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Original Contribution

CHENOPODIUM BONUS HENRICUS L. (PERENNIAL GOOSEFOOT) IN BULGARIA: I. POPULATION VARIABILITY

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ABSTRACT
Ten populations of *Chenopodium bonus-henricus* L., belonging to 6 different phytogeographic regions have been karyologically and morphologically tested. Intrapopulation and interpopulation variability have been traced. The relationship between morphological and karyological variability, the number, area, ecological, and geographic appurtenance of the studied populations has been explored.

Key words: *Chenopodium,* karyology, morphology, variation.

INTRODUCTION

The present study is a part of a multi-annual research on genus *Chenopodium* within the Bulgarian flora and it is concentrated on *Chenopodium bonus-henricus* L., one of the species with the broadest economic use. It has relatively numerous populations occurring in mountainous pasture grounds with grayish-brown, maroon and mountainous brown forest soils. In the recent years its natural habitats have been a subject of enhanced exploitation.

The objectives of our study were to estimate the karyological and morphological population variability of the species and to examine the relationship between the degree of morphological and karyological variability, the area, number, ecological, and geographic appurtenance of the studied populations.

MATERIAL AND METHODS

The subject of our study was 10 populations from 6 floristic regions **(Table 1).** The number and morphology of chromosomes have been used for karyological analysis. The karyotype characteristics have been based on metaphase plates obtained from root tops of seeds germinating in laboratory conditions. The roots were treated and squashed according to the accepted methods (1).

The morphological analysis comprises 24 quantitative characters reported in 30 specimens of each population: 1. plant height; 2. basal leaf length of; 3. basal leaf width; 4. length/width ratio; 5. basal leaf petiole length; 6. upper leaf length of; 7. upper leaf width; 8. length/width ratio; 9. upper leaf petiole length; 10. inflorescence length; 11. flower petiole length; 12. flower diameter; 13. perianth lobes length of bisexual flower; 14. perianth lobes width of bisexual flower; 15. perianth lobes length of female flower; 16. perianth lobes width of female flower; 17. seed length; 18. seed width; 19. length/width ratio; 20. seed thickness; 21. fruit length; 22. fruit width; 23. length/width ratio; and, 24. fruit thickness.

The morphological data have been processed by the program Statistica of Statsoft (2001). The population means and the coefficients of variation (V in %) have been estimated for each character of each population. Their values have been used in the comparison of various characters within the populations and between the populations. The Cluster analysis (CA) has been applied for exploration the hierarchical classification of the populations using the Unweighted pair-group average algorithm. The

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Euclidean distance (Ed) in the multivariate set of all morphological characters has been chosen as a measure of similarity between the populations. The one-way ANOVA method has been applied to determine the relative share of intrapopulation and interpopulation variability.

Table 1. *Studied populations of Chenopodium bonus-henricus* L.

Population No.	2n	Locality	Ecological conditions	Structure, number, area
No. 93	36	Stara planina (West), abandoned pasture ground above the town of Etropole.	Sloped terrain facing east, grayish-brown forest soils, well moistened, altitude 618 m, ruderal community dominated by *C. bonus- henricus*.	Mosaic spatial structure, number - 500 specimens, area 440 m^2.
No. 28**	36*	Stara planina (Central), Buzludzha, abandoned pasture ground to the right of the road for the village of Shipka.	Sloped terrain facing east, mountainous brown forest soils, well moistened, altitude 1398 m, ruderal community dominated by *Rumex alpinum* L.	Mosaic spatial structure, number - 300 specimens, area 560 m^2.
No. 111	36	Stara planina (Central), National Park "Central Balkan", summer sheep pen 500 m from Nezabravka hut.	Sloped terrain facing south-east, mountainous brown forest soils, altitude 1150 m, ruderal community dominated by *C. bonus-henricus* .	Mosaic spatial structure, number -700 specimens, area 940 m^2.
No. 113	36	Stara planina (Central), town of Troyan, abandoned pasture ground above town.	Sloped terrain facing south, grayish-brown forest soils, altitude 470 m, ruderal community dominated by *C. bonus-henricus*.	Mosaic spatial structure, number - 210 specimens, area 540 m^2.
No. 27	36	Vitosha region, Vitosha mountain, abandoned pasture ground.	Sloped terrain facing south-west, leached maroon forest soils, altitude 1077 m, ruderal community dominated by *Rumex alpinum*.	Mosaic spatial structure, number - 90 specimens, area 430 m^2.
No. 112	36	The Slavianka, above the village of Paril.	Sloped terrain facing south, mountainous brown forest soils, altitude 812 m, ruderal community dominated by *C. bonus-henricus.*	Mosaic spatial structure, number - 140 specimens, area 520 m^2.
No. 115	36*	The Pirin (South), abandoned pasture above the town of Razlog.	Sloped terrain facing south, mountainous brown forest soils, altitude 900 m, ruderal community dominated by *Rumex alpinum*.	Mosaic spatial structure, number - 220 specimens, area 650 m^2.
No. 110	36	The Rila, Resort Borovets in low places.	Sloped terrain facing south-east, mountainous brown forest soils, altitude 900 m, ruderal community dominated by *C. bonus-henricus.*	Mosaic spatial structure, number - 50 specimens, area 180 m^2.
No. 114	36*	The Rhodopes (West), hut Beglica, pasture.	Slightly sloped terrain, mountainous brown forest soils, well moistened, altitude 1500 m, ruderal community dominated by *C. bonus-henricus*.	Mosaic spatial structure, number above 1000 specimens, area 5,4 dka.
No. 301	36	The Rhodopes (Central), open meadow over Bachkovo monastery.	Slightly sloped terrain, mountainous brown forest soils, well moistened, altitude 1040 m, ruderal community dominated by *C. bonus-henricus* and subdominated by *Urtica dioica* L.	Mosaic spatial structure, number - 900 specimens, area 930 m^2.

* chromosome numbers are published by Grozeva & Stoeva (2)

** morphometric data about plants from this population are published by Grozeva (3)

RESULTS AND DISCUSSION

Karyology

Chromosome number 2*n* = 36 has been established for all studied populations **(Table 1)**. Our results confirmed both the data for a species population in Rila around Panitsite (4), in Stara planina, Pirin and West Rhodopes (2) and the ones in Western Europe (5, 6, 7, 8), from the former USSR (9), from Japan (10) and from the Slovak Republic (11). The karyotypes of 9 from all 10 studied species populations (No.28 from Buzludzha, No.93 above Etropole, No.111 from Nezabravka hut, No.113 above Troyan, No.115 from Pirin, No.37 from Vitosha, No.112 from Slavyanka, No.110 from Rila, No.301 from the Middle Rhodopes) are from 4 pairs of metacentric and 14 pairs of submetacentric chromosomes and in the first 4 populations (№28, №93, №111, №113) of one of the submetacentric chromosome pairs satellites have been established **(Fig. 1)**, and in the other 5 populations – №115, №37, №112, №110, №301 there are no satellites **(Fig. 2)**. The karyotype of population No.114 from the Western Rhodopes differs from that of the other 9 populations with extra 2 pairs of metacentric chromosomes and it comprises 6 pairs of metacentric and 12 pairs of submetacentric chromosomes.

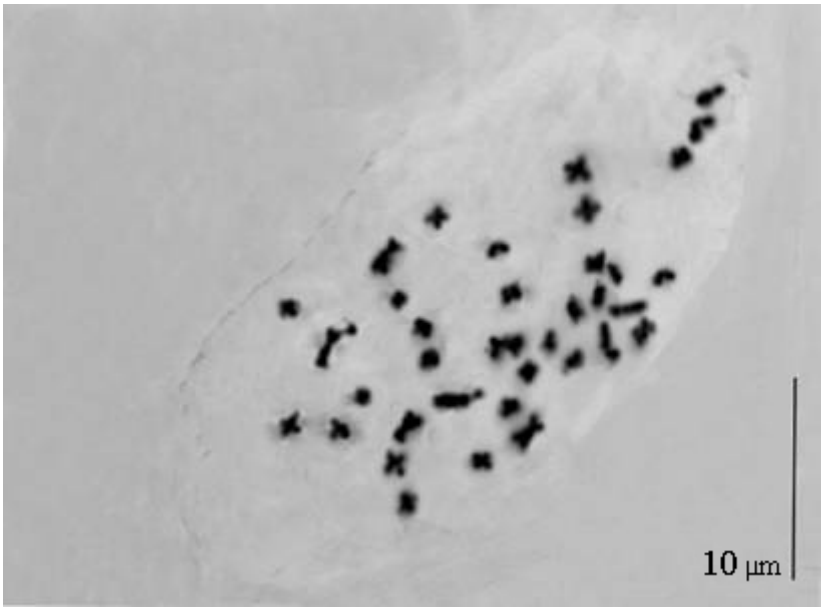

Fig. 1. Microphotograph of the metaphase plate of Population No.111 from National Park „Central Balkan", 2*n* = 36.

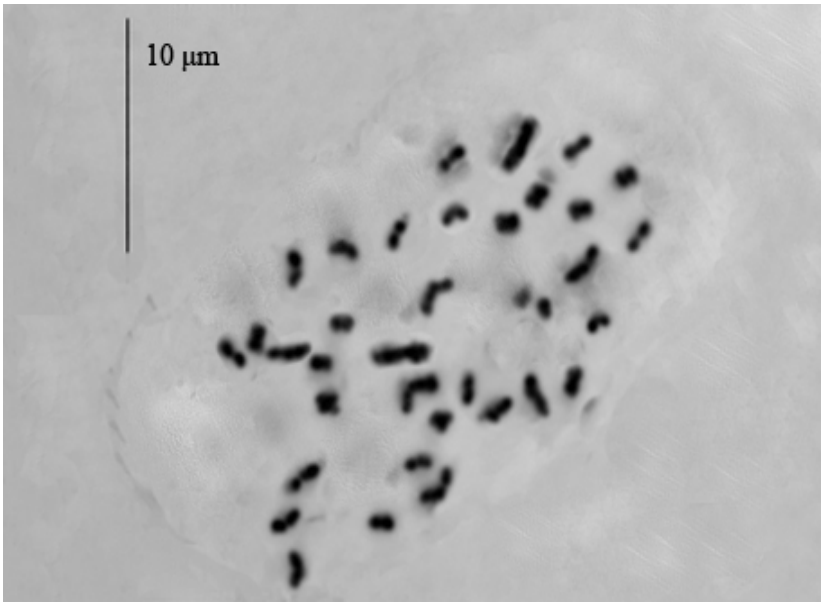

Fig. 2. Microphotograph of the metaphase plate of Population No.301 above the Bachkovo monastery, 2*n* = 36.

Morphology

Intrapopulation variability. The comparison of variation coefficients reveals that they are different both for the various characters within each population and among populations. Their values vary from 0% to 55.74% **(Table 2)**. The vegetative characters have higher level of variability than the generative ones within each population, and the most variable are the traits characterizing the leaves, the stem height and the inflorescence length. The least variable are the ones that characterize the seed and fruit. No variability has been established for the characters fruit width, seed length, and seed thickness of the Population No.112

The results from the ANOVA show that the intrapopulation variability is less pronounced than the interpopulation one for 19 of the characters **(Table 2)**. That corresponds with the smaller area and number of population, and the similar ecological conditions of its territory. The intrapopulation variability is dominating for the other 5 characters – stem height, inflorescence length, length and width of leaf lamina in basal leaves and correlation between them. According to our observations, this variability is strongly affected by the nature of the species habitats. It is spread mostly on mountainous pasture grounds and is grazed by animals. The uneven tramping and grazing delays the growth of some specimens in the population, and results in considerable variation in the values of traits characterizing stem height and respectively affects the inflorescence length and the leaves size.

Table 2. *Mean (first line) and coefficient of variation in % (second line) of C. bonus henricus populations. Percentage of the interpopulation variation in the overall morphological variation for each character (SSb).*

Population **Character number**	**93**	**28**	**111**	**113**	**27**	**112**	**115**	**110**	**114**	**301**	SSb
1	59,30	59,3	66,72	63,09	42,83	45,97	47,8	52,97	48,32	49,55	
	33,14	33,1	25,03	22,86	21,80	35,94	21,68	28,88	23,48	22,89	33,35
2	7,13	7,13	7,20	8,69	7,08	7,37	8,07	7,53	8,96	8,81	
	33,53	33,53	32,53	34,78	21,30	20,21	31,24	19,88	30,01	28,18	41,12
3	6,83	6,63	6,88	7,71	6,74	6,07	7,58	7,23	8,68	8,73	
	34,55	34,55	33,29	30,37	23,06	24,26	30,17	22,05	31,07	30,26	43,09
4	1,30	1,06	1,04	1,12	1,07	1,24	1,07	1,06	1,05	1,04	
	43,85	17,92	16,98	16,07	15,89	16,13	12,15	12,26	11,75	10,68	44,42
5	10,36	10,30	10,46	10,44	7,02	10,74	8,24	7,17	8,94	8,73	
	40,05	38,05	55,74	42,21	44,77	40,80	44,85	43,74	43,18	42,02	58,00
6	4,84	4,85	2,92	1,87	1,87	2,12	1,97	1,93	1,99	2,02	
	32,23	37,23	46,11	23,55	15,62	22,61	21,50	15,54	20,52	18,48	54,83
7	2,32	2,30	1,55	1,26	1,36	1,48	1,31	1,39	1,34	1,37	
	45,54	55,24	22,74	22,60	32,49	37,80	28,82	29,38	27,62	25,08	59,26
8	1,30	1,20	2,05	1,52	1,50	1,57	1,54	1,45	1,58	1,55	
	43,85	32,50	50,32	25,00	28,00	28,66	21,43	25,52	22,23	21,16	62,69
9	0,52	0,52	0,31	0,23	0,22	0,27	0,25	0,22	0,26	0,28	
	42,17	44,17	43,60	45,03	47,22	36,57	50,65	47,23	47,76	41,44	59,34
10	37,89	37,73	37,73	28,18	17,18	21,17	21,34	17,18	22,47	21,84	
	45,15	45,10	45,17	32,35	26,56	31,17	32,27	26,57	30,74	28,63	56,97
11	0,24	0,23	0,34	0,31	0,31	0,32	0,31	0,31	0,38	0,37	
	20,71	20,71	26,30	31,97	21,34	20,45	21,35	21,35	18,95	15,98	40,08
12	1,16	1,14	1,16	1,25	1,30	1,33	1,29	1,30	1,41	1,43	
	4,29	4,29	4,30	10,24	10,61	7,07	12,37	10,62	11,83	9,43	54,39
13	1,34	1,33	1,37	1,40	1,41	1,35	1,39	1,38	1,43	1,44	
	4,16	4,16	5,13	6,34	8,46	4,65	6,63	8,07	6,38	7,03	69,28
14	0,54	0,55	0,57	0,58	0,61	0,61	0,59	0,61	0,67	0,65	
	9,13	9,13	11,25	10,15	7,07	9,31	9,83	7,08	9,23	8,37	66,60
15	1,30	1,32	1,30	1,32	1,43	1,35	1,38	1,43	1,48	1,47	
	5,51	5,51	5,51	6,72	7,79	6,05	9,28	7,80	8,83	7,94	53,62
16	0,53	0,52	0,53	0,55	0,57	0,51	0,57	0,57	0,63	0,65	54,04

	8,98	8,99	8,99	10,33	9,64	6,74	11,43	9,65	10,37	11,76	
17	1,32	1,30	1,32	1,32	1,33	1,31	1,33	1,33	1,35	1,34	
	5,41	5,41	5,41	6,01	3,50	0,00	5,92	3,50	6,04	5,47	56,75
18	1,25	1,27	1,25	1,28	1,33	1,33	1,30	1,33	1,34	1,33	
	6,56	6,56	6,02	6,93	3,50	2,02	9,97	3,50	8,79	7,83	60,63
19	1,00	1,05	1,05	1,03	1,00	1,00	1,03	1,00	1,05	1,08	
	2,00	7,62	7,60	9,70	5,00	2,00	12,62	5,00	11,84	10,08	57,87
20	0,89	0,90	0,93	1,00	1,01	1,00	0,99	1,01	1,07	1,06	
	7,68	7,68	7,62	8,06	5,42	0,00	10,39	5,42	9,96	8,22	57,90
21	1,45	1,44	1,45	1,44	1,43	1,47	1,44	1,43	1,44	1,45	
	4,34	4,34	4,36	4,65	3,25	3,41	5,65	3,25	5,27	4,78	65,61
22	1,39	1,40	1,39	1,40	1,43	1,40	1,41	1,43	1,43	1,43	
	6,20	6,20	6,20	6,08	3,25	0,00	7,45	3,25	7,03	6,81	57,10
23	1,06	1,04	1,04	1,03	1,00	1,06	1,04	1,00	1,06	1,05	
	3,77	5,77	5,77	7,77	4,00	3,77	9,60	5,00	9,43	8,39	63,35
24	0,99	0,99	0,99	1,05	1,11	1,11	1,06	1,11	1,12	1,14	
	7,44	7,44	7,45	7,83	4,93	4,95	9,14	4,80	7,83	7,12	71,12

Interpopulation variability. The comparison of the values of the Euclidean distances **(Table 3)** reveals that interpopulation differences are the greatest between the two populations from the Rhodopes (No.114, No.301) and the 4 studied populations from Stara planina (No.93, No.28, No.111, No.113).

Table 3. *Values of Euclidean distance between the pairs of populations within C. bonus-henricus based on 24 characters.*

Number of population	**93**	**37**	**28**	**110**	**111**	**112**	**113**	**114**	**115**	**301**
93										
27	267									
28	5	267								
110	220	102	220							
111	77	317	77	250						
112	215	63	215	89	266					
113	109	234	109	154	104	186				
114	513	398	511	381	523	408	449			
115	204	67	204	68	252	35	169	405		
301	490	377	498	375	509	423	312	65	310	

The dendrogram generated as a result of the CA shows, that the 10 studied populations are grouped in three main clusters – A, B and C **(Fig. 3)**.

Cluster A comprises the populations from Stara planina – No.93 above Etropole, No.28 from Buzludzha, No.111 from Nezabravka hut and No.113 above Troyan. The similarity observed between them is accounted for by their appurtenance to the same floristic region and the lack of any differences in their karyotypes. Extremely high similarity has been registered between the population from Buzludzha and the one above Etropole (Ed=5), located at quite different altitudes, but situated on a slope with the exposure and their development takes place at soil moisture similar and higher than that of the other Stara planina populations.

Cluster B amalgamates 4 populations (No.112 from Slavyanka, No.115 from Pirin, No.27 from Vitosha, No.110 from Rila), which are from 4 different floristic regions but have identical karyotypes. The observed greater similarity between the first two populations corresponds to their similar area, number and slope exposure.

Cluster C consists of the two populations from the Rhodopes – No. 114 from Beglika area in the Western Rhodopes and No.301 above Bachkovo monastery in the Central Rhodopes. The similarity established between them is accounted for by their appurtenance to the same floristic region. They are located on a slightly sloped terrain and their vegetative development takes place in relatively high soil moisture. The plants from these populations

have larger basal leaves and bigger flowers. The recorded differences between them are most likely affected by the differences in their karyotypes.

The results from the ANOVA reveal that in 19 of the quantitative characters the interpopulation variability is more pronounced **(Table 2)**, which correlates with the differences in altitude and respectively the ecological conditions, and according to our data, it is also influenced by the differences in their area and number.

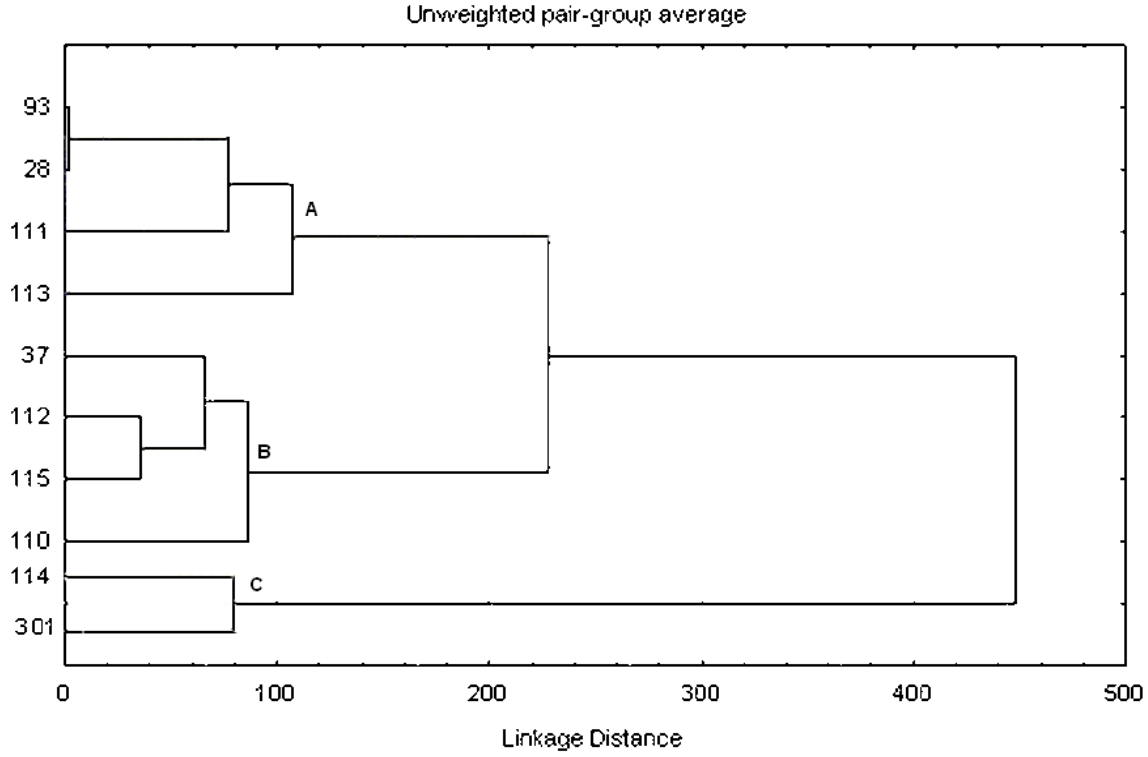

Fig. 3. Dendrogram of the studied populations of *C. bonus-henricus*

CONCLUSION

The conducted study reveals that the chromosome number in all Bulgarian populations of *Chenopodium bonus-henricus* is $2n = 36$, and the karyotype is variable and rather asymmetric. There is correlation between karyotype variability and the level of interpopulation and intrapopulation differences on one hand, and with the ecological conditions of the respective habitats on the other.

In all populations the vegetative characters are more variable than the generative ones. With the highest range of variability are the traits characterizing the stem height, length inflorescence, and dimensions of the leaf lamina. The least variable are the ones characterizing the seed and fruit.

The interpopulation variability is dominating in all populations. The recorded higher intrapopulation variability in 5 of the characters: stem height, inflorescence length, length and width of the leaf lamina of the basal leaves and the ratio between them is accounted for by the nature of the species habitats.

The interpopulation differences, according to the overall morphological trait complex, are influenced by the appurtenance of populations to various floristic regions, the ecological conditions of the various habitats, and the differences in their area and number. Among the 10 studied populations the two from the Rhodopes have the most pronounced interpopulation differences. Their specimens are characterized by the largest basal leaves and flowers.

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