

MORPHOLOGICAL AND CYTOTAXONOMIC  
INVESTIGATIONS OF *HELLEBORUS*  
*MULTIFIDUS* VIS.

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Introduction

Species of the genus *Helleborus*, widely distributed in Yugoslavia, are distinguished by morphological variability. This property makes it difficult to determine the taxonomic unit to which various specimens of this genus belong.

This paper contains some results obtained by morphological and cytotaxonomic investigations of the polymorphic species *Helleborus multifidus* Vis., widely distributed in the west and south-west parts of Yugoslavia. In our opinion this species comprises three groups, well differentiated from morphological, ecological and geographical points of view: subsp. *laxus* (Host) Martinis, *multifidus* and *intermedius* (Host) Martinis.

All the three subspecies were investigated cytologically and it was found that the chromosome numbers are  $2n = 20$  and  $2n = 30$  with a relatively large number of B chromosomes (1—11). According to information from the literature (Langlet 1927, 1932, Gregory 1941, Bolkovskikh et al. 1969), for *Helleborus* species only the chromosome number  $2n = 32$  has been noted, without any detailed data about the karyogram.

Material and Methods

Cytotaxonomic investigations have been carried out on plants brought from natural habitats and raised in Fran Kušan Botanical Garden in Zagreb. Morphological investigations were made on living material as well as on exsiccates kept in the proper herbarium.

The subspecies of *Helleborus multifidus* are widely distributed in the following areas: subsp. *laxus* — north-west part of Yugoslavia around the Sutla; subsp. *multifidus* — in the area of Istra, Kvarner and Gorski Kotar; subsp. *intermedius* — in the mountainous areas from Slovenija to western Hercegovina (fig. 1, tab. 1).

Table 1. Habitats and localities of *Helleborus multifidus*.

Tabela 1. Staništa i nalazišta vrste *Helleborus multifidus*.

Subspecies Podvrsta	Locality Nalazište	Habitat Stanište	2n	Collector Sakupio
<i>Helleborus multifidus</i> subsp. <i>laxus</i>	Zelenjak (Hrv. Zagorje, 1)	<i>Quercus-Carpinetum croaticum</i>	30+1-11 B	Martinis 1970
<i>Helleborus multifidus</i> subsp. <i>multifidus</i>	Učka (Istra, 2)	<i>Fagetum croaticum seslerietosum</i>	30+1-6 B	Kušan 1968
	Merag (Cres, 3)	<i>Quercus-Carpinetum submediterraneum</i>	30+1-6 B	Martinis 1965
	Omišalj (Krk, 4)	<i>Carpinetum orientalis croaticum</i>	30+1-9 B	Martinis 1963
	Otočac (Lika, 5)	<i>Quercus-Carpinetum croaticum</i>	30+1-3 B	Martinis 1970
<i>Helleborus multifidus</i> subsp. <i>intermedius</i>	Klek (G. Kotar, 6)	<i>Fagetum croaticum montanum</i>	20+1-4 B	Klapka 1964
	Perušić (Lika, 7)	<i>Quercus-Carpinetum croaticum</i>	30+1-3 B	Tomašević 1964
	Cincar (Bosna, 8)	<i>Fagetum croaticum montanum</i>	30+1-3 B	Kušan 1952

For purposes of cytotaxonomic investigations, root tips were pre-treated with  $\alpha$ -monobromnaphthalene at the temperature of  $-4^{\circ}\text{C}$  for about 20 hours, and fixed in 1:3 aceto-alcohol. The preparations were made by squashing, and stained with aceto-carmin. In the morphometric examination of leaves near the ground, the total number of leaflets was statistically investigated (20 samples from each locality) and classified in four groups: I — oligofid leaves with less than 10 leaflets, II — normofid 11—20, III — polyfid 21—40 and IV — multifid leaves with more than 40 leaflets.\*

\* The description of leaflet types as well as the nomenclature of leaflets have been used for the first time by Zlatan Martinis in the paper: "Some problems of polymorphism, geographical variation and taxonomic differentiation in the genus *Helleborus*". I. jugoslavenski simpozij iz sistematike, Sarajevo 1971.

## Results

Morphometric investigations have show that in populations of the *Helleborus multifidus* the ontogenetic and ecological gradient of polyfidity can be clearly observed; the young specimens as well as those in deeper shade have proportionally fewer leaflets, than the old specimens or specimens growing in conditions of strong insolation. Particular groups of populations show more or less clearly marked geographical gradient of leaflets number. Slightly lobed leaves — oligofid and normofid — dominate in the continental area between the Sava

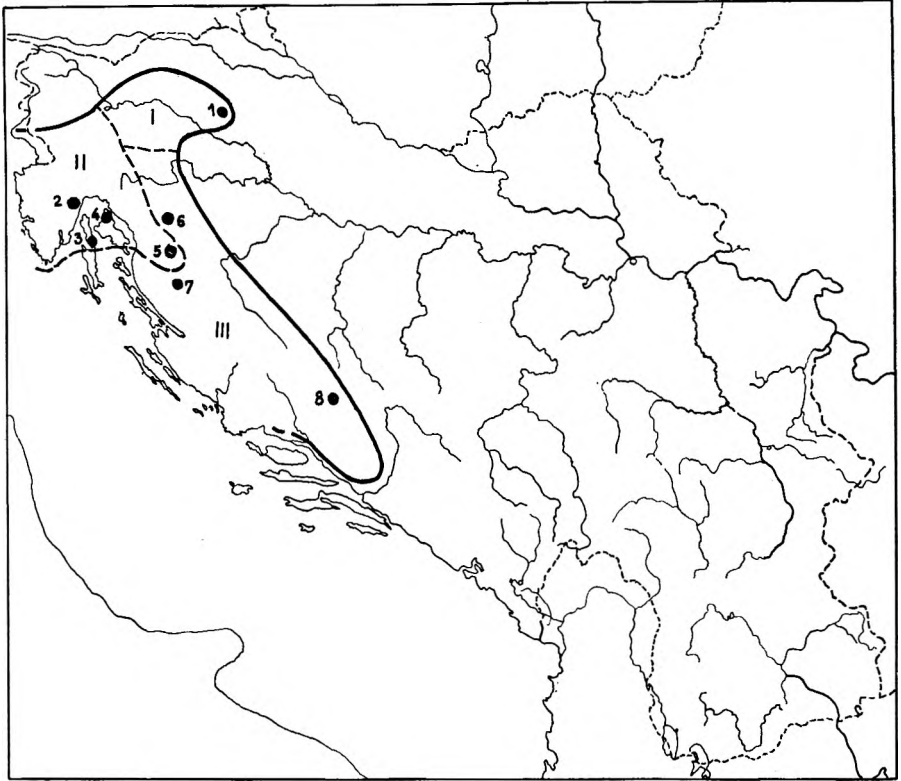


Fig. 1. *Helleborus multifidus*; localities of investigated samples (●). Approximate areas of subspecies: *laxus* — I., *multifidus* — II., *intermedius* — III.

Sl. 1. *Helleborus multifidus*; lokaliteti istraživanih primjeraka (●). Približni areali podvrsta: *laxus* — I., *multifidus* — II., *intermedius* — III.

and the Sutla, where the subsp. *laxus* is widely distributed. In the area of Istra, Kvarner and Gorski Kotar, where the subsp. *multifidus* is widely distributed the prevalence of normofid forms with an insignificant number of oligofid and polyfid forms can be established. In the area of the subsp. *intermedius* — Lika, Dalmacija and Western Bosna,

as well as in the south-east direction, the polyfid and multifid leaves predominate. Figure 2. shows the existence of four gradient classes of leaves in populations of *Helleborus multifidus* belonging to various geographical areas (fig. 2).

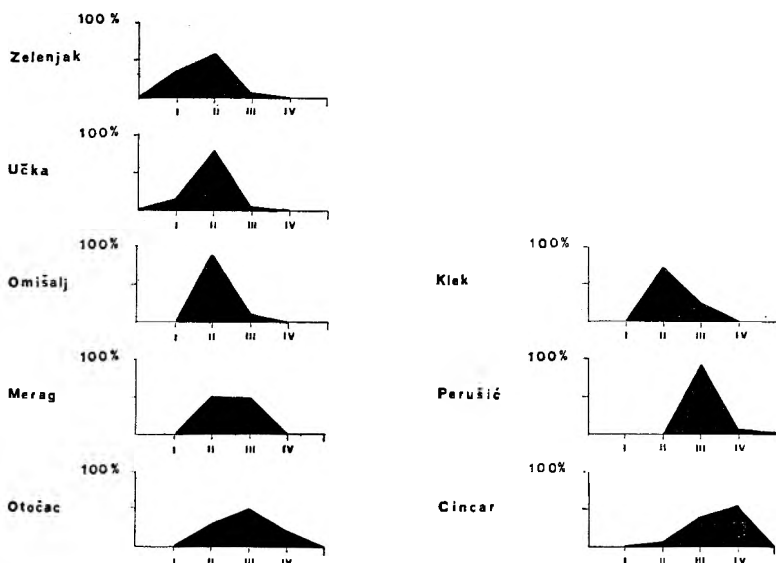


Fig. 2. Polygons of percentage share of gradient classes of leaflets in geographically different populations of *Helleborus multifidus*.

Sl. 2. Poligoni postotnog učešća gradijentnih razreda isjeckanosti listova u geografski različitim populacijama vrste *Helleborus multifidus*.

Preliminary results of cytotaxonomic investigations showed that the chromosome number is  $2n = 20 + 1 - 4B$ , and  $2n = 30 + 1 - 11B$  respectively. For the subspecies *intermedius* only (population from Klek) the chromosome number of  $2n = 20 + 1 - 4B$  was determined. The chromosome number of plants from populations on other localities (tab. 1) was exclusively  $2n = 30 + 1 - 11B$ . In the karyogram of the population from Klek three pairs of metacentric, three pairs of submetacentric and four pairs of subtelocentric chromosomes were determined, two of which with satellites on the short arm of the chromosome. There were also regularly  $1-4B$  chromosomes present, very likely acrocentric and with a satellite. Morphologically they are very similar to B chromosomes described in *Caltha palustris* (Reese 1954). The karyogram of plants of other populations with the chromosome number  $2n = 30 + 1 - 11B$ , showed 6 pairs of metacentric chromosomes, of which one with a satellite, 7 pairs of submetacentric chromosomes, of which two with a satellite and 2 pairs of acrocentric chromosomes with a satellite on the short arm. Figure 3 shows mitotic chromosomes of the population from Klek ( $2n = 20 + 2B$ ) and of the population from Otočac ( $2n = 30 + 3B$ ).

Table 2. *Helleborus multifidus*, somatic chromosomes measurement.  
 a) *H. multifidus* subsp. *intermedius*, population from Klek —  $2n = 20 + 2B$ .  
 b) *H. multifidus* subsp. *multifidus*, population from Otočac —  $2n = 30 + 3B$ .

Tabela 2. *Helleborus multifidus*, mjerenje somatskih kromosoma.  
 a) *H. multifidus* subsp. *intermedius*, populacija s Kleka —  $2n = 20 + 2B$ .  
 b) *H. multifidus* subsp. *multifidus*, populacija iz Otočca —  $2n = 30 + 3B$ .

Chromosomes	Length in $\mu\text{m}$	Relative length	F <sup>0</sup> %	Centromeres
Kromosomi	Dužina u $\mu\text{m}$	Relativna dužina		Centromeri
a) A	2,75+2,75=5,50	100	50	m
B	2,40+2,60=5,0	91	48	m
C	1,25+2,60=3,75	68	33	sm
Ds	0,75+3,0 =3,75	68	20	st
E	1,25+1,86=3,10	56	40	sm
Fs	0,90+1,70=2,60	47	35	sm
G	0,60+1,90=2,50	45	24	st
H	0,50+2,0 =2,50	45	20	st
I	0,50+2,0 =2,50	45	20	st
J	0,64+0,64=1,28	23	50	m
b) A	3,20+3,30=6,50	100	50	m
B	3,0 +3,0 =6,0	92	50	m
C	2,6 +2,4 =5,0	77	52	m
D	1,25+3,75=5,0	77	24	st
E	1,30+3,70=5,0	77	26	st
Fs	1,25+3,60=4,85	74	25	st
Gs	1,25+2,50=3,75	57	33	sm
H	1,25+2,50=3,75	57	33	sm
I	1,25+2,50=3,75	57	33	sm
Js	0,75+3,0 =3,75	57	20	a
Ks	0,50+3,20=3,70	57	13	a
L	1,0 +1,80=2,80	43	36	sm
Ms	1,20+1,30=2,50	38	48	m
N	1,25+1,25=2,50	38	50	m
O	1,10+1,15=2,25	34	48	m

Table 2 indicates the length of chromosomes in  $\mu\text{m}$  their relative length, and the position of the centromeres (F<sup>0</sup>% represents the relation in percentage between the average length of the short arm and the average total length of the chromosome). The chromosome pairs are given in alphabetic order, of values. Satellite chromosomes are marked by index s.

Figure 4 represents the schematic haploid karyogram of *Helleborus multifidus*, whose population has  $2n = 20 + 2B$  and  $2n = 30 + 3B$  chromosomes.

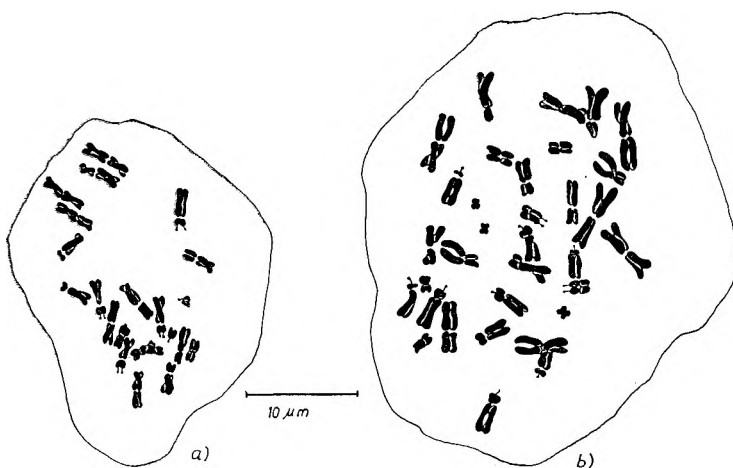


Fig. 3. a) *Helleborus multifidus* subsp. *intermedius*, population from Klek ( $2n = 20 + 2B$ ). Mitosis (acetocarmin). Drawing.  
 b) *H. multifidus* subsp. *multifidus*, population from Otočac ( $2n = 30 + 3B$ ). Mitosis (acetocarmin). Drawing.

Sl. 3. a) *Helleborus multifidus* subsp. *intermedius*, populacija sa Kleka ( $2n = 20 + 2B$ ). Mitoza (acetokarmin). Crtež.  
 b) *H. multifidus* subsp. *multifidus*, populacija iz Otočca ( $2n = 30 + 3B$ ). Mitoza (acetokarmin). Crtež.

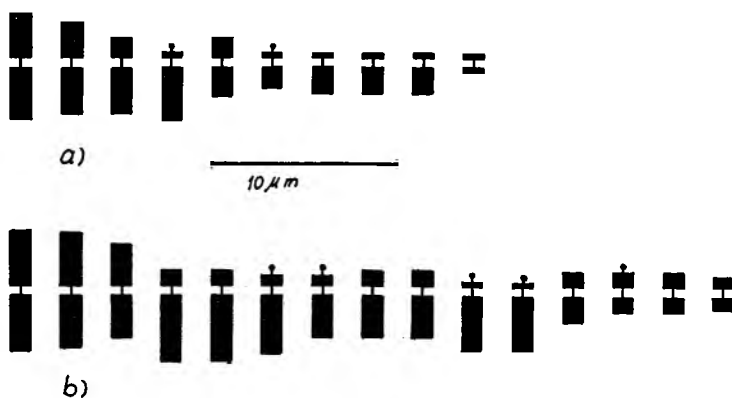


Fig. 4. *Helleborus multifidus*, schematic representation of a haploid karyogram.  
 a) *H. multifidus* subsp. *intermedius*, population from Klek ( $2n = 20 + 2B$ ).  
 b) *H. multifidus* subsp. *multifidus*, population from Otočac ( $2n = 30 + 3B$ ).

Sl. 4. *Helleborus multifidus*, shematski prikaz haploidnog kariograma.  
 a) *H. multifidus* subsp. *intermedius*, populacija s Kleka,  $2n = 20 + 2B$ .  
 b) *H. multifidus* subsp. *multifidus*, populacija iz Otočca ( $2n = 30 + 3B$ ).

The present investigations have shown that the number of B chromosomes within the *Helleborus multifidus* (for the populations with chromosome number  $2n = 30$ ) varies from 1—11. The most frequent were those with 1, 2, 4 and 11B chromosomes. The greatest number of B chromosomes has been noticed the populations of the north-west parts of Yugoslavia (Zelenjak), where we found 9—11B chromosomes exclusively. In the populations of south-west Yugoslavia 1—4B chromosomes were found (fig. 5). Shows the distribution of B chromosomes within *Helleborus multifidus*, in the population whose basic chromosome number is  $2n = 30$ . This distribution analysis was made on basis of 89 pictures of somatic mitosis.

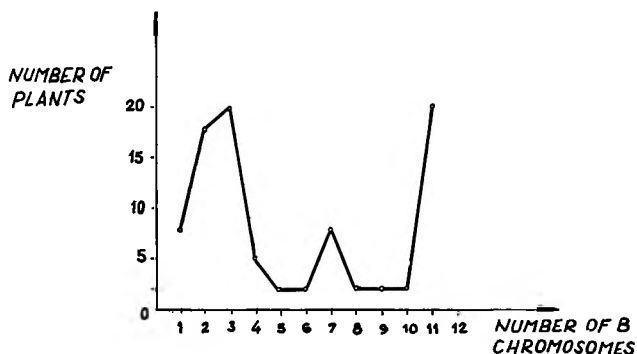


Fig. 5. Distribution of B chromosomes within the *Helleborus multifidus*, for populations with the chromosome number  $2n = 30$ .

Sl. 5. Distribucija B kromosoma unutar vrste *Helleborus multifidus*, za populacije kromosomskog broja  $2n = 30$ .

In the populations of *Helleborus multifidus* two types of B chromosomes have been found, the acrocentric one with a satellite and the small metacentric ones. The most usual are acrocentric forms. Both types of B chromosomes are very rarely found in the same population. Metacentric B chromosomes are mainly spread in the populations of Zelenjak and Otočac, while the acrocentric type only appears in other populations.

## Discussion

The morphologic variability, i. e. the polymorphism, is most clearly expressed in the form and number of leaflets on basal leaves. Even Visiani (1829), who was the first to describe *Helleborus multifidus* emphasized the considerable number of leaflets as an important systematic characteristic of the species. Later, he changed its name to *Helleborus viridis* var. *multifidus* (Visiani 1852) when he observed that in a relatively small area a specimen with a large number of leaflets can be found side by side with those with a small number of leaflets. He explained this fact by the existence of two varieties of *Helleborus viridis*, namely var. *viridis* and var. *multifidus*.

Later observations and our investigation have shown that these belonged to *Helleborus multifidus* which consists of specimens with different degrees of polyfidity.

The results of our investigations show a clear topoclinal variability of leaflets and an increase of geographic gradient from north-west to south-east. The constancy of this phenomenon under experimental conditions indicates the integrality of species *Helleborus multifidus* in the wider sense of the term, in which the intraspecies differentiation develops on basis of polyfidity and other morphologic characteristics such as for example: colour of flowers, indumentum shape etc.

Cytotaxonomic investigations brought forward the question of a very interesting species within which there is not only the variability of number but the variability of the morphologic qualities of the chromosomes too. The group *Helleborae* Stebbins (1950, 1971) reveals considerable morphologic variability of karyogram in which the satellite chromosomes are of particular interest. In *Helleborus multifidus* several types of satellite chromosomes were found — metacentric, submetacentric with 1 or 2 satellites, acrocentric with a satellite on the short arm and submetacentric with secondary constrictions and a satellite on the short arm. It was noticed in some populations with the chromosome number  $2n = 30 + 1 - 11B$ , that the number of metacentric chromosomes was reduced (from 6 to 2 pairs), while the number of various types of SAT chromosomes increased parallelly (from 3 to 6 pairs). In most cases the increase in the number of SAT chromosomes is connected with the reduction in the number of B chromosomes.

### S u m m a r y

1) The topoclinal geographical distribution of leaflet forms of basal leaves of *Helleborus multifidus* was established and it was found that the leaflet number gradient increases in the direction northwest-southeast. In the area where subsp. *laxus* is widely distributed, in the north-west part of Yugoslavia, oligofid and normofid leaves dominate; in the area of Istra, Kvarner and Gorski Kotar, where subsp. *multifidus* is widely distributed, the normofid forms are prevalent, while in the area where subsp. *intermedius* is widely spread, polyfid and multifid forms dominate.

2) The domination of one particular leaf form in the populations of certain geographic areas is constant, i. e. it does not change in culture under the same ecological conditions. This fact confirms the taxonomic value of this morphologic characteristic.

3) The chromosome number  $2n = 20 + 1 - 4B$ , and  $2n = 30 + 1 - 11B$  was determined. The karyogram of *Helleborus multifidus* was variable because of a change in the number of metacentric and SAT chromosomes.

4) Two types of B chromosomes were determined in populations: the acrocentric ones with a satellite, which predominated, and very small metacentric ones, which occurred in some populations only. Very rarely both types of B chromosomes were found in a population.



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## SADRŽAJ

### MORFOLOŠKA I CITOTAKSONOMSKA ISTRAŽIVANJA VRSTE *HELLEBORUS MULTIFIDUS* VIS.

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Morfometrijska analiza stupnja isjeckanosti plojke prizemnih listova većeg broja primjeraka vrste *Helleborus multifidus* pokazuje znatna odstupanja u isjeckanosti listova od tipičnog oblika na kojem se zasniva dijagnoza vrste (Schiffner 1890). Za morfometrijsku analizu isjeckanosti plojke prizemnih listova korišteni su razvijeni listovi fruktificirajućih primjeraka. Analizirani su uzorci od 20 listova sa svakog nalazišta, odnosno lokalne populacije. Prema ukupnom broju odsječaka plojke listovi su grupirani u 4 gradijentna razreda: I. do 10 odsječaka (oligofidni tip), II. 11—20 odsječaka (normofidni tip), III. 21—40 odsječaka (polifidni tip) i IV. više od 40 odsječaka (multifidni tip). Postotno učešće gradijentnih razreda isjeckanosti listova u geografski različitim populacijama vrste *Helleborus multifidus*, prikazano je poligonima (sl. 2).

Rezultati morfometrijske obrade pokazuju da je učešće pojedinih gradijentnih razreda u uzorcima populacija različitih geografskih područja različito. Uspoređivanjem poligona isjeckanosti listova može se jasno uočiti pravilnost u geografskoj distribuciji oblika listova, odnosno izraziti geografski gradijent isjeckanosti plojke. U kontinentalnom području između Save i Sutle, području rasprostranjenja subsp. *laxus*, do-

miniraju oligofidni i normofidni tipovi listova. U istarsko-kvarnersko-goranskom području, gdje je rasprostranjen subsp. *multifidus*, dominiraju normofidni, s neznatnim učešćem oligofidnih i polifidnih tipova. U području Pokuplja, Like, Dalmacije i zapadne Bosne prevladavaju polifidni i multifidni tipovi, osobito u jugoistočnom dijelu areala podvrste *intermedius*. Isti su odnosi primijećeni i na eksikatima s prirodnih staništa, kao i na materijalu u kulturi.

Rezultati naših istraživanja jasno pokazuju topoklinalni varijabilitet isjeckanosti listova, koji se očituje u porastu gradijenta isjeckanosti od sjeverozapada prema jugoistoku. Konstantnost te pojave i u eksperimentalnim uvjetima pokazuje ujedno da se radi o smjeni određenih topotipova, koji samim time imaju i izvjesno taksonomsko značenje. Kao što se vidi iz prikaza, visok stupanj isjeckanosti listova vrste *Helleborus multifidus* vrlo je jako izražen i stoji u vezi s ariditetom staništa. Vjerojatno se kod vrste *Helleborus multifidus* ekobiomorfogeneza i taksonomska diferencijacija odvija u pravcu kseromorfnosti polifidije. Sličan se proces može u izvjesnoj mjeri primijetiti i kod drugih vrsta roda *Helleborus*, sekcije *Helleborus*.

Preliminarna citotaksonomska istraživanja su pokazala da je najmanji nađeni broj kromosoma  $2n = 20 + 1 - 4B$ , populacija s Kleka. Biljke iz populacija s ostalih nalazišta imaju isključivo broj kromosoma  $2n = 30 + 1 - 11B$ . U kariogramu populacije s Kleka nađena su 3 para metacentričnih, 2 para submetacentričnih, te 5 parova suptelocentričnih kromosoma, od kojih su 2 para sa satelitom na kraćem kromosomskom kraku. Također se javljaju 1—4 B-kromosoma, koji su vrlo vjerojatno akrocentrični, a imaju satelit. Kod biljaka iz ostalih populacija, čiji je kromosomski broj  $2n = 30 + 1 - 11B$ , u kariogramu nalazimo 6 pari metacentričnih, od toga jedan sa satelitom, 7 pari submetacentričnih, od čega su 2 para sa satelitom, te 2 para akrocentričnih kromosoma sa satelitom na kraćem kraku. Unutar populacije vrste *Helleborus multifidus* nađena su 2 tipa B-kromosoma, akrocentrični sa satelitom, te mali metacentrični. Najčešći je akrocentrični tip, a vrlo rijetko u istoj populaciji nalazimo oba tipa B-kromosoma. U nekim populacijama kromosomskog broja  $2n = 30 + 1 - 11B$  primijećeno je da se smanjuje broj metacentričnih (od 6 na 2 para), a paralelno se povećava broj raznih tipova SAT-kromosoma (od 3 do 6 parova). Povećanje broja SAT-kromosoma u većini je slučajeva povezano sa smanjenjem broja B-kromosoma.

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