MICROSPOROGENESIS OF CERTAIN EDIBLE BANANAS*

by

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INTRODUCTION

Cheesman, Simmonds, Dodds, *et. al.* have published a series of papers under the subject of "Genetical and cytological studies of *Musa*" in the *Journal of Genetics* from 1932 on. They also have conducted the breeding program of bananas at Trinidad, British West Indies. Same work has been carried out in Jamaica.

Edible bananas are highly vegetatively parthenocarpic and sterile. The parthenocarpy is generally believed due to the higher auxin concentration in the developing ovary[4]. They belong to the subgenus *Eumusa* of the genus *Musa*. White[9] recorded the basic chromosome number of *Musa* in multiples of 4, while Cheesman and Larter[2] found that the haploid chromosome number in *Eumusa* is 11.

Majority of the edible bananas are triploids (3x=33)[2]. However, Dodds[4] reports five diploid edible varieties of bananas in the Banana Collection of the Trinidad Banana Institute. Among them, 4 are hybrids and 1 is a single reciprocal translocation. Apple and honey bananas of Jamaica are also diploids[6].

The present study confined to the chromosome study of the 3 commercial varieties of bananas widely cultivated on the Island of Formosa. They are Feng Cha (粉蕉) of the *Musa Cavendishii* and Pei Cha (北蕉) and Sen Jen Cha (仙人蕉) of the *M. sapientum*. Sen Jen Cha was reported to be a mutant of Pei Cha[8]. In this study, it is in attempt to discover the chromosome complexities of these 3 varieties and also to clarify the similarities and differences in chromosome complements among these varieties in order to lay a foundation for further survey and to offer some cytological knowledge needed for banana breeding on these varieties.

MATERIALS AND METHODS

The terminal bud of a banana inflorescence, after it has passed its femals stage, consists of a series of overlapping bracts, with a cluster of male flowers in each. Upon fixing the material, the bud was cut from the plant at about 5 o'clock p. m. and the bracts, with its subtended flowers, were dissected off and dropped in order into bottles. The fixing fluid used was acetic alcohol (one part glacial acetic acid and three parts absolute alcohol). Pollen mother cells were squared on the slide and stained with

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acetocarmine. Temporary mounts were made with the seal of paraffin bee wax. Permanent mounts were made by dropping slide in 95% ethyl alcohol and dried and mounted with Euparal.

Root tips were obtained from the rhizomes of the plants in the field and fixed in Craf's solution. Paraffin sections were cut at about 10 μ in thickness and stained in crystal violet.

Drawings were made under the microscope with the aid of a camera lucida. Photographs were taken with the E. Leitz "Makam" ×1 camera.

Materials were collected from the collections of both the Taiwan Agricultural Research Institute and its Chiayi Experimental Station.

DESCRIPTIONS

Meiosis of microsporocytes from metaphase I onwards had been examined in the 3 varieties in order to make a comparative study of their chromosome complexities. One fertile diploid species *Musa textilis* (2x=20), showing regular divisions was investigated for comparaison. Somatic chromosome counts were made to assist the accuracy of the chromosome numbers being observed in microsporogenesis. Earlier stages than metaphase I could not be satisfactorily analyzed owing to the smallness of the banana chromosomes. The features of microsporogenesis of these 3 varieties are rather unique.

Due to the smallness of the chromosomes and very irregularities of the chromosome movement at anaphase I, it is difficult to find out the differences in the the chromosome shape and size and to analyze the mode of chromosome movements among these 3 varieties. However, the difference in chromosome associations at metaphase I can be recognized and analyzed among these varieties.

All these 3 varieties studied are triploids, being with 33 somatic chromosomes as identified from both smear and section materials. At metaphase I, univalents, bivalents, and trivalents are in evidence. One quadrivalent was observed in one cell of Pei Cha. Although they are triploids, no one cell examined of these 3 varieties there are chromosomes arranged in 11 groups of three each. Trivalents are found only in few numbers. In Pei Cha they vary from 1 to 4; 0 to 2 in Sen Jen Cha; and 1 to 3 in Feng Cha. Bivalents are most common and vary from 6 to 14. More bivalents are found in Pei Cha and Feng Cha than in Sen Jen Cha. As shown in the following table, it is interesting to note that the number of univalents of sen Jen Cha is far greater than that of Pei Cha, the former being believed to be a mutant of the latter. The following table summarizes the chromosome associations of these 3 varieties at metaphase I:

Table I. Chromosome associations at MI

Var.	No. of cells examined	Average No. of		
		Univ.	Biv.	Triv.
Pei Cha	51 (Fr. 7 pts.)	2.94	12.55	1.64
Sen Jen Cha	60 (Fr. 8 pts.)	13.80	8.40	0.80
Feng Cha	54 (Fr. 6 pts.)	5.57	11.14	1.71

The univalents vary considerably in position. Sometimes they are near or in the equatorial plate (Text fig. 1). Sometimes they lie within the spindle some distance from the plate on either side. It is commonly observed that one or more univalents lie in the cytoplasm far apart from the spindle (Text fig. 5). Occasionally, microsporocytes were observed with sevaral univalents lying in a separate spindle distinctly apart from the major one (Text fig. 3). Here they are preparing to divide at the same time that the bivalents and trivalents of the major spindle are about to disjoin. The bivalents and trivalents are mostly arranged on the equatorial plate (Text figs. 1, 3, 4). However, in few microsporocytes, the bivalents show unequal tensions and lack of coincidence (Text fig. 5).

The distribution of the chromosomes at anaphase I appears to be a random one. Only in few figures the chromosomes pass to the poles regularly (Text figs. 7, 9). In some microsporocytes, with some dyads have moved to the poles, while others still remain on the equatorial plate, and with bivalents even not yet divide (Text fig. 6). Failure of anaphase movement of certain number of dyads is observed in a number of microsporocytes (Text figs. 8, 10, 11). Therefore it is often impossible to predict the exact number of dyads distributed at the two poles and can not verify the condition of disjunction at anaphase I among these 3 varieties.

As the result of many irregularities observed at both metaphase I and anaphase I, the appearence of the microsporocytes at telophase I and interphase is diverse. In some microsporocytes, two daughter cells formed are approximately equal in size and are separated by cell plate. But such cases are not common. Text figure 15 shows the formation of 2 small daughter cells between the two major daughter cells. These are apparently formed by chromosomes failured to be included in the major nuclei. In text figure 16, three daughter cells are found in a microsporocyte, one being very small and two large. In one of the two large daughter cells there appear several small nuclei which are not set off from the larger nucleus by the cell plate. Such small nuclei are organized by the chromosomes failured to be included in the major nucleus. In rare cases, persisted bridges are observed as the daughter nuclei are being formed and wall formation has initiated (Text fig. 14).

During the interkinesis, the dyads become invisible. Each daughter nucleus has one large or several rather small nucleoli. As the prophase II progresses, the characteristic X-and H-shaped chromosomes become evident. In one cell examined in Sen Jen Cha (Text fig. 17), there are 33 dyads at late prophase II. This is presumably due

to the failure of first division, either due to the failure of anaphase I movement as previousely described or due to something else. As observed, 4 dyads are rather far apart from the equatorial plane. They would fail to have normal orientation at metaphase II. However, in many cases, metaphase II and succeeding stages appear much regular than the preceeding stages. At metaphase II, the chromosomes are mostly arranged in the equatorial plate (Text fig. 20). The lagged chromosomes between the two daughter cells although appear again (Text fig. 19) at the second division, they are not orderly arranged. However, chromosomes of the small nuclei within the large cells show regular orientation and division (Text fig. 20) and regular anaphase II movement (Text fig. 18). Their stage may coincide with that of the major nuclei (Text figs. 18, 20). In some cases, failure of successful move- ment of the chromosomes in the second division was observed.

The microspores formed in a microsporocyte vary from 2 to 11, generally from 4 to 7 (Table II). The average number of cells per tetrad is about 6 in Pei Cha, 5 in Sen Jen Cha, and 5.4 in Feng Cha.

Table II. Cells per tetrad

Var.	No. of tetrads	Cells per tetrad										
		2	3	4	5	6	7	8	9	10	11	Peak
Pei Cha	63	—	—	12	12	20	5	10	—	3	1	6
Sen Jen Cha	63	4	1	18	21	14	3	1	1	—	—	5
Feng Cha	63	2	—	11	20	19	4	6	—	1	—	5—6

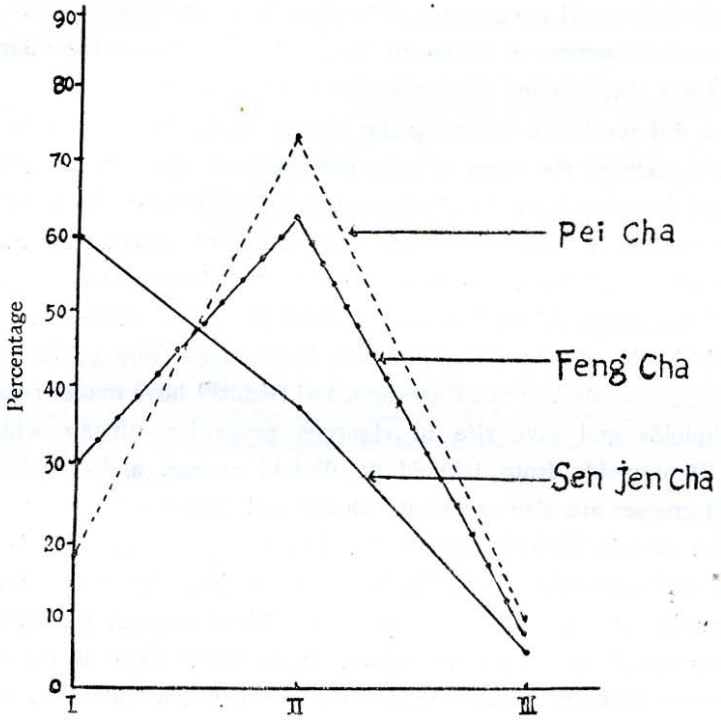
Normal tetrads have been observed in all of these 3 varieties but the frequency is very low. Thus, it can be assumed that many nuclei in the spore cells of these varieties are unbalanced owing to the irregular meiosis and that good pollens are rarely formed (Text figs. 24–26). Study of tetrad conditions among these varieties has comparative value to predict their chromosome behavior at meiosis.

DISCUSSION

Microsporogenesis of 3 varieties, Pei Cha, Sen Jen Cha and Feng Cha was observed and described. They are all triploids, being with 33 somatic chromosomes. Feng Cha belongs to the species *Musa Cavendishii*, while Pei Cha and Sen Jen Cha are included in the species *M. sapientum*. The plants of the former species are dwarf and with petioles 6 inches or less in length, while the plants of the latter species are tall and with petioles 1 foot or more in length. The variety Pei Cha is very similar to the variety Sen Jen Cha in external morphology. They are only slightly different in the length, width, and colorness of leaves, in the length of stems, and in the slenderness of the fruit stalks and of fruits. However, the latter, as generally believed to be a mutant of the former, is resistant to the wilt disease.

All these 3 varieties have 33 somatic chromosomes. As the banana chromosomes are very small and not differentiated at pachytene stage, it is impossible to distinguish the chromosome shape among these 3 varieties, neither the chromosome size. As the

chromosome movement is very irregular and the failure of anaphase movement occurs in high percentage, it is hard to analyze the discrepancies of chromosome distributions among these varieties. Yet the mode of the chromosome associations at metaphase I can be clearly observed and analyzed among these varieties. The chromosome configurations at metaphase I have been summerized in table I. The following graph summerizes the data in terms of percentage of different types of chromosome configurations.



Graph showing the percentage of different types of chromosome configuration at MI of the three varieties

Form this graph it is found that the types of chromosome configurations of these 3 varieties are quite different, particularly between Pei Cha and Sen Jen Cha. The highest peak of Pei Cha is bivalents, which is up to 73%; while in Sen Jen Cha, univalents are most prevalent, which reaches to 60%. About two-third chromosomes in Pei Cha are evidently associated into bivalents and with the rest unassociated or joined into trivalents. In Sen Jen Cha, about two-third chromosomes are remaining unassociated and with the rest paired into bivalents or trivalents. The condition of chromosome association of Feng Cha is intermediate between the above mentioned two varieties. The highest peak is bivalents; univalents come next; and trivalents are very rare. These differences are further evidenced in their tetrad condition. Classified according to the number of cells per tetrad, Pei Cha falls to the 6-celled class, Sen Jen Cha 5-celled class, and Feng Cha between 5- and 6-celled class (Table II).

As described in the above paragraph, chromosome configurations of the two varieties, Pei Cha and Sen Jen Cha, are very diverse, so that particular attention should be paid to these two varieties, because of the latter being conidered as a mutant of the former.

Triploid bananas are highly sterile and propagate by vegetative means. Hence the mutation occurred must be somatic. A number of somatic mutations is known among the banana complex[1,3,6]. Generally they only affect colour, habit, etc. Sen Jen Cha might exist as a real mutant of Pei Cha as the result of somatic mutations. If this had been the case, differentiation of chromosome sets in Sen Jen Cha would have taken place following the history of the variety, so that its chromosome structure, in accompanying the chromosome association, is different from its ancestor. Somatic mutation and chromosome differentiation would cause some differences between these 2 varieties, as in the length, width, and colourness of leaves, in the length of stems, in the disease resistance, as well as in their chromosome configurations at metaphase I.

Although chromosome differentiation following the history of Sen Jen Cha from its ancestor, Pei Cha, might explain as the cause of the difference in their chromosome associations at metaphase I between these 2 varieties, yet this seems not to be a sound one. There is possibility that these 2 varieties might have different origins, although they are similar in external morphology. As a matter of fact, interspecific crosses between diploid species of the genus *Musa* are with remarkable ease. Some kinds of edible bananas are hybrids[5]. At the same time, crosses between different ploids may yield progenies in the banana complex. Thus Cheesman and Dodds[3] have made crosses between triploids and diploids and give rise to vigorous progenies, among which tetraploids predominate. Heptoploids from triploid × diploid crosses and octoploids from triploid × tetraploid crosses are also recorded. Dodds and Simmonds[5] demonstrate that crosses between certain diploid interspecific hybrids and fertile diploids of *Musa* give rise triploids and pentaploids. Edible bananas, at least in some cases, originate from such crosses in nature. As the triploids of *Musa* express maximum plant vigor, the pentaploids would not survive in nature. Remarkable ease of crosses between diploids, and between different ploids will provide great potentialities of the diverse origins of the existing edible triploid bananas, and at the same time, make workers to study the chromosomes constitution of the triploids and the relationships among the banana complex rather difficult. Thus Sen Jen Cha might represent as a progeny produced by crosses among the certain species of *Musa* and shows external morphology similar to that of Pei Cha but has different chromosome constitution. By this means, it is easy to explain why the chromosome configurations of the 2 varieties are different. A survey in various directions of the wild and cultivated speices and varieties of *Musa* in Formosa, especially in Taichung area where Sen Jen Cha was reported to originate, and even breeding work are necessary in order to confirm this hypothesis, but this is very complex work and beyond the scope of the present available facilities.

SUMMARY

1. Microsporogenesis of the three most common varieties of edible bananas, Pei Cha, Sen Jen Cha, and Feng Cha on the Island of Formosa were studied and illustrated.

2. All of them are triploids, being with 33 somatic chromosomes.

3. The chromosome configurations of these 3 varieties are very diverse, although their chromosome size and shape can not be identified, owing to the smallness of the banana chromosomes.

4. Although Pei Cha and Sen Jen Cha are morphologically similar, thier chromosome configurations are very different. The cause of such difference and the possible origin of Sen Jen Cha were discussed.

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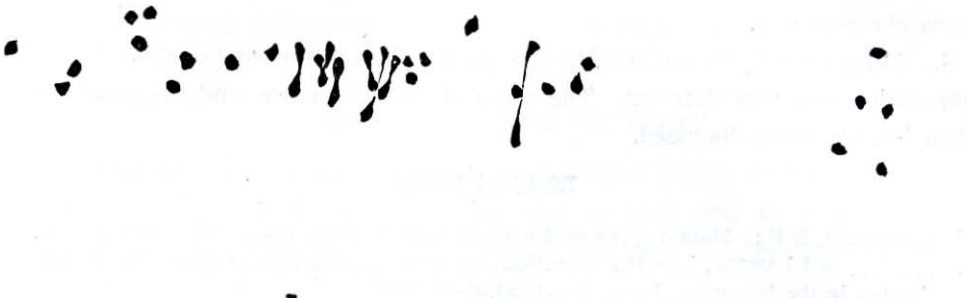

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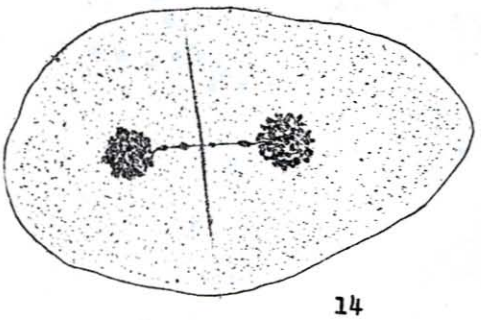
Text figs. 1-5. Metaphase I, 1. Sen Jen Cha ($18_{I} + 6_{II} + 1_{III}$). 2. Pei Cha ($10_{II} + 3_{III} + 1_{IV}$). 3. Pei Cha ($11_{I} + 9_{II} + 2_{III}$). 4. Feng Cha ($6_{I} + 9_{II} + 3_{III}$) 5. Feng Cha ($8_{I} + 11_{II} + 1_{III}$). 1-3 ×1260. 4-5 ×1360.

Text figs. 6-13. Anphase I. 6. Sen Jen Cha—with 5 lagged bivalents. 7. Sen Jen Cha—unequal distribution of chromosomes. 8. Sen Jen Cha—failure of anaphase I movement. 9. Pei Cha—unequal distribution of chromosomes. 10-11. Pei Cha—failure of anaphase I movement. 12-13. Feng Cha—irregular disjunction and stickiness of chromosomes. 6-8 ×1260. 9-13 ×1360.



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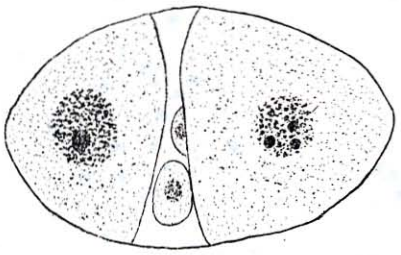

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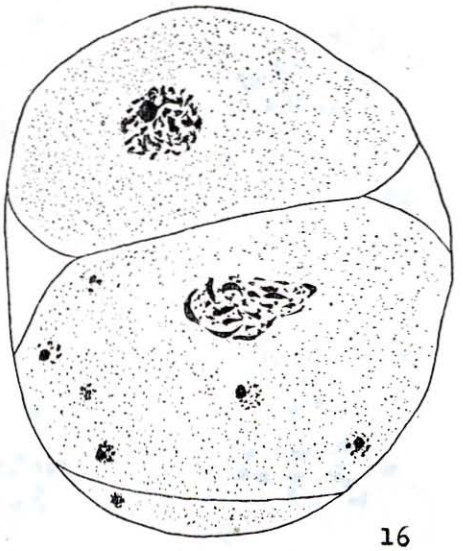

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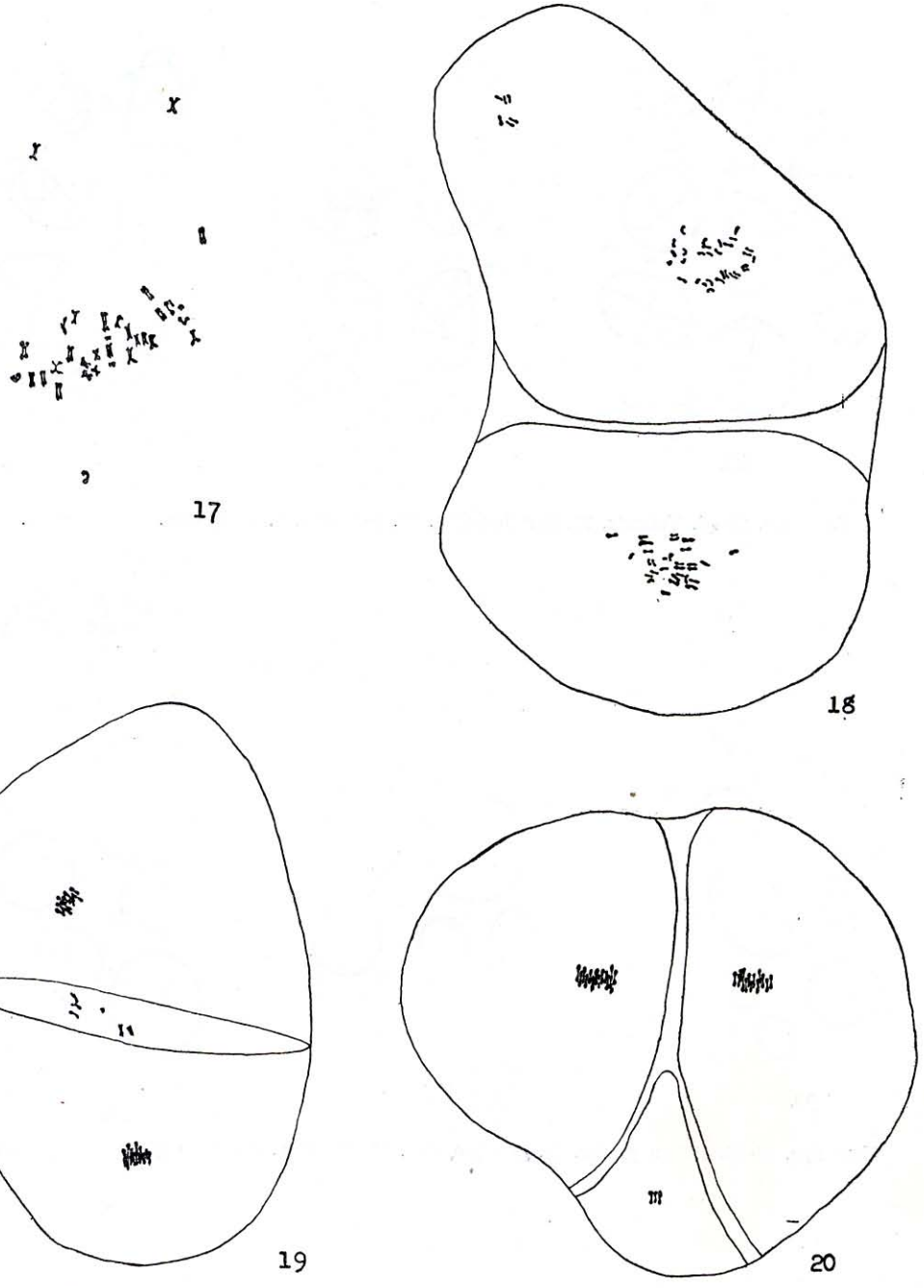
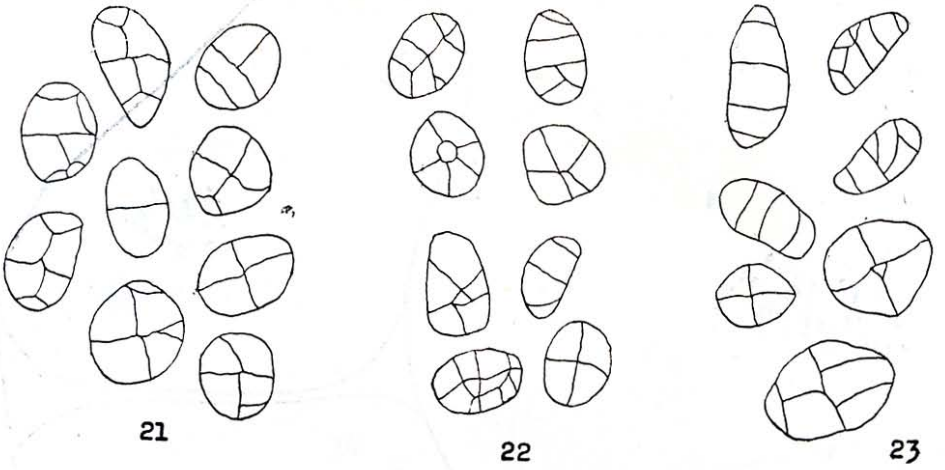
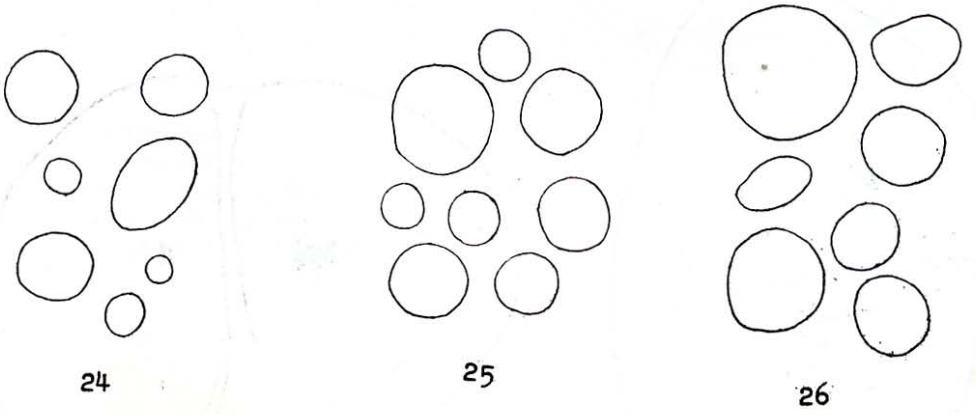
Text figs. 14–16. 14. Sen Jen Cha. Telophase I, showing one bridge. 15. Sen Jen Cha. Interphase, showing 2 small daughter cells formed between 2 major daughter cells. 16. Feng Cha. Interphase, showing scattered chromatic materials in one of the 2 major daughter cells. One small daughter cell formed besides 2 major ones. ×550.



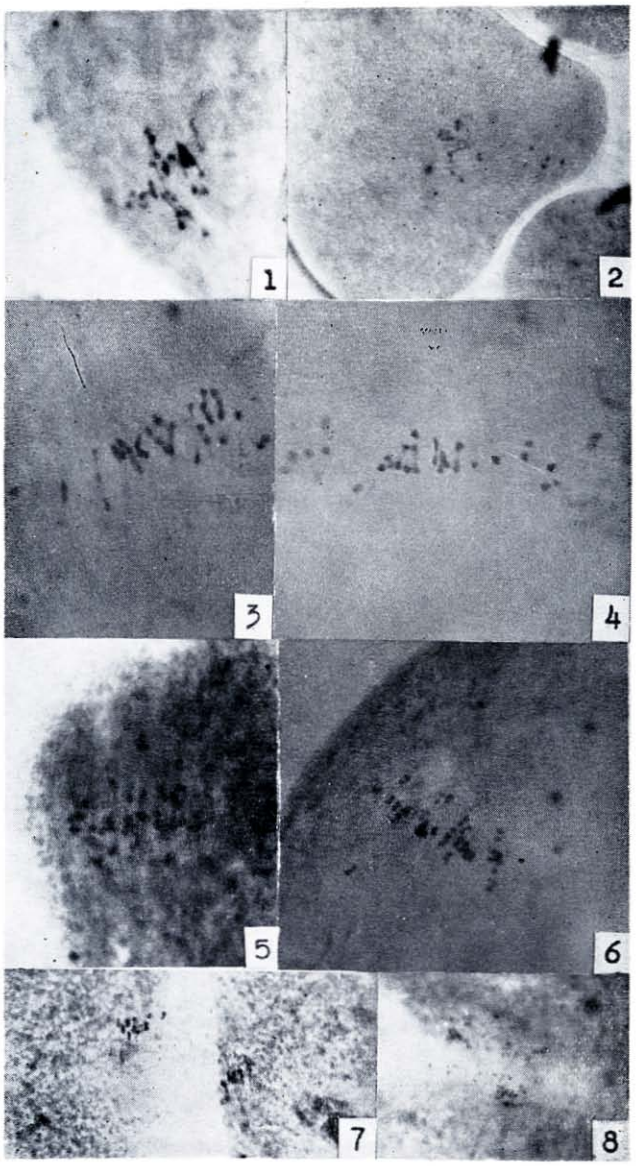
Text figs. 17-20. 17. Sen Jen Cha. Late prophase II, showing 33 chromosomes, probably due to the failure of the first meiotic division. 18. Feng Cha. Anaphase II, showing the normal anaphase movement of chromosomes of the minor nucleus in one of the 2 major daughter cells. 19. Pei Cha. Metaphase II, showing lagged chromosomes still persisted. 20. Pei Cha. Metaphase II, showing the small daughter cell at the same stage of division as major ones. 17 ×1260. 18-20 ×550.



Text figs. 21-23. Tetrads. 21. Sen Jen Cha. 22. Pei Cha.23. Feng Cha. ×134.



Text figs. 24-26. Pollen grains. 24. Sen Jen Cha. 25. Pei Cha. 26. Feng Cha. ×134.



EXPLANATION OF PLATE I

1. Feng Cha. Metaphase I, showing unequal tensions and lack of coincidence of bivalents (see text fig. 5). ×800.
2. Pei Cha. Metaphase I, showing a separate spindle with 6 univalents (see text fig. 3). ×720.
3. Feng Cha. Metaphase I, showing 6 univalents, 9 bivalents, and 3 trivalents (see text fig. 4). ×800.
4. Sen Jen Cha. Mepaphase I, showing 18 univalents, 6 bivalents, and 1 trivalent (see text fig. 1). ×800.
5. Pei Cha. Anaphase I, showing failure of anaphase movement of some dyads (see text fig. 10). ×800.
6. Sen Jen Cha. Late prophase II, showing 33 dyads (see text fig. 17). ×800.
7. Pei Cha. Metaphase II. ×800.
8. Pei Cha. Anaphase II. ×800.