Pl. Syst. Evol. 195: 45–59 (1995)

Plant Systematics and Evolution

Giemsa C-banded karyotypes of *Allium ericetorum* and *A. kermesinum* (*Alliaceae*)

WOLFANG WETSCHNIG

Received April 15, 1994; in revised version May 27, 1994

Key words: *Alliaceae, Allium ericetorum, A. kermesinum.* – C-banding, karyotype polymorphism. – Flora of the Steiner Alps, Slovenia.

Abstract: The widely distributed *Allium ericetorum* and the local endemic of the Steiner Alps (Slovenia), *A. kermesinum*, are two closely related species of sect. *Rhizirideum*, whose main distinguishing character is perianth colour. To obtain further evidence for species separation, karyotype morphology and C-banding patterns were examined in 10 populations. The chromosome number was 2n = 16. In some populations of *A. ericetorum* a B-chromosome occurred. Arm and satellite lengths and C-banding patterns were subjected to cluster analysis. Three different karyotype classes were observed and described. Karyotypes did not clearly discriminate between plants with different colours of perianth segments and therefore did not provide evidence for a taxonomic separation of *A. ericetorum and A. kermesinum*. There is polymorphism in number and patterns of C-bands within the populations. No correlation between B-chromosomes and particular banding patterns was observed.

A. ericetorum THORE (syn. *A. ochroleucum* WALDST. & KIT.) and *Allium kermesinum* REICHENB. are two closely allied species within sect. *Rhizirideum* G. DON ex KOCH of the relatively ancient subg. *Rhizirideum* (G. DON ex KOCH) WENDELBO.

A. ericetorum has a wide distribution ranging from the Carpathians across the former Yugoslavia, northern and Central Italy, southwestern France to northern Spain and northern Portugal (STEARN 1978), whereas *A. kermesinum* is a local endemic occurring only in the Steiner Alps (Slovenia), the south-easternmost range of the Alps.

According to STEARN (1980: 54), the most important morphological difference between these two species is the colour of the perianth segments (red in *A. kermesinum*, white or yellowish, sometimes tinged with pink in *A. ericetorum*). In the course of excursions to several mountains in the Steiner Alps, flower colours ranging from dark crimson to pink, yellowish and white were observed in individuals of various populations at different altitudes.

As karyological data on these two species were rather scarce, one objective of the present study is to document chromosome morphology of several populations of both species. As the taxonomy of the group is still uncertain it is also examined

whether karyotype morphology can serve as a distinguishing characteristic for delimiting the two taxa. Further, variation of C-banding patterns within and between populations is described. As 13% of the examined metaphase plates showed one B-chromosome, a possible correlation between C-bands and B-chromosomes as stated by BARBUJANI & PIGLIUCCI (1989) is discussed.

Material and methods

Species and collecting localities. Voucher specimens of the investigated populations will be deposited at the herbarium of the Institute of Botany, Karl-Franzens-University of Graz (GZU) and at the Kärntner Landesherbarium (KL).

4 populations of *Allium ericetorum*, 2 populations of *A. kermesinum* and 4 populations with intermediate flower colours were examined (Table 1).

Each population is referred to by an abbrevation. G stands for the Grintovez massif within the Steiner Alps (GN for the northern, GS for the southern foothills and slopes of this range), K for the Karawanken mountain range.

Allium ericetorum outside the Steiner Alps:

(BGL) Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Tax. 1547/90; material received from the Botanical Garden of the University of Leipzig (no further origin given). 21 metaphase plates of 4 pots were examined.

(KO) Austria, Carinthia, Karawanken, southern slopes of Mt Obir, 1100 m, July 1984, LEUTE, G. H.; 28 metaphase plates of 5 pots were examined.

(KF) Austria, Carinthia, Karawanken, Freibachschlucht (gorge) N of Zell Schaida, 900 m s.m., Oct. 1989, LEUTE, G. H., KOSCH, M. & PASSEGGER, R.; 17 metaphase plates of 3 pots were examined.

Allium ericetorum and *A. kermesinum* from the Steiner Alps: As populations in the Steiner Alps differ in composition of individuals with different flower colours, the composition of a population is marked by following abbreviations: (e) typical *A. ericetorum* (white or yellowish), (i) intermediate (different shades of pink), (k) typical *A. kermesinum* (crimson).

Table 1. Origin of populations of *Allium ericetorum*, *A. kermesinum*, and intermediate forms received from botanical gardens or collected in Slovenia and Austria (Carinthia). Flower colours: *e* like typical *A. ericetorum* (yellowish or whitish), *i* intermediate between *A. ericetorum* and *A. kermesinum* (different shades of pink), *k* like typical *A. kermesinum* (red)

Code	Locality	Altitude (m s.m.)	Flower colour	Number of pots	Number of metaphases examined
BGL	Bot. Garden, University of Leipzig		e	4	21
KO	Karawanken, Mt Obir	1100	e	5	28
KF	Karawanken, Freibachschlucht	900	e	3	17
GN 1	Steiner Alps, Grintovez-North	1180	e	4	20
GN 2	Steiner Alps, Grintovez-North	1200	i	10	64
GN 3	Steiner Alps, Grintovez-North	1570	e, i	3	18
GS 1	Steiner Alps, Grintovez-South	1350	i	2	12
GS 2	Steiner Alps, Grintovez-South	1700	k	6	35
GS 3	Steiner Alps, Grintovez-South	1950	k	5	27
GS 4	Steiner Alps, Grintovez-South	2000	i	3	12

Slovenia, Kamniške in Savinjske Alpe (Steiner Alps), Grintovez massif:

(GN 1) Along the hiking trail (NE of the material cable-railway) from Ravenska Kočna to Kranjska k. na Ledinah, 1180 m s.m., 18. 8. 1992, WETSCHNIG, W.; 20 metaphase plates of 4 pots (e) were examined.

(GN 2) Along the hiking trail (NE of the material cable-railway) from Ravenska Kočna to Kranjska k. na Ledinah, 1200 m s.m., 18. 8. 1992, WETSCHNIG, W.; 64 metaphase plates of 10 pots (i) were examined.

(GN 3) Along the hiking trail from Kranjska k. na Ledinah to Češka koča, 1570 m, 18. 8. 1992, WETSCHNIG, W.; 18 metaphase plates of 3 pots (e + i) were examined.

(GS 1) Along the hiking trail from Suhi Dol to Cojzova koča, 1350 m s.m., 22. 8. 1992, WETSCHNIG, W.; 12 metaphase plates of 2 pots (i) were examined.

(GS 2) Along the hiking trail from Suhi Dol to Cojzova koča, 1700 m s.m., 22. 8. 1992, WETSCHNIG, W.; 35 metaphase plates of 6 pots (k) were examined.

(GS 3) Along the hiking trail from Cojzova koča to the summit of Mt Grintovez, 1950 m s.m., 22. 8. 1992, WETSCHNIG, W.; 27 metaphase plates of 5 pots (k) were examined.

(GS 4) Along the hiking trail from Cojzova koča to the summit of Mt Grintovez, 2000 m s.m., 22. 8. 1992, WETSCHNIG, W.; 12 metaphase plates of 3 pots (i) were examined.

Cultivation. All plants were cultivated in the Botanical Garden of the Karl-Franzens-University of Graz (HBG) in clay pots (diameter: 10 cm) sunk in outdoor beds. For circumstantial reasons three bulbs on average were planted per pot. Each pot contained several morphologically similar individuals of a single population. Since root tips were taken from pots, individuals were not distinguished, and data refer to metaphases, pots and populations rather than to individuals. However, this should not invalidate the conclusions.

Cytology. Pretreatment and fixation. Meristems of root tips were used for karyotype studies. Root tips were pretreated for 12 hours in a 0.002 M solution of 8-hydroxyquinoline at room temperature. Then the root tips were fixed in ethanol : acetic acid (3 : 1). The material was stored in the fixative at about − 25 °C.

Carmine acetic acid staining. Root tips were boiled in carmine acetic acid for about 2 minutes and left in the dye for 3 to 5 hours before being transferred to acetic acid (45%). Meristems were then isolated, dissected, and squashed. The coverslips were sealed with paraffine : vaseline (1 : 1).

Giemsa C-banding was done according GREILHUBER & SPETA (1989) with minor modifications: enzymes for maceration were 1% cellulase (Sigma) and 10% pectinase (Sigma), staining was in 4% phosphate-buffered Giemsa for 30 min. Camera lucida drawings and photographs of squashed metaphase plates were taken before and after the banding procedure. After staining the slides were mounted in Entellan (Merck).

Microscopy and photography. Drawings of metaphase plates were made with a Reichert Polyvar microscope using the attached drawing device. Photographs were made with the same microscope using Agfa-Ortho film (15 Din/25 Asa).

Karyograms were taken from drawings of metaphase chromosomes which were measured using a measuring magnifying glass (units of 0.1 mm).

The following features were used to characterize chromosomes and karyograms: GL (total length of the diploid karyotype), s (length of the shorter arm), l (length of the longer arm), r (arm ratio of the chromosome: l/s), c (chromosome length: s + l), c_r [relative length of the chromosome: (l + s)/GL × 100], S_i [symmetry-index: (sum total of shorter arms/ sum total of longer arms) × 100], G_i [gradient of chromosome length-index (total length of the smallest chromosome/total length of the largest chromosome) × 100].

Drawings of the karyograms were produced and r-, c-, c_r-, G_i-, and S_i-value, %-values (in diploid karyograms GL is 100%, in haploid idiograms the total length of the haploid chromosome set is 50%) of long and short chromosome arms were calculated, first for each plate and then for the sample of plates, using the computer programme CHROM (WETSCHNIG 1992 b).

Within the karyograms (Figs. 3 b, 4 b, 5 b), chromosomes with NORs (asymmetric ones first) appear before those without NORs. The latter ones are arranged according to decreasing chromosome length. Length of satellites, short arms and long arms in the karyograms are given in percentages (total length of the haploid chromosome set is 50%) and in µm and were rounded to one digit behind the decimal point.

Chromosomes and centromeric positions are designated following LEVAN & al. (1964).

Numerical methods. All cluster analysis procedures were carried out using the NTSYS-pc 1.6 programme (ROHLF 1990). In order to attain a statistically valid cluster analysis of pots and populations (Figs. 1, 2 a, b), classes based on the length of satellites and short and long arms (in units of 0.33% of GL) were established. Frequencies of arm length associated with these classes were then used to calculate dissimilarity matrices. To attain a cluster analysis of C-banding results, percentages of C-bands at certain loci were used to produce a dissimilarity matrix of the 10 populations. Euclidean distance was used as dissimilarity value. Clustering was performed using the SAHN option and complete linkage.

Results

Interphase nuclei were rather uniform. They exhibited a relatively homogenous matrix of chromomeres and chromocentres varying in number and size. In interphase, nucleoli most frequently had fused to form one or two bodies. The number of primary nucleoli in telophases varied between two and four. Because of the low number of chromosomes with NORs, the number of nucleoli of *A. ericetorum* from BGL (Botanical Garden Gatersleben) never exceeded two. In telophases of *A. ericetorum* from KO (Karawanken, Obir) three or most often four primary nucleoli were visible. In all other populations 2 to 4 primary nucleoli were observed.

All material investigated (254 metaphase plates) had $2n = 16$. In 9 metaphase plates of population GN 1 (45% of the population) and in 25 plates of GN 2 (39% of the population), one accessory or B-chromosome was found. Thus in 18.1% of the 188 metaphases from the Grintovez populations and in 13.4% of all metaphases examined during this study one B-chromosome was present. The mode of cultivation precludes firm conclusions on intraindividual stability of this supernumerary element.

Using frequencies of chromosome arm length and satellite length to distinguish between the karyotypes of the pots (Fig. 1), four distinct clusters can be seen. Pots GS 3/1 and GS 4/2 form a cluster because in some plates of these two pots almost the entire short arm of one chromosome of pair no. 3 is lost. Without this deletion, karyograms of these two pots would belong to the cluster containing the pots from the KF and Grintovez populations. This conclusion was reached by carrying out a cluster analysis with the short arms of these chromosomes hypothetically completed. Thus three main clusters remain, each representing a clearly defined karyotype. The most compact is that of *A. ericetorum* from BGL (Botanical Garden Gatersleben). The karyotype of *A. ericetorum* from KO (Karawanken, Obir) is also well-defined. The pots of all the other populations (*A. ericetorum, A. kermesinum* and those with intermediate corolla colours) form one very large cluster. Within this cluster there are no obvious subclusters of populations according to any geographical criteria, flower colour or B-chromosomes (Fig. 1).

These three main clusters are also evident when populations are compared. As above, frequencies of arm length and satellite length were used. The results are shown in Fig. 2 a.

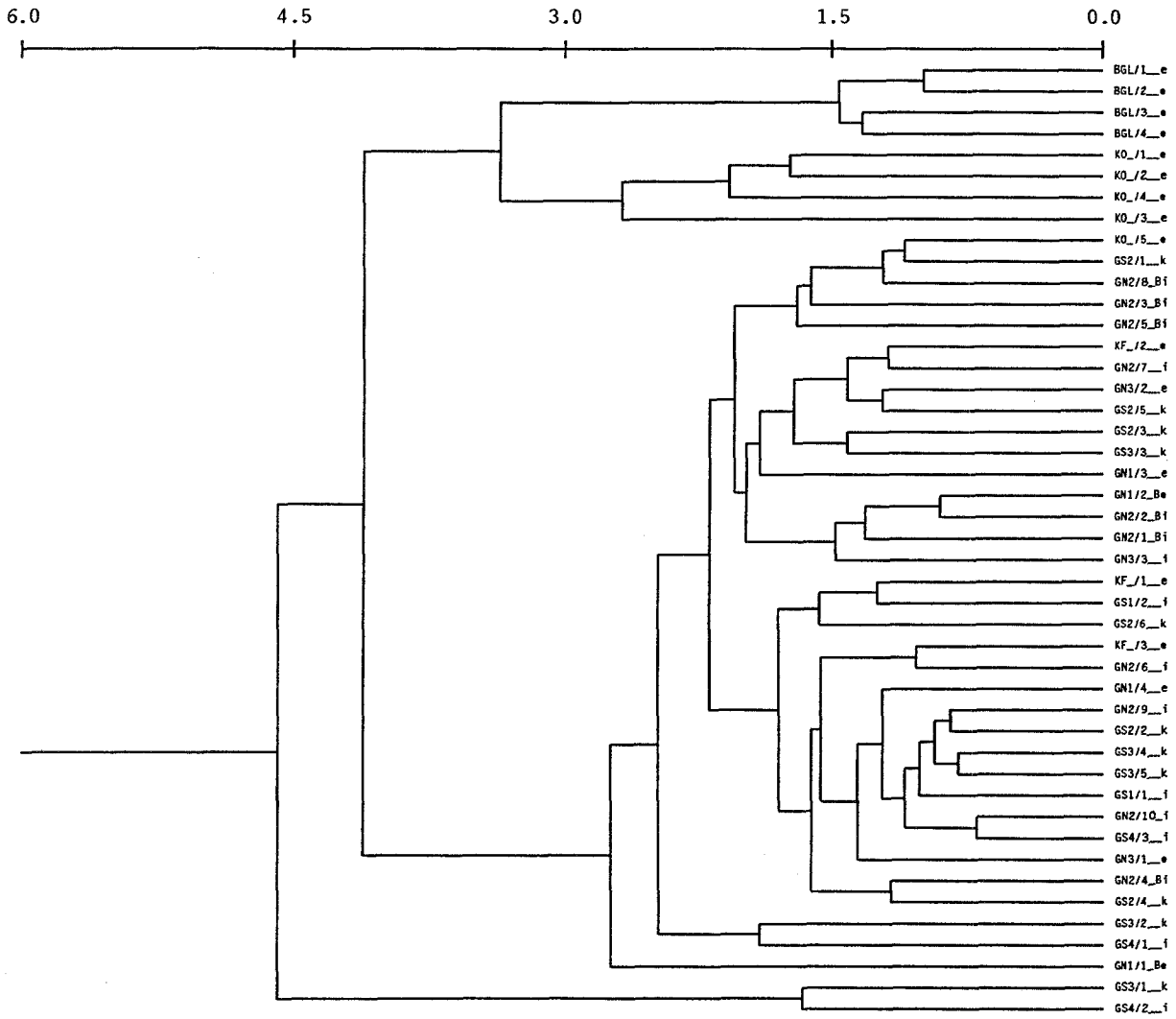

Fig. 1. Tree of the 45 *Allium* karyotype samples (from single pots) examined in the present study. Frequencies of arm lengths and satellite lengths of each sample were used to obtain a dissimilarity matrix. B-chromosomes were not included in the calculation of this matrix. Euclidean distance, and SAHN clustering with complete linkage was used. Explanations of the abbreviations are given in Material and methods

Additionally, a cluster analysis based on frequencies of C-bands at the various loci illustrated in Fig. 6 was carried out. The results (Fig. 2 b) confirmed the existence of the three main karyotypes described above. However, the cluster analysis of C-banding data (Fig. 2 b) shows a subcluster of KF and GN 1 within the KF and Grintovez cluster. Within this cluster, these two populations are also separated by their yellowish flower colour from the populations with pink, crimson, or white (in GN 3) perianth segments.

Karyotype of *A. ericetorum* (BGL). A metaphase plate of this karyotype is shown in Fig. 3 a. The cumulative karyogram of 21 metaphases of this population is shown in Fig. 3 b. Some particulars of the karyotype are listed in Table 2. All metaphase

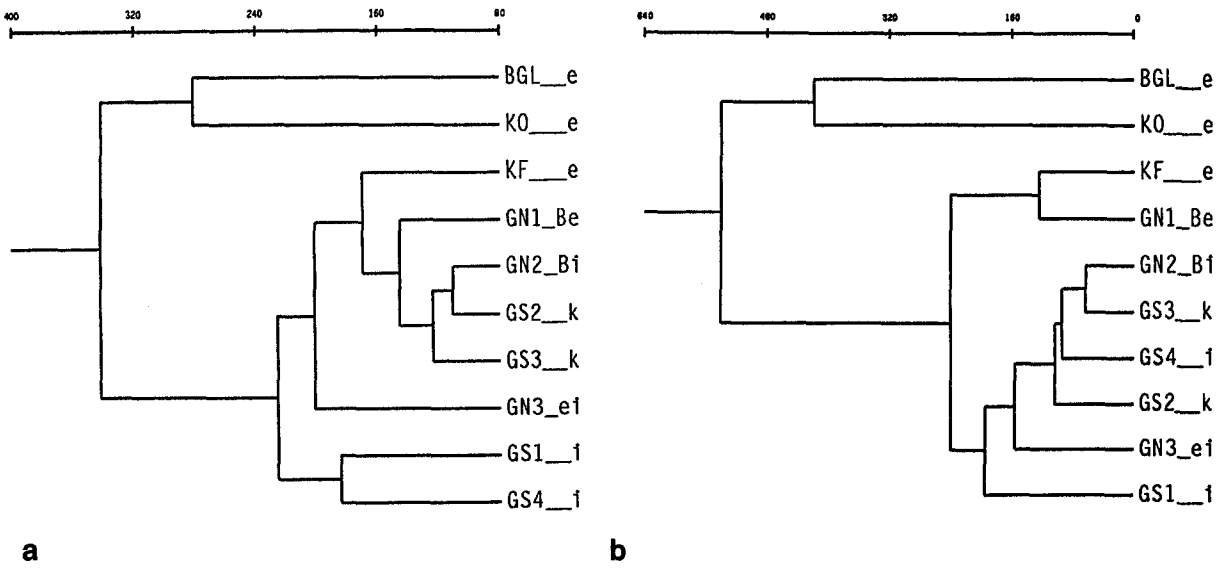

Fig. 2. Tree of the 10 *Allium* populations examined in the present study. *a* Frequencies of arm lengths and satellite lengths were used for obtaining a dissimilarity matrix. *b* Frequencies of C-bands at the different loci were used for the calculation of the dissimilarity matrix. B-chromosomes were excluded from the calculation of dissimilarity matrices. For clustering options see legend to Fig. 1

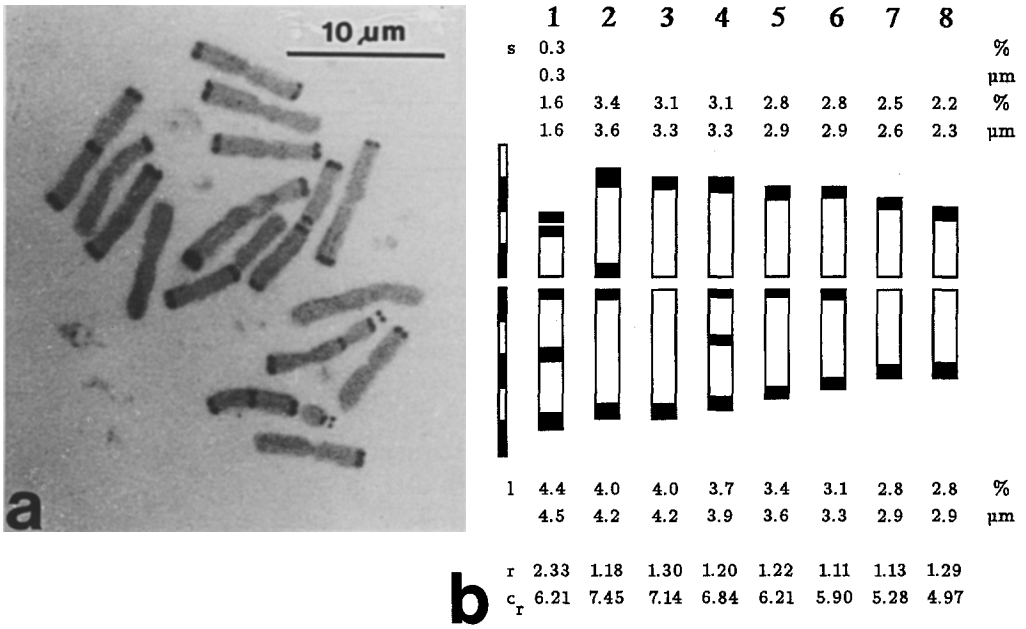

Fig. 3. Karyotype of *Allium ericetorum* from the Botanical Garden Gatersleben (BGL). *a* C-banded metaphase plate (bar: 10 µm), *b* cumulative theoretical haploid karyogram from 21 metaphase plates. Each chromosome is given with the maximal number of C-bands. For further explanations see Material and methods

Table 2. Characteristics of the three karyotypes found in *Allium ericetorum, A. kermesinum*, and intermediate forms. For codes see Table 1

	BGL	KO	KF + G
Diploid metaphases:			
Number of examined metaphases	21	28	205
GL (μm)	99.2 (92.6–102.4)	106.8 (99.6–113.7)	124.2 (93.4–153.2)
G_i	65.8 (63.8–69.0)	68.5 (66.5–70.2)	67.7 (58.2–73.7)
S_i	77.4 (76.8–78.7)	72.9 (71.4–75.0)	71.0 (66.2–74.2)
Primary nucleoli	2	3–4	2–4
C-bands	36 (33–45)	43 (41–47)	56 (49–64)
Metaphases with B-chromosomes (%)	0	0	16.6
Cumulative haploid karyogram:			
SAT-chromosomes	1	2	2
Centromeres in median region	7	7	7
Centromeres in submedian region	1	1	1
Loci of C-bands (excl. satellites)	24	31	42
Loci in distal position	16	16	16
Loci in intercalary position	2	5	11
Loci in proximal position	6	10	15

plates had 2n = 16. No B-chromosomes were found. The haploid karyogram consists of one pair of submetacentric (no. 1) and seven pairs of metacentric chromosomes (2–8). In all metaphase plates only one pair of satellite-bearing chromosomes was found (no. 1). This is the most characteristic feature of this karyotype. The satellited chromosome pair had submedian centromeres and small satellites (about 0.3 μm) at the shorter arms. The mean G_i-value is 65.76 (min.: 63.75, max.: 68.95), the mean S_i-value 77.42 (min.: 76.77, max.: 78.66). The mean length of the total chromosome set (GL) is 99.2 μm (min.: 92.6 μm, max.: 102.4 μm). Chromosome length varies from 7.8 μm (7.9% of GL) for the largest chromosome to 4.7 μm (4.8% of GL) for the smallest one.

The Giemsa C-banding pattern of this karyogram is characterized by comparatively few heterochromatic bands. None of the 8 pairs of chromosomes was homomorphic for C-banding patterns throughout the 21 metaphases examined. There also is a high degree of polymorphism in C-banding patterns among the metaphases. In diploid metaphase plates the number of C-bands varied between 33 and 45 (mean: 36). The theoretical haploid chromosome set (Fig. 3 b) shows a maximum of 25 loci of heterochromatic bands including NORs and satellites. Fig. 6 is showing the loci and the percentages of C-bands at the particular loci. With the exception of 6 terminal loci of chromosomes 1, 2, 5, 6, and 8, where all of the chromosomes had C-bands, a high degree of heterozygosity was found. No metaphase plate showed homozygosity over all the loci studied. Different pairs of chromosomes showed no obvious correlations in banding patterns. Only two intercalary loci of C-bands were observed in the haploid chromosome set (Fig. 3 b). They appeared in the long arms of pair 1 and 4. The banding pattern of pair 1 is very similar as in the other karyotypes (Figs. 4 b, 5 b). Compared to the other two karyotypes, intercalary bands in this karyotype are rare. Proximal C-bands were

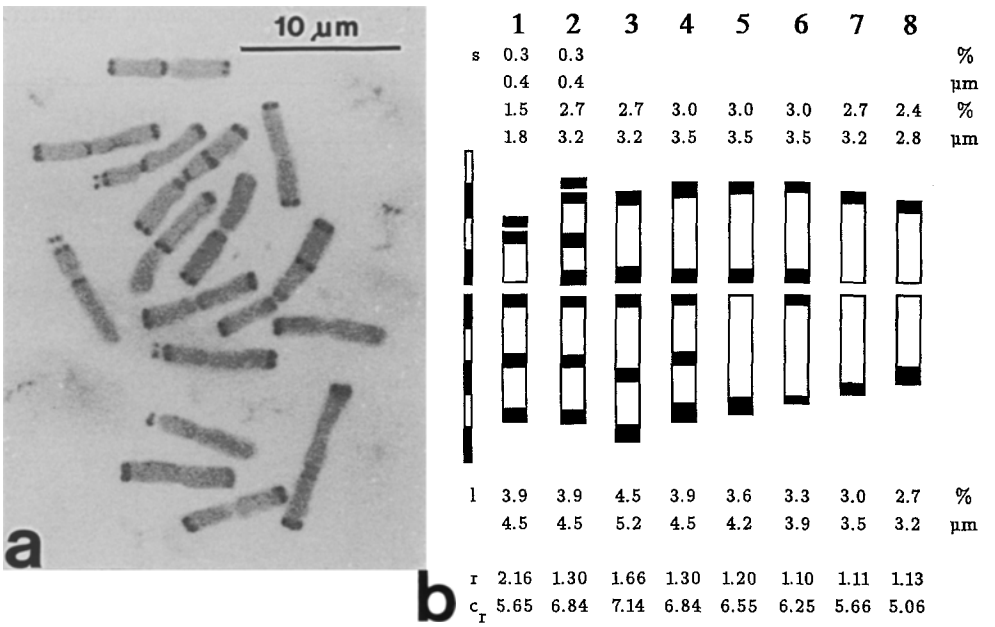

Fig. 4. Karyotype of *Allium ericetorum* from Mt Obir, Karawanken (KO). *a* C-banded metaphase plate (bar: 10 µm), *b* cumulative theoretical haploid karyogram from 28 metaphase plates. Each chromosome is given with the maximal number of C-bands. For further explanations see Material and methods

found in five pairs of chromosomes (1, 2, 4, 5, 6), mostly in the longer arms. Proximal C-bands generally occurred less often than C-bands at the distal position, but more often than intercalary ones (Fig. 6).

Karyotype of *A. ericetorum* (KO). A metaphase plate of this karyotype is shown in Fig. 4 a. The cumulative karyogram of 28 metaphases of this population is shown in Fig. 4 b. Some particulars of the karyotype are listed in Table 2. All metaphase plates had a diploid chromosome number of 2n = 16. No B-chromosomes were found. One pair of NOR-chromosomes (no. 1) always had a submedian centromere. The other pair of NOR-chromosomes (no. 2) had a median centromere. Depending on the number and distribution of C-bands, chromosome 3 had submedian or median centromeres. All the other chromosomes (4–8) had median centromeres. Two pairs of satellited chromosomes (1 and 2) were found. Pair 1 had submedian, pair 2 median centromeres. The small satellites (about 0.4 µm) were found at the shorter arms of the chromosomes. The mean G_i-value was 68.45 (min.: 66.53, max.: 70.20), the mean S_i-value 72.88 (min.: 71.36, max.: 74.95). The mean length of the total chromosome set (GL) was 106.8 µm (min.: 99.6 µm, max.: 113.7 µm). Chromosome length varied from 8.4 µm (7.9% of GL) for the largest chromosome to 5.4 µm (5.1% of GL) for the smallest one.

The Giemsa C-banding pattern of the cumulative haploid karyogram (Fig. 4 b) is characterized by 4 intercalary loci of C-bands in the longer arms (pairs 1–4) and 1 intercalary locus in the shorter arms of pair no. 2. In none of the 8 pairs of chromosomes both homologous had a homomorphic C-banding pattern throughout the 28 metaphases examined. There also is a high degree of polymorphism in C-

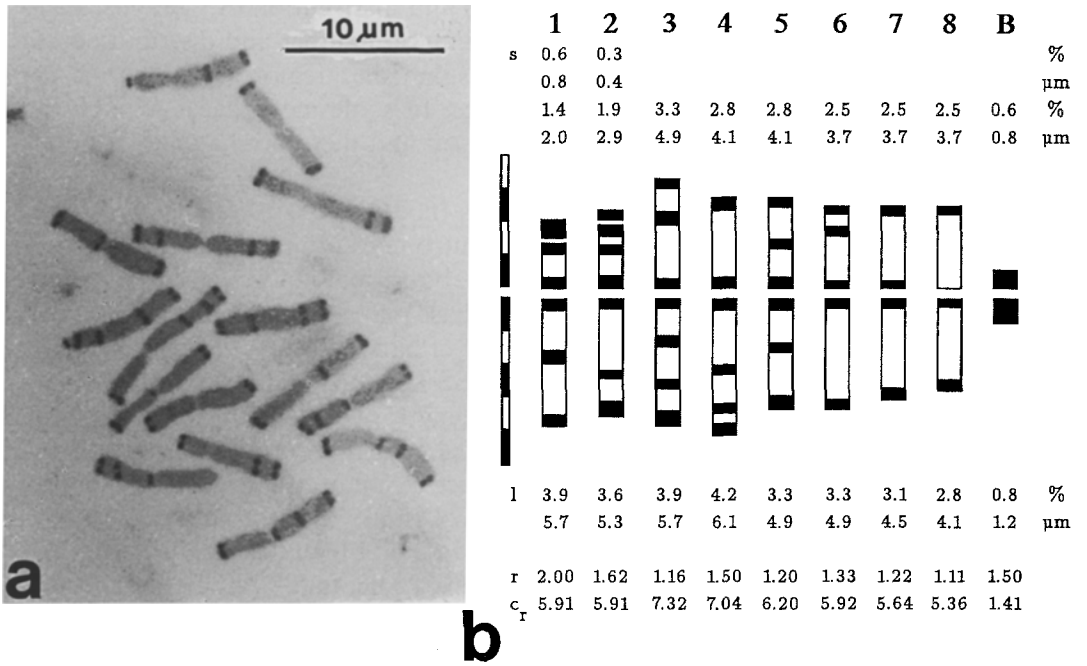

Fig. 5. Karyotype of *Allium* from KF and Mt Grintovez (KF, GN 1–GS 4). *a* C-banded metaphase plate of a plant from the GS 3 population (bar: 10 µm), *b* cumulative theoretical haploid karyogram from 205 metaphase plates. Each chromosome is given with the maximal number of C-bands. For further explanations see Material and methods

banding patterns of the metaphases. The number of C-bands in diploid metaphase plates varied between 41 and 47 (mean: 43). The theoretical haploid karyogram shows a maximum of 33 heterochromatic bands including NORs and satellites. Figure 6 is showing the loci and the percentages of C-bands at the particular loci. With the exception of 4 terminal loci of chromosomes of pairs 1, 4, 6, and 8 and the proximal locus of pair 4, where all chromosomes had C-bands, a high degree of heterozygosity was found. No metaphase plate showed homozygosity over all the loci studied. Different pairs of chromosomes showed no obvious correlations in banding patterns. Proximal C-bands were found in six chromosome pairs (1–6), equally in the longer and in the shorter arms. Proximal C-bands generally occurred less often than C-bands at distal position, but more often than intercalary ones (Fig. 6).

Karyotype of *A. ericetorum, A. kermesinum* and intermediate populations (KF, GN 1-GS 4). Karyograms of these populations of *A. ericetorum*, intermediate types, and *A. kermesinum* are very similar and are therefore treated as one karyotype which is distinct from the two others described above. A metaphase plate of this karyotype is shown in Fig. 5 a. The cumulative karyogram of 205 metaphase plates of these 8 populations is shown in Fig. 5 b. Some particulars of the karyotype are listed in Table 2. Most metaphase plates had $2n = 16$. In 34 plates (16.6% of all metaphase plates of this karyotype) one B-chromosome was found.

One pair of satellite-bearing chromosomes (pair 1) always had a submedian centromere. The other pair of satellited chromosomes (no. 2) had submedian or

median centromeres, depending on the number and distribution of C-bands. Pair 4 mostly had median centromeres. But, depending on number and distribution of C-bands, the centromere sometimes was in the submedian region. The other chromosome-pairs (3, 5–8) had median centromeres. In some metaphases, one chromosome of pair 3 lost almost the entire short arm and therefore had subterminal centromeres.

Two satellite-bearing pairs of chromosomes (no. 1 and 2) were found. Pair 1 had submedian, pair 2 median or submedian centromeres. In most metaphases the satellites of pair 1 were about 0.8 µm long and about two times longer than those of pair 2. In 3 metaphase plates (1.5% of metaphase plates showing this karyotype) almost the entire short arm of one chromosome of pair 3 was lost. Such a deletion was observed in 2 metaphase plates (7%) from GS 3 and in 1 metaphase plate (8%) from GS 4. The B-chromosomes had a mean length of 2 µm (1.6% of GL) and median centromeres. Excluding metaphase plates with B-chromosomes and those plates with an almost complete loss of the short chromosome arm of one chromosome of pair 3, the mean G_i-value was 67.74 (min.: 58.24, max.: 73.77), the mean S_i-value was 70.96 (min.: 66.19, max.: 74.24). Metaphase plates with B-chromosomes had a mean G_i-value of 22.44 (min.: 20.00, max.: 25.20) and a mean S_i-value of 71.80 (min.: 68.99, max.: 76.00). The mean length of the total chromosome set (GL) was 124.2 µm (min.: 93.4 µm, max.: 153.2 µm). The length of chromosomes without the loss of most of the short arms varied from 10.5 µm (8.5% of GL) for the largest chromosome to 6.5 µm (5.2% of GL) for the smallest one. The shortest A-chromosomes of all (3.8 µm, 3% of GL) were those of pair 3 when almost the entire short arm was lost.

The Giemsa C-banding pattern of the cumulative haploid karyogram is characterized by 3 chromosomes with one intercalary locus in the long arm (pairs 1, 2, and 5) and 4 chromosomes with one intercalary locus in the short arm (pairs 2, 3, 5, and 6). Two chromosome pairs (3 and 4) had 2 intercalary loci of C-bands in the long arms. In none of the 8 pairs of chromosomes both homologues had a homomorphic C-banding pattern throughout the 205 metaphases examined. There also is a high degree of polymorphism in C-banding patterns among the metaphases of the populations. The number of C-bands in diploid metaphase plates varied between 49 and 64 (mean: 56). The theoretical haploid chromosome set (Fig. 5 b) showed a maximum of 44 heterochromatic bands including NORs and satellites. Fig. 6 is showing the loci and the percentages of C-bands at the particular loci. With the exception of 3 terminal loci of pairs 4, 5, and 6, where all chromosomes had C-bands, a high degree of heterozygosity was found. Between different pairs of chromosomes no obvious correlations in banding patterns were found. There were no useable distinguishing characters between the banding patterns of the different populations. Proximal C-bands were found in all of the eight chromosome pairs of the theoretical haploid karyogram, almost equally in the long and short arms. Proximal C-bands generally occurred less often than C-bands at the distal or intercalary position (Fig. 6).

The ratio of the sum total of the long chromosome arms and the sum total of the short chromosome arms is 1.29 (min.: 1.27, max.: 1.3, S.D. 0.01) in diploid metaphase plates of BGL, 1.37 (min.: 1.33, max.: 1.40, S.D. 0.03) in those from KO and 1.41 (min.: 1.32, max.: 1.51, S.D. 0.04) in those from KF and the Grintovez Mountains. If sum totals of the long and short chromosome arms are subjected

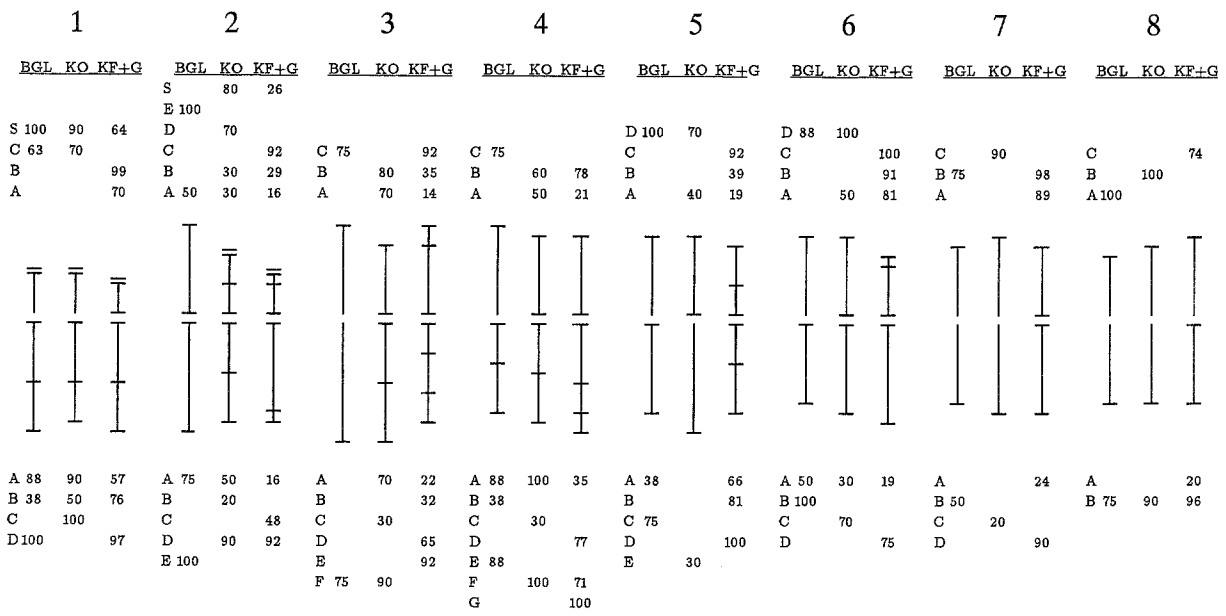

Fig. 6. Comparison of the three different *Allium* karyotypes distinguished in the present study (B-chromosomes are not considered). Chromosomes are reduced to their euchromatin lengths and drawn as vertical lines. Horizontal lines are indicating loci of C-bands. These loci are named according to their distance from the centromere. Percentages of C-bands at the particular loci are given for all chromosomes

to correlation analysis, a positive correlation within the populations and within the whole material ($r = 0.96$) is evident. This correlation is significant ($\alpha = 1\%$). If total numbers of C-bands within long and short arms are subjected to correlation analysis, there is also a positive correlation within populations and within the whole material. With a correlation coefficient of 0.52 this correlation is also significant ($\alpha = 1\%$). Thus, in the material of the present study, the distribution of additional C-bands seems to be balanced between the long and short chromosome arms.

B-chromosomes were observed in populations GN 1 and GN 2. For both populations correlation analyses between the number of B-chromosomes and the total number of C-bands were carried out using the point-biserial coefficient of correlation (BORTZ 1989). In population GN 1 ($r = 0.82$) as well as in GN 2 ($r = 0.25$) no significant correlation ($\alpha = 5\%$) between B-chromosomes and C-bands was evident.

Discussion

Allium ericetorum and *A. kermesinum* are two closely related species of *Allium* subg. *Rhizirideum* sect. *Rhizirideum*. *A. kermesinum* is a local endemic of the mountain range of the Kamniške in Savinjske Alpe (Steiner Alps) in Slovenia. It does not occur in the Karawanken Mountains as stated by STEARN (1978). *A. ericetorum* occurs in northern Portugal, northern Spain, southwestern France, northern and central Italy, the former Yugoslavia, and the Carpathians (STEARN 1978). In the Steiner Alps both species can be found. In the southeastern Alps *A. ericetorum*

generally prefers heaths of the lower alpine region up to 1200 m s.m., whereas *A. kermesinum* prefers alpine meadows up to 2200 m s.m. Since the two species are morphologically rather similar, flower colour is the only distinguishing characteristic mentioned by STEARN (1980). According to him, *A. ericetorum* has white or yellowish perianth segments, sometimes tinged with pink; those of *A. kermesinum* are red. CASTROVIEJO & FELINER (1986) state that *A. ericetorum* from El Pindo has a rosy corolla or at least a deep purple band on each tepal. Herbarium material from the Alpe Apuane in Italy (LJU, leg. WRABER, T.), also supports the observation that *A. ericetorum* can produce pink flowers. On northern and southern slopes of the Grintovez massif plants with perianth segment colours ranging from light pink to red were found by the author at altitudes from 1200–2000 m s.m. These plants formed populations with uniform or intermixed corolla colours.

Karyological data based on frequencies of arm lengths and satellite lengths do discriminate between three main karyotypes, but these karyotypes are not associated with particular flower colours and therefore species. One cluster contains populations KF and GN 1 with yellowish flowers and all the populations from Grintovez with pink and red colours. Within this cluster there are no agglomerations of populations with particular perianth colours. When a cluster analysis of populations is based on frequencies of C-bands, the same three main clusters are evident. But the largest cluster contains one subcluster with KF and GN 1, the only two populations within this cluster which consistently have yellowish perianth colours. Since this cluster is separated at a low agglomeration level and is based on statistical data from a fairly large set of metaphase plates, this result is of limited practical taxonomical value. Crossing experiments and more detailed studies of various characters, needed to elucidate the taxonomy of this alliance, are being carried out.

The number of primary nucleoli corresponds well to the number of satellite-bearing chromosomes found in metaphase plates of *A. ericetorum* from KO (3 or 4) and to the number found in the populations from KF and from Grintovez Mountains (2 to 4). The number of nucleoli supports the observation that in population KO one chromosome of pair 2, and in population KF and those from Mt Grintovez one or two chromosomes of pair 2 occasionally lack a satellite and a NOR. This can not be stated, however, with certainty since silver staining was not carried out.

To date, chromosome numbers of *A. ericetorum* have been reported by 11 publications. The chromosome number of $2n = 16$ is given for material from Spain (CASTROVIEJO & FELINER 1986), from the Alpi Apuane in Italy (CELA RENZONI 1964), from Central Slovakia (HOLUB & al. 1970), from garden material from the Uppsala Bot. Garden (JACOBSEN & OWNBEY 1977), from Slovenia (LOVKA & al. 1972), from Slovakia (MÁJOVSKÝ & al. 1974), from Mt Zlatibor in former Yugoslavia (VAN LOON & KIEFT 1980), and from the southeastern Alps (WETSCHNIG 1992a). FAVARGER (1965), investigating material from Cima Tombea in Italy, MÁJOVSKÝ & al. (1974) from Slovakia, and HOLUB & al. (1970) from Central Slovakia report one additional or B-chromosome. The diploid chromosome number of $2n = 19$ is given by KHOSHOO & SHARMA (1959) for garden material from the Jard. Bot. Mairie De Nantes in France. A diploid chromosome number of $2n = 32$ is given by MÁJOVSKÝ & al. (1970) for material from Slovakia and SKALIŃSKA & al. (1976) for plants from Poland.

The chromosome number of *A. kermesinum* has been recorded only once. LOVKA & al. (1971) reported a diploid number of 2n = 16 for this species.

All 254 metaphase plates investigated in the present study had 2n = 16. In 34 metaphase plates one B-chromosome was observed. B-chromosomes occurred not only in a population with yellowish flowers (GN 1), but also in a population with pink flowers (GN 2). B-chromosomes were not found in plants with crimson perianth segments, but the number of metaphase plates examined from such material was comparatively small (62).

Information concerning the chromosome morphology of *A. ericetorum* has been given by KHOSHOO & SHARMA (1959), CELA RENZONI (1964), and HOLUB & al. (1970). KHOSHOO & SHARMA (1959) examined root tips of *A. ericetorum* from the Jardin Botanique, Mairie De Nantes, France, and counted 19 chromosomes. 13 of these were metacentric, 6 were telocentric. They concluded that this karyotype evolved from a symmetric one (consisting of 16 metacentric chromosomes) by fragmentation of metacentric chromosomes. In the material of the present study not a single metaphase plate supported these observations. In all plates at least one pair of chromosomes with a submedian centromere was found. A drastic change in chromosome morphology of chromosomes with median centromeres was observed in 3 metaphase plates from population GS 3 and GS 4. In these cases the major part of the short chromosome arm of one chromosome of pair no. 3 was lost. However, no changes were found that lead to more than 16 A-chromosomes.

CELA RENZONI (1964) examined *A. ericetorum* from Pian della Fioba (Alpi Apuane, Massa), reports 2n = 16 and found 8 pairs of metacentric chromosomes. This is not supported by the present study. In all metaphase plates examined, at least one pair of chromosomes with submedian centromeres occurred.

The most detailed information available on chromosome morphology appears in HOLUB & al. (1970) who investigated *A. ericetorum* from a locality (800 m s.m.) east of the Vernár area in the Poprad distinct of Slovakia. They found 2n = 16 and observed one B-chromosome in about 75% of the seedlings studied. They found two pairs with submedian centromeres (r = 2.3), five pairs with median centromeres (r = 1.18–1.33) and one pair with strictly median centromeres (r = 1.09). The accessory chromosome had a median centromere and was only one third as long as the longest A-chromosome. These observations are very much in line with those of the present study. In the localities KF or GN 1 several metaphases had a similar karyotype.

Structural heterozygosity in the genus *Allium* has been known for decades. TSCHERMAK-WOESS (1947, 1964) observed it in *A. carinatum* and LOIDL (1981, 1983) found variability in the occurrence of heterochromatic C-bands in several *Allium* species. TARDIF & MORISSET (1991) carried out a thorough study of C-band variation in 7 populations of *A. schoenoprasum* from eastern North America. This species belongs to subg. *Rhizirideum* sect. *Schoenoprasum* DUMORT (HANELT & al. 1992). They studied 23 distinct bands (thus 23 loci) on 5 of the 8 chromosomes of the haploid set and found a high level of polymorphism per population. Polymorphism in the data of the present study was even higher. This is most likely due to the fact that all chromosomes and also centromeric bands were taken into consideration. TARDIF & MORISSET (1991) did not consider centromeric bands at all as they found them only sporadically and, when they did appear, they appeared in all chromosomes. In the material of this study, centromeric bands generally

occurred less frequently than terminal and intercalary bands but – unlike in the TARDIF & MORISSET (1991) study – they did not occur in all chromosomes. Similar to *A. schoenoprasum*, band widths in the present study were rather small (approximately 0.3–0.4 μm) and uniform. OHLE (1992) has carried out the most extensive C-banding study in sect. *Rhizirideum*. He studied C-banded karyotypes of 6 species of this section. However, as numbers of examined plates and comments on the variability of banding patterns are not given, his results can not be compared with data of the present study in some respects. Generally, his material exhibited a greater variability of the euchromatic parts between chromosomes of one pair. Furthermore, differences in band widths are more striking than in the material of the present study.

BARBUJANI & PIGLIUCCI (1989), in their study of *Ornithogalum montanum* CYR. ex TEN., found a strong negative correlation between the number of B-chromosomes and the number of Q-bands. TARDIF & MORISSET (1992) found 1 to 9 B-chromosomes in 5 of 7 populations from eastern North America. They found, however, a positive correlation between B-chromosome and C-band number in the overall tendency. Different populations behaved in different ways. In two populations (GN 1 and GN 2) of the present study metaphases with one B-chromosome were observed. In both populations there was no significant correlation between the number of B-chromosomes and C-bands. This result suggests that, as concluded by TARDIF & MORISSET (1992), there is no predictable relationship between B-chromosomes and the number of heterochromatic bands.

I would like to thank Prof. Dr R. FRITSCH, Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, who kindly provided material of *Allium ericetorum*, and Dr G. H. LEUTE, Kärntner Landesmuseum, who provided bulbs of this species. I am very grateful to Prof. Dr J. GREILHUBER, Institute of Botany, University of Vienna, for his kind help with the Giemsa C-banding method. Thanks also to F. NEWMAN, Institute of English Language and Literature, University of Graz, for his assistance with the English.

References

BARBUJANI, G., PIGLIUCCI, M., 1989: Geographical patterns of karyotype polymorphism in Italian populations of *Ornithogalum montanum* (*Liliaceae*). – Heredity **62**: 67–75.

BORTZ, J., 1989: Statistik für Sozialwissenschafter. – Berlin: Springer.

CASTROVIEJO, S., FELINER, G. N., 1986: Cytotaxonomic notes on some Spanish plants. – Willdenowia **16**: 213–219.

CELA RENZONI, G., 1964: Contributo alla cariologia della specie toscane del genere *Allium* (*Liliaceae*). Riassunto. – Nuovo G. Bot. Ital. **71**: 573–574.

FAVARGER, C., 1965: Notes de caryologie alpine IV. – Bull. Soc. Neuchatel. Sci. Nat. Ser. 3, **88**: 5–60.

GREILHUBER, J., SPETA, F., 1989: A Giemsa C-banding and DNA content study in *Scilla cilicica* and *S. morrisii*, two little known sibling species of the *S. siberica* alliance (*Hyacinthaceae*). – Pl. Syst. Evol. **165**: 71–83.

HANELT, P., 1992: Infrageneric grouping of *Allium* – the Gatersleben approach. – In HANELT, P., HAMMER, K., KNÜPFFER, H., (Eds): The genus *Allium* – taxonomic problems and genetic resources. Proceedings of an international symposium held at Gatersleben, Germany, June 11–13, 1991, pp. 221–232. – Gatersleben: Institut für Pflanzengenetik und Kulturpflanzenforschung.

HOLUB, J., MĚSÍČEK, J., JAVŮRKOVÁ, V., 1970: Annotated chromosome counts of Czechoslovak plants (1–15). – Folia Geobot. Phytotax. **5**: 339–368.

JACOBSEN, T. D., OWNBEY, M., 1977: In LÖVE, Á., (Ed.): IOPB chromosome number reports 56, pp. 271–274. – Taxon **26**: 257–274.

KHOSHOO, T. N., SHARMA, V. B., 1959: Structural hybridity in *Allium ochroleucum*. – Curr. Sci. **28**/1: 26–28.

LEVAN, A., FREDGA, K., SANDBERG, A. A., 1964: Nomenclature for centromeric position on chromosomes. – Hereditas **52**: 201–220.

LOIDL, J., 1981: Das Heterochromatin einiger *Allium*-Arten: cytochemische Charakterisierung und cytogenetische Aspekte. – Dissertation, University of Wien.

– 1983: Some features of heterochromatin in wild *Allium* species. – Pl. Syst. Evol. **143**: 117–131.

LOVKA, M., SUŠNIK, F., LÖVE, Á., LÖVE, D., 1971: In LÖVE, Á., (Ed.): IOPB chromosome number reports 34, pp. 788–791. – Taxon **20**: 785–797.

– 1972: In LÖVE, A., (Ed.): IOPB chromosome number reports 36, pp. 337–339. – Taxon **21**: 333–346.

MÁJOVSKÝ, J. & al. 1970: Index of chromosome numbers of Slovakian flora. Part 1. – Acta Fac. Rer. Nat. Univ. Comen. Ser. Bot. **16**: 1–26.

– 1974: Index of chromosome numbers of Slovakian flora. Part 3. – Acta Fac. Rer. Nat. Univ. Comen. Ser. Bot. **22**: 1–20.

OHLE, H., 1992: Karyotype analysis using Giemsa C-banding technique in *Allium* species of six sections of the subgenus *Rhizirideum*. – In HANELT, P., HAMMER, K., KNÜPFFER, H., (Eds): The genus *Allium* – taxonomic problems and genetic resources. Proceedings of an international symposium held at Gatersleben, Germany, June 11–13, 1991, pp. 221–232. – Gatersleben: Institut für Pflanzengenetik und Kulturpflanzenforschung.

ROHLF, F. J., 1990: NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 1.60. – New York.

SKALIŃSKA, M., JANKUN, H., WCISŁO, H. & al., 1976: Further studies in chromosome numbers of Polish angiosperms. XI. – Acta Biol. Cracov., Ser. Bot. **19**: 107–148.

STEARN, W. T., 1978: European species of *Allium* and allied genera of *Alliaceae*: a synonymic enumeration. – Ann. Musei Goulandris **4**: 83–198.

– 1980: *Allium*. – In TUTIN, T. G., HEYWOOD, V. H., BURGES, N. A., VALENTINE, D. H., WALTERS, S. M., WEBB, D. A., (Eds): Flora europaea, 5. – Cambridge: Cambridge University Press.

TARDIF, B., MORISSET, P., 1991: Chromosomal C-band variation in *Allium schoenoprasum* (*Liliaceae*) in eastern North America. – Pl. Syst. Evol. **174**: 125–137.

– 1992: Relation between numbers of B-chromosomes and C-bands in *Allium schoenoprasum*. – Cytologia **57**: 349–352.

TSCHERMAK-WOESS, E., 1947: Über chromosomale Plastizität bei Wildformen von *Allium carinatum* und anderen *Allium*-Arten aus den Ostalpen. – Chromosoma **3**: 66–87.

– 1964: Weitere Untersuchungen zum chromosomalen Polymorphismus von *Allium carinatum*. – Österr. Bot. Z. **111**: 159–165.

VAN LOON, J. C., KIEFT, B., 1980: In LÖVE, Á. (Ed.): Chromosome number reports 68, pp. 538–542. – Taxon **29**: 538–542.

WETSCHNIG, W., 1992 a: Chromosomenzahlen Kärntner Gefäßpflanzen (Teil 3): Karyologie und Verbreitung der *Allium*-Arten (*Alliaceae*) in Kärnten. – Carinthia II **182, 102**: 497–533.

– 1992 b: CHROM, ein neues Computerprogramm zur Darstellung chromosomenmorphologischer Daten. – Phyton (Austria) **31**: 251–256.

Address of the author: Dr WOLFGANG WETSCHNIG, Institute of Botany of the University of Graz, Holteigasse 6, A-8010 Graz, Austria.

Accepted May 30, 1994 by F. EHRENDORFER