Acta Bot. Croat. *31* (1972) 71—79

PENTAPLOIDS IN POPULATIONS OF VARIOUS *LEUCANTHEMUM* SPECIES

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Received February 3, 1972.

Introduction

Polyploidy, a common phenomenon in higher plants, is one of the most striking characteristics. The genus *Leucanthemum* is a well known example of this. It contains six ploidy levels: 2x, 4x, 6x, 8x, 10x and 12x all found in nature (Favarger and Villard 1965, Villard 1970, Mirković 1966, 1969 and others). There is not much evidence of the occurence in nature of plants with other ploidy levels, which represent unbalanced types with uneven somatic chromosome numbers such as triploids (3x), pentaploids (5x), heptaploids (7x) etc. Villard (1970) was the only author who described some natural (and also artifical) hybrids with these (5x, 7x) uneven chromosome numbers.

The results of several years of investigation in Yugoslavia of 463 plants from 95 populations of the *Leucanthemum* species, show that in the natural populations of the different species pentaploids are not rare.

Materials and Methods

The plants investigated were collected in nature in the period from 1962 to 1971. They were raised at the Botanic Garden in Zagreb. (Some plants had been transported to Kew Gardens in England where they were examined for a year).

Root tips were pretreated with α-monobromnaphtalene in a refrigerator for about 20 hours, fixed in 1 : 3 aceto-alcohol and stained with Feulgen. Studies of chromosome pairing were made from fixed anthers. The squashes were stained with aceto-orcein. All preparations were made permanent by the liquid-CO_2-method. Photographs were taken of permanent preparations.

The pollen size was determined by measuring 100—200 fresh pollen grains stained with aceto-carmine. For purposes of microphotography, the pollen grains were gently centrifuged for 3 minutes in ether (to extract fat-like substances) and then stained with aceto-carmine.

Results

Table 1. shows that from 463 investigated plants of the genus *Leucanthemum,* 35 are pentaploids with 2n =(5x) = 45 (Figs. 1 and 2). They occur in many populations of various species. Most of them are found in populations where hexaploids (6x) predominate (Table 2), as is the case of *L. vulgare* subsp. *amplifolium* Fiori in populations from the mountains of Galičica, Vranica, Inač, Maglič and Troglav, and also of *L. croaticum* var. *croaticum* Horvatić in two populations from Velebit. In some of these populations a few tetraploids have been found too. In two populations of *L. montanum* var. *heterophbyllum* (Willd.) Briqu. et Cav. pentaploids grow exclusively among octoploids (8x).

Ploidy	2 x	4 x	5 x	6 x	7 x	8 x	Total
2 n =	18	36 (— 1)	45	54 (∓ 2)	63 (∓ 4)	72 (∓ 3)	
No. of individuals	85	93	35	108	9	133	463

Table 1. Number of individuals of *Leucanthemum* species found at various ploidy levels.

Although pentaploids grow in different populations of various species, they have some common characteristics. They are selfsterile. The stainability of the pollen is quite high ranging from 58 to 88,3% (Table 2). The size of the pollen grains varies to a great extent in all pentaploids. The measurements were done in plants cyt. nos. 70.140, 70.908, 70.133, 70.48 and 70.88 and ranged from about 24 to 47 μm (Figs. 3 and 4). There are two peaks in Figs. 5 and 6. The first peak (27 to 29.7 μm) includes about 20% pollen grains, and the second one (in Fig. 5 from 35.1 to 37.8 μm and in Fig. 6 from 32.4 zo 35.1 μm) more than 30%. Some pollen grains of the size between 24.3 and 27 μm were found without extine.

Many of investigated *Leucanthemum* pentaploids produce a high percentage of fertile seeds by open pollination (e. g. the plant cyt. no. 66.1 from Vranica). The chromosome number of the seedlings varies considerably (2n = 35, 36, 45, 54, 69, 70 and 71).

All pentaploids are very similar in their pairing behaviour. Univalents are commonest. Bivalents, trivalents and quadrivalents are present in different numbers and consequently many PMC contain different chromosome numbers.

Morphological characteristics of the pentaploids do not differ from those of plants with other ploidy levels in the population. The only specific feature, which has been noticed, is that pentaploids flower rarely, e. g. once in three yars.

Discussion

Obviously the appearance of natural pentaploids in *Leucanthemum* populations is not so rare as the data in the literature may indicate (V i l l a r d 1970). This fact can be proved only by investigating many individuals from many populations. A good example of this is the work of J o n e s (1958) on *Holcus mollis,* in which after examining a great number of individuals from many populations, he found plants with four different chromosome numbers among which the sterile pentaploid (hybrid), reproducing only vegetatively, was very frequent.

There is no evidence to prove that *Leucanthemum* pentaploids are hybrids. They are not sterile since they produce seeds; the chromosome numbers of the seedlings, grown from these seeds vary, however, considerably. They also propagate vegetatively.

Leucanthemum pentaploids 2n = (5x) = 45, grow in different populations where representatives of higher polyploids are always predominant. In some, they are hexaploids (6x) and in others octoploids (8x). In both cases pentaploids seem to originate from plants with higher ploidy which would be contrary to the usual way of polyploidisation. Some examples are known in the tribe *Andropogoneae* of the grass family. De Wet (1965, 1968, cited after S t e b b i n s 1971) has shown that natural autopolyploids can produce diploids. Successful reversions of this sort, however, occur only in autopolyploid populations, which appear to be of relatively recent origin, and which live sympatrically with their diploid progenitors (S t e b b i n s 1971).

In *Leucanthemum* populations (Table 2), with predominant hexaploids, and with a few tetraploids, the pentaploids could be produced by hybridisation of both. In populations, however, where pentaploids grow exclusively among octoploids, it is impossible to recognize their ancestors. All that can be investigated here is the ability of *Leucanthemum* polyploids to produce gametes with chromosome numbers of those ploidy levels, which are successful in the process of fertilization.

As we can see the pollen stainability (of 58 to 88.3%) in pentaploids is rather high for an unbalanced type, but is still lower if compaired with that in balanced types of *Leucanthemum* species where pollen stainability is in the range of 75 and 95% (Papeš, unpublished).

The variations in the size of pollen grains may be related to their ploidy level. The two peaks, which appear on both histograms in Figs. 5 and 6, indicate that two chromosome numbers in the nuclei of pollen grains were most frequent. If we compare the size of pollen grains of pentaploids with that of plants with other ploidy levels (V i l l a r d 1970 and P a p e š, unpublished) the first peaks in Figs. 5 and 6 (27 — 29.7 μm) could correspond to diploids (or perhaps triploids), the second peak in Fig. 5 (35.1 — 37.8 μm) to hexaploids or octoploids, and the second in Fig. 6 (32.4 — 35.1 μm) to hexaploids (or perhaps pentaploids).

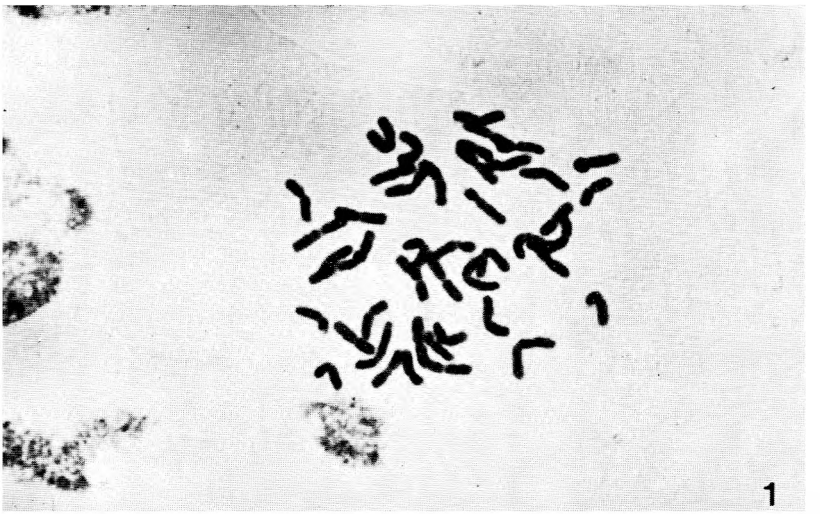
In addition to that, the analysis of various chromosome numbers of seeds (seedlings) from one plant (cyt. no. 66.1) shows that pentaploids have a great ability of producing progeny with many various chromosome numbers. How they survive, is still unknown.

Table 2. List of *Leucanthemum* populations containing pentaploids.

1	2	3	4	5	6	7	8
Species	Cyt. no.	Locality	Habitat	Altitudes in m	2 n	Pollen stainability	Collector
L. vulgare subsp. *amplifolium*	67.12	Makedonija, Galičica	Wodland, clearing, calcareus substratum	1600	36		S. Ungar
	67.13			"	45		"
	67.14			"	54		"
	67.15			"	54		"
L. vulgare subsp. *amplifolium*	63.8	Bosna and Hercegovina, above the Prokoško lake, Vranica mountain	Rocky mountain pastire, calcareous substratum	1800	36		Author
	63.10			"	36		"
	63.13			"	36		"
	64.2			"	36		"
	66.1			"	45		"
	66.12			"	45		"
	66.13			"	45		"
	66.14			"	45		"
	66.31			"	45		"
	66.32			"	45		"
	66.44			"	45		"
	66.54			"	45		"
	66.60			"	45		"
	66.65			"	45		"
	66.81			"	45		"
	66.83			"	45		"
	66.84			"	45		"
	66.87			"	45		"
	66.92			"	45		"
	67.1			"	45		"
	70.139			"	45		"
	70.140			"	45	58%	"
	64.30			"	54		"
	64.32			"	54		"
	64.35			"	54		"

1	2	3	4	5	6	7	8
	66.26			"	54		"
	66.28			"	54		"
	66.30			"	54		"
L. vulgare subsp. *amplifolium*	66.36	Vranica mountain		1600	54		Author and Č. Šilić
	66.37			"	54		"
	66.49			"	54		"
	66.62			"	54		"
	66.70			"	54		"
	66.73			"	54		"
	66.82			"	54		"
	66.86			"	54		"
	66.93			"	54		"
	66.94			"	54		"
	70.141			"	54		"
L. vulgare subsp. *amplifolium*	70.240	Bosna and Hercegovina, Inač mountain SE-exp. Kojsina	Rocky mountain pasture, calcareous substratum	1000	45		Č. Šilić
	70.1097			"	45		"
	69.14			"	54		"
	69.15			"	54		"
L. vulgare subsp. *amplifolium*	70.908	Bosna and Hercegovina, Maglić mountain near Dobre vode	Rocky mountain pasture, calcareous substratum	1800	45	63,6%	Author
	70.144			"	54		"
	70.152			"	54		"
	70.153			"	54		"
L. vulgare subsp. *amplifolium*	70.121	Hrvatska, Troglav mountain, above Velika Poljica	Woodland clearing, calcareous substratum	1350	36		Č Šilić
	70.185			"	45		"
	69.10			"	54		"

1	2	3	4	5	6	7	8
Species	Cyt. no.	Locality	Habitat	Altitudes in m	2 n	Pollen stainability	Collector
L. croaticum var. *croaticum*	70.138	Hrvatska, Velebit near Teminovac	Rocky mountain pasture, calcareous substratum	1000	36		Author
	70.486			"	45		"
	70.137			"	54		"
	70.870			"	54		"
L. croaticum var. *croaticum*	70.133	Hrvatska, Velebit near Ovčiće	Rocky mountain pasture, calcareous substratum	1100	45	81%	Author
	70.134			"	45		"
	70.487			"	45	88,3%	"
	70.526			"	45		"
	70.887			"	45	71%	"
	70.1090			"	45		"
	70.135			"	54		"
	70.136			"	54		"
L. montanum var. *heterophyllum*	68.15	Hrvatska, Risnjak mountain	Rock strown bank bordering a beech wood	1200	45		Author
	66.76			"	45		"
	70.184			"	45		"
	66.63			"	72		"
	66.66			"	72		"
	66.68			"	72		"
	66.71			"	72		"
	66.77			"	72		"
	66.78			"	72		
L. montanum var. *heterophyllum*	70.128	Hrvatska, Bijelolasica mountain	Rock strown bank bordering a beech wood	1500	45 + 2 B		V. Trinajstić
	70.129			"	45 + 1 — 2 B		"
	69.16			"	72		"
	69.17			"	72		"



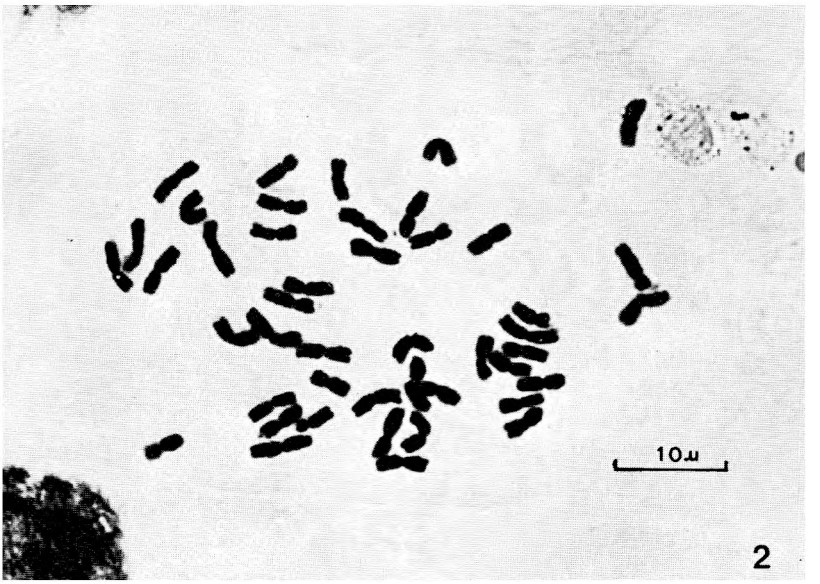
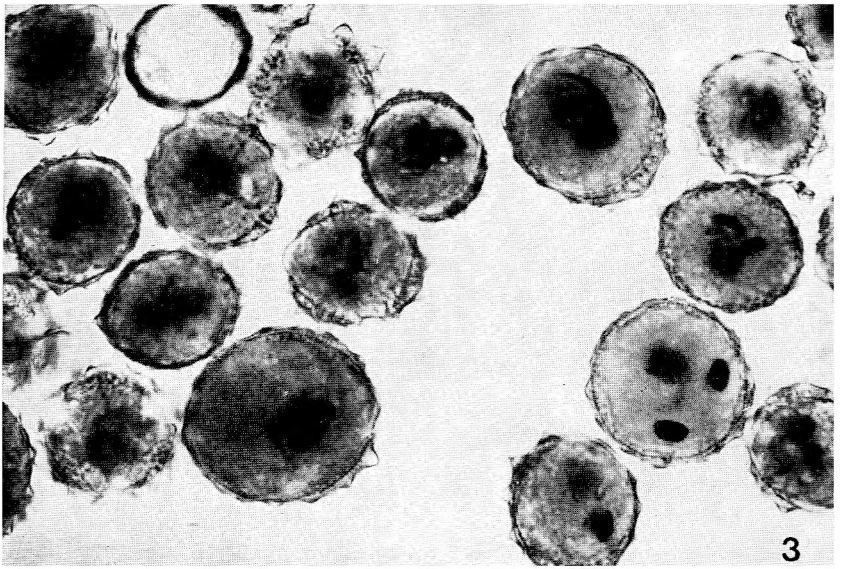


Plate I — Tabla I



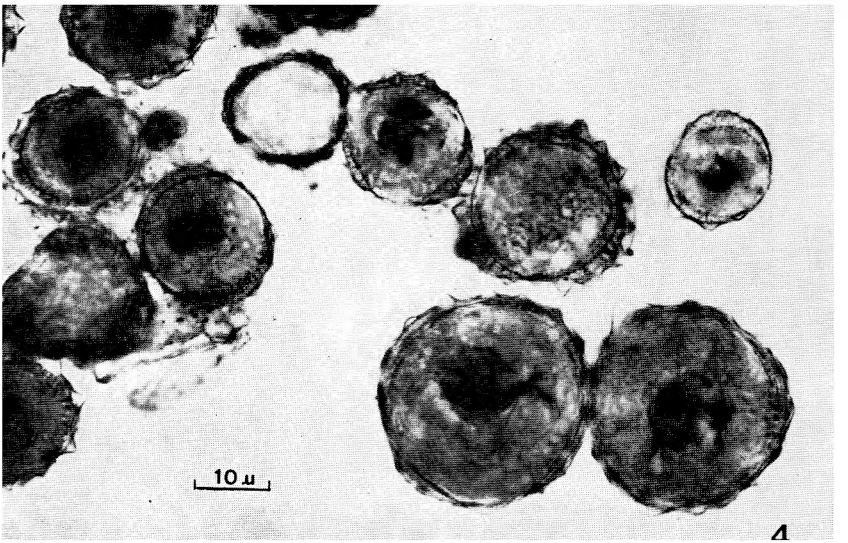


Plate II — Tabla II

Plate I — Somatic chromosomes of the *Leucanthemum* — pentaploids.
Tabla I — Somatski kromosomi u *Leucanthemum* — pentaploida.

Fig. 1. *L. vulgare* subsp. *amplifolium* 2 n = (5 x) = 45, (cyt. no. 70.1097).
Sl. 1. *L. vulgare* subsp. *amplifolium* 2 n = (5 x) = 45, (cyt. br. 70.1097).
Fig. 2. *L. croaticum* var. *croaticum* 2 n = (5 x) = 45, (cyt. no. 70.526).
Sl. 2. *L. croaticum* var. *croaticum* 2 n = (5 x) = 45, (cit. br. 70.526).

Plate II — Pollen grains of *Leucanthemum* - pentaploids.
Tabla II — Polenska zrnca u *Leucanthemum* - pentaploida.

Fig. 3. *L. vulgare* subsp. *amplifolium.*
Sl. 3. *L. vulgare* subsp. *amplifolium.*
Fig. 4. *L. croaticum* var. *croaticum.*
Sl. 4. *L. croaticum* var. *croaticum.*

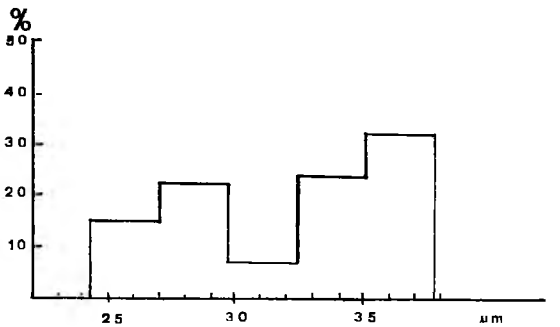


Fig. 5. Diameter of pollen grains in *L. vulgare* subsp. *amplifolium*, (cyt. no. 70.908).
Sl. 5. Promjer polenskih zrnaca u *L. vulgare* subsp. *amplifolium*, (cit. br. 70.908).

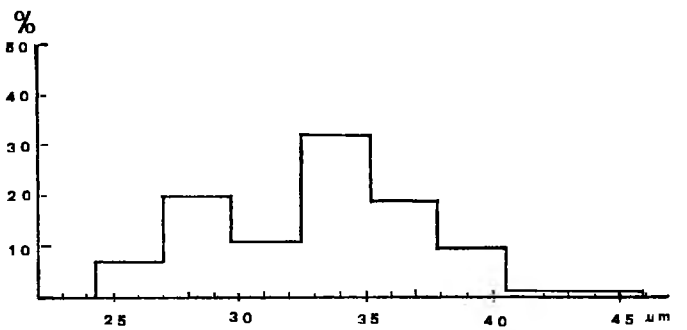


Fig. 6. Diameter of pollen grains in *L. croaticum* var. *croaticum*, (cyt. no. 70.487).
Sl. 6. Promjer polenskih zrnaca u *L. croaticum* var. *croaticum*, (cit. br. 70.487).

The appearance of individuals with three different ploidy levels in the same population, the morphological characteristics of which do not differ significantly, shows that in *Leucanthemum* species it is useless to make taxonomic conclusions only on basis of chromosome numbers, as many cytotaxonomists did.

Summary

Among 463 investigated plants from 95 populations of the genus *Leucanthemum* 35 plants were pentaploids (2n = (5x) =45). Most of them grow in populations where hexaploids (6x) predominate, but a few tetraploids (4x) have also been found (*L. vulgare* subsp. *amplifolium* Fiori and *L. croaticum* var. *croaticum* Horvatić). In two populations of *L. montanum* var. *heterophyllum* (Willd.) Briqu. et. Cav. pentaploids grow only among octoploids (8x). The origin of the *Leucanthemum* pentaploids is discussed.

Acknowledgments: A part of this work was prepared in Jodrell Laboratory, Royal Botanic Gardes, Kew, while the author was a recipient of a British Council Scholarship. I offer my special thanks to Dr. Keith Jones and the staff of the Cytology Department for their help and assistance in the work.

I am also grateful to Professor Z. Devidé for this helpful advice in preparing the paper.

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SADRŽAJ

PENTAPLOIDI U POPULACIJAMA RAZLIČITIH VRSTA RODA *LEUCANTHEMUM*

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Od 463 istražene biljke iz 95 populacija vrsta roda *Leucathemum* 35 biljaka bilo je pentaploidno s 2n = (5x) =45. Većina pentaploida nađena je u populacijama gdje su prevladavali heksaploidi; u istim populacijama također je nađeno i nekoliko tetraploida. Takav slučaj uočen je u populacijama s vrstama L. *vulgare* subsp. *amplifolium* Fiori i L. *croaticum* var. *croaticum* Horvatić. U dvije populacije L. *montanum* var. *heterophyllum* (Willd.) Briqu. et Cav. pentaploidi su rasli isključivo između oktoploidnih ivančica.

Svi *Leucantheumum*-pentaploidi, iako su rasli u različitim populacijama i bili pripadnici različitih vrsta, imali su mnogo zajedničkih karakteristika.

Po morfološkim karakteristikama pentaploidi se nisu razlikovali od ostalih ivančica u istoj populaciji. Konstatacija da pripadnici jedne vrste u jednoj populaciji s različitim brojem kromosoma, odnosno s različitim stupnjem ploidije (kao npr. 4x, 5x i 6x u jednoj populaciji i 5x i 8x u drugoj) čija se izvanja morfologija međusobno ne razlikuje, upućuje na to da je nemoguće klasificirati vrste isključivo na temelju kromosomskog broja, a isto tako zaključivati o broju kromosoma na osnovi samo jednog brojenja.

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