- HO, KWEI-CHI and H. W. LI. Cytogenetical Studies of *Oryza sativa* L. and Its Related Species, 10. Study on Meiosis and Unreduced Gametes Formation of the Hybrid *O. sativa* L. \times *O. australiensis* Domin. (in press).
- MAGOON, M. L. and P. L. MANCHANDA. A cytological study of some species in the genus *Paspalum*. Ind. Jour. Genet. Plant Breed. **21**: 212-221. 1961.
- SMITH, B. W. Hybridity and apomixis in the perennial grass *Paspalum dilatatum*. Genetics **33**: 628-629 (abstract). 1948.
- SNYDER, L. A. Apomixis in *Paspalum sedans*. Amer. Jour. Bot. **44**: 318-324. 1957.
- SNYDER, L. A. Asyndesis and meiotic non-reduction in microsporogenesis of apomictic *Paspalum secans*. Cytologia **26**: 50-61. 1961.
- WALTERS, M. S. Aberrant chromosome movement and spindle formation in meiosis of *Bromus* hybrids: An interpretation of spindle organization. Amer. Jour. Bot. **45**: 271-289. 1958.