

flow rate during the analysis was 10–20 nuclei/s. The fluorescent signals, proportional to the quantity of DNA per nucleus, were measured with the multichannel distribution analyzer 2103 (Ortho Diagnostic Systems) and presented as histograms in which nuclear DNA contents were expressed as C-values.

Measurement of pollen size

Pollen size of the *L.* × 'Enchantment' × *L. pumilum* hybrids was measured in random samples of 200 pollen using a light microscope at a magnification of 1000 ×.



Fig. 1. The flower of the *L.* × 'Enchantment' × *L. pumilum* hybrid.

Table 1. The *Lilium* hybrids and species with corresponding chromosome and IVT*-numbers

Hybrid/species	Chromosome number	IVT-number	Hybrid group**
<i>L. auratum</i> × <i>L. henryi</i>	2n=24	82111	O
<i>L. longiflorum</i> 'Gelria'	2n=24	78372-1	S
<i>L. longiflorum</i> 'Gelria' tetraploid	2n=4x=48	84184	S
<i>L.</i> × 'Enchantment' × <i>L. pumilum</i>	2n=24	79418-1/6	A

*IVT=Instituut voor de Veredeling van Tuinbouwgewassen.

**A=Asiatic hybrid, O=Oriental hybrid, S=Species.

Results

Flow cytometry—general remarks

Figs. 2a–f and 3a–f show histograms obtained with flow cytometry of nuclei from various tissues of the plants listed in Table 1. On the X-axis the channel numbers of the flow cytometer multichannel distribution analyzer are plotted ranging from 40 to 500, the Y-axis represents the number of fluorescence signals recorded per channel.

Damaged nuclei as well as cell-wall fragments smaller than 20 μm in size gave rise to background signals which were registered predominantly at lower channel numbers. For this reason it was decided not to record counts below channel 40. Beyond channel 500 no counts were recorded as well. By this the flow cytometer was adapted to register the signals of all intact nuclei. In the histograms of Figs. 2a–f and 3a–f the channel number in which most counts are found, indicated with P, is used to mark a peak's position on the X-axis. Channel numbers approximately halfway in between two P-channels are used to

level. The maximum number of counts is found in channel 127. Nuclei isolated from the diploid *L. longiflorum* 'Gelria' give rise to one large peak at the 2C-level (Fig. 2b) with most counts in channel 120 and a small peak at the 4C-level with most counts in channel 241. Nuclei isolated from the tetraploid *L. longiflorum* 'Gelria' give rise to a single large peak at the 4C-level (Fig. 2c) with most counts in channel 253. Therefore the 2C-amount of nuclear DNA of *L. longiflorum* is the same as that of the Asiatic hybrid. Analysis of the diploid Oriental hybrid *L. auratum* × *L. henryi* shows one peak at the 2C-level (Fig. 2d) with the maximum number of counts in channel 103. Apparently, the 2C-DNA amount in nuclei of the Oriental hybrid *L. auratum* × *L. henryi* is about 25% less than in diploid nuclei of both *L. longiflorum* and the Asiatic hybrid *L.* × 'Enchantment' × *L. pumilum*.

Flow cytometry with pollen

Pollen of the diploid *L. longiflorum* 'Gelria' (Fig. 2e) gives rise to two peaks of virtually identical size. When compared with the histogram of nuclei from roots of the diploid and the tetraploid *L. longiflorum* 'Gelria' (Figs. 2b-c) these peaks are located at the 1C- and the

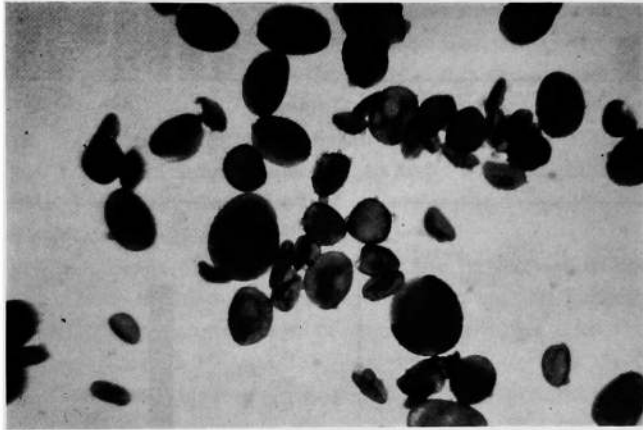


Fig. 4. A microscopic view of a pollen sample from *L.* × 'Enchantment' × *L. pumilum* 79418-2, with small (empty), normal and large pollen grains. ×100.

2C-level respectively. An additional peak at the 4C-level is found when pollen from plants of the diploid interspecific Asiatic hybrid *L.* × 'Enchantment' and *L. pumilum* is analyzed (Figs. 3a-f; cf. Fig. 2a). The percentage of counts covered by the 4C-peak is different for individual hybrid plants, varying from 5.6% (Fig. 3d) to 16.9% (Fig. 3b).

Peaks obtained with nuclei from pollen of the diploid interspecific hybrid between *L. auratum* and *L. henryi* (Fig. 2f) are of identical size and located at the 2C- and 4C-level respectively when compared to Fig. 2d.

Pollen size of *L.* × 'Enchantment' × *L. pumilum*

Microscopic observations of pollen from the *L.* × 'Enchantment' × *L. pumilum* hybrids, showed a large variation in pollen size (Fig. 4). Length of the pollen grains varied between 45 and 137 μm. Several hybrid plants had a relatively high percentage of large pollen (>90 μm). This is shown in Figs. 5a-f representing histograms in which pollen lengths are grouped in classes of 7.2 μm. These data were obtained with the same plants that were used for the flow cytometric determinations. Therefore, Figs. 5a-f correspond with Figs. 3a-f.

