The Karyotype of *Petunia hybrida* and the Differential Chromosome Condensation

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The somatic metaphase karyotype of *Petunia hybrida* was first studied by Marthaler (1936) with ordinary paraffin-sectioned specimens. Several other workers, Dermen (1931), Steere (1932), Malinowski (1935), Wergin (1936), Levan (1937), and Cooper (1946), also observed the somatic chromosomes of *Petunia hybrida* in usual paraffin-sectioned specimens. Most of them, however, except Malinowski and Levan, failed to show positions of kinetochore in their camera-lucida drawings. In Malinowski's and Levan's drawings the positions of the kinetochore and nucleolar constrictions were shown distinctly, but their karyotypes were slightly different from Marthaler's. The species of *Petunia* employed by Malinowski was recorded as *Petunia violacea* in his paper, but today it is believed to be *Petunia hybrida* (cf. Stout, 1952). Thus the presence of different karyotypes in *Petunia hybrida* naturally requires a re-examination of the karyotype of this plant.

Material and Method

Materials were a number of commercial varieties of *Petunia hybrida* ($2n=14$); a strain with small and white flowers which showed sporadically variegation, "Snow Ball" (SW), one with large and white flowers (LW), one with large and pink-rose flowers (LFC), one with large and white double flowers (DW), one with small and reddish-purple flowers, "Fire Chief" (FC), one with small and violet flowers (V), and intercrossed progenies among them.

The observation of somatic chromosomes was carried out by the same method as applied by Sullivan (1947), though he did not try the detailed examination of chromosome morphology. The young leaves surrounding a young vigorous flower bud were fixed with acetic-alcohol (1:3) and stained by Feulgen method after they were pretreated for 3-4 hours either with 0.05% colchicine, or with 0.278 g/ml 8-hydroxy-quinoline, or with single solution containing 0.5g podophyllin and 0.05% chloral

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hydrate in 100 ml of tap water (cf. Sullivan, 1947). Basal third of the stained entire leaf was squashed in aceto-carmin to discriminate the nucleolar chromosomes by staining prophase nucleolus. The measurement of the chromosomes was made on the basis of camera-lucida drawings at a magnification of 2000 times in diameter.

Results

To clarify whether Marthaler's karyotype is applicable to the present material or not, the individual chromosomes of a somatic complement were roughly classified into two groups, the metacentric (M) and the submetacentric chromosomes (SM), and numbered in subscript from the largest to the smallest, and they were compared with Marthaler's karyotype (cf. Fig. 1). It was found the idiograms to be composed of, irrespective of the strains, the largest chromosome pair which was sometime submedian

Fig. 1. Karyotype analysis of *Petunia hybrida*. M: karyotype reported by Marthaler (1936). LW, LFC, SW, and DW: karyotypes from respective strains indicated by abbreviations. $\times 1500$.

(SM₁), and another time median (M₁); the second was M₁ when the largest pair was SM₁, or it was SM₁ when the largest pair was M₁; the 3rd was always the SM₂ which was subterminal or submedian; the 4th was M₂; the 5th and 6th pairs were submedian SM₃ and SM₄, respectively, and the smallest pair was M₃ (Fig. 1).

The series of the chromosome length made clear by the present karyotypic analysis is as follows:

SM₁ ~ M₁ > SM₂ > M₂ > SM₃ > SM₄ > M₃
(Present study)

M₁ > SM₁ > SM₂ > M₂ > SM₃ > SM₄ > M₃
(Marthaler, 1936)

The same series constructed from the drawings of Marthaler (1936) is given under the series obtained by the present writer. As is obvious, these two karyotypes are quite similar with one another. However, the present analysis limited to metaphase configuration is not yet decisive as to lengths of SM₁ and M₁. In order to clarify this point, the lengths at late prophase of SM₁ and of M₁ were further investigated.

According to Marthaler (1936) and Malinowski (1935) the chromosome SM_1 is satellited at the short arm. As pointed out by Sullivan (1947) it is not easy to ascertain the very minute satellite. However, in some of metaphase cells chromosome SM_1 was found undoubtedly attached with a minute satellite at the long arm (Plate 1, Figs. 4, 5, C, D). At late prophase only a pair being one of subterminal chromosomes had a secondary constriction locating on the long arm (Plate 1, Figs. 2,

Plate 1. Photomicrographs of somatic chromosomes of *Petunia hybrida*. $\times 1500$. Arrows indicate SAT-chromosomes. A in 2, B in 3, C in 4, and D in 5, are arranged in right of the bottom of the plate after enlarged at the same magnification ($\times 2700$).

3, A, B.). This nucleolus organizing chromosome was always the longest in the late prophase complement, that is chromosome SM₁ at metaphase. Malinowski (1935) reported that in the individuals with flower color variegation a pair of SAT-chromosomes are heteromorphic, i.e. one of the two SAT-chromosomes is lacking the satellite. In the present study, however, the strain characterized by the same variegation as described by Malinowski has been found to be homomorphic as to the presence of the satellite. There arises a question why the chromosome, which is the longest at late prophase, is sometimes not the longest at metaphase. A probable answer is that chromosomes might be different from one other in the degree of contraction from late prophase to metaphase. Indeed, ratio of lengths between SM₁ and M₁ varies from late prophase to metaphase within a cell (Fig. 2). Further, ratios of lengths of SM₁ and M₃ were compared between the two mitotic stages mentioned above (Table 1). The difference

Fig. 2. Two marker chromosomes, SM₁ and M₁, from seven different late prophasic cells showing different degrees of condensation. The chromosomes at the right extremity was picked out from the metaphasic cell DW-2. ×1000.

Table 1. Variation in ratio of lengths between the nucleolus organizing (SM₁) and the shortest median chromosomes (M₃)

Stage in mitosis	Cell No.	Length of chromosome*						Mean ratio M ₃ /SM ₁
		M ₃		mean	SM ₁		mean	
Late prophase	1	10.5	8.5	9.5	22.5	17.5	20.0	0.475
	2	7.0	7.0	7.0	12.0	12.5	12.3	0.569
	3	10.5	13.5	12.0	23.5	22.5	23.0	0.522
	4	7.0	7.0	7.0	16.5	15.5	16.0	0.438
	5	9.0	8.0	8.5	14.0	14.5	14.3	0.594
	6	8.5	7.5	8.0	17.0	13.5	15.3	0.523
	7	10.0	9.0	9.5	21.0	16.0	18.5	0.514
mean				8.8			17.1	0.519
Metaphase	1	7.5	6.5	7.0	10.0	8.0	9.0	0.778
	2	4.0	4.0	4.0	6.0	6.0	6.0	0.667
	3	5.5	6.5	6.0	11.5	7.0	9.3	0.645
	4	5.0	5.5	5.3	10.0	8.5	9.3	0.570
	5	4.5	4.5	4.5	7.5	6.5	7.0	0.643
	6	6.5	5.5	6.0	10.0	10.5	10.3	0.583
	7	5.5	6.0	5.8	8.5	9.0	8.8	0.659
mean				5.5			8.5	0.649

* Length (mm) estimated by the camera-lucida drawings.

in the mean values of M_3/SM_1 is significant between the two stages ($F_0=16.1$, $F=4.75$; $n_1=1$, $n_2=12$, $\alpha=0.05$). Thus it is convincing that the differential chromosome condensation takes place between the two stages, i.e. the length of SM_1 at metaphase being 49.7% (8.5/17.1) of the prophase length, and that of M_3 at metaphase 62.5% (5.5/8.8). The degree of chromosome contraction is further different between long and short arm as recognized in a variation in arm ratio of chromosome SM_1 (Fig. 3). Further to say, the degree of contraction seems not uniform among all members of the complement. For example, M_1 , the longest or the second in the length at metaphase, was in one of late prophase nucleus the fifth in the length (cf. Fig. 4), but in other cases (cf. Fig. 2) the fourth (FC-1), the third (SW-3, FC-2), or the second (LW-3, -4, DW-3).

At any rate, according to Sinoto's formulas (1944), the karyotype of *Petunia hybrida* at metaphase will be expressed as follows.

$$K(2n)=14=2A_1^m(n)+2A_2^m+2B^m+2C_1^m+2C_2^m+2D_1^m+2D_2^m$$

Discussion

Historically in 1835 or so the species

Fig. 3. Variation in length of the nucleolus organizing chromosome (SM_1) following chromosome condensation. Vertical line; length of SM_1 . Horizontal line; arrangement of the chromosome following degree of condensation. Nos. correspond to those in Fig. 2. DE; distal end of chromosome. C; centromere. N; nucleolus organizing region.

Fig. 4. Idiograms of *Petunia hybrida* at the different stages of the mitotic cycle. Upper idiogram taken from the late prophase cell which is shown in 2 of Plate 1. Lower idiogram from the metaphase cell shown in 6 of Plate 1. $\times 1500$.

Petunia hybrida has been derived from a cross between *Petunia integrifolia* ($2n=14$) and *Petunia axillaris* ($2n=14$), the latter being only one species having SAT-chromosomes (Sullivan, 1947). And more than a century has elapsed since the time of hybridization. The *Petunia hybrida* has been through selection after selection during this term. Therefore, the present day strains of *P. hybrida* should be heterogeneous in their genetic constitution. However, the karyotypes are the same, irrespective of the strains, as far as the present study has concerned. They conform well to the karyotype reported by Marthaler (1936). This implies that the species *Petunia hybrida* is nowadays karyotypically unifying. Yet according to Marthaler (1936), Malinowski (1935), and Levan (1937), the secondary constriction of SAT-chromosomes locates in the short arm of SM_1 . In contradiction to the observations of the authors mentioned above, it locates on the long arm of SM_1 as verified by the present writer. Then, two types of *Petunia hybrida* differing in the position of the secondary constriction may have been still existing today.

The most interesting point revealed by the present study is that the differential chromosome condensation takes place from late prophase to metaphase in all the chromosomes of a complement and even in arms of a chromosome. Sasaki (1961) observed in tissue-cultured mammalian cells that the degree of chromosome contraction is not uniform among the chromosomes, and it varies by the duration of colchicine treatment. He considered the differential chromosome condensation to be the colchicine modification in size and shape of metaphase chromosomes. In the present study the differential chromosome condensation was confirmed by comparing the chromosome lengths at two different stages, late prophase and metaphase, on which colchicine had equally an effect. From this finding it is evident that the differential chromosome condensation is a manifestation of the different condensating abilities intrinsic to the chromosome itself and not a result of the modification due to colchicine treatment.

The differential chromosome condensation has been reported hitherto in *Salmo alpinus* (Svårdson, 1945), in *Bufo bufo*, *Salamandra salamandra*, and *Hyla arborea* (Wickbom, 1949). On the other hand, Bajer (1959) reported that in *Haemaphysalis katherinae* and *Leucospermum aestivum* all the chromosomes shorten in a similar way in all the stages independently of their sizes. These facts suggest that the differential condensation is not recognizable in some species. Total amount of heterochromatin per complement differs in different species, and the degree of chromosome contraction is different between the eu- and heterochromatic chromosome or chromosome segment in meiosis (cf. Eberle, 1957). Heterochromatin intimately relates to the differential chromosome condensation in meiosis as confirmed in *Gesneriaceae* (Eberle, 1957) and in interspecific hybrids of *Gossypium* (Brown, 1961), in which paired chromosomes differ in their amounts and distributions of heterochromatin but equal in length. Further, in the somatic chromosomes of *Cypripedium*, Miduno (1962) has found, using his differential staining technique, that the heterochromatin may be responsible for the differential

chromosome condensation. From the facts referred to above it may be safely concluded that the differential chromosome condensation has certain relationship to heterochromatin.

Summary

1. The karyotype of *Petunia hybrida* was re-investigated and was determined as follows. $K(2n) = 14 = 2A_1^{im}(n) + 2A_2^{im} + 2B^{im} + 2C_1^{im} + 2C_2^{im} + 2D_1^{im} + 2D_2^{im}$.

2. The present study has revealed the differential chromosome condensation to take place from late prophase to metaphase in somatic mitosis. And the differential chromosome condensation probably has its relationship to heterochromatin.

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