VARIATIONS IN THE SATELLITED CHROMOSOMES OF *CICER ARIETINUM* LINN.

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Introduction

In a report on the presence of tandem satellites in *Cicer arietinum* (Meenakshi and Subramaniam, 1960) it was indicated that considerable variation in the morphology of the satellites was observed in different cells of one of the roots. A comparison of the differing satellite configurations in the cells of a single root with the range of variations observed in different specimens was, therefore, of academic interest in the context of the interpretations offered for such diversity.

Historical

The close association between the satellites and nucleoli during telophase reported by Heitz in 1931, stimulated an interest in the role of these chromosomal parts in nucleolar organization. Fifteen decades had, however, to intervene between the discovery of the nucleolus (Fontana, 1781; Wagner, 1835; Valentin, 1839; *see* reviews by Montgomery, 1899; Ludford, 1922; Gates, 1942; Vincent, 1955) and a demonstration of its origin during telophase in the satellited regions of the chromosomes. As far back as 1902 Conklin observing the constancy in the number of nucleoli surmised that there may be definite centres in the nucleus for their formation. The discovery of Heitz itself was anticipated by Navashin (1912) who saw the globular ends of the satellites of *Galtonia* attached to the prophase nucleoli. Failing to observe the tenuous threads connecting these grains to the chromosomes, he suggested that they became associated with the chromosomes after detachment from the nucleoli.

Heitz (1931) found that the nucleoli arose in contact with specific regions of particular pairs of chromosomes. These were the secondary constrictions. Often, these regions appeared drawn out into an achromatic filament with a chromatin knob at its end. The nucleoli corresponded in number to the satellites and their size to the length of the filament. The filament was Feulgen

negative in his preparations and the prefix SAT (sine acido thymonucleinico) to the thread was intended to indicate its negative reaction to the Feulgen test. Resende (1937, 1939) was undecided whether it was Feulgen positive and even suggested that the thin filament in material preserved in chromic fixatives may stain positively with Feulgen even though it does not contain DNA (Gates, 1942, p. 365). Fernandes (1937) it was, who first adduced evidence that the filament is really Feulgen positive (*cf.* Mensinkai, 1939).

As early as 1934 McClintock deduced that the nucleolus arose not on the SAT-filament but in association with a nucleolar organizer at the tip of the chromosome giving origin to that filament. Contrary to Heitz (1931), McClintock suggested that the length of the SAT-filament is determined by the size of the nucleolus. The fact that she could break the nucleolar organizer by X-raying the material and that its parts produced nucleoli of different sizes necessitates acceptance of her observations, since she could demonstrate a competition between the organizers as judged by the size of the nucleoli formed in different situations. Heitz observed nucleoli in micro-nuclei lacking SAT-chromosomes. McClintock reported that while a deficiency of the organizer region resulted in an accumulation of droplets resembling nucleoli on chromosomes, other genomic deficiencies produced the same condition when the organizer was present.

DEFINITIONS

The original connotation of the prefix SAT used by Heitz to indicate the absence of DNA in the filament lost its significance when the filament was shown to be Feulgen positive (Mensinkai, 1939; Berger, 1940). But the usage of the prefix SAT has continued as an abbreviation of the word satellite (Gates, 1942; De Robertis, Nowinski and Saez, 1948; Swanson, 1960). While originally it indicated the nature of the filament, the term satellite now refers to the very short part which is separated from the body of the chromosome by a thin chromatic thread if it is terminal, and by two short constrictions if it is intercalary (*cf.* Darlington, 1937). When viewed from a broader perspective, the region in question is only a secondary constriction where the short segment of the chromosome has a tendency to assume a rounded shape, while the filament is drawn out into a tenuous thread during the development of the nucleolus. In the tandem condition, therefore, two or sometimes more short segments of a chromosome are connected to one another and the body of the chromosome by thin filaments, the gap adjacent to the main body of the chromosome representing the secondary constriction (Knight, 1948).

Material and Methods

Root tips of 24-hour-old germinated seeds of *Cicer arietinum* Linn. were fixed in acetic alcohol (1 : 3) for 24 hours and then stored in 70% alcohol. As and when required, they were washed in distilled water, hydrolysed in N HCl at 60° C. for 6 min. and then stained in bulk with Heidenhain's hæmatoxylin (Marimuthu and Subramaniam, 1960). The schedule followed was: water 10 min.; 4% ferric ammonium sulphate 10 min.; water 10 min.; 0·5% hæmatoxylin (BDH) 20 min.; and wash in repeated changes of distilled water.

The tip of each root was transferred to a drop of 45% acetic acid on a slide and then squashed. The slides and coverslips used were coated with a thin layer of Mayer's albumen. The differentiation was controlled by gently warming the slide over a spirit lamp. When the desired grade of staining was obtained, the coverslip was released in tertiary butyl alcohol and if the cells were attached to the slide as well as the coverslip, each was mounted separately in Canada balsam.

Though the squash technique enables a study of the spread-out chromosome complement of individual cells, its inherent limitation is that the flattening of the cells will not be uniform in the different regions of a preparation. The individual small bits, into which the roots are teased out initially, have varying number of layers of cells in their different regions. The pressure applied to spread out the cells cannot, therefore, be presumed to be uniform. This precludes any accurate comparison of the sizes of the satellites and their filaments in different cells of the same preparation (*cf.* Schulz-Schaeffer, 1960). The length of the filament may depend on the pressure applied. The descriptions that follow are based only on meta- and ana-phases.

Observations

The large number of preparations examined indicated that the somatic chromosome number is 16 (Iyengar, 1939). Contrary to Iyengar's experience, usually, a pair of chromosomes alone were satellited (arrows, Photo 1). The satellite was borne by the longest pair of chromosomes with median constrictions and the filament as well as the satellite appeared well-defined. The nucleolus of *C. arietinum* persists often up to metaphase (Iyengar, 1939). In favourable examples the satellited chromosomes were seen in association with the nucleoli (Photo 2). The interesting tandem condition was seen only in some roots. The two tandem satellites were uniform in size in the meta- and ana-phases of only one root (Photos 3 and 4). Such uniformity was

lacking in the other roots. The variations observed relate to the number of SAT-grains in each chromosome and their relative sizes.

VARIATIONS IN THE NUMBER OF SAT-CHROMOSOMES IN A COMPLEMENT

In Different Roots.—Rare instances of more than two SAT-chromosomes in a complement were observed. Photos 5 and 6 taken at two different foci exhibit an extra SAT-chromosome in each of the anaphase groups. These are clear in Photo 6 where the usual pair is out of focus. It is as a rarity that two pairs of SAT-chromosomes occur in each anaphase set (Photo 7). Photo 7 shows in addition, two others, one in each anaphase group, exhibiting a " segmented " appearance.

In a Single Root.—Two instances of extra SAT-chromosomes were observed in a root where the different cells exhibited a variety of satellite configurations. An odd chromosome indicated by an arrow in Photo 15 is seen at the right extremity of the top anaphase group. The end of the chromosome was knobbed but the constriction was not clear. Its sister in the other anaphase set had no such dilatation at its tip. The satellites of the two chromosomes, one in each anaphase cluster in Photo 16, show the tandem condition (Photos 16 A and 16 B). The grains appear well defined in only one of them (Photo 16 B).

VARIATIONS IN SATELLITE MORPHOLOGY

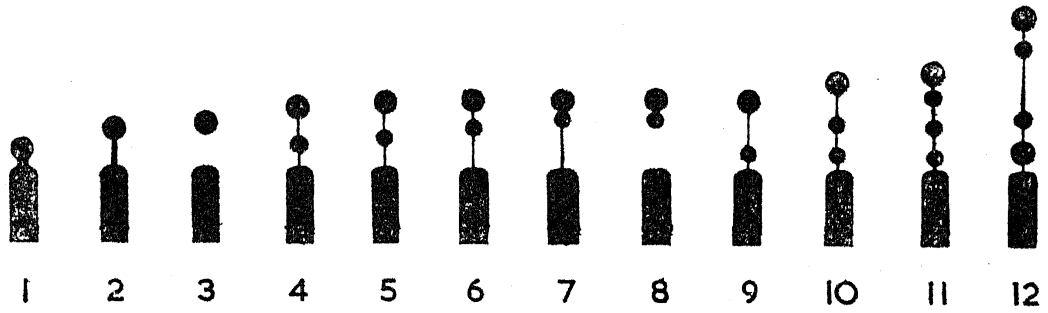
In Different Roots.—In Photo 8 one of a pair of SAT-chromosomes alone exhibits the tandem condition (Photo 8 A) while the other (Photo 8 B) appears normal. Both the chromosomes associated with the persisting nucleolus in Photo 3 show the tandem condition. The intercalary SAT-grain is separated from the terminal one and the chromosome body by well-defined filaments.

The grains vary in number even in a pair of SAT-chromosomes. The chromosome A in Photo 9 has two intercalary grains, while B has only one. The lightly stained nucleolus is in association with this pair of chromosomes. Chromosome B in Photo 10 has also two intercalary grains and that adjacent to the body of the chromosome is separated from it only by a constriction. The terminal SAT-grains in Photos 9 and 10 are bigger than the intercalary ones and they appear bifid in Photo 10.

The two grains of a tandem satellite may lie close together and the tenuous thread connecting them to the chromosome is often difficult to demonstrate (arrow, Photo 11). The difference between the homologues is more marked in Photo 12 where chromosome A (Photo 12 A) shows three grains in addition

to the terminal one. The sister chromosomes differ in Photos 13 and 14 where three bear normal satellites while the fourth has a tandem one (arrow, Photo 14).

In a Single Root.—A normal and a tandem SAT-chromosome are shown in Photo 17. The bi-partite condition of the satellite is illustrated in Photo 17 A. This difference between the homologues is more marked in Photo 18 where one of the chromosomes (Photo 18 A) has two intercalary grains apart from the terminal one. The SAT-thread appears well stained and the grain near the chromosome is the smallest. The normal satellite of the homologue (B), on the other hand, is not very well-defined. The persisting nuceolus appears attached to this chromosome. A similar situation is found in Photo 19 also, where one chromosome alone bears two SAT-grains arranged in tandem. The homologue, on the other hand, appears to have a small normal satellite as judged from the presence of a secondary constriction. In two metaphases both the chromosomes had tandem satellites. The intercalary grain in each homologue was separated from the chromosome arm only by a constriction (Photo 20; *see also* Photo 3 of Meenakshi and Subramaniam, 1960).



Text-Figure. Variations in Satellite Morphology

The variations observed in anaphases were not all a mere duplication of the metaphase configurations. There were intergrades between the two pairs of chromosomes with normal satellites (Photo 21) and those with tandem ones (Photo 22). The normal condition is illustrated in Photo 21 and as the enlargement Photo 21 A would show, the SAT-grain is separated from the chromosome body only by a neck of lesser diameter. Normal replication of a metaphase complement of one normal and one tandem (1 N: 1 T) SAT-chromosome (Photo 17) or both tandem (2 T; Photo 20) would result in the anaphase configurations exemplified by Photo 15 (2 N: 2 T in addition to the odd SAT-chromosome) and Photo 22 (4 T). While the homologues may (photo 15) or may not differ (Photos 16 and 22) in their morphology, the sister chromosomes resemble each other.

When the sister chromosomes in the separated anaphase sets differ occasionally in their morphology it has to be presumed that non-reciprocal translocations may have taken place. Two alternatives in such a situation would be the occurrence of (*a*) one tandem with three normal SAT-chromosomes (Photos 13 and 14) and (*b*) three tandem with one normal SAT-chromosome (Photo 23). In photo 23 three chromosomes show the tandem condition while one alone is normal. The sister chromosomes have also been observed to differ in their complement of SAT-grains (Photo 24). Chromosome A in Photo 24 has only two such grains (Photo 24 A) while chromosome B (Photo 24 B) has four.

The variations relating to the number and size of grains of the satellites are presented in Text-figure.

DISCUSSION

The discovery of tandem satellites and their variations in *C. arietinum* was solely due to their crisp delineation in hæmatoxylin squashes. Squashes are superior to sections and hæmatoxylin to acetic carmine and orcein. It is not surprising, therefore, that the tandem satellites had not been reported so far from this species. The accidental detection of variations in satellite morphology in cells of one of the roots was suggestive of a mutable condition conducive to the occurrence of translocations.

Losses, inversions and translocations of satellites have all been surmised from chromosome configurations of various organisms (Gates, 1942; Srinath, 1942; Sharma and Chatterjee, 1961; Ohno, Kaplan, Trujillo and Kinosita, 1961). Translocations may be between chromosomes or from the short to the long arm of the same chromosome (Gates, 1942). The presence of a tandem and a normal satellite in a metaphase group of *Allium cepa* led Mensinkai (his Fig. 23, 1939) to doubt Taylor's interpretation of such a condition as the result of an interchange (Gates, 1942). Should a translocation be only between homologous chromosomes ?

A metaphase group of *C. arietinum* showing a normal and a tandem satellite is illustrated in Photos 8 and 17. To interpret the tandem condition as the result of a non-reciprocal translocation, evidence has to be adduced from some other direction. The anomalies during anaphase illustrated as Photos 13, 14 and 23 are interesting in this connection. When replicated chromosomes differ in their morphology it has to be presumed that non-reciprocal translocations may be between sister chromosomes. Further, since the sister chromosomes differ in the number as well as the size of the

SAT-grains (Photos 12, 12 A, 24, 24 A, 24 B) it would appear that translocations may be of parts of grains and not of entire ones.

Summary

The diversity in the morphology of the SAT-chromosomes of *C. arietinum* in different roots are analysed in the context of similar variations observed in the different cells of one of the roots. Intergrades between the two pairs of chromosomes with normal satellites and those with tandem ones were observed in anaphases. The differences in the number as well as the size of the SAT-grains of replicated chromosomes indicate that non-reciprocal translocations between sister chromosomes may be of parts of SAT-grains and not of entire ones.

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EXPLANATION OF PLATES

PLATE I

PHOTOS 1-14. General Variations

PHOTO 1. Metaphase plate with 16 chromosomes. The two SAT-chromosomes are indicated by arrows.

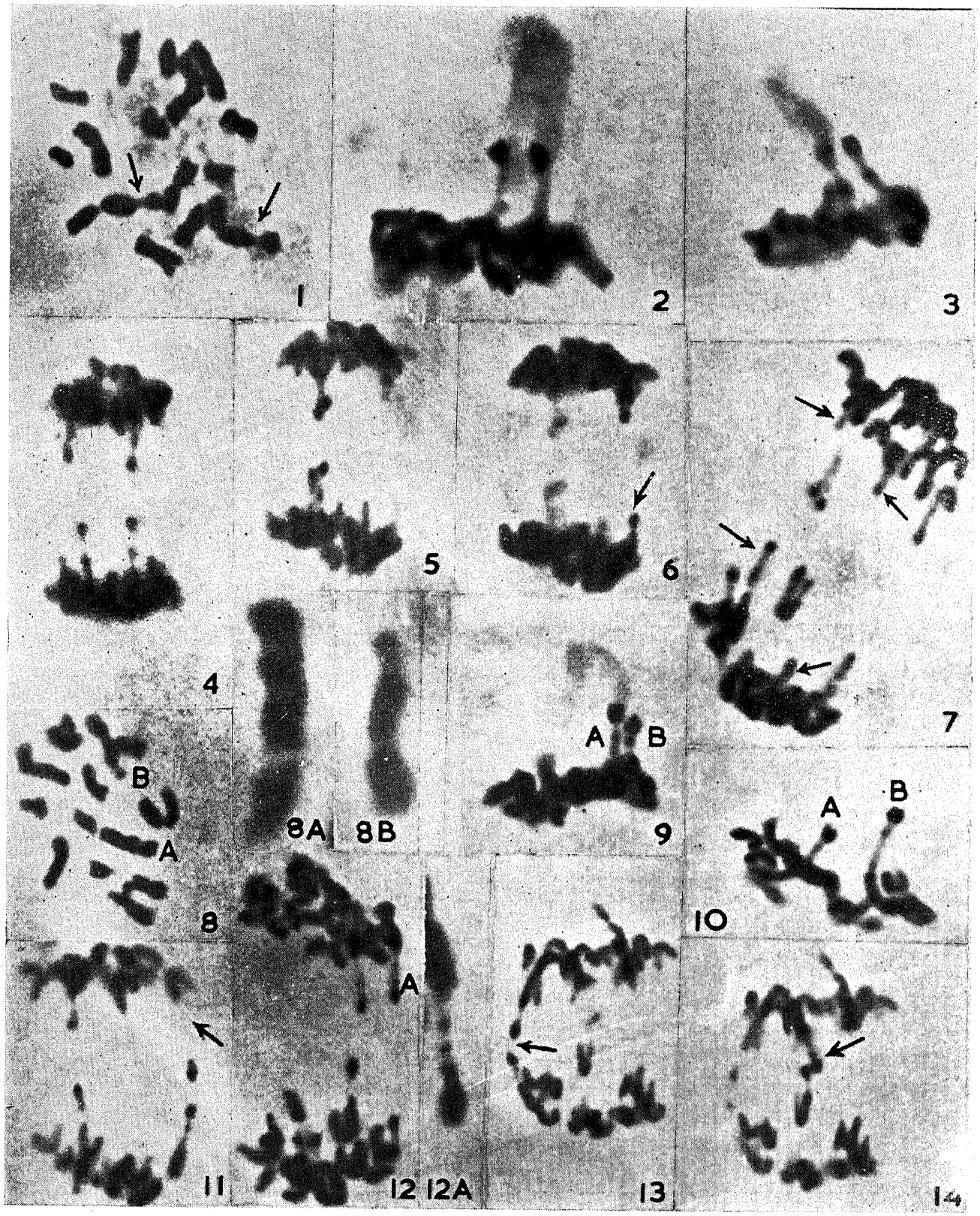
PHOTO 2. The metaphase SAT-chromosomes are in contact with the nucleolus.

PHOTO 3. Both the SAT-chromosomes have tandem satellites. The SAT-filaments are well-defined. The nucleolus is seen.

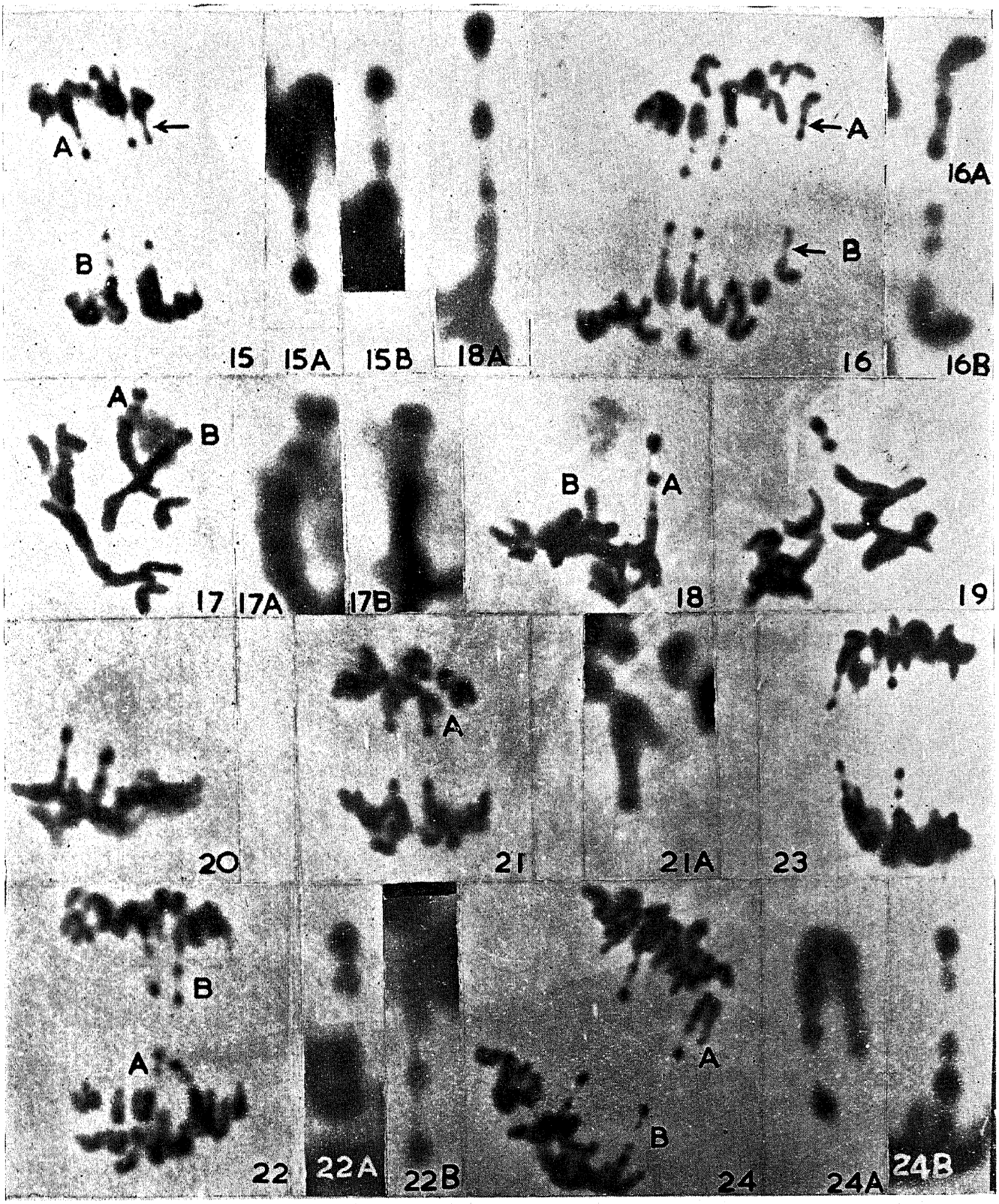
PHOTO 4. Four anaphase chromosomes have well-defined tandem satellites.

PHOTOS 5 and 6. Anaphase photographed in two foci. The normal SAT-chromosomes are seen in Photo 5, while the extra pair of SAT-chromosomes is in focus in Photo 6.

PHOTO 7. Anaphase showing four pairs of SAT-chromosomes. There are two others which present a "segmented" appearance.



FIGS. 1–14


A
B
15
15A
15B
18A
A
B
16
16A
16B
A
B
17
17A
17B
B
A
18
19
20
A
21
21A
23
B
A
22
22A
22B
A
B
24
24A
24B

FIGS. 15–24

PHOTO 8. Metaphase plate with a pair of SAT-chromosomes. One is normal (8 B) while the other shows the tandem condition (8 A).

PHOTO 9. Metaphase group with SAT-chromosomes and a persisting nucleolus. Chromosome A has two intercalary grains while B has only one.

PHOTO 10. Metaphase. The terminal SAT-grains are bifid. Chromosome B shows two intercalary grains, the one near the chromosome body being separated by a secondary constriction.

PHOTO 11. Anaphase. The two grains in the tandem satellite lie close together. The SAT-thread is unstained.

PHOTO 12. Anaphase. Homologous SAT-chromosomes are not alike. One of them (12 A has four grains, the terminal one being bigger than the rest.

PHOTOS 13 and 14. Anaphase (3 N: 1 T) photographed under two foci. A tandem and a normal SAT-chromosome are seen in Photo 14. In Photo 13 the chromosomes bear only normal satellites.

PLATE II

PHOTOS 15-24. Variations in cells of a single root tip.

PHOTO 15. Anaphase (2 N: 2 T) showing an extra SAT-chromosome indicated by an arrow. Enlargements of the tandem SAT-chromosomes are presented as 15 A and 15 B.

PHOTO 16. A pair of extra chromosomes indicated by arrows show the tandem condition as illustrated in 16 A and 16 B.

PHOTO 17. One tandem (17 A) and one normal satellite (17 B) are seen in association with the persisting nucleolus.

PHOTO 18. Metaphase. Chromosome A (18 A) has three grains, the one near the chromosome arm being the smallest. The satellite is not well-defined in the homologue (B in photo).

PHOTO 19. Metaphase. One chromosome with tandem and the other with a normal but ill-defined satellite.

PHOTO 20. Metaphase. Both the chromosomes show the tandem condition. The intercalary grain in both is separated from the chromosome body by the secondary constriction.

PHOTO 21. Anaphase with two pairs of normal satellites. The SAT-filament is short and thick.

PHOTO 22. Two pairs of tandem satellites at anaphase. Chromosomes A and B are enlarged to illustrate the position of the grains (22 A and 22 B).

PHOTO 23. Anaphase with 1 normal and 3 tandem satellites.

PHOTO 24. Anaphase. Chromosome A (24 A) has only one intercalary grain very near the chromosome arm while its sister B (24 B) has four grains, that next to the chromosome arm being the biggest.

MAGNIFICATIONS

PHOTOS 1–3, 8–10, 17–20, × *ca.*, 3,200.

PHOTOS 4–7, 11–16, 21–24, × *ca.*, 2,600.

PHOTOS 8 A and B, 12 A, 15 A and B, 16 A and B, 17 A and B, 18 A, 21 A, 22 A and B, 24 A and B, × *ca.*, 6,600.