

DEVIATING BASIC GENOME SIZE IN A HEXAPLOID POPULATION OF SCILLA
BIFOLIA AGG. IN THE VALLEY KREUTTAL (WEINVIERTEL; LOWER AUSTRIA)

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Abstract.

Deviating basic genome size in a hexaploid population of *Scilla bifolia* agg. in the valley Kreuttal (Weinviertel, Lower Austria). Key words: *Scilla*, Liliaceae; polyploidy, genome size.

DNA content determinations in a hitherto unique hexaploid population ($2n = 54$) of *Scilla bifolia* agg. in the valley Kreuttal, Weinviertel, Lower Austria, revealed a basic DNA content of $1 C_x = 5,01$ pg ($1 C = 15,03$ pg), which is significantly less than the values of about $1 C_x = 5,7$ pg which are typical for the close allies *Scilla bifolia* subsp. *danubialis* ($2n = 18, 2x$), *Scilla drunensis* ($2n = 36, 4x$), or *Scilla buekkensis* ($2n = 36 - 54, 4x - 6x$). New measurements in *S. drunensis* from northern Lower Austria, and from the Vitoscha mountains near Sofia, Bulgaria, as well as reassessments of previous reports in *S. bifolia* subsp. *danubialis*, *S. drunensis* and *S. buekkensis* confirmed the previously reported basic genome size of about $1 C_x = 5,7$ pg. As to now, $1 C_x = 5,01$ pg is the lowest value recorded in *Scilla* sect. *Scilla*. The findings on the Kreuttal - population demand a search for other such populations and for possible ancestors of lower degree of polyploidy, until the biological and taxonomic significance of the present data can be reasonably assessed.

Zusammenfassung.

Abweichende Grund - Genomgröße in einer hexaploiden Population von *Scilla bifolia* agg. im Kreuttal (Weinviertel, Niederösterreich).

DNA - Mengenbestimmungen an einer bisher einzigartigen hexaploiden Population von *Scilla*

bifolia agg. im Kreuttal, Weinviertel, Niederösterreich, ergaben eine Grund - Genomgröße von $1 C_x = 5,01 \text{ pg}$ ($1 C = 15,03 \text{ pg}$). Dies ist signifikant weniger als die Werte von etwa $1 C_x = 5,7 \text{ pg}$, die für die nahen Verwandten *Scilla bifolia* subsp. *danubialis* ($2n = 18, 2x$), *Scilla drunensis* ($2n = 36, 4x$), oder *Scilla buekkensis* ($2n = 36 - 54, 4x - 6x$) typisch sind. Neue Messungen an *S. drunensis* aus dem nördlichen Niederösterreich und von den Vitoscha - Bergen bei Sofia, Bulgarien, sowie Überprüfungen früherer Befunde an *S. bifolia* subsp. *danubialis*, *S. drunensis* und *S. buekkensis* bestätigten die bereits früher publizierte Grundgenomgröße von etwa $1 C_x = 5,7 \text{ pg}$. Gegenwärtig stellt $1 C_x = 5,01 \text{ pg}$ den niedrigsten bei *Scilla* sect. *Scilla* gefundenen Wert dar. Die Befunde über die Kreuttal - Population machen die Suche nach weiteren solchen Populationen und nach möglichen Ahnen mit niedrigerem Polyploidiegrad erforderlich, ehe die biologische und taxonomische Bedeutung der vorliegenden Daten vernünftig eingeschätzt werden kann.

Introduction.

There exist only a few dispersed Lower Austrian populations of *Scilla bifolia* agg. north of the Danubian valley, which attracted attention not earlier than recently when Greilhuber and Karrer communicated the occurrence of $2n = 54, 6x$, for plants collected in the valley Kreuttal (Fig. 1,2). In the meantime, the remaining three populations, at Goggendorf, at Wischathal, and at Sonnberg near Hollabrunn, were identified, not quite expectedly, as *S. drunensis*, and the chromosome number $2n = 36, 4x$, was stated (Speta 1983). As is well known since the studies of Speta (1973), the Lower Austrian part of the Danubian valley below Ybbs/Donau is populated by *Scilla vindobonensis*, $2n = 18, 2x$, with the only exceptional disjunct population of *Scilla bifolia* subsp. *danubialis*, $2n = 18, 2x$, at Stopfenreuth (Greilhuber and Speta 1977). The Kreuttal - plants are habitually, and in regard of the basic karyotype morphology, similar to *S. bifolia* subsp. *danubialis* or *S. drunensis*, and have brown seeds (an essential group character) like these taxa too. However, we report here that these plants have significantly less DNA per basic genome than *S. bifolia*, *S. drunensis*, or the tetra - hexaploid *S. buekkensis*. During the course of this study, for comparison new DNA determinations have also been performed in *S. drunensis* from Goggendorf, Lower Austria, and from the Vitoscha mountains near Sofia, Bulgaria. Furthermore, previously published values in *S. bifolia* subsp.

danubialis, *S. drunensis*, and *S. buekkensis* were reproduced in this study. These data corroborate a fairly stable and consistently higher basic DNA value in these taxa than we find in the Kreuttal - population.

Material and methods.

Chromosome counts were performed with colchicinated root tip and ovule material. DNA contents were determined by Feulgen two - wavelength cytophotometry on a Leitz MPV II cytophotometer. Root tips, and bisected ovaries of flowering plants were fixed together with *Allium cepa* cv. 'Centennial' root tips as an internal standard in methanol - acetic acid (3 : 1) overnight at + 4°C, stored in 96% ethanol in the deep - freezer, and processed a few days later. Ovule material was scrupulously teased into pieces as small as possible with needles prior to the first wash before hydrolysis to avoid incomplete penetration of reagents due to epidermal cuticles. The classical Feulgen - reaction was performed in Eppendorf centrifuge tubes, because numerous centrifugations were required. The 50 min. 5N HCl hydrolysis at 20°C was applied. Air dried squash preparations were measured within 1 or 2 days. Immersion oil was used as mountant medium without a cover slip. From each slide the relative *Scilla* value, $\bar{q} \pm s_q$, was calculated, and the weighted means and standard deviations of all slides of a provenance, $\bar{q}_w \pm s_{q_w}$, formed (see Sachs 1978, pp. 63, 64, 77). Relative values were transformed to absolute ones taking 1 C = 16,75 pg for *Allium cepa* (Van't Hof 1965, Bennett and Smith 1976). Statistical tests were performed as indicated in the text (see Sachs 1978, pp. 209 - 216, 386 - 395). The species and provenances are as follows:

1. *Scilla bifolia* agg. Lower Austria, the valley Kreuttal, between Hetzmannsdorf and Unterolberndorf, 205 m to 225 m alt., flore mapping field square 7564/4. Plant material was collected at three points (see Table 1), in the western, the central and the eastern part of the valley: (a) long. 16° 26' 16" E, lat. 48° 26' 26,4" N, (b) long. 16° 27' 12" E, lat. 48° 26' 41,3" N (c) long. 16° 28' 00" E, lat. 48° 26' 36,4" N.
2. *Scilla bifolia* L. subsp. *danubialis* Speta. Lower Austria, water meadows along the Danube near Stopfenreuth, 144 m alt.
3. *Scilla drunensis* (Speta) Speta. Lower Austria, Goggendorf, hill "Kasperlberg", approx. 335m alt., leg. F. Speta.

4. *Scilla drunensis* (Speta) Speta. Upper Austria, Pucking near Traun, near the cottage farm "Oberreiter", 285 m alt.
5. *Scilla drunensis* (Speta) Speta. Bulgaria, Vitoscha mountains, near Sofia, approx. 1 200m.
6. *Scilla buekkensis* Speta. Hungary, Bükk mountains, 1,5 km NW Répàshuta, leg. F. Speta 23.3.1974, type material !

Herbarium specimens are preserved in the herbarium of F. Speta (Sp), Linz. Specimens of provenance (1) are also preserved in the herbarium of the Institute of Botany, University of Vienna (WU).

Results and discussion.

The data of the present investigation are indicated in Tables 1 and 2, and present evidence that the basic DNA content $1 C_x$ is significantly lower in the hexaploid Kreuttal population than in the diploid *Scilla bifolia*, the tetraploid *S. drunensis*, or the tetra- to hexaploid *S. buekkensis*. Analysis of variance shows that the basic DNA contents in the provenances 2 to 6 of *S. bifolia*, *S. drunensis*, and *S. buekkensis* are not significantly different from each other ($F = 2,14 < F_{4,217;0,05} = 2,42$), while inclusion of the Kreuttal plants indicates a highly significant difference ($F = 12,04 > F_{5,369;0,01} = 3,07$). The Scheffé - test indicates a highly significant difference of the Kreuttal mean and the mean of the other values ($S = 41,625 > S_{0,01} = 3,915$). Within the Kreuttal - population, analysis of variance indicates that some values may be different from each other ($F = 2,39 > F_{9,144;0,05} = 1,95$), but the "modified LSD (lowest significant difference) - test" indicates homogeneity of these values. One should also note that the values obtained from root tips are not different from those obtained from ovules ($t = 0,253 < t_{151;0,05} = 1,976$) and that the means of the three Kreuttal subpopulations, (a), (b), and (c), are not significantly different from each other ($\hat{F} = 0,17 < F_{2,150;0,05} = 3,06$). Therefore, there is every reason to assume that the significant part of the variation within the Kreuttal plants is methodical and not genuine. There is also no evidence that the lower $1 C$ value in this population is a methodical artifact due to glare as a result of a presumably higher chromatin compaction in the hexaploids, since no such effects are observed, for instance, in the $2x$ - $4x$ - $6x$ -

Table 1. DNA measurements in plants of *Scilla bifolia* agg. from three subpopulations (a - c) of the valley Kreuttal (Weinviertel, Lower Austria).

Subpopulation	Plant no.	Chromosome number	N	1 C (pg), $\bar{q}_w \pm s_{q_w}$
a	1	2n = 54	10	15,26 \pm 0,82
	2	2n = 54	10	14,85 \pm 0,67
	3	2n = 54	10	14,87 \pm 0,63
	4		9	15,37 \pm 0,81
		sum of a:	39	15,08 \pm 0,74
b	5		16	15,52 \pm 0,68
	6		10	14,88 \pm 0,67
	7		10	14,78 \pm 0,36
	8		10	14,48 \pm 0,63
		sum of b:	46	14,99 \pm 0,72
c	9	2n = 54	10	15,10 \pm 0,76
	10 + 11	2n = 54	58	15,01 \pm 0,68 *
		sum of c:	68	15,02 \pm 0,69
		sum of 1-9:	95	15,04 \pm 0,73
		sum of a-c:	153	15,03 \pm 0,71

* Values obtained from root tips; in the other plants the values were obtained from ovules.

Table 2. DNA measurements in provenances (as listed under materials and methods) of the *Scilla bifolia* alliance.

Species	Provenance	Chromosome number	1C (pg), $\bar{q}_w \pm s_{q_w}$	N	1C _x (pg)
<i>Scilla bifolia</i> agg.	1	2n = 54, 6x	15,03 \pm 0,71	153	5,01
<i>Scilla bifolia</i> subsp. <i>danubialis</i>	2	2n = 18, 2x	5,80 \pm 0,26	50	5,80
<i>S. drunensis</i>	3	2n = 36, 4x	11,22 \pm 0,50	55	5,61
<i>S. drunensis</i>	4	2n = 36, 4x	11,37 \pm 0,53	56	5,69
<i>S. drunensis</i>	5	2n = 36, 4x	11,62 \pm 0,45	11	5,81
<i>S. buekkensis</i>	6	2n = 36, 4x	11,55 \pm 0,59	50	5,78

polyploid series of *S. subnivalis*, *S. reuteri*, and *S. pneumonanthe* (Greilhuber et al. 1981, see also Seal 1983). The main problem arising from the present data is the phylogenetic position of the Kreuttal population. Is it a hexaploid derivative of the geographically neighbouring allies *S. bifolia* or *S. drunensis* - or of a common ancestor of these - with a lower DNA

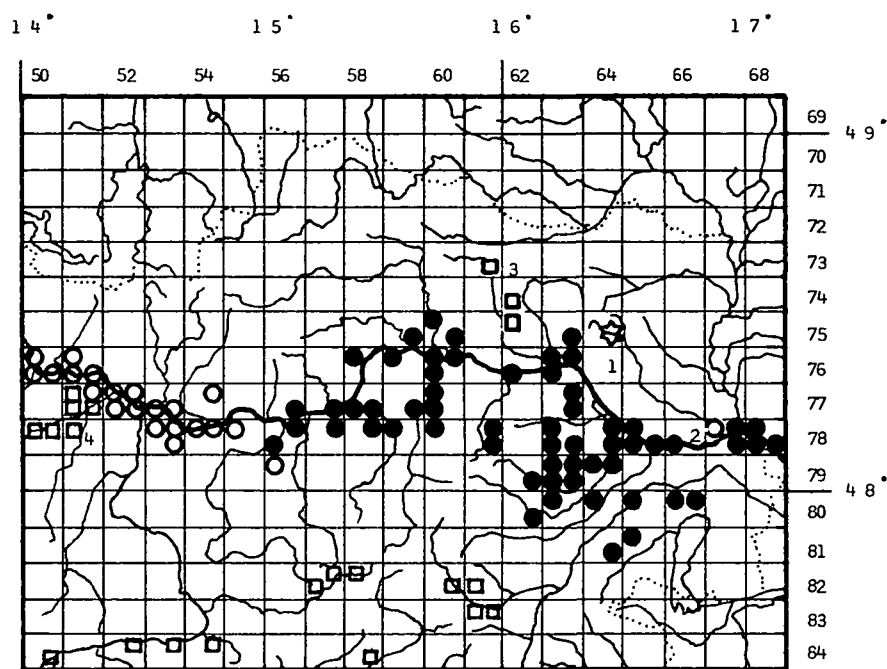


Fig. 1: The Austrian provenances of *Scilla bifolia* agg. measured (1 - 4) in the context of species distribution. Empty circles: *S. bifolia* subsp. *danubialis* ($2n = 18$); empty squares: *S. drunensis* ($2n = 36$); asterisk: *S. bifolia* agg. ($2n = 54$), Kreuttal; black circles: *S. vindobonensis* ($2n = 18$). Distributional map according to Speta (1983). The *S. vindobonensis* locality, field square 6375/4, is our addition: Rohrwald near Stockerau, (a) long. $16^{\circ}18'24,4''$ E, lat. $48^{\circ}24'38,4''$ N, (b) long. $16^{\circ}18'42,6''$ E, lat. $48^{\circ}24'48,0''$ N; chromosomal identification of fruiting plants.

content, or are these plants more directly phylogenetically related to the morphologically somewhat distinct *S. laxa*, known as tetra- and hexaploids, with a DNA content of about $1 C_x = 5,2$ pg? The published DNA amount for *S. laxa* ($4x$) from Talmaciu at Sibiu, Rumania, (Greilhuber 1979), given here as $\bar{q}_w \pm s_{q_w}$ in pg DNA, is $1 C = 10,43 \pm 0,54$, which amounts to a significant difference to the Kreuttal genome ($t = 5,158 > t_{201;0,001} = 3,340$). New unpublished measurements from other provenances of *S. laxa* rather reinforce this significant difference. Similar low DNA contents of about 5,3 pg or 5,4 pg ($1 C_x$) are found in the black seeded taxa *S. subnivalis*, *S. reuteri*, *S. pneumonanthe*, *S. xanthandra* and *S. longistyllosa*, which form a monophyletic group together with *S. nivalis* and *Scilla* ser. *Chionodoxa* according to the present view of phylogenetic relationships in *Scilla* (Greilhuber, in press; Greilhuber 1979, Greilhuber et al. 1981).

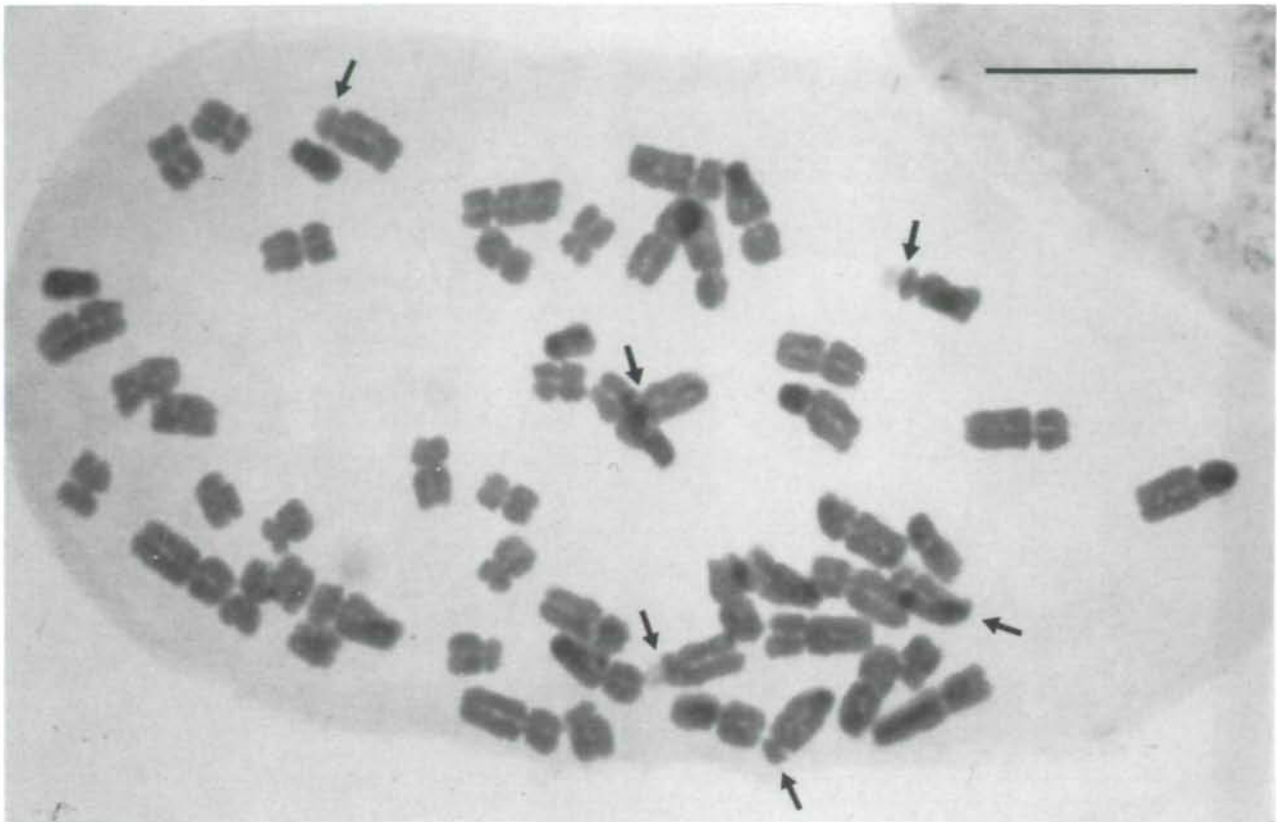


Fig. 2: *Scilla bifolia* agg. ($2n = 54$, $6x$), Kreuttal. Root tip metaphase plate. Aceto - carmine. Nucleolar organizing chromosomes arrowed. Bar = $10\mu\text{m}$.

Another interesting taxon to be considered is *S. bulgarica* Speta, a provisional diploid member of *Scilla* sect. *Luteoscilla* (Speta 1980) with a surprisingly low DNA content of $1C = 5,15 \pm 0,40$ pg ($\bar{q}_w \pm s_{q_w}$, 67 nuclei measured; unpublished data by J. Greilhuber). As to now this is the value most close to the value of the Kreuttal population, but is nevertheless significantly different ($t = 2,668 > t_{87;0,01} = 2,633$). It is therefore obvious, that at present statements about the phylogenetic position of the Kreuttal population would be premature. It appears that these plants represent a hitherto unique element in the *Scilla bifolia* aggregate and therefore possibly a new biological species. The DNA-amount seems to be a sensible specific marker for this phylogenetic line, and offers the possibility for a more extensive geographical screening. It is, however, clear that in terms of the present taxonomic grouping (Speta 1980) the Kreuttal plants are a member of *Scilla* sect. *Scilla* by definition, since they have dark brown seeds, as the authors could observe.

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