Karyotype Alteration and Phylogeny, V. New types of SAT-chromosomes in *Nothoscordum* and *Nerine*¹

By

D. Satô

Received November 24, 1941.

Introduction

According to Heitz nucleolar chromosomes are SAT-chromosomes or chromosomes with satellite or secondary constriction (Sine Acido Thymonucleino), but recent investigations with regard to the nucleolar chromosomes clearly indicated two types of the nucleolar chromosomes, i.e., terminal and intersitial types. In the case of the terminal type a nucleolus-organizing body consisted of heterochromatin is either present or absent and the former is found in some plants such as *Narcissus bulbocodium* var. *genuinus* (Fernandes 1935) and *Oryza sativa* (Nandi 1937) and the latter in other plants such as *Trillium kamtschaticum* (Matsuura 1938) and *Paris hexaphylla* (Haga 1940; Satô 1941). In the case of the interstitial type it is reported that not only a stalk of so called SAT-chromosome (satellited chromosome, chromosome with a secondary constriction) includes thymonucleic acid, but also primary constrictions participate in nucleolus organization in some samples. This new type of SAT-chromosome may be explained by either coincidence of primary constriction with secondary one or inversion of chromosome arms with satellite or terminal end bearing on the nucleolus organization.

If a differential capacity of these various nucleolar chromosomes for nucleolus condensation is assumed, the relation between the nucleolar chromosomes and the nucleoli in both hybrids and polyploids may be reasonably be explained. The balance of nucleolar chromosomes is generally maintained after competition of the total chromosomes with the differential capacity for nucleolus condensation present in a complement, but sometimes a trace of such competition of nucleolar chromosomes is observed in the root-tip cells and in the pollen grains of *Tricyrtis* (Satô 1939b), that is, many chromosomes remained attached to the surface of nucleoli in the prophase besides the nucleolar chromosomes. According to this balance hypothesis of nucleolar chromosomes diploid plants with only one nucleolus is expected to exist and actually the writer (1941) found it in *Brodiaea uniflora* ($2n=12=2b$). Then karyotype alteration as well as hybridization may change the balance of nucleolar chromosomes

¹ Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 283.

and indicate the differential capacity of nucleolar chromosomes which contrariwise may indicate a trace of derivation of karyotypes.

Material and Methods

The bulbs of *Scilla sibirica* and *S. sibirica* var. *alba* were obtained from the nursery, Tôkyô-Nôsan-Syôkai. Other plants *Disporopsis ari-sanensis*, *Lycoris sanguinea*, *L. squanmigera*, *Nothoscordum fragrans*, *Allium obliquum*, *A. condensatum* and *Nerine undulata* were also used, these having been raised in the Koisikawa Botanic Garden of the Tôkyô Imperial University. The root-tips were fixed chiefly with Bonn modification of Flemming's solution which proved sufficiently favourable in making preparations to enable observation of the nucleoli in *Scilla* and *Nerine*. While in the cases of *Disporopsis*, *Lycoris*, *Nothoscordum* and *Allium* Navashin's solution was exclusively employed as a fixative.

The acetocarmine and permanent smear methods were also employed for pollen grains. For staining of preparations Newton's gentian-violet method was exclusively applied.

Observation

Scilla sibirica has twelve somatic chromosomes one pair of which are newly defined SAT-chromosomes, that is, nucleoli are organized at

Fig. 1. Somatic mitosis of root-tip cells in *Scilla sibirica* ($2n=12=8L+2S^s+2S$). *a*, metaphase especially showing two SAT-chromosomes (S^s) with only primary constriction. *b*, anaphase. *c*, resting nuclei with two corresponding nucleoli. $\times 1700$.

the primary constriction (Fig. 1). The attachment of the S^s chromosome to nucleolus was ascertained in the root-tip cells and also in the pollen grains (Fig. 2). Sometimes two nucleoli are found in large pollen grains which are diploid in nature having two S^s chromosomes (cf. Fig. 2, d).

This type of SAT-chromosomes were found in other species, i.e., in *Scilla japonica* (Satô 1940), *Diporopsis arisanensis* (Fig. 3) and *Nerine undulata* (Figs. 9–11).

Lycoris squanmiger has twenty-seven somatic chromosomes, that is, six V-shaped chromosomes (L) and twenty-one rod-shaped chromosomes (S) of which three SAT-chromosomes were clearly observed and these corresponded to three nucleoli in the telophase. Inariyama reported that both arms of L-chromosomes were homologous to two S-chromosomes.

Fig. 2. The primary division of pollen grains in *Scilla sibirica* var. *alba*. a, metaphase. b, c, prophase especially showing one SAT-chromosome (S^s) attached to nucleolus by the primary constriction. d, diploid pollen grain showing two SAT-chromosomes (S^s). $\times 1450$.

Consequently we can expect karyotype alterations such as fragmentation of long chromosome or fusion of two short chromosomes in *Lycoris*. Kinetochores observed in the metaphase and anaphase often showed a similar morphology to a small satellite (Fig. 4) (cf. Satô 1937 in Aloinae) and the SAT-chromosome in *Lycoris* has a satellite at the proximal end and its stalk coincides with the primary constriction. Based upon the karyotype analysis in other *Lycoris* species, it is probable that the L-chromosome in *Lycoris* is derived by either fusion or translocation of two S-chromosomes.

For instance *L. sanguinea* has twenty-two somatic chromosomes with subterminal constrictions one pair of which has each a satellite at the proximal end. SAT-chromosome of this species is clearly different from that of *squanmiger* and short chromosomes of the latter have extremely subterminal constrictions (Fig. 5).

While on the other hand, *Nothoscordum fragrans* has nineteen somatic chromosomes of which thirteen chromosomes have median constrictions and six chromosomes have terminal constrictions (Fig. 6), and the latter chromosomes seem to be derived from fragmentation of the former ones (cf. Levan and Emsweller 1938). According to this explanation six short chromosomes have terminal kinetochores two of which were derived respectively from one kinetochore of long chromosomes. Among these six chromosomes four resemble the satellited chromosomes of

Figs. 3-8. Somatic metaphases of root-tip cells. 3, *Disporopsis arisanensis* ($2n=40=2L+2S^s+36S$). 4, *Lycoris sanguinea* ($2n=22=2S^t+20S$). 5, *L. squamigera* ($2n=27=6L+3S^t+18S$). 6, *Nothoscordum fragrans* ($2n=19=13L+4S^t+2S$). 7, *Allium obliquum* ($2n=16=12L+2M+2S^t$). 8, *A. condensatum* ($2n=17=11L+2M+2S^t+2S$). $\times 1600$.

Lycoris and take part usually in the nucleolus condensation. Satellite-like body of these chromosomes seems to be a kinetochore and homologous with the terminal body of two remaining short chromosomes. According to Levan and Emsweller six short chromosomes each had a satellite proximally attached, but the present observation indicated only four SAT-chromosomes and corresponding four nucleoli in the telophase. Then the kinetochores of only four short chromosomes are lengthened by nucleolus condensation at these regions and show an appearance of a satellite, while two remaining ones show a terminal kinetochore.

This terminal kinetochore was found in *Allium condensatum* ($2n=17=11L+2M+2S^t+2S$) which had eleven long chromosomes with median

or submedian constrictions, two medium chromosomes with subterminal constrictions, two short chromosomes each with a satellite at the proximal end and two short chromosomes each with a terminal kinetochore (Fig. 8). This chromosome seems to be derived from fragmentation of long chromosomes at the kinetochore and is not mobilized for nucleolus condensation because one pair of SAT-chromosomes is actually present in a complement. The original karyotype of this species seems to be similar to that of *A. oblongum* ($2n=16=12L+2M+2S^s$) (Fig. 7).

In *Nerine curviflora* ($2n=22=2L+10M+2M^s+8S$) one pair of medium chromosomes have secondary constrictions at the proximal arms and the other species, *humilis*, *flexuosa*, *sarniensis* and *pudica* have similar karyotypes, while *N. undulata* ($2n=22=2L+11M+1M^s+1S^s+7S$) has peculiar

Fig. 9. Somatic mitosis of root-tip cells in *Nerine undulata* ($2n=22=2L+11M+1M^s+1S^s+7S$). a, prophase especially showing two SAT-chromosomes (M^s and S^s) attached to nucleolus. b, metaphase. c, resting nuclei with two corresponding nucleoli. a, b $\times 1900$, c $\times 1450$.

SAT-chromosomes, that is, one medium chromosome with a secondary constriction at the distal arm and one short chromosome with an extremely lengthened primary constriction. These newly formed SAT-chromosomes relate to nucleolus organization and are found attached to nucleoli one of which is large and formed at the neighbourhood of the pole and a remaining small one is formed

at a distance from the pole in the telophase (Fig. 9 c). This suggests that the S^s - and M^s -chromosomes correspond to large and small nucleoli respectively and this inference is partially proved by the observation in the primary division of pollen grains (Fig. 10 a).

In the primary division of pollen grains of *Nerine undulata* four combinations of SAT-chromosomes are expected to exist, that is, both

Table 1. The ratio of four combinations of SAT-chromosomes found in the metaphase of the primary pollen grain division in *Nerine undulata*.

SAT-chromosomes	M^s+S^s	M^s	S^s	0
Frequency	13	18	14	9

M^s and S^s , M^s only, S^s only and no SAT-chromosomes. The ratio of these combinations is expected to be 1:1:1:1 (Table 1).

According to the balance hypothesis of nucleolar chromo-

some the nucleolus is expected to be organized by other chromosomes in the case of the combination of no SAT-chromosomes. From this expectation the relation between chromosomes and nucleoli was carefully searched in the prophase. Both M^s - and S^s -chromosomes are present

Fig. 10. The primary division of pollen grains of *Nerine undulata*. a-f, prophase showing nucleolar chromosomes attached to nucleoli. Besides two SAT-chromosomes (M^s and S^s), the other chromosomes (M and S) also attached to nucleoli. g-i, metaphases and anaphase showing the combinations of SAT-chromosomes, M^s and S^s (g), M^s only (h) and none (i). $\times 1000$.

in the case of two nucleoli (Fig. 10 a) and either M^s - or S^s -chromosomes is present in the case of one nucleolus (Fig. 10 b-e). In the case of no SAT-chromosomes one nucleolus was found attached to the short chromosomes (Fig. 10 f).

Based upon the present observation it is evident that the S^s chromosome is the most efficient and the M^s is more efficient for nucleolus con-

Fig. 11. Diagram of nucleolar chromosomes in *Nerine*. *a*, one pair of SAT-chromosomes in *Nerine* species. *b*, new SAT-chromosomes (M^s and S^s) and mobilized nucleolar chromosomes (M and S) in *Nerine undulata*.

densation, and M and S have also relation to the nucleolus organization. This M chromosome seems to be the M^s chromosome deficient of a distal segment of a secondary constriction at the proximal end. The M^s chromosome of *N. undulata* seems to be derived from translocation of a distal segment of a secondary constriction found commonly in other species to the distal arm (Fig. 11 *a, b*). The S^s chromosome seems to be derived from inversion of the chromosome arm with this terminal end. The origin of this S chromosome is not clear, but it may be said to be a mobilized nucleolar chromosome in the case of combination of less efficient chromosomes for nucleolus condensation.

Discussion

(1) Various types of nucleolar chromosomes.

If we take the karyotype alteration into consideration, various types of nucleolar chromosomes are reasonably explained. Four types of SAT-chromosomes (interstitial types of nucleolar chromosome) were reported by the present writer (1939 *a*), namely, (1) one with a secondary constriction, (2) one with a satellite, (3) one with a secondary constriction which coincides with the primary constriction and (4) one with a satellite the stalk of which coincides with the primary constriction. The relation between (1) and (2) or between (3) and (4) is easily explained by the chromosome alteration such as translocation or inversion, but an origin of (3) and (4) has not been clearly explained.

Meanwhile a new type of SAT-chromosome in *Nothoscordum* is found in the present work which can explain the derivation of these types. The fragmentation of long chromosomes at the point of a kinetochore may result in two short chromosomes with terminal constrictions. If this terminal kinetochore participates in the nucleolus condensation, this kinetochore may give an appearance of a satellite by stretching of the adjoining chromonema which resulted by the nucleolus condensation at that region. If this terminal kinetochore has no relation to the nucleolus organization, it remains unaffected at the terminal end of the chromosome (cf. *Allium condensatum*). By the discovery of this SAT-chromosome we can easily explain origins of (3) and (4), that is, these may be formed with no difficulty by chromosome alteration in this SAT-chromosome.

Terminal types of nucleolar chromosome may be said the nucleolar

chromosomes in a narrow sense and divided into two types by presence or absence of the nucleolous-organizing body consisting of heterochromatin, that is, the former is known in *Narcissus bulbocodum* var. *genuinus* (Fernandes 1935) and *Oryza sativa* (Nandi 1937) and the latter is reported in *Trillium kamschaticum* (Matsuura 1938) and *Paris hexaphylla* (Haga 1940; Satô 1941).

(2) The balance hypothesis of nucleolar chromosomes.

As if sex determination is controlled by the balance between sex chromosomes and autosomes, nucleolus condensation is assumed to be controlled by the balance between nucleolar chromosomes and other chromosomes present in a complement. This balance may be altered by hybridization, karyotype alteration and other chromosomal aberrations. Consequently polyploidy accompanied by no increase of nucleolus number, differential amphiplasty, heteromorphic SAT-chromosomes, and also a diploid plant with only one nucleolus may be explained by this balance hypothesis of nucleolar chromosomes. If differential capacity for the nucleolus condensation is assumed at the SAT-constriction, the primary constriction and chromosome end, various types of nucleolar chromosomes may be reasonably explained and the mobilization of extra nucleolar chromosomes may be understood in the case of less efficient nucleolar chromosomes.

The relation between the primary constriction and the nucleolus condensation was emphasized in the present paper and it has been neglected by many cytologists in this line for a long time. Bearing the balance hypothesis of nucleolar chromosomes in mind, the relation between chromosomes and nucleoli in *Antirrhinum majus* (Ernst 1938; Resende 1940) may be explained as follows; the extra nucleolar chromosomes were mobilized in the case of races 50 and trier. Resende observed four nucleoli in the pachytene and diakinesis one of which was free from the chromosomes and three attached to the nucleolar chromosomes. This free nucleolus may be comparable with a persistent nucleolus. Apparently abnormal relations between chromosomes and nucleoli must be traced from the view point of karyotype alteration and they may be explained in future by the balance hypothesis of nucleolar chromosome.

Summary

1) Nucleoli are organized at the primary constriction in *Scilla sibirica* and *S. sibirica* var. *alba*. This new type of SAT-chromosome was observed in *Disporopsis arisanensis* and *Nerine undulata*.

2) SAT-chromosome in *Lycoris squamigera* ($2n=27=6L+3S'+18S$) has a satellite at the proximal end and its stalk coincides with the primary constriction. While SAT-chromosome with a subterminal

constriction of *L. sanguinea* has a satellite at the proximal end and then the relation between these SAT-chromosomes is not so simple.

3) *Nothoscordum fragrans* ($2n=19=13L'+2S$) has thirteen long and six short chromosomes and each two of the latter chromosomes seem to be derived from fragmentation of one long chromosome at the kinetochore. Consequently six short chromosomes has terminal kinetochores four of which have an appearance of a satellite by bearing on the nucleolus condensation at that region. The short chromosome with a terminal kinetochore was found also in *Allium condensatum* ($2n=17=11L+2M+2S'+2S$). These two chromosomes also have an origin in the fragmentation of one long chromosome, judging from a karyotype of *A. obliquum* ($2n=16=12L+2M+2S'$).

4) *Nerine undulata* ($2n=22=2L+11M+1M^s+1S^s+7S$) has two SAT-chromosomes, that is, one medium chromosome with a secondary constriction at the distal arm and one short chromosome with an extremely lengthened primary constriction. The relation between nucleoli and SAT-chromosomes was carefully traced in the primary pollen grain division, and a certain chromosome was found attached to nucleolus besides SAT-chromosomes and had relation to the nucleolus condensation in the case of combination of no SAT-chromosomes.

In conclusion the writer wishes to express his sincere thanks to Prof. Y. Sinotô for his kind advice and valuable criticism through the course of this work.

The cost of this research has partly been defrayed from the Scientific Research Expenditure of the Department of Education.

Literature Cited

- Fernandes, A. 1936. Les satellites chez Narcisses. II. Les satellites pendant la mitose. Bol. Soc. Broteriana 11 (II sér.): 87-146.
- Haga, T. 1940. Nucleolar chromosomes in *Paris*. Bot. & Zool. 8: 1769-1771.
- Levan, A. and Emsweller, S. K. 1938. Structural hybridity in *Nothoscordum fragrans* and the origin of terminally attached chromosomes. Jour. Hered. 29: 291-294.
- Matsuura, H. 1938. Chromosome studies on *Trillium kamtschaticum* Pall. VI. On the nucleolus-chromosome relationship. Cytologia 9: 55-77.
- Nandi, H. K. 1937. Cytological investigations of rice varieties. Cytologia 8: 277-305.
- Resende, F. 1940. Die Nukleolen bei *Antirrhinum majus* L. Ber. deut. bot. Ges. 58: 469-470.
- Satô, D. 1937. Karyotype alteration and phylogeny, I. Analysis of karyotypes in Aloinae with special reference to the SAT-chromosome. Cytologia, Fujii Jub. Vol., 80-95.
- 1939a. Karyotype analysis and nucleolar chromosomes. A review. Jap. Jour. Genet. 15: 36-43.
- 1939b. Cyto-genetical studies on *Tricyrtis*, II. Karyotype analysis in *Tricyrtis* and *Brachycyrtis* with special reference to SAT- and nucleolar chromosomes. Cytologia 10: 127-157.
- 1940. Polyploidy and nucleolus. Bot. Mag. (Tokyo) 54: 66-72.
- 1941. A diploid plant with only one nucleolus and its bearing on the balance hypothesis of nucleolar chromosome. Bot. Mag. (Tokyo) 55: 159-163.