Cytogenetics in the Genus *Linum*¹⁾

By

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The genus *Linum* comprises many species and Kikuchi (1926) was the first to give some account of the chromosome numbers of these species. This investigation was followed by those of Reynders (1926), de Vilmorin and Simonet (1927), Martzenitzina (1927), Emme and Schlapeljeva (1927), Inouye (1929), Kikuchi (1929) and Simonet (1929). Recently Richharia and Kalamkar (1939), Sugiura (1940) and Nagao (1938, 1940) also reported on the number and behaviour of chromosomes in some species of this genus.

The number of chromosomes hitherto reported in this genus has been enumerated by Sugiura in his paper "Studies on the chromosome numbers in higher plants, V" (Cytologia Vol. 10, 1940, p. 336), to which readers are referred. According to Martzenitzina (1927), the species of *Linum* may be grouped into five sections according to the number, dimension and shape of chromosomes. It may be summarized as follows:—

Section	Chromosome numbers (2n)	Species	Section	Chromosome numbers (2n)	Species
I	18	<i>L. austriacum</i>	III	32	<i>L. usitatissimum</i>
		<i>L. tenuifolium</i>	IV	30	<i>L. angustifolium</i>
		<i>L. corymbiferum</i>			<i>L. flavum</i>
		<i>L. punctatum</i>			<i>L. catharticum</i>
		<i>L. perenne</i>	V	over 57	
II	16	<i>L. grandiflorum</i>			

According to the writer who has carried out the crossing experiments between two different species, the *Linum* species may be classified into the following groups as seen from the results of the experiments and those of the cytological studies which were made by previous authors and the writer himself (Nagao 1940).

Group I. An annual species which has 8 chromosomes in gametic cells may be involved in this group, presently only one species *L. grandiflorum* being known. This species was found to have 8 chromosomes in gametic cells by all the investigators except Kikuchi (1926) who has found 9 to be the

1) Contributions from the Plant Breeding Institute, Faculty of Agriculture, Hokkaido Imperial University.

haploid number. The writer also ascertained the number to be 8 and 16 in gametic as well as somatic cells. (Fig. 1).

Group II. In this group are involved the following three species, namely, *L. usitatissimum*, *L. crepitans* and *L. angustifolium*, all of these species being homostylous, a characteristic by which the species of this group are clearly distinguished from those which belong to the other four groups.

According to Reynders (1926), Kikuchi (1926, 1929), de Vilmorin and Simonet (1927), *L. usitatissimum* has 15 chromosomes in haploid number. Recently Richharia and Kalamker (1939) obtained the same results in Indian varieties of *L. usitatissimum*. Whereas Emme and Schepeljeva (1927) found in general $2n=32$ in great number of different varieties of *L. usitatissimum*, the plant with 30 ($2n$) chromosomes, however, being exceedingly rare and for *L. crepitans* both 15 and 16 chromosomes in pollen mother cells of one

Fig. 1. *L. grandifolium*. Homotype metaphase, showing 8 chromosomes at each nuclear plate. Original. **Fig. 2.** *L. campanulatum*. Heterotype metaphase, showing 14 bivalent chromosomes. Original. **Fig. 3.** *L. flavum*, showing 14 chromosomes. Original. **Fig. 4.** *L. maritimum*. Heterotype metaphase, showing 9 chromosomes. Original. **Fig. 5.** *L. hirsutum*. Homotype metaphase, showing 9 chromosomes at each nuclear plate. Original. **Fig. 6.** The same, showing 9 bivalent chromosomes at the heterotype nuclear plate. Original.

single flower are counted. Inouye (1929) also found 16 chromosomes in pollen mother cells of *L. usitatissimum* with only a few exceptions in which 15 chromosomes were counted.

It is reasonable to say from these statements that there are two different numbers, that is, 15 and 16 as haploid number of chromosomes in *L. usitatissimum*, the latter number being considered to be derived secondarily by cross segmentation of an element of the 15 chromosomes as Emme and Schepeljeva have already pointed out.

It is reported in *L. angustifolium* also that the same difference regarding the number of chromosomes occurs as in the case of *L. usitatissimum* (cf. Reynders 1926, Martzenitzina 1927).

Group III. Belonging to the third group are the species with 14 chromosomes in gametic cells, namely *L. flavum* and *L. campanulatum*. According to Martzenitzina (1927), Nikolaewa (1927) and Kikuchi (1929) *L. flavum* has 15 chromosomes, Nagao (1940) has found, however, 14 to be the haploid chromosome number in this species and also in *L. campanulatum* (Figs. 2 and 3).

Group IV. Two yellow flowered species, *L. maritimum* and *L. hirsutum*, may be placed in this group. According to the writer's observations the chromosomes in pollen mother cells are 9 in number in *L. maritimum* as well as *L. hirsutum* as shown in Figs. 4, 5 and 6 though the number has been reported to be 10 and 8 in *L. maritimum* and *L. hirsutum* respectively by de Vilmorin and Simonet (1927).

Group V. In the fifth group may be placed perennial, blue flowered flax species as *L. perenne*, *L. tenuifolium*, *L. Lewisii*, *L. montanum*, *L. Narbonne* and so on. The number of chromosomes of these species is found to be 9 in haploid number. In *L. alpinum*, Kikuchi (1926, 1929) has found 18 chromosomes in the pollen mother cells. This is an only tetraploid species hitherto reported in the genus *Linum* (cf. Figs. 7 and 8).

Fig. 7. *L. perenne*, showing 7 bivalent chromosomes. Original. **Fig. 8.** *L. alpinum*. Homotype metaphase, showing 18 chromosomes at each nuclear plate.

According to the writer's observations on the behaviour of chromosomes in F_1 hybrids between *L. perenne* and *L. alpinum*, it is possible to say that *L. alpinum* is of an autopolyploid (Nagao 1938).

The facts mentioned above will be enumerated in the following list.

Groups	Species	Chromosome number (n)	Groups	Species	Chromosome number (n)
I	<i>L. grandiflorum</i>	8	V	<i>L. austriacum</i>	9
II	<i>L. usitatissimum</i>	15(16)		<i>L. extraaxillare</i>	9
	<i>L. crepitans</i>	15(16)		<i>L. hologynum</i>	9
	<i>L. angustifolium</i>	15(16)		<i>L. Lewisii</i>	9
				<i>L. montanum</i>	9
III	<i>L. campanulatum</i>	14		<i>L. muelleri</i>	9
	<i>L. flavum</i>	14		<i>L. Narbonense</i>	9
VI	<i>L. maritimum</i>	9		<i>L. perenne</i>	9
	<i>L. hirsutum</i>	9		<i>L. sibiricum</i>	9
V				<i>L. tenuifolium</i>	9
	<i>L. altaicum</i>	9		<i>L. alpinum</i>	18

The relative difficulty of obtaining hybrid seeds in species crossing gives an important clue to know the affinity among species with each other. According to the results of experiment which are carried out by the writer and his co-worker¹⁾ it may seem sufficiently sure that crosses between two species which belong to the different groups are always unsuccessful, while the hybrid seeds are successfully obtained when the crossing is carried out between species of the same group.

The results of the cytological observations of the species hybrid between *L. perenne* and *L. alpinum* were first reported by Kikuchi (1929). The F_1 plants and their progenies obtained by him have been grown under cultivation ever since in the experimental garden of Hokkaido Imperial University. Recently these material plants were re-examined by the writer who have reported the results so far newly obtained (Nagao 1938).

The F_1 plant of this cross has 27 chromosomes in somatic cells where 9 from *L. perenne* and 18 from *L. alpinum* were contributed. In the hetero-type metaphase of pollen mother cells there can be found several numbers of trivalent, bivalent and univalent chromosomes. In Figs. 9–12, metaphasic views are reproduced where the following chromosomal constitutions are shown respectively:

$$7_{III} + 3_{II} \quad (\text{Fig. 9})$$

$$4_{III} + 6_{II} + 3_I \quad (\text{Fig. 10})$$

$$5_{III} + 5_{II} + 2_I \quad (\text{Fig. 11})$$

$$2_{III} + 8_{II} + 5_I \quad (\text{Fig. 12})$$

1) Wachi, T.: Crossing experiments in the genus *Linum*. Graduation thesis. (Unpublished).

From these constitutions of the chromosome sets, it may not be unreasonable to assume that the two parent species *L. perenne* and *L. alpinum* are homologous in genomic constitution, the latter species carrying twice as many genomes as the former species.

While it is not yet obvious whether the conjugation of chromosomes occurs between the two parent chromosomes or it occurs between chromosomes of a parent, we must have assume the taking place of an autosynopsis between non-homologous chromosomes in a genome, by these premises the conjugation of chromosomes above described being explainable. If we deal with, as an example, the case where $7_{III} + 3_I$ is found the following two types of chromosome mating are considerable, namely¹⁾ :—

Fig. 9. Heterotype metaphase of F_1 hybrid between *L. perenne* and *L. alpinum*, showing 7_{III} and 3_{II} chromosomes. Original. **Fig. 10.** The same showing 4_{III} , 6_{II} and 3_I chromosomes. Original. **Fig. 11.** The same, showing 5_{III} , 5_{II} and 2_I chromosomes. Original. **Fig. 12.** The same, showing 2_{III} , 8_{II} and 5_I chromosomes. Sectioned material. Original. **Fig. 13.** The same showing 16 and 11 chromosomes in each homotype nuclear plate respectively. Sectioned material. Original. **Fig. 14.** The same, showing 13 and 14 chromosomes. Sectional material. Original. **Fig. 15.** Heterotype metaphase of a F_2 plant of *L. perenne* $\times$ *L. alpinum*, showing 18 chromosomes loosely associated. Original. **Fig. 16.** The same, showing $30(4_{III} + 6_{II} + 6_I)$ chromosomes. Original.

1) By the latters a_{1-9} and p_{1-9} were denoted the chromosomes of *L. alpinum* and of *L. perenne* respectively.

$ \begin{array}{cccccccc} & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ a & a & a & a & a & a & a & a & a \\ 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \\ a & a & a & a & a & a & a & a & a \\ 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \\ p & p & p & p & p & p & p & p & p \\ 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \end{array} $								
or								
$ \begin{array}{cccccccc} & & & & & & & & \\ & & & & & & & & \\ & & & & & & & & \\ a & a & a & a & a & a & a & a & a \\ 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \\ a & a & a & a & a & a & a & a & a \\ 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \\ p & p & p & p & p & p & p & p & p \\ 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \end{array} $								

The cases as shown in Figs. 10–12 will be able to elucidate in similar manners. In the heterotype anaphase some irregularities regarding the migration of chromosomes which are commonly found in triploid plant are shown (Kikuchi 1929, Nagao 1938). The distribution figures of chromosomes at the homotype metaphase are reproduced in Figs. 13 and 14 where 11:16 and 14:13 chromosomes are seen respectively.

Kikuchi has obtained F_2 plants by crossing inter se in both natural and artificial ways among which the five F_2 plants were determined to have 18, 20, 28, 30 and 34 chromosomes respectively in somatic numbers (cf. Kikuchi 1929, and Figs. 15 and 16). By the F_2 plant with 30 chromosomes were produced a number of F_3 progenies, among which six individuals are now living and employed by the writer for cytological observations. Although a full and particular account will be published later, the number and the conjugation of chromosomes observe by the writer will be shown in the following table.

Plant number	Chromosome numbers
F_3A_6 - 1	44 <i>Fig. 17</i>
- 3	34 ($2_{III}+11_{II}+6_I$) <i>Figs. 18, 19</i>
- 7	42 ($3_{III}+15_{II}+3_I$) <i>Fig. 20</i>
- 8	36 ($2_{III}+11_{II}+8_I$) <i>Fig. 21</i>
- 9	32 ($1_{III}+11_{II}+7_I$) <i>Fig. 22</i>
-12	38 <i>Fig. 23</i>

Recent progress in cytogenetics is opening to us the colchicine method by which the polyploidal multiplication of chromosomes is artificially induced, and there exists now an extensive literature on the subject. In *Linum* species, Lutkov (1938) has successfully obtained a tetraploid by subjecting plants to an unusually high temperature. The experiments of inducing polyploids by means of colchicine treatment has been carried out by Simonet and Chopinet (1939) in *L. grandiflorum* and by Györfy (1938, 1940), Rybin (1939), Lutkov (1939) and Müntzing (1939) in *L. usitatissimum*. According to Müntzing, the seed treatment with 0.025% solution for two and four days was successful in obtaining tetraploids in *Linum*.

Figs. 17-23. Chromosomes of F_3 plants derived from F_2 having 30 chromosomes in diploid number. **17.** A_6-1 . Homotype metaphase, showing 20 and 24 chromosomes in each nuclear plate respectively. Original. **18.** A_6-3 . Heterotype metaphase, showing 34 ($2_{III} + 11_{II} + 6_I$) chromosomes. Original. **19.** The same. Homotype metaphase, showing 17 chromosomes in each nuclear plate. Original. **20.** A_6-7 . Heterotype metaphase, showing 42 ($3_{III} + 15_{II} + 3_I$) chromosomes. Original. **21.** A_6-8 . The same, showing 36 ($2_{III} + 11_{II} + 8_I$) chromosomes. Original. **22.** A_6-9 . The same, showing 32 ($1_{III} + 11_{II} + 7_I$) chromosomes. Original. **23.** A_6-12 . Homotype metaphase, showing 21 and 17 chromosomes at each nuclear plate respectively. Original.

It is an important problem in cytogenetics not only its scientific interest but also from the point of view of breeding to study the method of inducing polyploids in *L. usitatissimum* which is an important fibre crop plant.

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