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CHROMOSOME ASSOCIATION AND POLLEN FERTILITY IN SOME INTERSPECIFIC HYBRIDS OF *LILIUM*

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INDEX WORDS

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SUMMARY

Meiotic observations in PMCs were made in eight kinds of interspecific hybrids in *Lilium*. Three hybrids, '6134-S' × *L. cernuum*, *L. regale* × *L. leichtlinii maximowiczii* and *L. auratum platyphyllum* × *L. henryi* showed respectively the mean chromosome association frequencies of 1.4_{II}, 0.8_{II} and 1.5_{II} per cell at MI, which were the lowest values hitherto reported in *Lilium* hybrids. In *L. longiflorum* × *L. henryi*, 33.3% of the cells had 1 or 2 multivalents of three to five chromosomes in addition to uni- and bivalents. 12 bivalents and 12 univalents were invariably observed in the triploid hybrid *L. longiflorum* × *L. cernuum*. The hybrids between the species belonging to the different sections of the genus generally showed high pollen sterility, with some exceptions. A more or less remote genomic homology was found between the different sections in *Lilium*.

INTRODUCTION

For over a hundred years a large number of horticultural varieties of *Lilium* have been produced by crossing between different species. Except for some exceptions most of those interspecific hybrids hitherto produced were raised between the species within the sections, after the botanical classification established by COMBER (1949). The hybrid lilies are classified horticulturally into eight divisions according to their parental species (ANONYMOUS, 1964). Both classifications are in good agreement with each other.

With embryo culture, often in combination with the 'intrastylar (amputated style) pollination technique', it has recently become possible to obtain hybrids between the different sections in certain cross combinations (SKIRM, 1942; ASCHER, 1973, 1977; STIMART & ASCHER, 1974; ASANO & MYODO, 1977a, b; ASANO, 1978, 1980a, b; BARBER, 1977; MCRAE & MCRAE, 1979; VAN TUYL, 1980). The work of these researchers has thrown light on the production of superior hybrid lilies by combining some desirable characters found in species of different sections.

Making use of such interspecific hybrids as material, chromosome association and pollen fertility were observed for the estimation of genomic homology between the parental species.

Tabel 1. Frequency of chromosome association at metaphase I in eight interspecific hybrids in *Lilium*.

	Configurations/cell														Others including multivalents	Total cells obs.	Mean association frequency	Pollen fertility (%)
	II: 12	12	11	10	9	8	7	6	5	4	3	2	1	0				
	I: 12	0	2	4	6	8	10	12	14	16	18	20	22	24				
L. longiflorum × *L. leichtlinii maximowiczii*			1	8	9	25	12	14	4	3						76	$7.5_{II}+9.0_{I}$	52.0
L. longiflorum × *L. henryi*				3	4	10	24	17	16	11	8	9	2	2	53*	159	$04_{III\text{-}V}+5.4_{II}+12.0_{I}$	0
L. longiflorum × *L. cernuum***	52															52	$12.0_{II}+12.0_{I}$	0
'Royal Gold' × *L. speciosum*						1	3	16	5	27	18	9	10	2		91	$3.7_{II}+16.6_{I}$	0
'6134-S' × *L. cernuum*										4	8	28	29	19		88	$1.4_{II}+21.2_{I}$	0
L. regale × *L. leichtlinii maximowiczii*											2	9	45	28		84	$0.8_{II}+22-.4_{I}$	0
L. formosanum × *L. speciosum*		1	1	3	3	3	3	4	6	6	13	20	28	49		140	$2.2_{II}+19.6_{I}$***	0
L. auratum platyphyllum × *L. henryi*										2	19	34	39	23		117	$1.5_{II}+21.0_{I}$	83.6

*See Table 2; **Allotriploid; *** + f; I = Univalents, II = Bivalents, III-V = Multivalents.

MATERIALS AND METHODS

Meiotic observations in PMCs were made in eight kinds of interspecific hybrids in *Lilium*, which were produced at our laboratory (Table 1). Of the parents used, 'Royal Gold' is a horticultural variety reputed to be a yellow flowered mutant of *L. regale* (DE GRAAFF, 1955) and '6134-S' was raised from 'Royal Gold' × *L. regale*. Seven out of the eight interspecific hybrids are diploids (2n = 24) and the remaining one, *L. longiflorum* × *L. cernuum*, is an allotriploid (2n = 36) with two genomes from the female parent and one from the male parent (ASANO, 1978, 1980b).

The hybrid plants were cultivated at the experimental farm of our laboratory. Young anthers were fixed in NEWCOMER's fluid (1953). The metaphase I in PMCs of buds that were 19.5 to 35 mm in length, occurred 20 to 29 days before flowering. Iron aceto-carmine smears were employed for the meiotic observations. Pollen fertility was measured by the percentage of well-stained pollen grains with acetocarmine at anthesis.

RESULTS AND DISCUSSION

Chromosome association. Frequencies and configurations of chromosome association observed in the eight kinds of interspecific hybrids are shown in Table 1 and Fig. 1. All of the hybrids revealed various frequencies of univalents. Among the diploid hybrids, the mean frequency of bivalents per cell was highest in the hybrid of *L. longiflorum* × *L. leichtlinii maximowiczii*, 7.5_{II}, and lowest in *L. regale* × *L. leichtlinii maximowiczii*, 0.8_{II}. In the hybrid lilies previously reported, chromosome association has been very high with frequencies of bivalents ranging from 11 to 12 (MATHER, 1935; RICHARDSON, 1936; RIBBANDS, 1937; KUMAZAWA & KIMURA, 1946; BROCK, 1954; NODA, 1971). An exceptional example with a very low association frequency was seen in 'Black Beauty' (*L. speciosum* × *L. henryi*): on average $2.2(0–5)_{II}$ per cell (EMSWELLER & UHRING, 1966). In the present study, however, '6134-S' × *L. cernuum*, *L. regale* × *L. leichtlinii maximowiczii* and *L. auratum platyphyllum* × *L. henryi*, showed respectively 1.4_{II}, 0.8_{II} and 1.5_{II} per cell. As far as the writer is aware, these values are the lowest hitherto reported in *Lilium* hybrids. The hybrid, *L. formosanum* × L. speciosum, is characterized by a wide range of bivalent formation from 0 to 12 per cell in spite of its low mean frequency. In *L. longiflorum* × *L. henryi*, 53 out of 159 cells (33.3%) had 1 or 2 multivalents of three to five chromosomes in addition to uni- and bivalents (Table 2, Fig. 2). Multivalent formation in an interspecific hybrid is considered to indicate the existence of interchromosomal translocation or duplication (STEBBINS, 1971; MOORE, 1976). The presence of the multivalents with the maximum of five chromosomes in this hybrid may indicate that complicated structural changes took place during the speciation of the parental species.

12 bivalents and 12 univalents were invariably observed in the triploid hybrid *L. longiflorum* × *L. cernuum*. Since 24 (two genomes) out of the 36 chromosomes were karyotypically ascertained to be derived from *L. longiflorum* and 12 (one genome) from *L. cernuum*, the 12 bivalents were possibly formed by autosyndesis between the homologous chromosomes from *L. longiflorum* and the remaining 12 univalents were of *L. cernuum*. As in other species, trivalent formation of a majority of the chro-

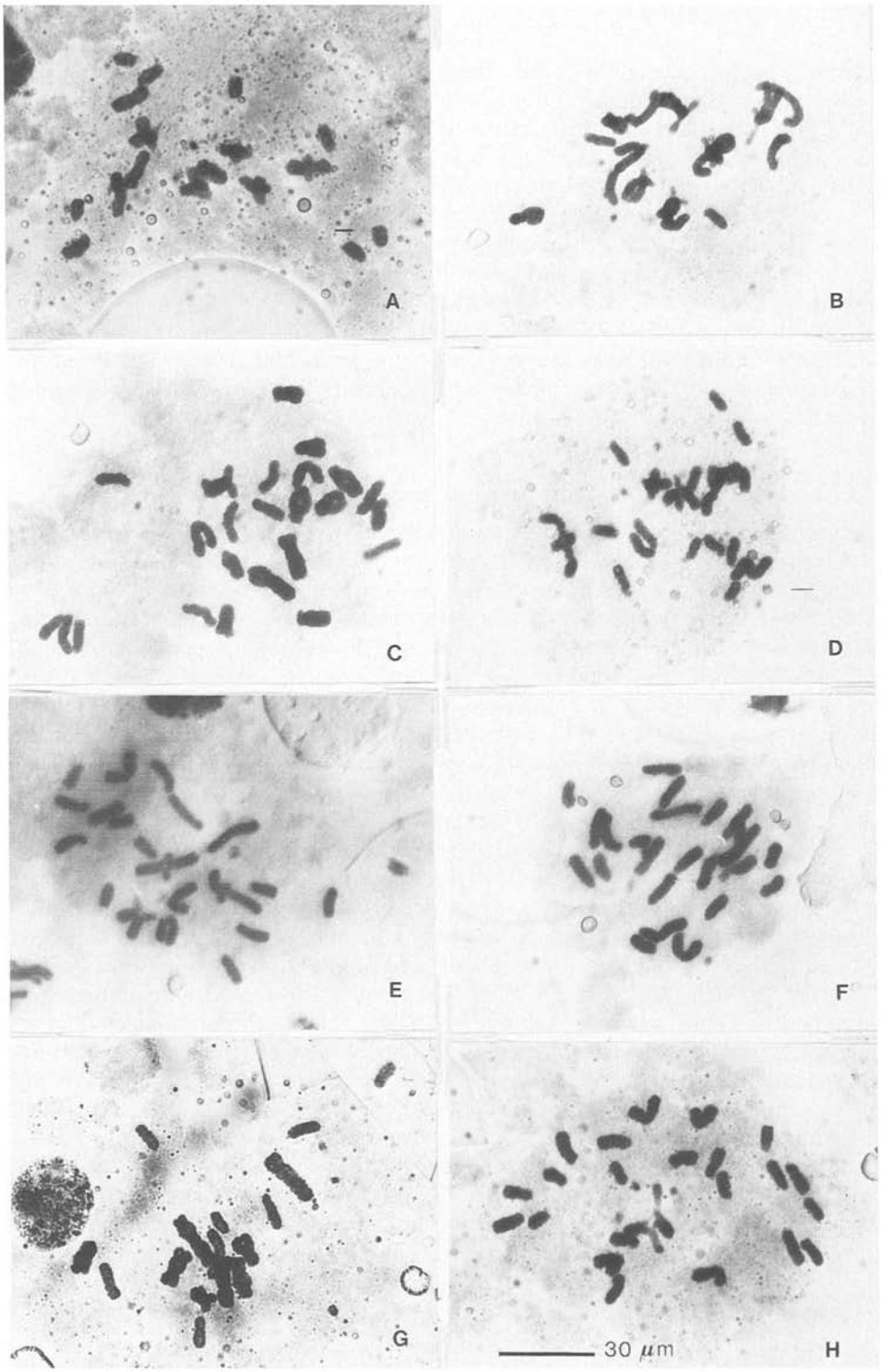
A
B
C
D
E
F
G
30 μm
H

Table 2. Frequency of chromosome association at metaphase I in the hybrid, *Lilium longiflorum* × *L. henryi*.

	Configurations/cell																					Total
V:	1	1	1																			
IV:				2	1	1	1	1	1	1												
III:	1	1			1						2	2	1	1	1	1	1	1				
II:	6	4	4	5	7	8	6	5	4	3	4	1	8	7	6	5	4	3	10-0			
I:	4	8	11	6	3	4	8	10	12	14	10	16	5	7	9	11	13	15	4-24			
Cells obs.	1	1	4	1	1	1	1	3	3	2	1	1	2	4	9	11	4	3	106	159		

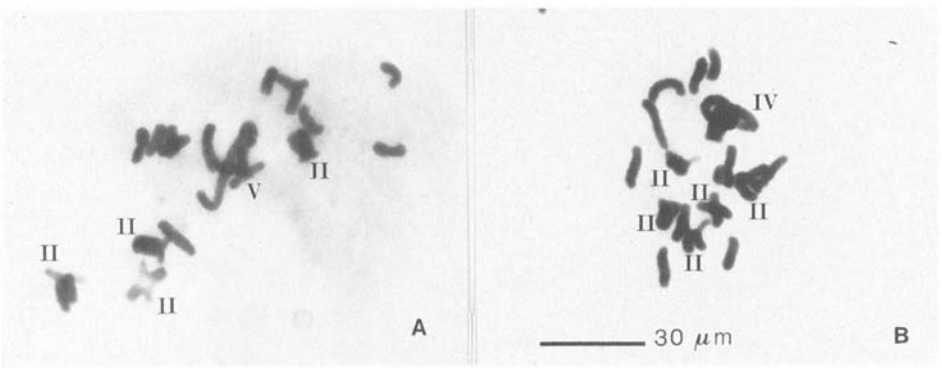

Fig. 2. Meiotic metaphase I including multivalent chromosomes in PMCs of the hybrid, *Lilium longiflorum* × *L. henryi*.
A: $1_{IV}+4_{II}+11_{I}$; B: $1_{IV}+5_{II}+10_{I}$.

mosomes at meiosis in allotriploids of *Lilium* indicates a high genomic homology between the parental species (NODA, 1966, 1971). Such differential pairing in this hybrid therefore shows that *L. longiflorum* and *L. cernuum* have genetically been well differentiated.

These observations may indicate a more or less remote genomic homology between the species belonging to the different sections in *Lilium*.

Pollen fertility. In interspecific hybrids of *Lilium* species, it is likely that a high association frequency generally causes the hybrids to be fertile. For example, 'Tsukiha' (*L. maximowiczii* × *L. pumilum*), in which the association frequency is $11.96_{II}+0.08_{I}$

Fig. 1. Meiotic metaphase I in PMCs of eight interspecific hybrids in *Lilium*.
A: *L. longiflorum* × *L. leichtlinii maximowiczii* ($6_{II}+12_{I}$); B: *L. longiflorum* × *L. henryi* ($8_{II}+8_{I}$); C: *L. longiflorum* × *L. cernuum* ($12_{II}+12_{I}$); D: 'Royal Gold' × *L. speciosum* ($4_{II}+16_{I}$); E: '6134-S' × *L. cernuum* ($3_{II}+18_{I}$); F: *L. regale* × *L. leichtlinii maximowiczii* (24_{I}); G: *L. formosanum* × *L. speciosum* ($8_{II}+8_{I}+f$); H: *L. auratum platyphyllum* × *L. henryi* ($1_{II}+22_{I}$).

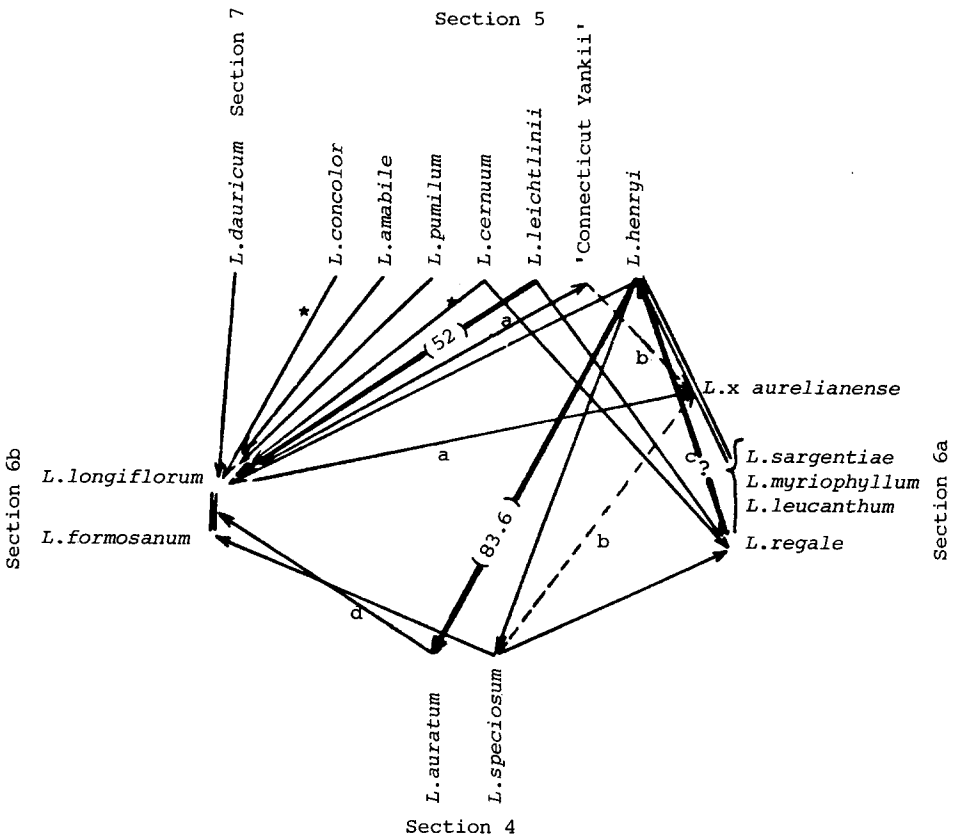

Fig. 3. Pollen fertility in the hybrids between different sections in *Lilium*. (Taxonomic sections following COMBER, 1949).
Explanation of signs:
Male parent → Female parent.
Pollen fertility: —— sterile; ——— (partially) fertile, a figure in parenthesis represents the fertility (%); --- unknown.
═══ Hybrid between two or more species.
a: ASCHER (1973, 1977); b: RONALD (1977, 1979); c: SKIRM (1942); d: TAKIZAWA (1977).
* Triploid.

per cell, has a pollen fertility of 91.0% (NODA, 1971); 'Marhan' (*L. martagon album* × *L. hansonii*), $11.64_{II} + 0.72_{I}$, and 'Dalhansonii' (*L. martagon dalmaticum* × *L. hansonii*), $11.86_{II} + 0.28_{I}$, have a pollen fertility of 51.5% and 34.5% respectively (BROCK, 1954). Also, such hybrids as *L.* x *umbellatum* (a hybrid between *L. croceum* and *L. thunbergii*?) (MATHER, 1935), 'Marhan' (RICHARDSON, 1936) and *L.* x *testaceum* (*L. candidum* × *L. chalcedonicum*) (RIBBANDS, 1937) have their mean bivalent frequencies between 11 and 12 per cell and apparently are fertile. These are examples of interspecific hybrids within the same sections with fertile pollen due to the almost regular meiotic chromosome association and the following division.

On the other hand, pollen fertility in some hybrids between the different sections

which have till now been produced by us and some other investigators are shown in Fig. 3. As seen in Fig. 3. these hybrids generally show high pollen sterility. In many of these cases it may be chromosomal sterility as seen in the present study rather than genic or cryptic, i.e., the pollen sterility may be primarily caused by the irregular chromosome association and the various frequencies of following abnormalities such as unbalanced chromosome separation, longitudinal splitting of non-associated chromosomes, chromatin bridge formation, appearance of lagging chromosomes, and so forth. One of the remarkable results is the partial fertility (52%) in the hybrid of *L. longiflorum* × *L. leichtlinii maximowiczii*. This is possibly related to the relatively high chromosome association at meiosis: 7.5_{II} per cell. If the frequency of trivalent formation in the triploid hybrid is high, the hybrid is rather fertile in the pollen formation (NODA, 1966, 1971). The triploid hybrid *L. longiflorum* × *L. cernuum* formed no trivalents at meiosis and was completely pollen sterile. According to these facts, it can be said that meiotic chromosome association in the interspecific hybrids of *Lilium* is a prerequisite for high frequency of good pollen grains. An exceptional case is the hybrid of *L. auratum platyphyllum* × *L. henryi*, where, in spite of the very low chromosome association, $1.5_{II} + 21.0_{I}$ per cell, the hybrid had a high pollen fertility: 83.6%. This high frequency of fertility appeared to be caused by an unusual meiotic division with the omission of a second division (unpublished).

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