Karyotype Morphology of Some Diploid Subspecies of *Dactylis glomerata* L. *(Poaceae)*

By

Wolfgang WETSCHNIG *)

With 13 Figures

Summary

WETSCHNIG W. 1991. Karyotype morphology of some diploid subspecies of *Dactylis glomerata* L. *(Poaceae)*. – Phyton (Horn, Austria) 31 (1): 35–55, with 13 figures. – English with German summary.

Chromosome numbers and morphology of metaphase chromosomes (idiograms!) of 11 diploid subspecies out of approximately 16 diploid taxa of *Dactylis glomerata* L. are given.

Based on chromosome morphology three groups of haploid idiograms can be formed: One group consists of the idiograms of *D. g.* subsp. *smithii* (LINK) STEBBINS & ZOHARY, *D. g.* subsp. *ibizensis* STEBBINS & ZOHARY, *D. g.* subsp. *aschersoniana* (GRAEBNER) THELLUNG, *D. g.* subsp. *judaica* STEBBINS & ZOHARY and *D. g.* subsp. *juncinella* (BORY) BOISSIER. A further group is formed by the idiograms of *D. g.* subsp. *lusitanica* STEBBINS & ZOHARY and *D. g.* subsp. *santai* STEBBINS & ZOHARY. The third group is formed by *D. g.* subsp. *"parthiana* PARKER & BORRILL*"* and *D. g.* subsp. *himalayensis* DOMIN. The idiograms of the remaining two subspecies – *D. g.* subsp. *mairei* STEBBINS & ZOHARY and *D. g.* subsp. *reichenbachii* (HAUSMANN) STEBBINS & ZOHARY – are rather different, but both own a certain kind of chromosome which is not present in any other subspecies.

A quantitative comparison of the idiograms using the cluster analysis method underlines the similarity between the five idiograms of the first group. There is a remarkable difference between the idiograms of *D. g.* subsp. *aschersoniana* and *D. g.* subsp. *himalayensis*, two subspecies rather similar in morphology as well as in chemistry.

---

*) Dr. Wolfgang WETSCHNIG, Institut für Botanik der Universität Graz, Holteigasse 6, A-8010 Graz (Austria).

Zusammenfassung

WETSCHNIG W. 1991. Karyotypmorphologie einiger diploider Unterarten von *Dactylis glomerata* L. *(Poaceae).* – Phyton (Horn, Austria) 31 (1): 35–55, mit 13 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Chromosomenzahlen und Morphologie der Metaphasechromosomen (Idiogramme!) von 11 diploiden Subspecies der schätzungsweise 16 diploiden Taxa von *Dactylis glomerata* L. werden angegeben.

Aufgrund der Chromosomenmorphologie ergeben sich drei Gruppen von haploiden Idiogrammen: Eine Gruppe bilden die Idiogramme von *D. g.* subsp. *smithii* (LINK) STEBBINS & ZOHARY, *D. g.* subsp. *ibizensis* STEBBINS & ZOHARY, *D. g.* subsp. *aschersoniana* (GRAEBNER) THELLUNG, *D. g.* subsp. *judaica* STEBBINS & ZOHARY und *D. g.* subsp. *juncinella* (BORY) BOISSIER. Eine weitere Gruppe bilden die Idiogramme von *D. g.* subsp. *lusitanica* STEBBINS & ZOHARY und *D. g.* subsp. *santai* STEBBINS & ZOHARY. Die dritte Gruppe wird von *D. g.* subsp. *"parthiana* PARKER & BORRILL" und *D. g.* subsp. *himalayensis* DOMIN gebildet. Die Idiogramme der restlichen beiden Unterarten – *D. g.* subsp. *mairei* STEBBINS & ZOHARY und *D. g.* subsp. *reichenbachii* (HAUSMANN) STEBBINS & ZOHARY – passen nicht gut zusammen; beide haben jedoch einen Chromosomentyp, der in den Idiogrammen der übrigen Subspezies nicht zu finden ist.

Ein quantitativer Vergleich der einzelnen Idiogramme mittels Clusteranalyse unterstreicht die Ähnlichkeit der fünf Idiogramme der ersten Gruppe. Bemerkenswert ist die Verschiedenheit zwischen den Idiogrammen von *D. g.* subsp. *aschersoniana* und *D. g.* subsp. *himalayensis,* zwei Unterarten, die sowohl in ihrer Morphologie als auch in ihren Inhaltsstoffen sehr ähnlich sind.

1. Introduction

Within the genus *Dactylis* L. – one of the best-known examples of a natural polyploid complex – four levels of ploidy are known up to now. Hexaploid (2n = 42) populations of *Dactylis* were found in Western Egypt and in the Cyrenaica (BORRILL & JONES 1961). Tetraploids (2n = 28) are widley distributed in the northern hemisphere and have been spread almost worldwide by man. Natural triploids (2n = 21) have also been found by SYLVÉN (MÜNTZING 1937) in Sweden. With exception of *D. g.* subsp. *aschersoniana* the diploids (2n = 14) occure in rather small, particular areas (Fig. 12). B-chromosomes have been found in tetraploid as well as in diploid taxa (JONES, CARROLL & BORRILL 1961, BORRILL & CARROLL 1969).

The present study is restricted to the chromosome morphology of the diploid subspecies. 12 diploid subspecies have been described up to now. Four further names of diploid subspecies – *D. g.* subsp. *"castellata", D. g.* subsp. *"galician diploid", D. g.* subsp. *"lebanotica"* and *D. g.* subsp. *"parthiana"* – are frequently used in literature, but are – as far as I know – not published till now (BORRILL & CARROLL 1969 announced the publication of *D. g.* subsp. *"castellata", D. g.* subsp. *"lebanotica"* and *D. g.* subsp. *"parthiana"* by PARKER & BORRILL). Out of these 16 taxa, 11 were available for my karyological studies. No seeds of *D. g.* subsp. *"castellata", D. g.* subsp. *"galician diploid", D. g.* subsp. *"lebanotica"* and *D. g.* subsp. *sinensis* were

available and all seeds of *D. g.* subsp. *woronowii* proved to produce tetraploid plants.

Although *Dactylis* is one of the best-documented examples of a polyploid complex (see list of references in MIZIANTY 1986 and LUMARET 1988), there are only a few articles dealing with chromosome morphology. For the diploids LEVAN 1930 investigated the karyotype of *D. g.* subsp. *aschersoniana*. The same subspecies has also been studied (idiograms, giemsa C-banding) by WETSCHNIG 1983, 1984 and MIZIANTY 1985. Chromosome morphology of *D. g.* subsp. *reichenbachii* has been studied by WETSCHNIG 1983.

The object of this paper is to describe and compare the chromosome morphology of 11 diploid subspecies of *Dactylis glomerata* L. and to contribute this feature to the evolutionary studies within this genus.

2. Material

With exception of *D. g.* subsp. *juncinella* (caryopses were kindly provided by R. LUMARET), *D. g.* subsp. *aschersoniana* and *D. g.* subsp. *reichenbachii* (living plants were collected and taken into cultivation by the author) all *Dactylis*-plants were grown from seeds obtained by the international seed exchange of botanical gardens.

All plants werde cultivated in the Botanical Garden of the Karl-Franzens-University of Graz in clay pots (diameter: 10 cm) with garden mould, which were sunk in outdoor-garden-beds. Because of lack of space, three to five seedlings had to be planted in one pot, so that each pot contained several individuals of a single subspecies.

Voucher specimens of the subspecies investigated will be deposited at the herbarium of the Institute of Botany, University of Graz.

List of sites of original collections

D. g. subsp. *aschersoniana* (GRAEBNER) THELLUNG
  Austria: Carinthia, NW of Klagenfurt, 600 m; (46°38′N/14°17′E).

D. g. subsp. *himalayensis* DOMIN
  India: Gangi, Tehri; (30°20′N/78°30′E). Caryopses received from the Bot. Gard. Aberystwyth.

D. g. subsp. *ibizensis* STEBBINS & ZOHARY
  Ibiza: Island of Ibiza; (38°55′N/1°31′E). Caryopses received from the Bot. Gard. Aberystwyth.

D. g. subsp. *judaica* STEBBINS & ZOHARY
  Lebanon: Yuntah; (34°22′N/36°00′E). Caryopses received from the Bot. Gard. Aberystwyth.

D. g. subsp. *juncinella* (BORY) BOISSIER
  Spain: Top of Sierra Nevada, 3200 m; (37°03′N/3°6′E); leg. R. LUMARET.

D. g. subsp. *lusitanica* STEBBINS & ZOHARY
  Portugal: Sierra de Cintra; (38°47′N/9°25′W). Caryopses received from the Bot. Gard. Aberystwyth.

D. g. subsp. *mairei* Stebbins & Zohary

> Algeria: Gorges de Kerrata, SE of Bougie; (36°34′N/4°52′E). Caryopses received from the Bot. Gard. Aberystwyth.

D. g. subsp. *"parthiana* Parker & Borrill"

> Iran: Elburz Mountains; (36°00′N/51°00′E). Caryopses received from the Bot. Gard. Aberystwyth.

D. g. subsp. *reichenbachii* (Hausmann) Stebbins & Zohary

> Italy: Province Bolzano (Bozen), Tires (Tiers) E of Bolzano, 1200 m; (46°29′N/ 11°33′E).

D. g. subsp. *santai* Stebbins & Zohary

> Algeria: Above Zemmora on Relizane-Tiaret road; (35°35′N/0°21′E). Caryopses received from the Bot. Gard. Aberystwyth.

D. g. subsp. *smithii* (Link) Stebbins & Zohary

> Tenerife: Icod el Alto; (28°24′N/16°30′W). Caryopses received from the Bot. Gard. Aberystwyth.

3. Methods

Meristems of root-tips were used for chromosome morphology studies. Root-tips were pretreated for 4 hours in a 0,002 molare solution of 8-hydroxyquinoline (0,058 g/200 cm³; soluted in aqua destillata at 60°C; Löve & Sarkar 1956) at room temperature.

After pretreatment, the root-tips were fixed in ethanol:acetic acid (3:1). After fixation the material was stored in the fixative in the refrigerator at about –25°C.

For chromosome staining carmine acetic acid was used. Root-tips were boiled in carmine acetic acid for about 2 minutes, and were then left there for 3 to 5 hours. After staining the root-tips were moved into acetic acid (45%). Meristems than were isolated, dissected and finally squashed.

Drawings of the metaphase plates were made with the Reichert Polyvar microscope (Ok. 10×; Obj. 100×/Ap. 1,32), using the drawing-equipment of this microscope. Photographs were made with the same microscope using an Agfa-Ortho film (15 Din/25 Asa).

All measurements for the idiograms were taken from drawings of metaphase chromosomes. After the production of a diploid idiogram, a haploid one was constructed out of this. For each subspecies 5 well scattered metaphase plates were investigated and haploid idiograms of them were used for the construction of a middled idiogram of the subspecies. Calculation of r-, Lr-, G_l- and S_l-value, %-values of long and short chromosome arms and drawing of the idiograms were done by the computer programme CHROM, originally written in Fortran for a DEC/VAX computer by the author; the programme then was transformed into Basic for an Atari Mega ST2 microcomputer.

Concerning the presentation of idiograms, the method used by Teppner in teaching and in his studies of *Onosma* and *Poaceae* (Teppner 1974, 1991, Teppner & Wetschnig 1980) was modified and adapted by me for the purposes in *Dactylis*.

Within the haploid idiograms (Figs. 1a–11a) the chromosomes were numbered and arranged according to their r-value. Within the chromosomes, the longer arms were always positioned below the shorter ones.

The following features were used to characterize chromosomes and idiograms:

s...length of the shorter arm.

l...length of the longer arm.

r...arm ratio of the chromosome: longer arm of the chromosome/shorter arm of the chromosome.

L_r...relative length of the chromosome: $((l+s)/\text{sum total of all chromosomes}) \times 100$.

S_i...symmetry-index: (sum total of shorter arms/sum total of longer arms) $\times$ 100.

G_i...gradient of chromosome length index: (total length of the smallest chromosome/total length of the largest chromosome) $\times$ 100.

The following values are given in the idiograms: Above the chromosomes the figures of the uppermost row indicate the numbers of the chromosomes. If there are satellites at the shorter chromosome arm, their length-values are given in µm in the first row; below these values, the %-values of the satellites – (satellite/sum total of all chromosomes) $\times$ 100 – are given in brackets. The values of the next row indicate the length-values of the shorter arms (or satellite-bearing parts of the shorter arms) in µm (%-values in brackets below the µm-values). Beneath the box-diagramms of the chromosomes, the length-values of the longer arms (or satellite-bearing parts of the longer arms) are given in µm- and in %-values (%-values in brackets below the µm-values). If there are satellites at the longer arms of the chromosomes, their values are given in further rows. The next row of boxes shows the r-values, with the numeric r-values below. At last the L_r-values are given as boxes with their numeric values beneath them.

In Figs. 1a–11a the chromosomes are drawn according to their µm-values. In Fig. 12 chromosomes are plotted according to their %-values to make comparison more easily.

Nomenclature of chromosomes and centromeric positions are given according to LEVAN, FREDGA & SANDBERG 1964.

For the quantitative comparison of the haploid idiograms the chromosomes were arranged within the idiograms as shown in Fig. 12. In this arrangement, the sum of differences between all features of all idiograms is a minimum (tested and proved by a computer programme written by the author). Each subspecies was characterized by 42 features: length of the longer and shorter arm of chromosome, length of the satellite at the longer and shorter chromosome arm, value of arm ratio und relative length of each of the 7 chromosomes of the haploid idiogram were used. Comparison of the idiograms was done by the cluster analysis method. The routine 2M of the software package BMDP (DIXON 1977, BOLLINGER, HERRMANN & MÖNTMANN 1983) was used on a DEC/VAX785 computer of the computer centre at the University of Graz. Euclidian distance was used and no standardization of data was made. Several other combinations of features (for example r- and L_r-value were excluded or satellite and satellite-bearing arm were added up) were tested, as well as standardization of data, but the options mentioned above proved to be appropriate.

4. Morphology of Metaphase Chromosomes

4.1. *D. glomerata* L. subsp. *aschersoniana* (GRAEBNER) THELLUNG

Karyotype morphology of this subspecies has already been investigated by LEVAN 1930, WETSCHNIG 1983, 1984 and MIZIANTY 1985; the idiogram
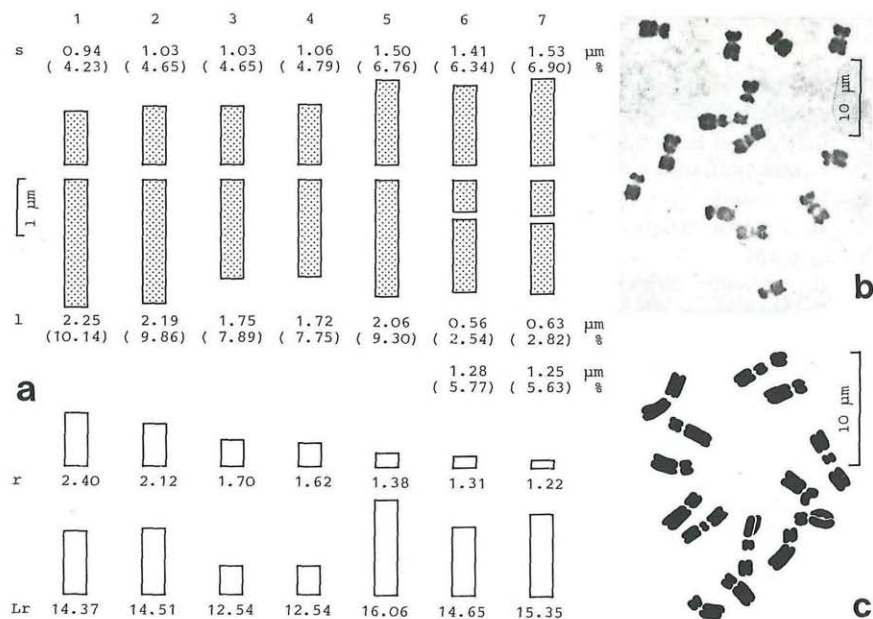
	1	2	3	4	5	6	7	
s	0.94	1.03	1.03	1.06	1.50	1.41	1.53	µm
	(4.23)	(4.65)	(4.65)	(4.79)	(6.76)	(6.34)	(6.90)	%

b

l	2.25	2.19	1.75	1.72	2.06	0.56	0.63	µm
	(10.14)	(9.86)	(7.89)	(7.75)	(9.30)	(2.54)	(2.82)	%
						1.28	1.25	µm
						(5.77)	(5.63)	%

a

r	2.40	2.12	1.70	1.62	1.38	1.31	1.22

Lr	14.37	14.51	12.54	12.54	16.06	14.65	15.35

Fig. 1. *Dactylis glomerata* subsp. *aschersoniana. a. Idiogram of the haploid chromosome set. – b, c metaphase plates.*

	1	2	3	4	5	6	7	
s	1.11	1.07	1.37	1.33	1.55	1.54	1.30	µm
	(4.43)	(4.27)	(5.46)	(5.31)	(6.18)	(6.14)	(5.19)	%

b

l	2.33	1.93	0.81	0.56	0.69	2.42	1.96	µm
	(9.29)	(7.70)	(3.23)	(2.23)	(2.75)	(9.65)	(7.82)	%
			1.65	1.62	1.83			µm
			(6.58)	(6.46)	(7.30)			%

a

r	2.10	1.80	1.80	1.64	1.63	1.57	1.51

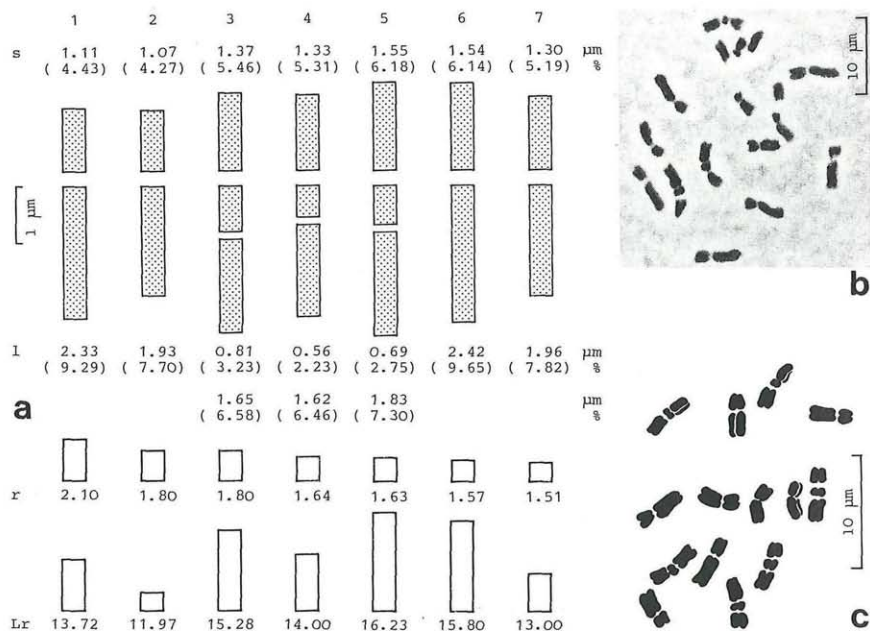| Lr	13.72	11.97	15.28	14.00	16.23	15.80	13.00

Fig. 2. *Dactylis glomerata* subsp. *himalayensis. – a. Idiogram of the haploid chromosome set. – b, c metaphase plates.*

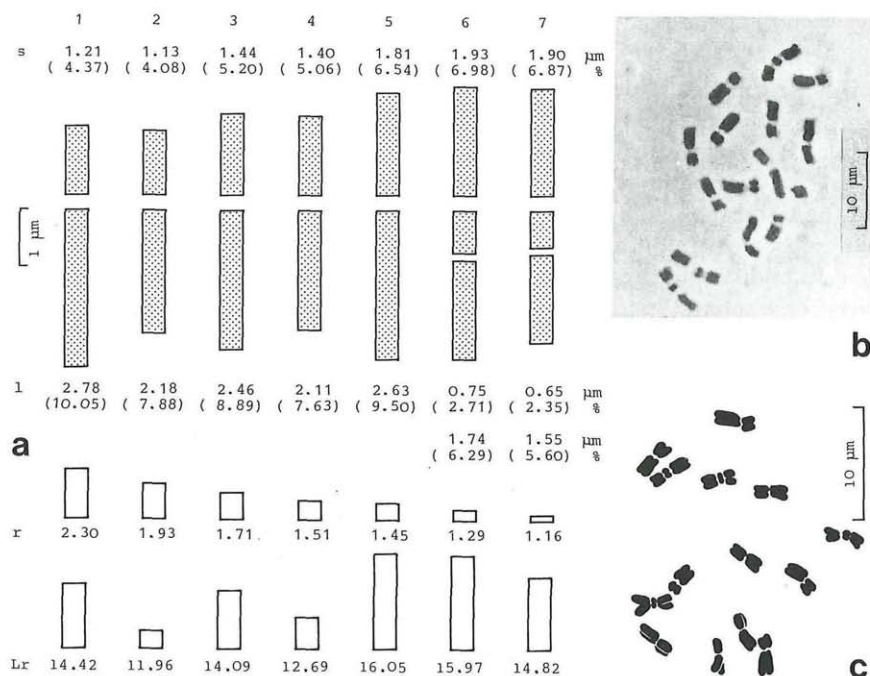|	1	2	3	4	5	6	7	
s	1.21	1.13	1.44	1.40	1.81	1.93	1.90	µm
	(4.37)	(4.08)	(5.20)	(5.06)	(6.54)	(6.98)	(6.87)	%
l	2.78	2.18	2.46	2.11	2.63	0.75	0.65	µm
	(10.05)	(7.88)	(8.89)	(7.63)	(9.50)	(2.71)	(2.35)	%
						1.74	1.55	µm
						(6.29)	(5.60)	%
r	2.30	1.93	1.71	1.51	1.45	1.29	1.16	
Lr	14.42	11.96	14.09	12.69	16.05	15.97	14.82	

Fig. 3. *Dactylis glomerata* subsp. *ibizensis*. – a. Idiogram of the haploid chromosome set. – b, c metaphase plates.

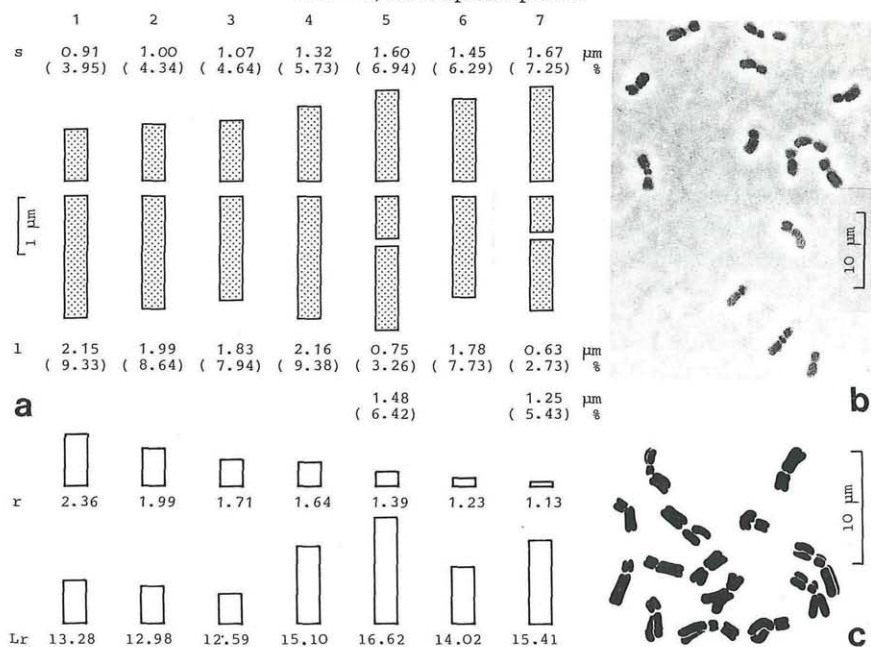|	1	2	3	4	5	6	7	
s	0.91	1.00	1.07	1.32	1.60	1.45	1.67	µm
	(3.95)	(4.34)	(4.64)	(5.73)	(6.94)	(6.29)	(7.25)	%
l	2.15	1.99	1.83	2.16	0.75	1.78	0.63	µm
	(9.33)	(8.64)	(7.94)	(9.38)	(3.26)	(7.73)	(2.73)	%
					1.48		1.25	µm
					(6.42)		(5.43)	%
r	2.36	1.99	1.71	1.64	1.39	1.23	1.13	
Lr	13.28	12.98	12.59	15.10	16.62	14.02	15.41	

Fig. 4. *Dactylis glomerata* subsp. *judaica*. – a. Idiogram of the haploid chromosome set. – b, c metaphase plates.

42

presented in this paper is based on further metaphase plates. The diploid chromosome set (2n = 2x = 14) is shown in Figs. 1b, c. The haploid idiogram (Fig. 1a) consists of two satellite-bearing chromosomes (6, 7) with centromeres in the median region, and five chromosomes without satellites. Three of the latter (3, 4, 5) have the centromere in the median region, and in two chromosomes (1, 2) the centromere is inserted in the submedian region.

In addition to the two satellite-bearing chromosomes, chromosome 5 is very characteristic for the chromosome set of this subspecies. With a relative length of 16,06 it is the longest chromosome of the set, and with an r-value of 1,38 it is the most symmetric of the five chromosomes without satellites.

Without giemsa C-banding it is rather difficult to seperate chromosome 1 from 2 and 3 from 4.

The G_i-value of this subspecies ist 78,07, the S_i-value is 62,10.

4.2. *D. glomerata* L. subsp. *himalayensis* DOMIN

Metaphase plates of the diploid chromosome set (2n = 2x = 14) are shown in Figs. 2b, c. The haploid idiogram (Fig. 2a) contains three satellite-bearing chromosomes (3, 4, 5). Two of the four remaining chromosomes have the centromere in the median region (6, 7), whereas the centromere of chromosome 1 and 2 is situated in the submedian region.

Among all the idiograms investigated till now, those of *D. g.* subsp. *himalayensis* and *D. g.* subsp. *"parthiana"* are the only two having three satellite chromosomes, whose satellites exceed the length of the satellite-bearing arms. The longest chromosome of the set is chromosome 5 ($L_r = 16,23$), followed by chromosome 6 ($L_r = 15,80$).

The chromosomes of this subspecies are rather easy to distinguish with the exception of the satellite chromosomes 3 and 5.

The G_i-value is 73,71, the S_i-value is 58,67.

4.3. *D. glomerata* L. subsp. *ibizensis* STEBBINS & ZOHARY

Metaphase plates of the diploid chromosome set (2n = 2x = 14) are shown in Figs. 3b, c. The haploid idiogram (Fig. 3a) consists of two satellite chromosomes with the centromere in the median region (6, 7). A centromere in the median region is also evident in chromosome 4 and 5, whereas the chromosomes 1, 2 and 3 have the centromere in the submedian region.

Characteristic for this chromosome set is chromosome 5. With an L_r-value of 16,05 it is the longest, and with r = 1,45 also the most symmetric chromosome of the set.

It is rather difficult to distinguish the chromosomes 2 and 3. This is most easily possible by means of the length of the short arms.

The G_i-value is 74,55, the S_i-value is 64,21.

4.4. *D. glomerata* L. subsp. *judaica* STEBBINS & ZOHARY

The morphology of the diploid chromosome set (2n = 2x = 14) is shown in the metaphase plates of Figs. 4b, c. The haploid idiogram is shown in Fig. 4a. It contains two satellite-bearing chromosomes (5, 7) with the centromere in the median region. With L_r-values of 16,62 and 15,41 they are the longest chromosomes of the set. Two of the remaining satelliteless chromosomes have the centromere in the median region (4, 6). The chromosomes 1, 2 and 3 have the centromere inserted in the submedian region.

In addition to the satellite chromosomes, the symmetric chromosomes (4, 6) are characteristic for the idiogram of this subspecies.

The G_i-value is 75,72, the S_i-value is 64,34.

4.5. *D. glomerata* L. subsp. *juncinella* (BORY) BOISSIER

The diploid chromosome set (2n = 2x = 14) is shown in Figs. 5b, c. The haploid idiogram (Fig. 5a) contains two satellite chromosomes (4, 7) with the centromere in the median region and five satelliteless chromosomes. Three of them (3, 5, 6) have the centromere in the median region, whereas the centromere of the chromosomes 1 and 2 is inserted in the submedian region.

Most characteristic are the two satellite chromosomes (4, 7) which are the longest chromosomes of the set (L_r = 16,74 and 15,57).

The G_i-value is 74,94, the S_i-value is 65,70.

4.6. *D. glomerata* L. subsp. *lusitanica* STEBBINS & ZOHARY

Figs. 6b, c are showing metaphase plates of the diploid chromosome set (2n = 2x = 14). The haploid idiogram is shown in Fig. 6a. Only one satellite-bearing chromosome (7) has been found. With an r-value of 1,19 this chromosome is the most symmetric of the set. Among the six satelliteless chromosomes two (5, 6) possess the centromere in the median region, while the rest (1, 2, 3, 4) have the centromere inserted in the submedian region.
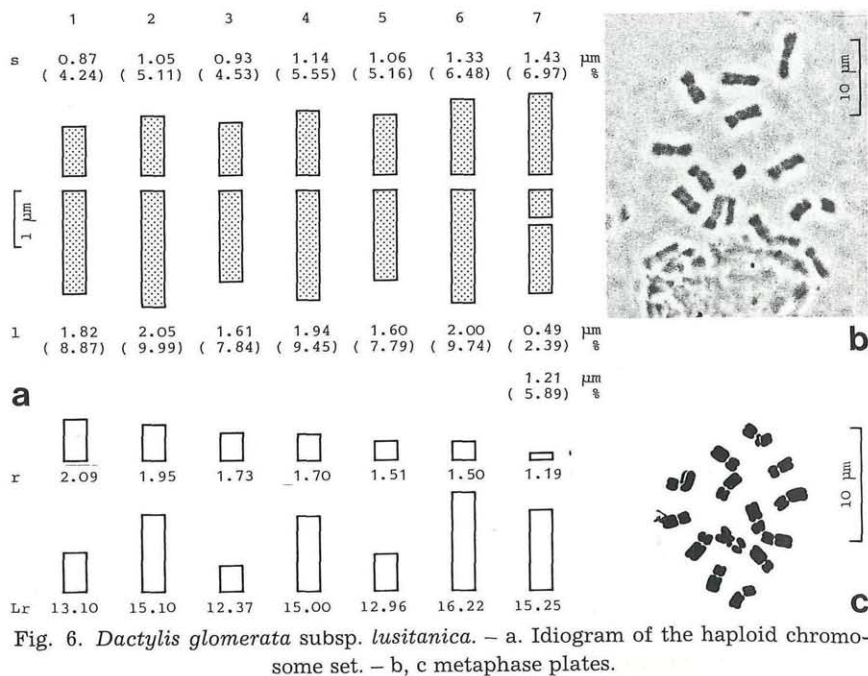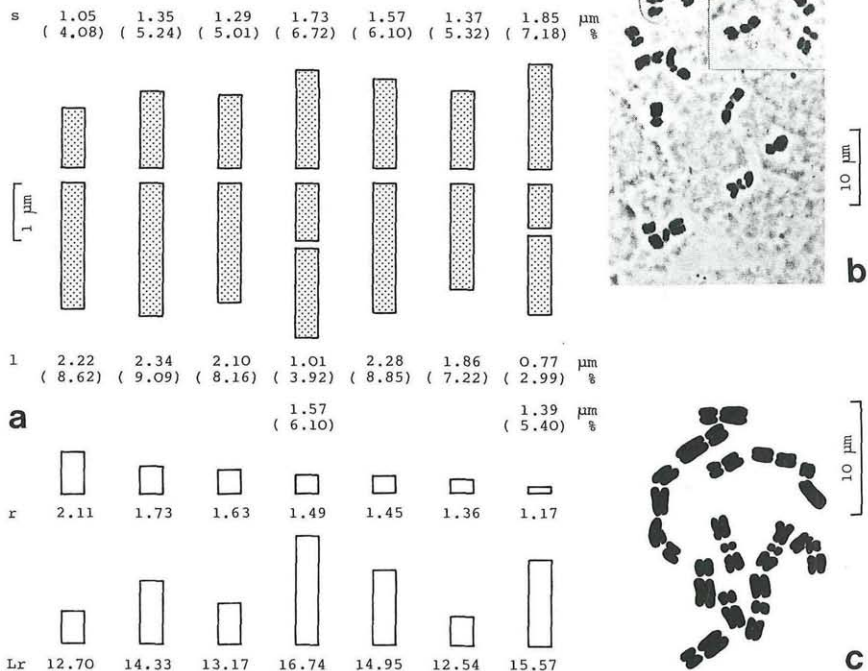
The chromosomes 1, 3 and 5 are rather similar and hard to distinguish as well as the chromosomes 2 and 4.

The G_i-value is 76,28, the S_i-value is 61,40.

4.7. *D. glomerata* L. subsp. *mairei* STEBBINS & ZOHARY

The diploid chromosome set (2n = 2x = 14) is shown in Figs. 7b, c. The haploid idiogram (Fig. 7a) consists of two satellite chromosomes (5, 7) with the centromere in the median region and five satelliteless chromosomes. Two of the satelliteless chromosomes (4, 6) have the centromere in the median region, three (1, 2, 3) have the centromere inserted in the submedian region.

The two satellite chromosomes are very characteristic. Chromosome 5 has a long arm consisting of a bearing arm which is shorter than the satellite

Fig. 5. *Dactylis glomerata* subsp. *juncinella*. – a. Idiogram of the haploid chromosome set. – b, c metaphase plates.

Fig. 6. *Dactylis glomerata* subsp. *lusitanica*. – a. Idiogram of the haploid chromosome set. – b, c metaphase plates.
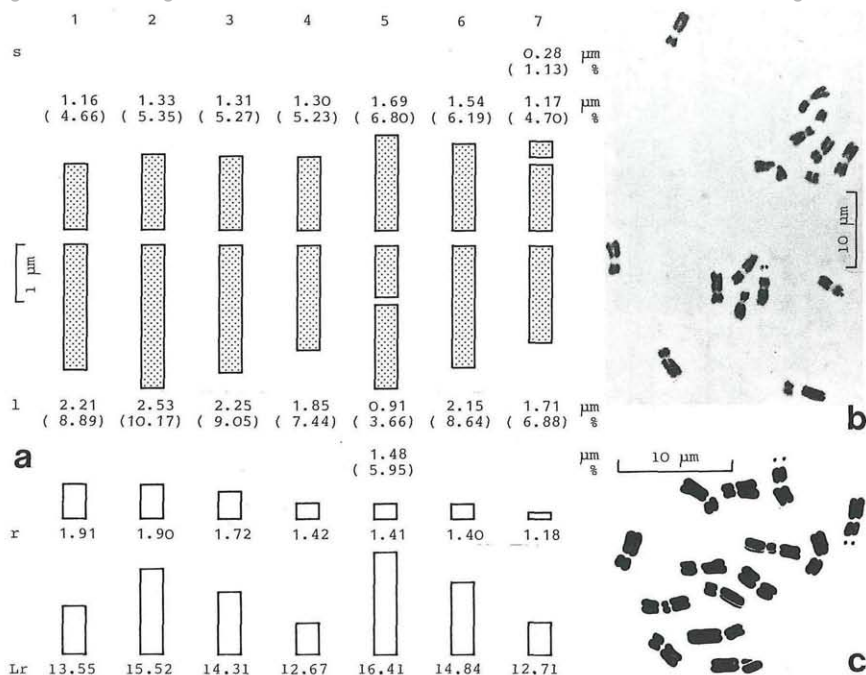
Fig. 7. *Dactylis glomerata* subsp. *mairei*. – a. Idiogram of the haploid chromosome set. – b, c metaphase plates.

on it. In opposition to that, the shorter arm of chromosome 7 bears a very short satellite (mean value = 0,3 μm). Chromosome 7 is the most symmetric chromosome of this subspecies (r = 1,18) too. Among the satelliteless chromosomes the symmetric chromosome 6 (r = 1,40) and the chromosome 2 with its distinctive long arm are remarkable.

The G_i-value is 77,21, the S_i-value is 64,81.

4.8. *D. glomerata* L. subsp. *"parthiana* PARKER & BORRILL"

Metaphase plates of the diploid chromosome set (2n = 2x = 14) are shown in Figs. 8b, c. The haploid idiogram (Fig. 8a) consists of three satellite-bearing chromosomes (5, 6, 7) with the centromere in the median region and four satelliteless chromosomes (1, 2, 3, 4) with the centromere inserted in the submedian region.

Two of the satellite chromosomes (5, 6) are the largest chromosomes (L_r = 16,18 and 16,30) of the set.

The G_i-value is 70,41, the S_i-value is 63,80.

4.9. *D. glomerata* L. subsp. *reichenbachii* (HAUSMANN) STEBBINS & ZOHARY

The karyotype morphology of this subspecies has already been investigated in a previous work (WETSCHNIG 1983). The idiogram in the present
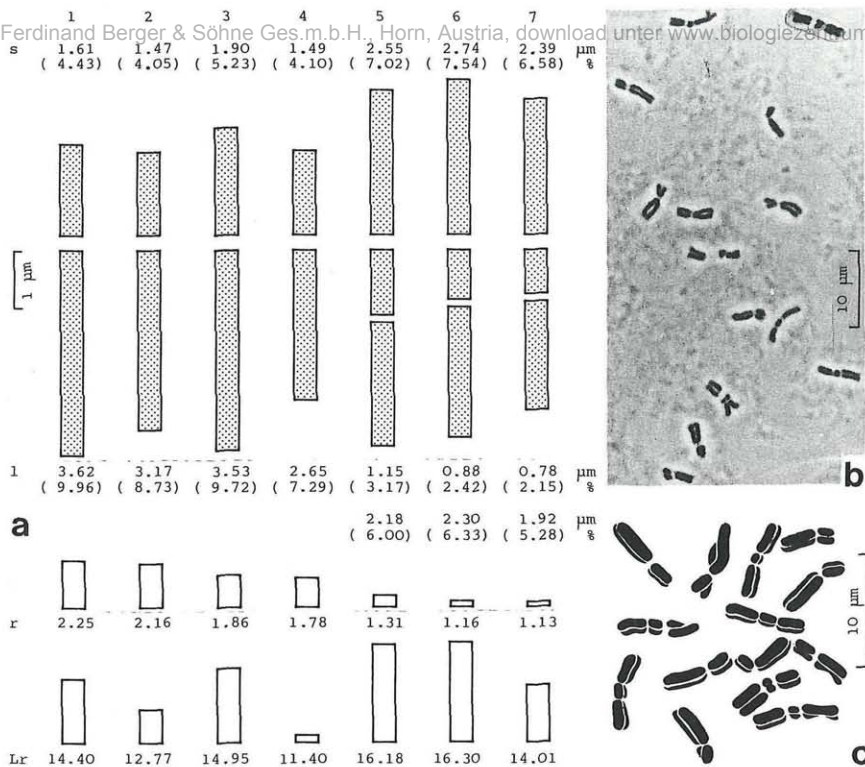
s	1	2	3	4	5	6	7	
	1.61	1.47	1.90	1.49	2.55	2.74	2.39	μm
	(4.43)	(4.05)	(5.23)	(4.10)	(7.02)	(7.54)	(6.58)	%

l	3.62	3.17	3.53	2.65	1.15	0.88	0.78	μm
	(9.96)	(8.73)	(9.72)	(7.29)	(3.17)	(2.42)	(2.15)	%
					2.18	2.30	1.92	μm
					(6.00)	(6.33)	(5.28)	%

r	2.25	2.16	1.86	1.78	1.31	1.16	1.13

Lr	14.40	12.77	14.95	11.40	16.18	16.30	14.01

Fig. 8. *Dactylis glomerata* subsp. *"parthiana"*. – a. Idiogram of the haploid chromosome set. – b, c metaphase plates.

s	1	2	3	4	5	6	7	
				0.22			1.28	μm
				(0.97)			(5.68)	%
	0.94	1.06	1.06	1.03	1.38	1.34	0.50	μm
	(4.16)	(4.71)	(4.71)	(4.57)	(6.09)	(5.96)	(2.22)	%

l	2.09	2.03	1.91	2.00	0.66	1.84	1.91	μm
	(9.28)	(9.00)	(8.45)	(8.86)	(2.91)	(8.17)	(8.45)	%
					1.31			μm
					(5.82)			%

r	2.23	1.91	1.79	1.60	1.43	1.37	1.07

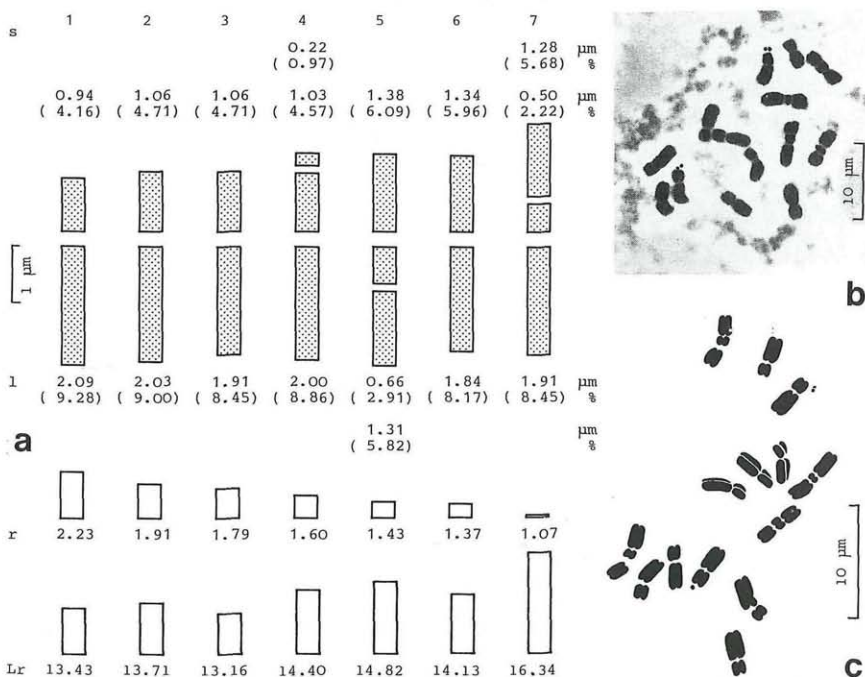| Lr	13.43	13.71	13.16	14.40	14.82	14.13	16.34

Fig. 9. *Dactylis glomerata* subsp. *reichenbachii*. – a. Idiogram of the haploid chromosome set. – b, c metaphase plates.
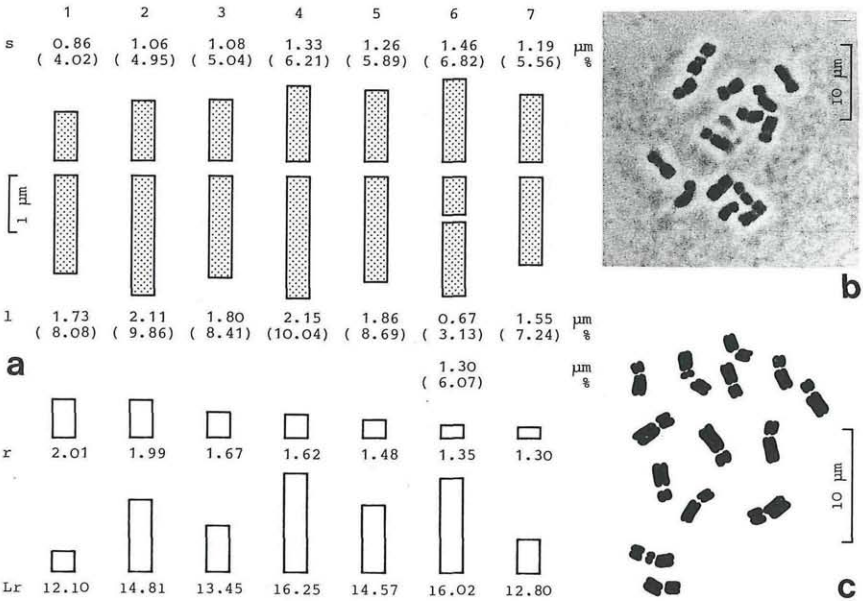
	1	2	3	4	5	6	7	
s	0.86 (4.02)	1.06 (4.95)	1.08 (5.04)	1.33 (6.21)	1.26 (5.89)	1.46 (6.82)	1.19 (5.56)	µm %
l	1.73 (8.08)	2.11 (9.86)	1.80 (8.41)	2.15 (10.04)	1.86 (8.69)	0.67 (3.13)	1.55 (7.24)	µm %
						1.30 (6.07)		µm %
r	2.01	1.99	1.67	1.62	1.48	1.35	1.30	
Lr	12.10	14.81	13.45	16.25	14.57	16.02	12.80	

Fig. 10. *Dactylis glomerata* subsp. *santai.* – a. Idiogram of the haploid chromosome set. – b, c metaphase plates.

	1	2	3	4	5	6	7	
s	1.04 (4.03)	1.14 (4.41)	1.31 (5.07)	1.57 (6.08)	1.27 (4.92)	1.85 (7.16)	1.93 (7.47)	µm %
l	2.37 (9.18)	2.23 (8.63)	2.26 (8.75)	2.58 (9.99)	1.89 (7.32)	0.76 (2.94)	0.71 (2.75)	µm %
						1.50 (5.81)	1.42 (5.50)	µm %
r	2.28	1.96	1.73	1.64	1.49	1.22	1.10	
Lr	13.20	13.05	13.82	16.07	12.23	15.72	15.91	
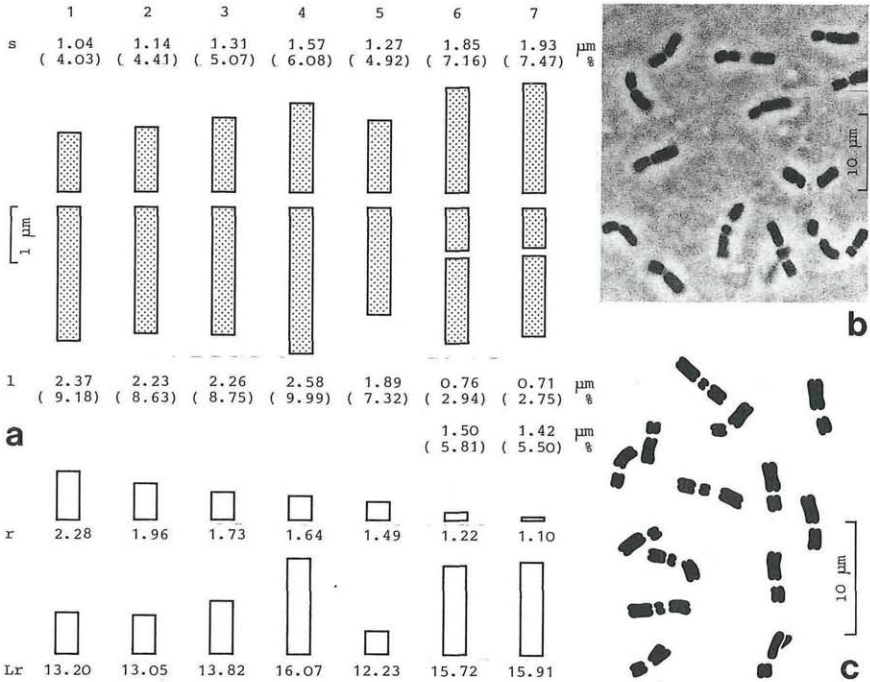
Fig. 11. *Dactylis glomerata* subsp. *smithii.* – a. Idiogram of the haploid chromosome set. – b, c metaphase plates.

48

paper is based on further metaphase plates. Figs. 9 b, c are showing meta-phase plates of the diploid chromosome set (2n = 2x = 14). The haploid chromosome set (Fig. 9 a) contains three satellite chromosomes with the centromere in the median region (4, 5, 7) and four chromosomes without satellites. One of the satelliteless chromosomes (6) has the centromere in the median region, whereas all the others (1, 2, 3) have the centromere inserted in the submedian region.

The satellite chromosomes are characteristic for the chromosome set of this subspecies. In two of them (4, 7) the satellite-bearing arm plus the satellite is shorter then the other arm. The satellite is very small in chromo-some 4, whereas in chromosome 7 the satellite exceeds the length of the satellite-bearing arm. In case of chromosome 5 the satellite-bearing arm plus the satellite is longer than the other arm.

The G_i-value is 80,51 and the S_i-value is 64,00.

4.10. *D. glomerata* L. subsp. *santai* STEBBINS & ZOHARY

The diploid chromosome set (2n = 2x = 14) is shown in Figs. 10 b, c. The haploid idiogram (Fig. 10 a) consists of only one satellite chromosome (6) with the centromeric position in the median region and of five satelliteless chromosomes. Four of them (3, 4, 5, 7) have the centromere in the median region, the remaining two (1, 2) have the centromere in the submedian region.

Besides the idiogram of *D. g.* subsp. *lusitanica* this is – so far as I know – the only subspecies investigated till now with only one satellite-bearing chromosome in the haploid set.

The G_i-value is 74,43, the S_i-value is 62,57.

4.11. *D. glomerata* L. subsp. *smithii* (LINK) STEBBINS & ZOHARY

Metaphase plates of the diploid chromosome set (2n = 2x = 14) are shown in Figs. 11 b, c. The haploid idiogram (Fig. 11 a) contains two satellite chromosomes (6, 7) with the centromeric position in the median region and five satelliteless chromosomes. Two of them (4, 5) have the centromere in the median region, whereas the centromere of the remaining (1, 2, 3) is inserted in the submedian region.

The satellite-bearing chromosomes (6, 7) are rather similar in their morphology. With an r-value of 1,22 and 1,10 they are the most symmetric chromosomes of the set. Among the satelliteless chromosomes the longest (4) with an L_r-value of 16,07 and the smallest (5) with L_r = 12,23 are noteworthy.

The G_i-value is 76,14, the S_i-value is 64,31.

5. Comparison of Middled Idiograms

For a qualitative comparison of the haploid idiograms of the eleven subspecies (Fig. 12) number and morphology of the satellite-bearing
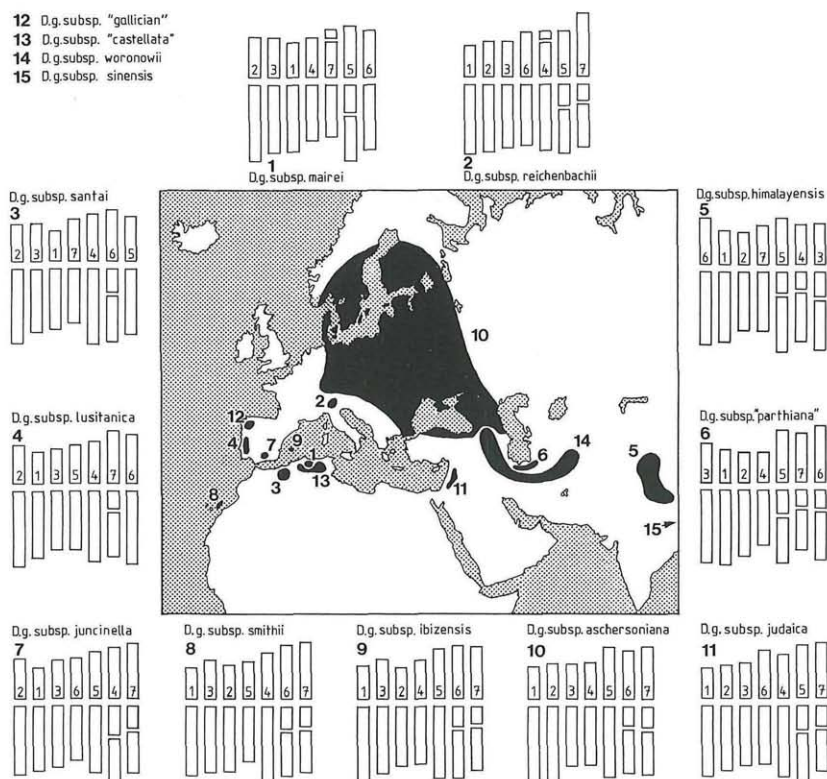
49

Fig. 12. Primary distribution of diploid *Dactylis glomerata* subspecies and haploid idiograms of eleven diploids. In case of taxa no. 12–15 no material has been available for karyological investigations.

chromosomes are most useful. The most common type of these chromosomes has the centromere in the median region and a longer satellite on a shorter bearing arm; the length of the bearing arm plus the satellite exceeds the length of the other arm.

Within the haploid idiograms three distinct groups may be formed: The first group (Fig. 12, idiograms number 7–11) consists of the idiograms of *D. g.* subsp. *aschersoniana, D. g.* subsp. *ibizensis, D. g.* subsp. *judaica, D. g.* subsp. *juncinella* and *D. g.* subsp. *smithii*. The idiograms are rather similar and are characterized by two satellite chromosomes. One of these chromosomes is always the most symmetric of the set. The other one is also rather symmetric with the centromere in the median region.

The second group (Fig. 12, idiograms number 3 and 4) consists of the idiograms of *D. g.* subsp. *santai* and *D. g.* subsp. *lusitanica*. The idiograms of these two subspecies are characterized by only one satellite chromosome

which represents the common type of morphology of the satellite chromosomes.

The idiograms of *D. g.* subsp. *himalayensis* and *D. g.* subsp. *"parthiana"* may be regarded as a third group (Fig. 12, idiograms number 5 and 6). Both possess three satellite chromosomes, but on the other side they differ in many aspects, for example in the length of the short arms of the satellite chromosomes.

The idiograms of the remaining two subspecies – *D. g.* subsp. *mairei* and *D. g.* subsp. *reichenbachii* (Fig. 12, idiograms number 1 and 2) – are showing only little correspondence. They both own a type of satellite chromosome not to be found in other subspecies. In these satellite chromosomes the length of the short satellite plus the bearing arm does not exceed the length of the other arm.

These observations based on qualitative features were checked up by a quantitative procedure, namely by cluster analysis. For this reason the sequence of the chromosomes within the idiograms was changed by a computer programme until the sum of differences between all features of all idiograms proved to be a minimum. The result of this procedure is shown in the arrangement of chromosomes within the idiograms of Fig. 12.

The results of the cluster analysis made afterwards are shown in Figs. 13 a and 13 b. The existence of the first group of idiograms (consisting of the idiograms of *D. g.* subsp. *aschersoniana, D. g.* subsp. *ibizensis, D. g.* subsp. *judaica, D. g.* subsp. *juncinella* and *D. g.* subsp. *smithii*) as statet above (qualitative observation) is now confirmed by the cluster analysis (quantitative observation). Within this group *D. g.* subsp. *ibizensis* and *D. g.* subsp. *smithii* are most similar (agglomeration value of 53). Together with .*D. g.* subsp. *aschersoniana* they form a subcluster within this group at an agglomeration value of 55. A second subcluster within group 1 is formed by *D. g.* subsp. *juncinella* and *D. g.* subsp. *judaica* agglomerating at a value of 54.

The existence of the second group of idiograms (consisting of the idiograms of *D. g.* subsp. *santai* and *D. g.* subsp. *lusitanica*) is also confirmed by cluster analysis. The agglomeration value of these two idiograms is 58.

Group 1 and group 2 agglomerate at a level of 68, forming a single cluster. The idiograms of the remaining subspecies are forming no further clusters, but agglomerate with the cluster formed by group 1 and 2 one after another at the following agglomeration levels: *D. g.* subsp. *reichenbachii* at 73, *D. g.* subsp. *himalayensis* at 81, *D. g.* subsp. *mairei* at 91 and with *D. g.* subsp. *"parthina"* all subspecies are agglomerated.

6. Discussion

As WETSCHNIG 1983 pointed out, it is possible to distinguish certain subspecies of *D. glomerata* by idiograms of their chromosomes (carmine
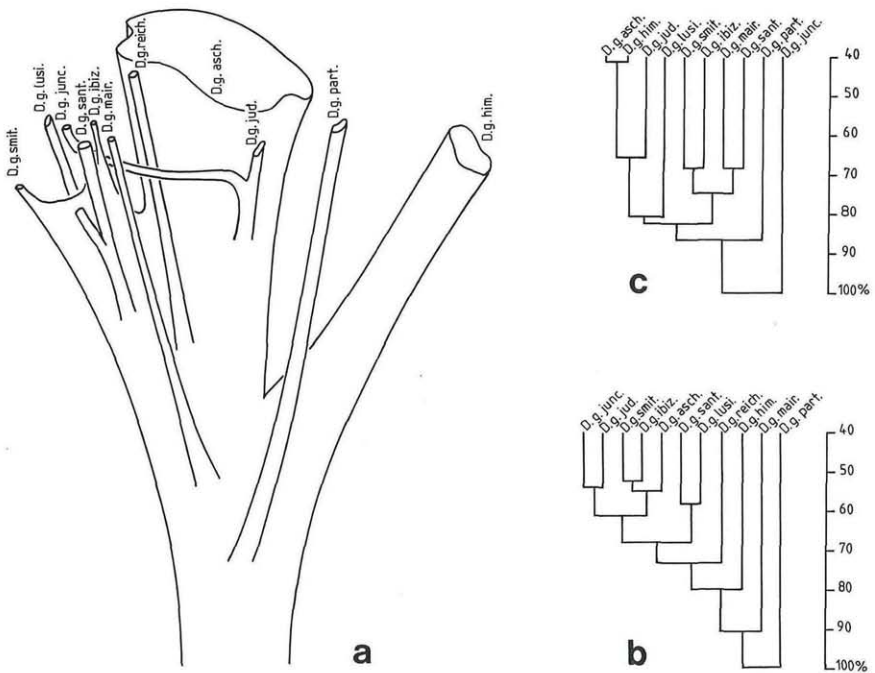
Fig. 13. a. Three dimensional phenogram showing relations of eleven diploid sub-species based on chromosome morphology. The positions of the subspecies are reflecting their geographical distribution, the places of branching off sign the agglomeration levels. – b phenogram showing relations of eleven diploid subspecies based on chromosome morphology. – c phenogram showing relations of ten diploid subspecies based on enzymatic polymorphism (redrawn and transformed from LUMARET 1984).

stained as well as giemsa C-banded); the differences of chromosome morphology between several populations within *D. g.* subsp. *aschersoniana* and *D. g.* subsp. *reichenbachii* proved to be very slight. MIZIANTY 1985 studied the chromosomes of *D. g.* subsp. *aschersoniana* from Poland using giemsa C-banding. She found minor differences to the material studied by WETSCHNIG 1983 in the gradient of chromosome length and in the content of heterochromatin in two pairs of submedian chromosomes. On principle it is desirable to study the chromosome morphology of several populations of each subspecies. For lack of seed-material this has to be delayed to further studies, which will include chromosome banding of the different diploid subspecies too.

Despite the differences in chromosome morphology, all diploid sub-species can be crossed and fertile seeds will be obtained (BORRILL 1961, PARKER 1968, PARKER & BORRILL 1968 and JONES & BORRILL 1962), although a decrease in fertility was recorded over two (F1 and F2) generations.

52

LUMARET 1988 gave an excellent summary and discussion of previous studies on *Dactylis* of various fields of botany. Therefore it is not necessary to discuss the whole spectrum of previous studies in detail. So I restrict the discussion to a comparison of the results of the present study with the classical observations on morphology, plant geography and evolution of the genus made by STEBBINS & ZOHARY 1958, and with the studies of enzymatic polymorphism and diversity for flavonoid compounds presented by LUMARET 1988.

STEBBINS & ZOHARY 1958 stated that morphologically four extreme types can be recognized within the diploid subspecies of *D. glomerata: D. g.* subsp. *himalayensis, D. g.* subsp. *juncinella, D. g.* subsp. *ibizensis* and *D. g.* subsp. *smithii.* The remaining subspecies are intermediate between these extreme forms. Based on the geographical distribution and the plants with which the various diploid subspecies are associated, they arrived at the conclusion, that *D. g.* subsp. *aschersoniana, D. g.* subsp. *himalayensis* and *D. g.* subsp. *smithii* are the oldest subspecies within the genus and probably date back from the Miocene period. According to them, there is reason to believe, that *D. g.* subsp. *ibizensis* also dates from the Miocene. *D. g.* subsp. *juncinella* may be derived from *D. g.* subsp. *ibizensis,* but it is probably also of Tertiary age. In the opinion of LUMARET 1988 *D. g.* subsp. *"parthiana"* and *D. g.* subsp. *sinensis* also belong to the old diploid taxa and maybe date from the Miocene. All the other diploids grow in floras with a more modern aspect and with or near to the more aggressive and abundant tetraploids.

More recently, the study of flavonoid variability (for a list of references see LUMARET 1988) has provided a phylogenetic groundplan for the *Dactylis* complex. As result of these studies three groups may be formed within the diploids. A first primitive group consists of *D. g.* subsp. *aschersoniana, D. g.* subsp. *himalayensis, D. g.* subsp. *woronowii* and *D. g.* subsp. *smithii.* Within this group *D. g.* subsp. *aschersoniana* and *D. g.* subsp. *himalayensis* evidently have more primitive molecules than the other two subspecies. A second group is formed by *D. g.* subsp. *ibizensis, D. g.* subsp. *juncinella, D. g.* subsp. *mairei, D. g.* subsp. *"castellata"* and *D. g.* subsp. *santai.* Probably *D. g.* subsp. *ibizensis* is a rather primitive element, whereas *D. g.* subsp. *santai* and *D. g.* subsp. *"castellata"* likely are of a more recent origin. A third group with the most advanced molecules consists of *D. g.* subsp. *judaica, D. g.* subsp. *reichenbachii, D. g.* subsp. *lusitanica* and the *"galician diploid".* There is biochemical evidence, that *D. g.* subsp. *judaica* has undergone important evolutionary change quite recently. LUMARET 1988 suggests, that *D. g.* subsp. *judaica* may be derived from a northern subspecies e. g. *D. g.* subsp. *aschersoniana.* In *D. g.* subsp. *"parthiana"* two different phenolic pathways, one close to *D. g.* subsp. *aschersoniana,* the other close to *D. g.* subsp. *juncinella* are existing.

Enzymatic polymorphism was also used to compare the diploid subspecies (e. g. LUMARET 1984). Fig. 13c (redrawn and transformed from

LUMARET 1984) shows the result of a numerical comparison of enzymatic polymorphism between 10 diploid subspecies. Like in morphology and in flavonoid composition, *D. g.* subsp. *himalayensis* shows many similarities with *D. g.* subsp. *aschersoniana*. In allozyme characters *D. g.* subsp. *juncinella* is very distinct from all the other subspecies (Fig. 13 c).

As a result of the present study of chromosome morphology at least two groups can be formed within the observed diploids (Fig. 13 a, b). The group 1 consists of *D. g.* subsp. *aschersoniana, D. g.* subsp. *ibizensis, judaica, D. g.* subsp. *juncinella* and *D. g.* subsp. *smithii*. With the exception of *D. g.* subsp. *judaica* (which probably is a rather young taxon as shown by flavonoid variation (LUMARET 1988)), all the subspecies of this group are believed to be of ancient origin, dating back to the Miocene period (STEBBINS & ZOHARY 1958). The type of idiogram represented by the subspecies of group 1 (Fig. 12, idiograms no. 7 to 11) is therefore probably the more primitive one and the other types have derived from it. Within group 1 *D. g.* subsp. *aschersoniana* and *D. g.* subsp. *smithii* are belonging to LUMARET's first group of subspecies (LUMARET 1988), which is characterized by the most primitive molecules. *D. g.* subsp. *himalayensis,* which also belongs to this primitive group has an idiogram differing from those of the other members of LUMARET's first group and from those of the subspecies regarded as the oldest ones by STEBBINS & ZOHARY 1958. *D. g.* subsp. *ibizensis* and *D. g.* subsp. *juncinella,* two members of my group 1, are regarded as old by STEBBINS & ZOHARY. They belong to LUMARET's second group with advanced molecules, which differ from the members of her first group by degree of the expression of a flavonol pathway. *D. g.* subsp. *judaica,* a further member of my group 1, has most advanced molecules (LUMARET's third group) and is regarded as a rather young subspecies. The idiograms of group 2 (*D. g.* subsp. *lusitanica* and *D. g.* subsp. *santai* (Fig. 12, idiograms no. 3, 4)) with only one satellite chromosome maybe descended from the *D. g.* subsp. *smithii* − *D. g.* subsp. *ibizensis* group or from *D. g.* subsp. *juncinella.* According to LUMARET 1988 *D. g.* subsp. *santai* belongs to the group of taxa with advanced molecules, while *D. g.* subsp. *lusitanica* is one of the taxa of LUMARET's third group with most advanced molecules. The chromosome morphology of *D. g.* subsp. *mairei* (Fig. 12, idiogram no. 1) could have derived from that of *D. g.* subsp. *santai,* a subspecies rather close to the former e. g. in enzymatic polymorphism. In contrast to that, the idiogram of *D. g.* subsp. *reichenbachii* probably is a more direct descent of one of the members of group 1.

The most striking difference in the results of chromosome morphology and other (morphological and biochemical) investigations is, that *D. g.* subsp. *himalayensis* has a chromosome morphology rather different from that of *D. g.* subsp. *aschersoniana,* whereas in all other studies these two subspecies proved to be the closest relatives. In this context *D. g.* subsp. *"parthiana"* is also worth to mention. This subspecies is regarded to be very

54

closely related to *D. g.* subsp. *aschersoniana* and could have derived from it as LUMARET 1988 stated, but the chromosome morphology of this subspecies is rather different from *D. g.* subsp. *aschersoniana*. Like in *D. g.* subsp. *himalayensis*, the idiogram of *D. g.* subsp. *"parthiana"* is characterized by three satellite chromosomes. Biochemically *D. g.* subsp. *"parthiana"* is marked by an extraordinary differentiation of flavonoid compounds, showing two phenolic pathways (LUMARET 1988). In enzymatic polymorphism this subspecies is also rather different from the rest of the diploids (LUMARET 1984) as shown in Fig. 13 c.

7. References

BOLLINGER G., HERMANN A. & MÖNTMANN V. 1983. BMDP Statistikprogramme für die Bio-, Human- und Sozialwissenschaften. – Stuttgart, New York.

BORRILL M. 1961. Chromosomal status, gene exchange and evolution in *Dactylis*. I. Gene exchange in diploids and tetraploids. – Genetica 32: 94–117.

— & CARROLL C. P. 1969. A chromosome atlas of the genus *Dactylis* (Part two). – Cytologia 34: 6–17.

— & JONES K. 1961. Hexaploid *Dactylis*. – Nature 190: 469–470.

DIXON W. J. (Ed.) 1977. BMDP Biomedical Computer Programs. – Los Angeles.

JONES K. & BORRILL M. 1962. Chromosomal status, gene exchange and evolution in *Dactylis*. III. The role of the inter-ploid hybrids. – Genetica 32: 296–322.

—, CARROLL C. P. & BORRILL M. 1961. Chromosome atlas of the genus *Dactylis*. – Cytologia 26: 333–343.

LEVAN A. 1930. Beitrag zur Kenntnis der Chromosomen in der Gattung *Dactylis* L.- Bot. Notiser 1930: 95–104.

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

LÖVE A. & SARKAR P. 1956. Cytotechnology; Some methods and recipes for modern cytology. (hektographied).

LUMARET R. 1984. Further contribution to the knowledge of evolution in *Dactylis glomerata* (polyploid complex) from enzymatic polymorphism studied in several diploid and tetraploid subspecies. – In: WHYTE R. O. (Ed.), Proc. int. Conf. The Evolution in the East Asian Environment, 2: 651–663. – University of Hong Kong Press.

— 1988. Cytology, genetics, and evolution in the genus *Dactylis*. – CRC Critical Reviews in Plant Sciences 7 (1): 55–91.

MIZIANTY M. 1985. Banding patterns in plant chromosomes. III. *Dactylis glomerata* subsp. *aschersoniana* (GRAEBNER) THELL. from Poland. – Acta Soc. Bot. Polon. 54 (2): 169–178.

— 1986. Biosystematic studies on *Dactylis* L. 1. Review of previous studies. 1.1. Systematics, variability, ecology, biology and cultivation problems. – Acta Soc. Bot. Polon. 55 (3): 467–479.

MÜNTZING A. 1937. The effects of chromosomal variation in *Dactylis*. – Hereditas 23: 113–235.

PARKER P. F. 1968. Genetic variation in diploid *Dactylis*. I. Cross compatibility and seedling characters. – Heredity 23: 279–300.

—   & BORRILL M. 1968. Studies in *Dactylis*. I. Fertility relationships in some
        diploid subspecies. – New Phytologist 67: 649–662.
STEBBINS G. L. & ZOHARY D. 1958. Cytogenetic and evolutionary studies in the genus
        Dactylis; I. Morphology, distribution and interrelationship of the diploid
        subspecies. – Univ. Calif. Publ. Botany 31: 1–40.
TEPPNER   H.   1974.   Karyosystematik   einiger   asiatischer   *Onosma*-Arten
        (Boraginaeceae), inkl. *O. inexspectatum* TEPPNER, spec. nov. – Plant Syst. Evol.
        123 (1): 61–82.
—   1991. Karyology of some Greek *Onosma* species *(Boraginaceae).* – In: OPTIMA.
        Sixth Meeting, Delphi 1989 (in press).
—   & WETSCHNIG W. 1980. Zur Karyologie von *Poa hybrida, P. chaixii, P. sylvicola*
        und *P. stiriaca (Poaceae)* unter besonderer Berücksichtigung von B-Chromo-
        somen. – Phyton (Horn, Austria) 20 (1–2): 47–63.
WETSCHNIG W. 1983. Zur Karyologie von *Dactylis glomerata* L. *(Poaceae)* am Südost-
        Rand der Alpen. – Phyton (Horn, Austria) 23 (2): 271–305.
—   W. 1984. Zur Morphologie, Karyologie und Verbreitung von *Dactylis glome-
        rata* L. *(Poaceae)* in Kärnten. – Carinthia II 174 (= 94): 107–130.