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CHROMOSOMAL VARIATION IN THE *BRACHYPODIUM* GENUS IN BULGARIA WITH REGARD TO ITS EVOLUTION AND TAXONOMY[1]

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Summary. Four species of *Brachypodium* distributed in Bulgaria were karyologicaly investigated. The following chromosome numbers were established: *B. pinnatum* — $2n=14$, $2n=16$, $2n=28$; *B. glaucovirens* — $2n=16$; *B. silvaticum* — $2n=18$, $2n=28$, $2n=42+2$, $2n=56$; B. *distachyon* — $2n=10$.

No correlation has been established at this stage of knowledge between the common morphology and the chromosome numbers in the different cytodemes. Euploidy as well as increasing or decreasing disploidy are supposed to play simultaneously an evolutionary role in the genus which seems to be undergoing secondary speciation.

Some objections are put forth against splitting of the genus.

The *Brachypodium* genus is represented in the flora of Bulgaria by three species, all of which show morphological variation. This variation has found its expression in seven intraspecific taxa (B. Kitanov, 1963, Stojanov, Stefanov and Kitanov, 1966). Some of these taxa were described originaly as species (Host, 1809; Velenovsky, 1892; Hayek, 1933). This morphological variation has provoked, however, only few cytotaxonomic studies on material of a Balkan origin. This is because of the little taxonomic value the Balkan authors attribute to the intraspecific taxa of this genus. Many other authors, though, (Krause, 1912; Avdulov, 1931; Prat, 1936; Hitchcook, 1927; Löve and Löve, 1961 and the others) have found a considerable heterogeneity — morphological, chromosomal or anatomical — which has given to some of them reasons for splitting the genus, as well as for thinking it is not in its right place, in the family *Gramineae*.

Having in mind the above mentioned considerations and the large distribution of this genus in Bulgaria over various habitats we have studied representative populations of the species, both karyologicaly and morphologicaly.

The investigation was aimed firstly at finding out to what extend the phenotypic plasticity is genotypically determined and secondly — at finding answers to questions comprised in a large research topic on the tribe *Brachypodieae* in the Balkans.

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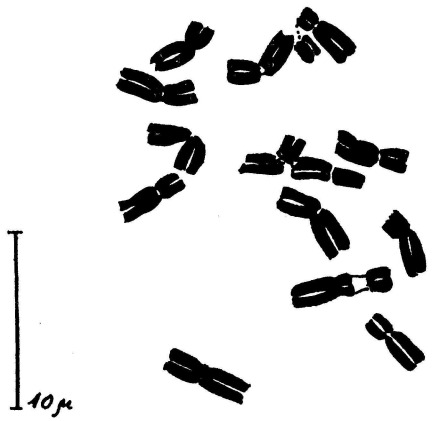

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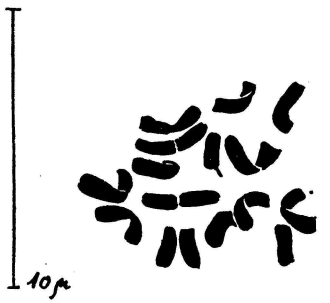

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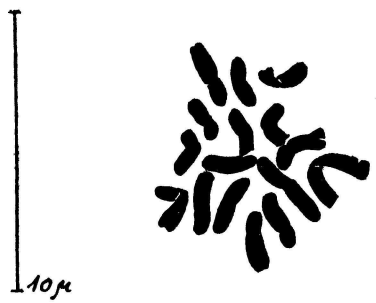

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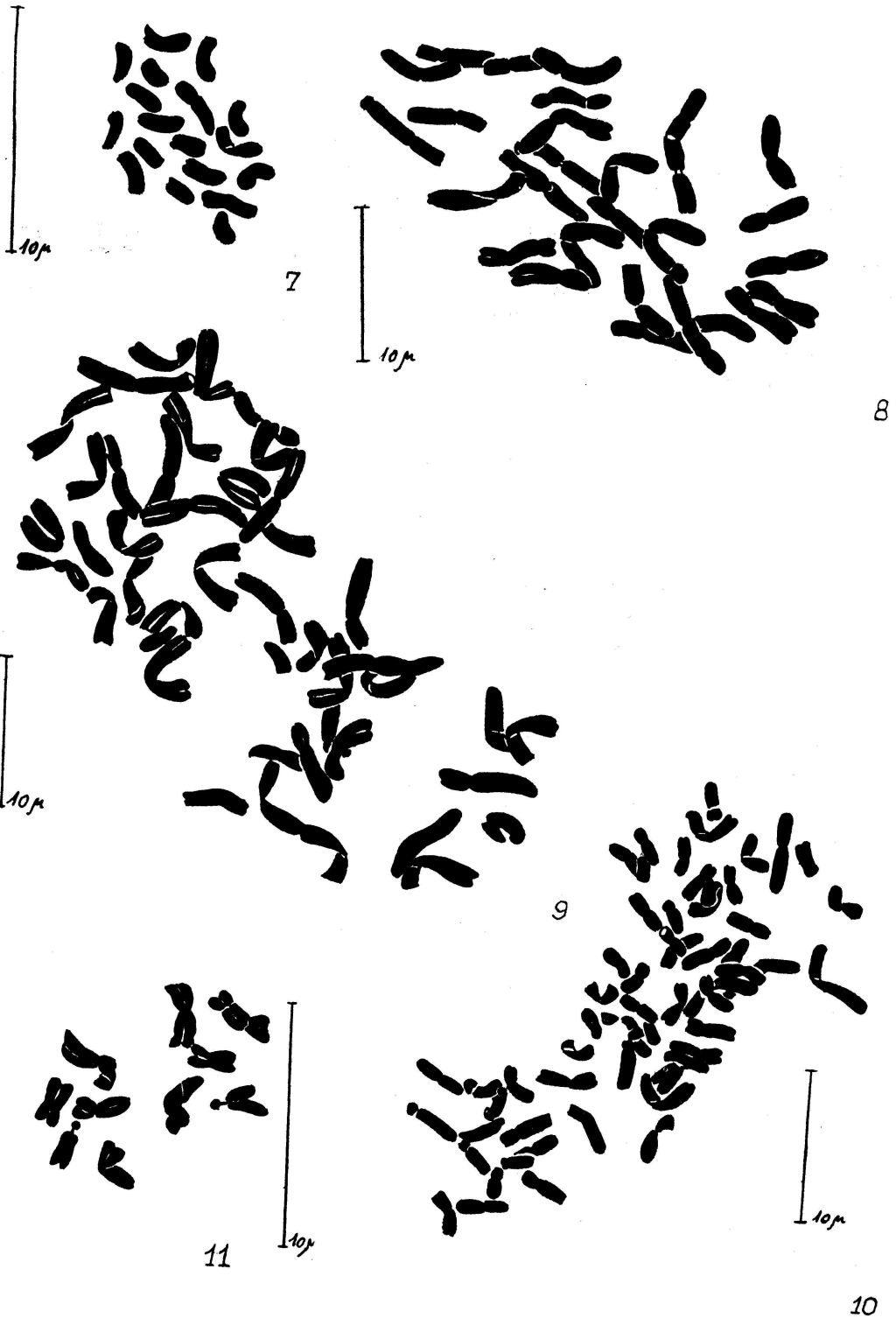
Drawings of mitotic metaphase root-tip cells Figs. 1 — 11.

Brachypodium pinnatum (L.) P. B. — Fig. 1. $2n=28$ (2285); Fig. 2. $2n=14$ (22365); **Fig. 3. $2n=16$** (221076); Fig. 5. $2n=16$ (1178)

***Brachypodium glaucovirens* (Murb.) Fritsch.** — *Figs. 4. and 5* $2n=16$



Brachypodium *silvaticum* (Huds.) P. B. — Fig. 6. $2n=18$ (221489); Fig. 7. $2n=18$ (221157); Fig. 8. $2n=28$ (22944); Fig. 9. $2n=44$ (221625); Fig. 10. $2n=56$ (22900)
Brachypodium distachyon (L.) P. B. — Fig. 11. $2n=10$

MATERIAL AND METHODS

The material studied originates from natural habitats in different parts of the country. The plants were dug out with soil, reared in pots in a greenhouse; or else seeds (*B. distachyon*) were germinated in Petri dishes at room temperature. The root tips were treated with 0.1% colchicine for 1.30 - 2.30 hrs, or with 0.002 M oxychinolin for 0.30 hours, fixed in Klarke for 24 hrs, macerated in 1n HCL for 6 - 14' in 60°C and stained with a mixture of aceto-orcein + Schiff; for *B. distachyon* hematoxylin after Gomory (Pearse, 1962) was used, and squashed.

Voucher specimens are deposited in the Herbarium of the Botanical Institute of the Bulgarian Academy of Sciences (SOM).

DISCUSSION AND RESULTS

1. *B. PINNATUM* (L.) P. B.

Largely distributed species on the grassy and stony habitats and in openings and shrubs in the lowlands, as well as in the mountains where it grows to 2200 m a.s.l. (B. Kitanov, 1963).

The populations studied show considerable variation of the chromosome numbers.

1.1. $2n=2x=14$ (Fig. 2)

Voucher specimen: Tundża hilly region, Dervent hill, in the šibljak, 23 V 1972, SK — 22365.

The diploid chromosome number is reported for the first time. The karyotype has submetacentric chromosomes: two pairs with arms of the same length; a pair of the same type but with somewhat shorter arms; two pairs of the same lenght but with a short or small arm; a pair of shortest chromosomes in respect of both arms; a pair of SAT-submetacentric chromosomes.

The voucher specimen belongs to a populations with scabrous leaves and very unequal glumes as well as very long and straight spikelets consisting of (13) 15 - 17 flowers. The plant has no leaves in its upper part and has very short rhizomes, the leaves all unrigid and glabrous. On grounds of this morphological syndrome it might be referred to Velenovsky's species *B. tenerum*.

1.2. $2n=2x=16$ (Fig. 3)

Voucher specimen: Black Sea coast, about four km south wards from the town Mičurin, 25 VI 1972, SK-221076.

A new cytodeme having a diploid chromosome number and different basic number, $x=8$. The karyotype consists of following submetacentric chromosomes of medium size: a pair with the longest arms of almost equal size; two pairs with shorter arms of the same type; a pair with a long arm similar to the previous pair, the small arm half as long as the long one; a pair of shorter chromosomes than the previous pair, having the short arm less than half as long as the long one; a pair of shorter chromosomes of the same type; a pair of the same length but almost acrocentric which sometimes seems to be SAT; a pair of shortest chromosomes in the karyotype with a nearly submedian centromere.

The population where the voucher specimen comes from belongs to var. *pinnatum* but shows some characteristics of *B. tenerum* Vel.: not very rigid and setose to certain extent on the margin leaves as well as very unequal glumes.

The same diploid chromosome number was established in a population originating from the central sea coast (voucher specimen — the coast between Cape Emine and the Sunny Beach, 16 IX 1967, SK-1778). It consists mainly of the same types of chromosomes, but some of them are of medium size having very short small arms and an acrocentric appearance. The longest pair of them shows secondary constrictions on the long arms (Fig. 5).

1.3. $2n=4x=28$ (Fig. 1)

Voucher specimen: Strandża Mountain, village Velika, 19 V 1972, SK-2285.

The chromosome number coincides with the data from the literature (see Fedorov edit. et al., 1969, and Fernandes and Queiros, 1969).

The karyotype is almost symmetrical and consists of submetacentric chromosomes: three pairs having almost a median centromere, one pair being a little shorter; three pairs having one of the arms half as long as the other, one having a secondary constriction on the long arm; four pairs shorter than the previous ones of the same type; three pairs as long as the previous one having a shorter small arm, and a pair shortest in the karyotype.

The karyotype seems rather homogeneous, the chromosomes do not differ very much in length.

The voucher specimen belongs to the typical variety which is largely distributed in the lowlands.

The comparison of all cytodemes studied reveals a close similarity between all of them, especially concerning the submetacentric chromosomes. In spite of this fact a biological isolation should be expected owing to the two extra long chromosomes in comparison with the 14th chromosome cytodeme and the 28th chromosome cytodeme. Morphologicaly they differ in many respects but not strongly enough to give them a specific rank. The $2n=14$ cytodeme and $2n=16$ cytodeme seem to belong to the same taxon *B. tenerum* Vel. It probably consists of many morphodemes and requires further taxonomic studies.

The most primitive basic number seems to be $x=7$ giving rise in one direction to $4x=28$ and to nother $x=8$ $(7+1)$ by which it shows trends to an increasing disploidy. The fundamental role of the basic number 7 in the subtribe *Brachypodieae* is assumed by Avdulov (1931) and is in accordance with the development of most genera in the subtribe.

2. *B. GLAUCOVIRENS* (MURB.) FRITSCH. (*B. PONTICUM* VEL.)

Established with certainty mainly along the sea coast as well as in the southern parts of the country (Achtarov, 1960). Most of its localities inside the country should be referred mainly to *B. rupestre* Roem. et Schult. as well as *B. tenerum* Vel., both included in a single variety *B. pinnatum* var. *rupestre* (Host.) Reichenb. (Kitanov, 1963).

($2n=2x=16$ (Figs. 4 and 5)

Voucher specimen: Black Sea coast, in the surroundings of the town Mičurin, 8 VII 196 SK-1716.

The karyotype shows the following characteristics of chromosomes: all of the are submetacentric, with a pair of longest almost metacentric chromosomes; a pa of the same size but with the shorter arm still shorter than in the previous pai a pair of chromosomes with shorter both arms; with two pairs shorter; two pai without well visible centromere; and a pair of the same size — SAT.

The leaves of the voucher specimen are very hard and hairy. The glumes a very unequal. In some respects it is closely similar to *B. tenerum* by the morphol gical syndrome mentioned above.

Voucher specimen corresponds also to the overall syndrome of *B. ponticum* Velenovsky whose taxonomical status was not studied by us particulary. Kitanc (1963), Stojanov, Stefanov and Kitanov (1966) assign *B. glaucovirens* a *B. ponticum* to the variety *rupestre* (Host.) Reichenb., which according to o views does not agree with the main characters of *B. ponticum*. Its awn is almo as long as the glabrous glumellules (glumellules are puberulent) and almost twice long as the awn in *B. pinnatum*), the length of the glumes — 10 mm (7 - 9 in *B. pinn tum*).

These taxa should be assigned, if they deserve at all intraspecific rank, to *silvaticum*; or if considered as synonymous to *B. glaucovirens*, a very close positi to *B. silvaticum* as they were arranged by Hayek (1933) and Achtarov (196 whose oppinions we follow in the present paper.

This species seems to have an intermediate position between the two speci *B. pinnatum* and *B. silvaticum* and is most closely related with the first of them variety *tenerum* Vel., whose rank was admited by Hayek (l. c.) as well as Achtarc (l. c.). This results probably from the common chromosome number $2n=16$. Evident the two species *B. pinnatum* and *B. silvaticum* might hibridize on a diploid lev

3. *B. SILVATICUM* (HUDS.) P. B.

The species is distributed over shady forests and shrubs mainly in the mountai up to 1600 m a.s.l. (Kitanov, 1963). Our impression is that this species is not ra neither in the lower altitudes. That is why we have studied populations growing different altitudes finding a considerable variation in the chromosome numbers a structure.

3.1. $2n=2x=18$ (Fig. 6)

Voucher specimen: Central Stara Planina Mt., locality "Beklemeto" near town Troja in grassy habitats 1300 m a.s.l., 27 VII 1972, SK-221489.

This diploid chromosome number has been reported by all authors who studi this species (see Fedorov edit. et al., 1969), Fernandes and Queiros (1969), well as Kožuharov and Kuzmanov (1970) for this country. The karyoty consists of small metacentric and submetacentric chromosomes as well as of tv fragments. Metacentric chromosomes consist of a pair of middle size; two submet centric pairs, the longest in the karyotype, two pairs of shorter chromosomes, tv

pairs still shorter of the same type with slight difference in the length of the short arm, two pairs of short chromosomes, one of them shorter than the other, probably of a fragmentary origin.

3.2.2n=18(221157) (Fig. 7)

Voucher specimen: Strandža Mountain, near village Kosti, in locality "Keresov dol", 26 VI 1972, SK-221157.

The karyotype differs from the previous one by the lack of metacentric chromosomes. The first of both our karyotypes differs from this one studied by Kožuharov and Kuzmanov (l. c.) by the lack of one long metacentric pair — having instead of it a long submetacentric pair; and by the absence of a SAT telocentric pair. The second karyotype (221157) differs by the lack of metacentric chromosomes as well as of SAT chromosomes. Generally, the three karyotypes are not very much different and we may state that the 18th chromosomes cytodeme is not very heterogenous.

All cytodemes studied show little variation in their general morphology, but the great differences in the chromosome number and in some repects also in ploidy should cause many abnormalities in the mejosis and induce sterility. In this case we might consder them as cryptospecies or siblings.

The most interesting fact four us is the finding of a new basic chromosome number within this species, x=7.

Löve and Löve (1961) splitted the genus described a new one, with the basic chromosome number x=9—g. *Brevipodium*. However, having in mind that cytodemes with x=7 are also common in it, we find such a splitting not satisfactory. For the evolution of this species increasing diploidy as well as euploidy seem to be of importance.

3.3 $2n=4x=28$ (Fig. 8)

Voucher specimen: Balkan Foothill Region, hill "Vráska ćuka", calcareous rocks, 690 m a.s.l., 15 VI 1972, SK-22944.

The tetraploid chromosome number is reported for the first time. It consists of long Bromus-type metacentric or submetacentric chromosomes, some of them being secondary constricted: metacentric — four pairs of long chromosomes two of them slightly shorter; submetacentric — two pairs of long chromosomes having almost submedian centromere and two pairs of the same length, but having secondary constrictions on their long arms; a pair of the shortest chromosomes in the almost metacentric karyotype; five pairs shorter than the previous long chromosomes, one them being slightly shorter than the others — all of them tipically submetacentric, having small arms at most half as long as the long ones. The karyotype seems to be stricly multiplied.

3.4 $2n=6x=42+2$ (Fig. 9)

Voucher specimen: Belasica Mountain, near village Kolorovo, at the foot of the mountain, in shrubs on rocky silicous habitats at about 200 m a.s.l., 5 VIII 1972. SK-221625.

A hexaploid chromosome number which is reported for the first time having (except the multiples) two B chromosomes.

The karyotype consists of following long or short chromosomes: metacentric -a pair of long chromosomes and three pairs of slightly shorter ones; submetacentric — two pairs of long chromosomes with an almost median centromere; a pair of the same size but with the short arm shorter than the previous pair; two pairs of shorter chromosomes; ten pairs of almost the same kind, shorter than the previous, its short arm half as long as the long one; seven pairs the shortest in the karyotype of the same kind.

There are ten pairs in this almost metacentric karyotype — no trends to acrocentricity being visible. This is a specialized karyotype, but not stricly multiplied and to a great extent showing structural heterogenity. This seems to indicate that the number 44 might be due to hybridization between tetraploid cytodemes with different basic numbers, 7 and 8 ($2n=16$ and $2n=28$).

3.5. $2n=8x=56$ (Fig. 10)

Voucher specimen: Balkan Foothill Region, the hill near village Zgorigrad, district Vraca, in grassy habitats, on rocky silicous ground, 14 VI 1972. SK-22900.

The octoploid chromosome number is reported for the first time. The karyotype has long chromosomes. Metacentric or almost metacentric — four pairs of long chromosomes; seven pairs having an almost median centromere; eight pairs of medium-size typicaly submetacentric chromosomes; eight pairs almost acrocentric; two of them having secondary constrictions on the long arm, a pair of SAT telocentric in appearance.

This chromosome complement is not strictly multiplied — only the group of submetacentric chromosomes of the medium size as well as the group of submetacentric showing tendency to acrocentricity are strictly multiplied. The complement is thus rather heterozygous.

4. *B. DISTACHYON* (L.) P. B.

The species is distributed mainly in South Bulgaria and in one locality in the Balkan foothill region, near town Tărnovo (Kitanov, 1963). It shows no considerable variation in the common morphology.

$2n=2x=10$ (Fig. 11)

Voucher specimen: Black Sea coast, to the south of town Mičurin, 25 VI 1972, SK-221069.

Our diploid chromosome number is reported for the second time after Roux (1957), see Fernandes a. Queiros, 1969), while Avdulov (1931) and Fernandes and Queiros (l. c.) report $2n=30$, and Mimeur (1950)—$2n=28$.

The karyotype has comparatively small chromosomes, submediatly constricted: a pair of long almost metacentric chromosomes: a pair with a slightly shorter one arm and with a secondary constriction on the long arm; a pair of almost acrocentric chromosomes of the same size; a pair of short almost metacentric chromosomes and a pair of SAT — telocentric chromosomes.

It is easily seen that the karyotype is the most specialized among all karyotypes studied. In comparison, though with the studied one (Avdulov, l. c. and Fernandes a. Queiros l. c.), which is hexaploid, $x=5$, the karyotype described by us has longer

chromosomes with well visible centromeres, most of them similar to the longer chromosomes of the complements $2n=30$ studied by the authors cited. With respect to this fact as well as on the base of two basic numbers within this species ($x=5$ and $x=7$, see Fedorov a. al., 1969) the question of separating this species within the genus *Trachinia* by Link (1827) approved by Avdulov (l. c.) and Fernandes a. Queiros (l. c.) seems at least still open to further studies on different populations, untill they give enough reason to consider number $2n=28$ (Mimeur, 1950) as a result of a miscount, as Fernandes and Queiros (l. c.) suppose.

CONCLUSIONS

From this study the following conclusions can be drawn:

1. Chromosomal variation in the genus *Brachypodium* in Bulgaria shows a recent development of the genus under conditions of the Balkan Peninsula. The variation due both to chromosomal multiplication — euploidy, and to increasing diploidy (Table 1) and hybridization.

Table 1. A comparative table to show the chromosomal changes in *Brachypodium* genus

Increasing diploidy	Basic numbers		*Brachypodium* genus		
			pinnatum	*silvaticum*	*distachyon*
	$x=9$		*2n=18*	2n=18(2x) **2n=18**	
Decreasing diploidy	$x=8$		**2n=16(2x)**	**2n=16(2x)**	
		8x	*2n=56*	**2n=56**	
		6x	2n=42	**2n=42+2**	
	$x=7$	4x	2n=28 **2n=28**	**2n=28**	2n=28
		2x	**2n=14**	**2n=14**	*2n=14*
	$x=5$	6x			2n=30
		2x			2n=10 **2n=10**

The light figs. — old counts (lit.); the heavy figs. — our counts; the figs. in italics — chromosome numbers expected.

B. glucovirens ($2n=16$) is included in the evolutionary line of *B. silvaticum* from which it is separated.

Both types of chromosomal changes are of evolutionary significance to the genus, but they seem not to be correlated with the general morphology of the species. Additional studies should give more information about the connection between chromosomal variation and the epidermal structure, vascular bundles, etc.

2. The basic chromosome number of the genus seems to be $x=7$; it has given rise to some euploid complexes as well as some increasing diploid series in the perennial species and a dicreasing diploid trend in the annual species.

In all of the perennial species the following basic chromosome numbers should be expected: $x=7$, $x=8$, $x=9$ and in the annual: $x=5$, $x=6$ and $x=7$.

The karyotype of the annual *B. distchyon* seems more advanced in that it is less symmetrical than the karyotype of the other perennial species.

3. The basic chromosome number $x=7$ in *B. silvaticum* which is primary to all of the species, shows it is unreasonable to give this species the rank of a genus. The presence of this basic number in *B. pinnatum* as well, disproves the scheme of Fernandes a. Queiros (1969). The tetraploid number $2n=28$ seems to rise more simply: $2x=14 \rightarrow 2x=28 \rightarrow 2x=56$, the last number is found in *B. silvaticum* and is expected in *B. pinnatum* (see Table 1). The *Brachypodium* genus appears to be a good example of the Vavilov's law of homologous variation (Vavilov, 1922).

The relative uniformity of the chromosomal types of *B. distachyon* in comparison with the other species as well as the possibility of having $2n=28 (x=7)$ does not support the idea for delimiting this species into a genus.

In spite of many contradictionary views about the place of the genus as a whole, so far we cannot see enough reasons to consider it heterogeneous and to split it.

REFERENCES

1. Achtarov B. (1960). Mitteilung. Bot. Inst. bd. VII: 9 - 36.
2. Avdulov N. P. (1931). Karyo-Systematische Untersuchung der Familie Gramineen. Suppl. 43th of Applied Bot. Genet. and Plant-breed., 44. Leningrad.
3. Fedorov A. edit. et al. (1969). Chromosome numbers of Flowering Plants. Leningrad.
4. Fernandes A. and Queiros M. (1969). *Gramineae* I., Bol. Soc. Broteriana, 43, ser. 2A: 20 - 140.
5. Hayek A. (1933). Prodromus Florae Peninsulae Balcanicae, Berlin, 3.
6. Hitchcook A. (1927). The grasses of Ecuador, Peru and Bolivia. Contrib. from the Unit St. National Herbarium, vol. 24, part 8.
7. Host N. (1809). *Gramineae austriaca* IV, 10, Wien.
8. Kitanov B. (1963). Flora Reip. Popul. Bulgaricae, vol. 1: 433 - 436.
9. Kožucharov S. and Kuzmanov B., (1970). Mitteilung. Bot. Inst. bd. 20 : 99.
10. Krause E. (1912). Systematik Beh. zum Bot. Zentralbl. bd. 20, hf. 2.
11. Link J. H. F. (1827). Hortus regius botanicus Berolinensis descriptus Berolini, 1 : 43.
12. Löve A. and Löve D. (1961). Bot. Notiser, 114, 1 : 33 - 47.
13. Mimeur G., (1950). Bull. Mus. Natl. Hist. Nat. (Paris) 22, ser. 2, 1 : 130.
14. Pearse E. (1962). Chistochimia, Moskva.
15. Prat H. (1936). Ann. Sci. Nat. ser. 10, bot. 17 - 18: 202.
16. Stojanov N., Stefanov B. and Kitanov B. (1966). Flora of Bulgaria, ed. IV, part 1, Nauka i iskustvo, Sofia.
17. Vavilov N. J. (1922). Journ of Genet., 12 : 47 - 90.
18. Velenovsky I. (1893). Flora Bulgarica. Pragae.

ZRÓŻNICOWANIE CHROMOSOMÓW W OBRĘBIE BUŁGARSKICH GATUNKÓW *BRACHYPODIUM* Z UWZGLĘDNIENIEM ICH EWOLUCJI I TAKSONOMII

Streszczenie

U 4 gatunków rodzaju *Brachypodium* stwierdzono następujące liczby chromosomów: *B. pinnatus* — $2n=14$, $2n=16$, $2n=28$; *B. glaucovirens* — $2n=16$; *B. silvaticum* — $2n=18$, $2n=28$, $2n=42+2$, $2n=56$; *B. distachyon* — $2n=10$.

W wyniku dotychczasowych badań nie zdołano ustalić korelacji między morfologią i liczbą chromosomów u różnych cytotypów.

Zakłada się, że euploidy i disploidy odgrywają jednocześnie rolę ewolucyjną dla rodzaju, który wydaje się znajdować w stanie wtórnej specjacji.

ДИФФЕРЕНЦИРОВАНИЕ ХРОМОСОМ В БОЛГАРСКИХ ВИДАХ *BRACHYPODIUM* С УЧЕТОМ ИХ ЭВОЛЮЦИИ И ТАКСОНОМИИ

Резюме

В 4 видах рода *Brachypodium* констатированы следующие количества хромосом: *B. Pinnatus* – $2n=14$, $2n=16$, $2n=28$, *B. glaucovirens* – $2n=16$; *B. silvaticum* – $2n=18$, $2n=28$, $2n=42+2$, $n=56$; *B. distachyon* – $2n=10$.

В результате проведенных до сих пор исследований не установлена взаимозависимость морфологии и количества хромосом в разных цитотипах.

Предполагается, что эуплоиды и дисплоиды играют одновременно эволюционную роль для рода, который находится, по всей вероятности, в состоянии вторичной специации.

Translated by M. Podgórska M. Sc.