

Polyplody and hybridization in Bulgarian *Brassicaceae*: distribution and evolutionary role

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Abstract. The results from the investigation and analysis of the chromosome numbers and ploidy levels of 141 species and not nominal subspecies of *Brassicaceae*, their distribution and phytogeographical relations are discussed. Nearly 65 % of the taxa are diploids, most of them perennials, Mediterranean, Anatolian and Pontic floristic elements. Another 35 % are polyploids, c. 2/3 of them with tetraploid cytotypes. The rest of the polyploids have chromosome numbers ranging from hexa- to dodecaploid level. The polyploid cytotypes in *Erysimum*, *Rorippa*, *Cardamine*, *Alyssum*, and *Iberis*, occurring mostly in the Rilo-Rhodope Massif, are discussed in the light of the position of these mountains in a transitional climatic zone between continental European and Mediterranean climates, as well as of the role of the Quaternary climatic changes for stimulation of the hybridogenesis and species differentiation in the Southern Balkans, one of the major glacial refugia in South Europe.

Key words: *Alyssum*, *Brassicaceae*, Bulgaria, *Cardamine*, *Erysimum*, hybridization, *Iberis*, patterns of speciation, polyploidy, *Rorippa*, triploid hybrid

Introduction

The progress of biosystematic and phylogenetic research has increased the interest in hybridogenesis and polyploidy as major factors of speciation and evolution of higher plants. The use of molecular methods, isozyme and DNA techniques have significantly contributed to the knowledge of the polyploid genome, to its establishment and evolution (Soltis & Soltis 1993, 1995, 2000; Levin 2002; Marhold & Lihova 2006).

Polyplody, the possession of at least three complete sets of chromosomes, is a basic pattern for speciation in plants. Stebbins (1950, 1971) estimated that approximately 30–35 % of the flowering plants and much more of the ferns are polyploids. Greilhuber & Ehrendorfer (1988) assumed that at least 50 % of the higher plants consist of polyploid taxa. Reviewing the distribution of polyploidy in monocots, Goldblatt (1980) concluded

that, “at least 70 %, and most likely above 80 % of monocots are in some sense polyploid”. In *Brassicaceae*, on the basis of 42 % of studied species out of the total number in the family, it was estimated that 37 % were polyploids (Warwick & Al-Shehbaz 2006).

Polyploid plants may arise through fusion of two unreduced gametes (Harlan & de Wet 1975; Lewis 1980). Most often polyploidization results from fusion of diploid gametes and more seldom of unreduced with reduced ones, producing triploid plants. Such triploids can generate tetraploid progeny, or higher-numbered ploidal races (Levin 2002). According to their origin, polyploids relate to the categories of autoploids (intraspecific polyploids) or allopolyploids, which are products of interspecific hybridization. Segmental allopolyploids form a range of intermediate conditions arising from parents with partially divergent chromosome arrangements (Stebbins 1950; Soltis & Soltis 2000).

The outcrossing mating system, along with the perennial habit and vegetative reproduction are among the important factors for hybridization, formation and establishment of the polyploids. Among the other factors contributing to the success of polyploid plants is availability of new ecological niches, geographically and ecologically related to their parents (Stebbins 1950; Ehrendorfer 1980; Soltis & Soltis 1993, 2000). Equally important is the increased genetic diversity through multiple formations of a polyploid species and genome rearrangements. Molecular data demonstrate that both autopolyploids and allopolyploids exhibit a high frequency of recurrent formation. The multiple polyploidization events within species have significant genetic and evolutionary implications, and contradict the traditional view of autopolyploidy as being rare and maladaptive (Soltis & Soltis 1993, 1995, 2000; Levin 2002).

Polyploids and modes of hybridization in Bulgarian *Brassicaceae*

Crucifers are represented in the Bulgarian flora by 183 species and 30 not nominal subspecies. The karyological studies in the family comprise 76 % of the species and subspecies occurring in the country. Almost 35 % of these taxa are polyploids (Anchev 1997, 2001) (Fig. 1). The correlation of polyploid with diploid taxa in the studied sample is very close to the percentage of polyploids in the Bulgarian flora (cf. Kuzmanov 1993). The tetraploid species and tetraploid races dominate among the polyploids of *Brassicaceae*. The presence of hexaploid and higher cytotypes is more limited.

In order to evaluate the distribution and significance of polyploidy for speciation in the Bulgarian *Brassicaceae*, we have analyzed the probable modes of hybridization and the patterns of polyploidization of species and species groups of *Erysimum*, *Rorippa*,

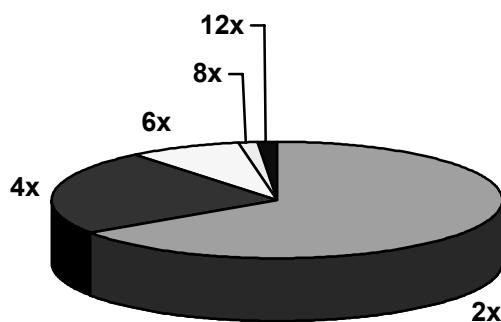


Fig. 1. Ploidy levels and ploidal structure of 142 species of *Brassicaceae* distributed in Bulgarian flora.

Cardamine, *Alyssum*, and *Iberis*, on the basis of our investigation of these genera, as well as of the preliminary results and hypotheses.

***Erysimum drenowskii* group and *E. diffusum* group**

The distribution of polyploidy in *Erysimum* and its role in the evolution of the polyploid species groups has been of interest in terms of the cytogenetics, taxonomy and speciation within these groups. It was considered that the main reason for this variability was the interspecific hybridization, with participation of plants with unbalanced meiosis, forming reduced and unreduced gametes (Favarger & Goodhue 1977; Favarger 1978, 1981; Correvon & Favarger 1979).

In the Bulgarian flora, considering the morphology, distribution and geography, chromosome numbers and ploidy levels, there are two groups of species close to *E. drenowskii* Degen and *E. diffusum* Ehrh., both with basic number $x=7$. Members of the first group are the diploid/tetraploid *E. drenowskii*, the hexaploid *E. pseudoatticum* Ančev & Polatschek and the dodecaploid *E. slavjankae* Ančev & Polatschek (Fig. 2). They are perennial, calciphylous, entomophylous plants, with morphologically protogynous, more or less fragrant flowers.

Erysimum drenowskii frequents open south-facing slopes, glades and grazed meadows, occurs in scattered localities from the oak-hornbeam and beach forest belt, up to the coniferous forests. The diploid cytotype of *E. drenowskii* has an area of distribution ranging from E Central and NE Greece, northwards to the high mountains of the Rilo-Rhodope Massif, and extending to the ridges of the Central Stara Planina. The diploid number was counted in two populations, one in the southern and another one in the northern part of its range of distribution (Anchev 1995; Ančev & Polatschek 2006). The tetraploid cytotype occurs in the N Pirin Mts. The plants are growing along edges of forests of *Pinus heldreichii*, which in N Pirin Mts reaches the northern limit of its distribution. Morphologically, the tetraploid plants are very close to the diploid ones and with their restricted distribution suggest a local autopolyploid origin. The hexaploid *E. pseudoatticum* is a Bulgarian endemic, distributed from W Rhodope Mts northwards to the Rila Mts and Stara Planina. In the Rhodopes and C Stara Planina it occurs sympatrically with *E. drenowskii*. The species prefers open

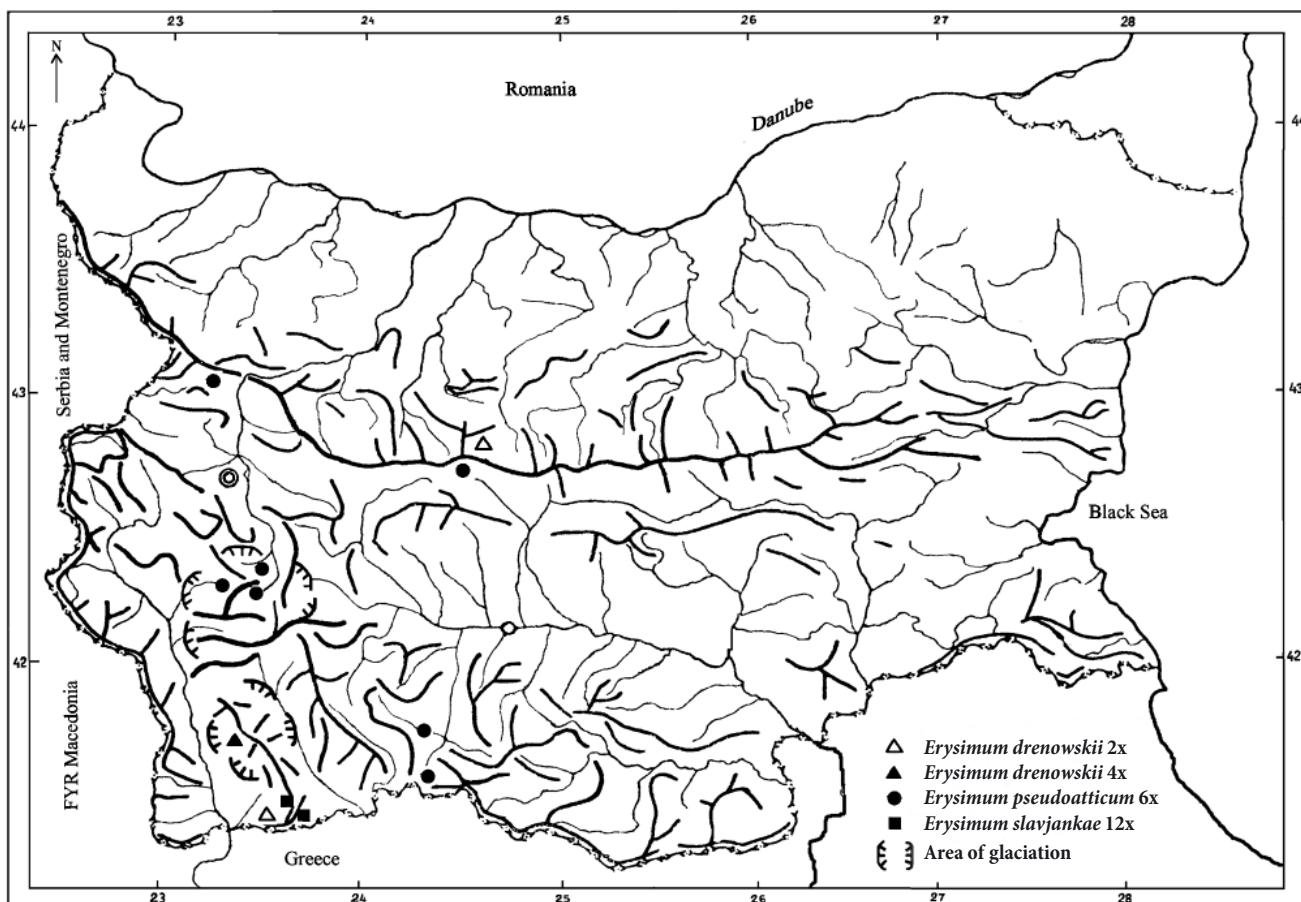


Fig. 2. Distribution of the karyologically studied populations of the species members of *Erysimum drenowskii* group. The area of glaciation during the Late Glacial period, based on data of Bozilova (1990), is roughly outlined.

slopes, grows along wood margins and river banks. Its localities are scattered from the xeromesophilous oak forests, up to the coniferous forests and the alpine vegetation belt (Ančev & Polatschek 2006). In this area of distribution *E. pseudoatticum* is differentiated into two ecotypes: one of loosely caespitose plants with scattered localities in Stara Planina, the Rhodopes and Rila Mts, ranging between 700 m and 1600 m a.s.l., and another of densely caespitose low plants, growing in the subalpine-alpine zone of the Rila Mts, at altitudes up to 2600 m.

The dodecaploid *E. slavjankae*, the third member of the polyploid complex, is a typical calcicolous plant, with large balmy fragrant flowers. It is a local endemic with narrow distribution on the slopes of the summits of Mt Slavyanka and on the southernmost ridges of the N Pirin Mts. There it occurs in the upper zone of the coniferous belt and in the subalpine area, up to 2200 m. The range of distribution of *E. slavjankae* is situated in the southern part of the range of the polyploid complex of *E. drenowskii*.

The polyploid species group of *E. drenowskii* reflects the development of an evolutionary line of mountainous and high-mountainous mesic ecosystems, realized at four different ploidy levels (Fig. 3). Whereas the diploid cytotype of *E. drenowskii* has an area ranging from EC Greece to Central Stara Planina, its tetraploid race is restricted to the N Pirin Mts. The polyploids *E. pseudoatticum* and *E. slavjankae* are local endemics, which together with the tetraploid cytotype of *E. drenowskii*, are products of hybridization in the mountains of the Rilo-Rhodope Massif. These species have characteristics of members of a neopolyploid complex, which southwards, in the mountains of Greece, is related to the group of *E. pussilum* with 10 perennial species, of which eight are diploids, and two polyploids, all with the same basic number $x=7$ (cf. Polatschek & Snogerup 2002).

The species of the polyploid group of *E. diffusum* (4x) and the closely related *E. crassistylum* C. Presl (2x) and *E. welcevii* Urumov (6x, 8x) reflect another evolutionary line, related to the xerothermic vegetation of

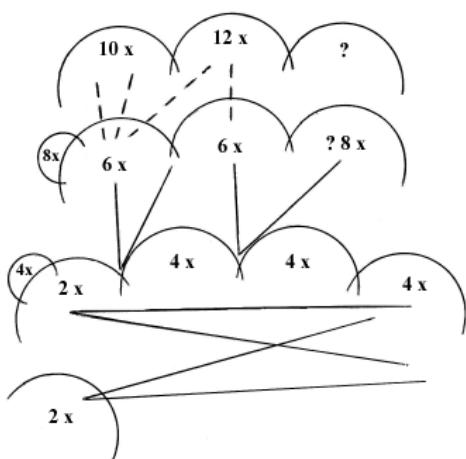


Fig. 3. Model of the origin of polyploid groups of *Erysimum drenowskii* & *E. diffusum*, with putative and unknown diploid parental species and hybrid polyploids.

the plains and foothills of South, East and Northeast Bulgaria. All three species are biennial plants with autogamous flowers. They are confined to open, often anthropogenically disturbed habitats. While the polyploid cytotype and perennial species from the mesophilous *E. drenowskii* group have restricted local distribution, the polyploids from the xerophilous evolutionary line have broader ranges in the open steppe and forest steppe plant communities, supposedly as a result of the biennial life form and better competitive abilities.

Rorippa pyrenaica group

This species group comprises the morphologically related perennial herbs *R. pyrenaica* (L.) Rchb., *R. lippizensis* (Wulfen) Rchb. and *R. thracica* (Griseb.) Fritsch (Ančev & Tomšović 1999). They have slender rhizomes, pinnate or pinnatifid auriculate caudine leaves, many small entomophyllous flowers associated in racemose inflorescences.

The diploid *R. pyrenaica* has the widest area of distribution, ranging from the Ukraine and the Balkan Peninsula westwards to the Iberian Peninsula. The other two members of the group are Balkan endemics. *Rorippa lippizensis* is a diploid with narrow distribution along the northeastern Adriatic coast. The diploid/tetraploid *R. thracica* is spread in the central and south Balkan countries from Albania to European Turkey. In Bulgaria *R. thracica* occurs in open mountain plant communities of the oak vegetation belt, up to the coniferous belt. In this area the species is

known with diploid and tetraploid chromosome races, as most localities of the polyploid cytotype are in the mountains of the Rilo-Rhodope Massif. Tetraploid chromosome number was found also in plants from N Greece.

Analyzing the area of distribution of the species group, the range of the Balkan endemic species with areas sympatric on the Balkans, the chromosome numbers and ploidy levels, we consider that the evolution of the group resulted from differentiation and geographical speciation on diploid level in the flora of the Balkan Peninsula. The polyploid race of *R. thracica* arose comparatively later, in result of intraspecific polyploidization in the mountains of the Rilo-Rhodope Massif and N Greece.

Cardamine ×rhodopaea (*Cardamine matthioli* × *C. rivularis*): new triploid species

The European polyploid group of *Cardamine pratensis* is known for its morphological and taxonomic complexity (Lövkvist 1956; Jones & Akeroyd 1993; Marhold 1994, 1996). In the southern part of the Balkan Peninsula the group is represented by *C. penzesii* Ančev & Marhold, *C. matthioli* Moretti and *C. rivularis* Schur. *Cardamine penzesii* is distributed along the S Black Sea Coast of Bulgaria and NW Turkey (Marhold & Ančev 1999; Marhold & Tan 1999). *Cardamine matthioli* is the most widely distributed member of the group, as southwards from the Carpathians occurs in Bulgaria and almost all other Balkan countries. The southernmost species localities are situated in Central Greece, and the southeastern ones are in European Turkey. *Cardamine rivularis* is restricted to the high Bulgarian mountains and the South Carpathians.

In Bulgaria *C. matthioli* is frequent in damp habitats along forests and in meadows, at altitudes from 700 m up to about 1500 m. *Cardamine rivularis* occurs in wet places, mostly in peat bogs and along streams, from 1400 m up to about 2500 m.

In the Western Rhodope Mts, *C. matthioli* and *C. rivularis* are comparatively frequent species. In the catchment area of the rivers Chepinska, Dospat and Stara Reka, between 1200 m and 1700 m, where both species occur sympatrically or parapatrically, populations with triploid plants ($2n=24$) were found. They have intermediate morphological characteristics between *C. matthioli* and *C. rivularis* (Fig. 4). The stem



Fig. 4. *Cardamine matthioli* (A), *C. rivularis* (B), and *C. ×rhodopaea* (C) with details of rosette leaves (Aa, Ba, Ca); in *C. ×rhodopaea* with young daughter plants on the leaf midrib (Cb).

is 25–50 cm high; rosette leaves are pinnate, glabrous or with dense or sparse hairs, with terminal leaflets 2–3 times larger than the lateral ones, lateral leaflets are in 7–13 pairs; caudine leaves are 5–8 (–10); anthers are usually pale-yellow, occasionally slightly pale-purplish before anthesis; pollen grains are 3–4-colporate, rather different in size. The hybrid plants are characteristic with a great leaf variability and comparatively large pale-white-lilac balmily fragrant flowers. The morphology and triploid chromosome number reveal distribution of an earlier unknown hybrid

species, here provisionally named *Cardamine x rhodopaea*, with putative parental species *C. matthioli* and *C. rivularis* (Ančev & al. unpubl.).

The tentative embryological studies of plants of the triploid *C. ×rhodopaea* and diploid *C. rivularis* have demonstrated that the embryological processes in the hybrid, as compared to the diploid *C. rivularis*, are unbalanced with higher plasticity of embryological structures. *C. ×rhodopaea* seems to have a high reproductive potential sustained by vegetative propagation and probably by agamospermy.

Distribution of the putative parental species and the triploid hybrid in the W Rhodope Mts outline a hybrid zone (cf. Petit & al. 1999) about 25 km long, situated along the valleys of rivers Chepinska, Dospat and Stara Reka and their feeders. Triploid populations were found in seven localities along this zone, 2-3 km to 12 km from each other. The diploid parental species *C. matthioli* (2x) and *C. rivularis* (2x) occur in scattered localities in the north, south and west parts of the hybrid zone, sympatrically or parapatrically with hybrid populations within a hypsometrical range from 1200 m up to about 1700 m a.s.l. (Fig. 5). Ecologically, the hybrid species is closer to *C. rivularis*. Considering the wider distribution of *C. rhodopaea* and the more restricted distribution of *C. rivularis*, we suppose that in the common habitats the hybrid species have replaced the parental diploid *C. rivularis*.

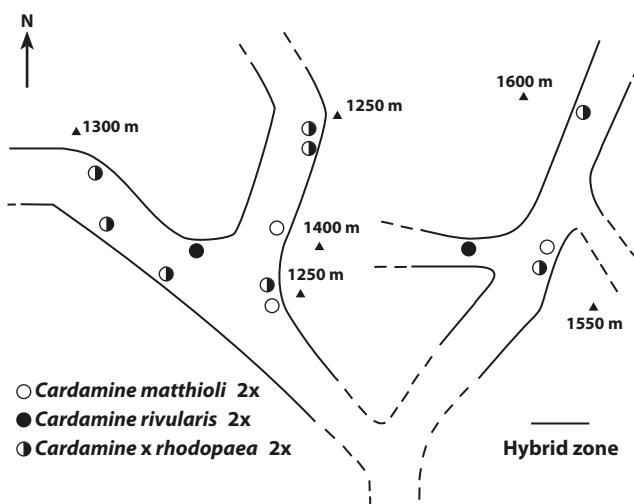


Fig. 5. Scheme of a hybrid zone with distribution of the triploid hybrid *Cardamine* *x* *rhodopaea* ($2n=24$) and its diploid putative parental species *C. matthioli* ($2n=16$) and *C. rivularis* ($2n=16$) in W Rhodope Mts.

This is the third case of a triploid hybrid in the *Cardamine* sect. *Cardamine*, after *C. ×insueta* (*C. rivularis* auct. *×C. amara*) (Neuffer & Jahncke 1997; Urbanska & al. 1997) and reported by Perny & Marhold (2006) *C. matthioli* *×C. acris* subsp. *pindicola* found in NW Greece. Two of them arose in the Southern Balkans, both with the participation of *C. matthioli*, in a region known as a glacial refuge, characteristic with dynamic processes of plant successions in the Late Glacial and the Holocene (cf. Bozilova 1990; Hewitt 1999; Bozilova & al. 2000; Bozilova & Tonkov 2000; Palamarev 2003).

Alyssum montanum polyploid group

Six perennial species of sect. *Alyssum* occurring in Bulgaria pertain to the polyploid group of *A. montanum* L. Two of them, *A. reiserii* Velen. and *A. pirinicum* (Stoj. & Acht.) Ančev, are diploid local endemics. *Alyssum reiserii* occurs in scattered localities in open xerothermic communities in the vegetation belt of mixed *Quercus* forests. It reflects the differentiation of the group at lower altitudes, probably in the earlier stages of its evolution. *Alyssum pirinicum*, which is morphologically close to the South European *A. cuneifolium* Ten. (Ančev & Goranova 2006), is a diploid glacial relict, an endemic restricted to the alpine belt of the N Pirin Mts, with localities lying in a zone of the Late Pleistocene glaciations (cf. Stefanova & Amann 2003). The other four members of the group are polyploids. *A. pulvinare* Velen. (4x) is a Balkan endemic distributed from the S Carpathians to Bulgaria and N Greece. *Alyssum stibrnyi* Velen. (4x) ranges from the central part of the Balkan Peninsula eastwards to Asia Minor. *Alyssum montanum* is represented in Bulgaria by three subspecies: *A. montanum* subsp. *gmelinii* (Jordan) E. Schmid (4x), which occurs mostly in the zone of the oak forests up to about 1400 m a.s.l., *A. montanum* subsp. *ali-botushicum* (Degen & Dren.) Stoj. & Stef. (4x), which is a local polyploid race restricted to Mt Slavyanka and the S Pirin Mts; and *A. montanum* subsp. *montanum* (4x) with scattered localities mostly in the mountains of SW Bulgaria.

In the N Pirin Mts, above the forests of *Pinus heldreichii* in the transition to the subalpine vegetation belt, *A. montanum* subsp. *montanum* (4x) occurs sympatrically with a local hexaploid cytotype (6x), which is morphologically close to *A. trichostachyum* Rupr. These are low, loosely caespitose plants with comparatively large orange-yellow flowers. The hexaploid race is ranging from (1900) 2000 m up to 2500 m a.s.l. Above 2200 m it occurs together with *A. pirinicum* (2x). Presumably, *A. montanum* and *A. pirinicum* have played a role in the evolution of this hexaploid race.

Alyssum orbelicum: an octoploid glacial relict

Alyssum orbelicum Ančev & Uzunov is a member of sect. *Odontarrhena* represented by eight perennial species in Bulgaria. It is an octoploid, recently described from the alpine zone of the N Pirin Mts (Ančev & Uzunov 2002). Its small population is scat-

tered on the slopes of the Banski Souhodol Circus. The species occurs at altitudes from about 2350 m, up to 2450 m, on gravelly marble terrains, together with other calciphilous chasmophytes. Among them are such Balkan and local endemics as *Dianthus microlepis* Boiss., *Alyssum pirinicum*, *Saxifraga ferdinandi-coburgi* Kellerer & Sünd., *Asperula aristata* subsp. *condensata*, etc.

According to our hypothesis, the octoploid *A. orbelicum* is a glacial relict that has evolved in the result of local processes of hybridogenesis in sect. *Odontarrhena*. We assume that it is a product of interspecific hybridization among cytotypes of related *A. corymbosoides* (4x), *A. obtusifolium* (4x), and *A. sibiricum* (*A. tortuosum*) (4x), presently distributed in the zone of the mixed oak forests. Probably *A. orbelicum* had a wider area and vertical range of distribution. Changes in borderline and structure of the forest belts have isolated the high mountainous populations of the species, now restricted to the subalpine belt of the N Pirin Mts. This assumption, based on the arguments of the open structure of the Balkan Pine formations and the fluctuations of the upper forest limit, is supported by the results of the palynological studies in the N Pirin Mts, where in the late Boreal-Atlantic times the mixed oak forests had expanded upward to 1900 m and had retreated to lower altitudes in the Subatlantic time (Stefanova & Amann 2003).

Remarkably, in the same high mountainous area of the N Pirin Mts, lying in the Submediterranean transitional Climatic Zone and refugial area, at the southern limit of the Pleistocene glaciations (cf. Bozilova 1990; Stefanova & Amann 2003), the areas of another three Bulgarian endemics also range: *Alyssum pirinicum*, *Arabis ferdinandi-coburgii* and *Brassica jordanofii* O.E. Schulz, all diploids and characteristic elements of the autochthonous flora of these mountains.

Iberis saxatilis subsp. *longistyla*: a triploid race

In Europe the genus *Iberis* contains 20 species (Pinto da Silva & do Amaral Franco 1993). Four basic numbers ($x=7, 8, 9, 11$) are known for them, as the polyploidy is not of common occurrence. Diploid and polyploid cytotypes have been found in taxa with the basic number $x=11$: *I. saxatilis* L. subsp. *cinerea* (Poiret) P.W. Ball & Heywood ($2n=22, 44$) (Moreno 1985), *I. sempervirens* L. ($2n=22, 66, 70$) (Ančev 1978; Jalas & al. 1996), and *I. semperflorens* L. ($2n=22, 44$) (Jalas & al. 1996).

The distribution area of *I. saxatilis* subsp. *saxatilis* ranges from the Iberian Peninsula eastwards to the Balkan Peninsula, with the easternmost localities in the E Stara Planina and Dobrudzha, reaching estwards up to the Crimea (Pinto da Silva & do Amaral Franco 1993).

In Bulgaria *I. saxatilis* subsp. *saxatilis* occurs in the E Stara Planina, in the transition area between the oak-hornbeam and beech forest belt, from 900 m up to about 1100 m a.s.l., as well as in the N Pirin and Slavyanka Mts in the zone of coniferous forests, at altitudes from 1900 m up to 2300 m. The plants of the population in the E Stara Planina are diploid ($2n=22$) (Ančev 2001; Ančev & Goranova 2002). The chromosome number coincides with that reported from S France (cf. Jalas & al. 1996), Spain (Moreno 1985) and N Greece (Gustavsson 1978).

The plants in three populations – one in Mt Slavyanka and two in the N Pirin Mts – turned to be triploid ($2n=33$) (Ančev 2001; Ančev & Goranova 1997, 2002). They differ from *I. saxatilis* subsp. *saxatilis* by some morphological characters and triploid chromosome number, and are distinguished here as a new subspecies:

Iberis saxatilis L. subsp. *longistyla* Ančev, subsp. nova.

Holotype: Bulgaria, Mt Slavyanka, open rocky grassland near peak Tsarev, 41°23'N, 23°37'E, 2100 m, 12.06.1995, coll. M. Ančev, A9549 (SOM 3538).

Stylo 1.5–2.0 mm longo, pollen grains: 11.50–33.75 µm, chromosomatum numerus $2n=33$ a *I. saxatilis* subsp. *saxatile* diversum.

Iberis saxatilis subsp. *longistyla* differs from *I. saxatilis* subsp. *saxatilis* by its longer style (in subsp. *saxatilis* 0.8–1.2 mm), different pollen diameter (26.00–27.00 µm in subsp. *saxatilis*), and triploid chromosome number, as well as by more or less puberulent leaves, and some longer inner petals. Diploid *I. saxatilis* subsp. *saxatilis* was not found in Slavyanka and N Pirin Mts.

Both diploid and triploid races reproduce by seeds and vegetatively, forming underground rhizomes, rooting at the nodes and developing flowering stems (Ančev 2001). The flowers are proterandrous, as the flower development and position of the inside-turned anthers characteristic of the autogamous Crucifers lead to the assumption that at least some seeds result from autogamy. A comparative embryological investigation of the diploid and triploid cytotypes has proved that the dip-

loid from E Stara Planina is amphimictic. The triploid plants of the populations in Slavyanka and N Pirin Mts are amphiapomictic, with sexual reproduction combined with apomixis (diplospory and somatic apospory) (Yurukova-Grancharova & al. 2004). The pollen morphology and fertility/sterility pollen ratios of the diploid and triploid cytotypes correlate with their diploid, respectively polyploid levels. Thus the fertile pollen of the diploid plants of *I. saxatilis* subsp. *saxatilis* is over 90 %, tricolpate, more or less equal in size. The fertile pollen of the polyploid *I. saxatilis* subsp. *longistyla* is under 70 %, different in size, and the tetracolpate pollen are 4.8 % – 6.2 % (Ančev 2001; Ančev & Goranova 2002; Yurukova-Grancharova & al. 2004).

Analyzing the distribution of *I. saxatilis* subsp. *saxatilis* in its South European range and the geography of diploid cytotype, we have launched a hypothesis that the triploid cytotype of *I. saxatilis* subsp. *longistyla* in Slavyanka and N Pirin Mts is a product of postglacial local processes of intraspecific polyploidization. The distribution of *I. saxatilis* from the mountains of C and N Greece northwards to the phytogeographically related Slavyanka and Pirin Mts reflects a south-north oriented migration, a spread stimulated by the Late Glacial to Post Glacial climatic oscillations. The triploid populations in Slavyanka and N Pirin Mts might have resulted from two separate events of recurrent autoploidization in diploid populations of *I. saxatilis* subsp. *saxatilis* in these mountains. It is also possible that the recent distribution of the polyploid *I. saxatilis* subsp. *longistyla* in the Slavyanka and N Pirin Mts reflects a south-north disjunction of a common triploid cytotype (Fig. 6).

We believe that the diploid race of *I. saxatilis* subsp. *saxatilis* in the E Stara Planina is a remnant of an ancient range of its East Mediterranean area of distribution, up to the Dobroudza and the Crimea. It was

probably broken in the preglacial times by the interrupting of the continental link between the Crimea and the Balkan Peninsula. This hypothesis is congruent with Stojanov's view (Stojanov 1956) on the ancient connections between the flora of the Western Black Sea Coast, the contiguous east ridges of the Stara Planina and the Crimean flora.

Conclusions

In *Erysimum*, *Rorippa*, *Cardamine* and *Alyssum*, some closely related taxa form species groups, which are differentiated at one, two or higher ploidy levels. According to their distribution, ecology and chromosome numbers, the polyploids of the species groups and the triploid race of *I. saxatilis* subsp. *longistyla* refer to the category of neopolyploids (cf. Greilhuber & Ehrendorfer 1988). They have probably evolved as a result of ongoing hybrid processes in close relation with the climatic changes in the Balkan Peninsula during the Late Glacial and the Holocene. Our hypothesis is congruent with the Stebbins' secondary contact hypothesis about the relationship between the Quaternary climatic changes, secondary contacts between previously isolated populations and sympatric speciation via hybridization and polyploidization (Stebbins 1984).

We bring the hybridization and polyploidy in Bulgarian *Brassicaceae*, considered in the manner of a secondary contact hypothesis and the role of the multiple polyploidization for the speciation of plants (Soltis & Soltis 1993), in conjunction with the changes in the composition and structure of the flora and the vegetation in the Late Glacial and Holocene. The climatic oscillations during the Quaternary had become a reason for multiple successions in the vegetation in the mountains of Bulgaria, had affected the composition and vertical distribution of deciduous and coniferous forest communities, increasing and decreasing the upper forest limit. Many herb species took part in these successions, including Crucifers, which are presented in the pollen spectra in most of the palynological profiles of the mountains in the Rilo-Rhodope Massif (Bozilova 1990; Bozilova & al. 2000; Bozilova & Tonkov 2000; Tonkov & al. 2002, in press; Stefanova & Amann 2003).

The triploid hybrid *Cardamine ×rhodopaea* demonstrates a mode of speciation with comparatively re-

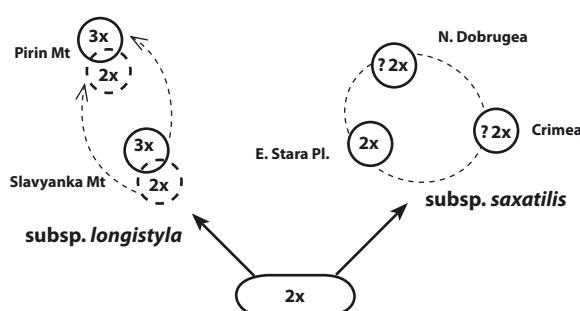


Fig. 6. Probable origin of the triploid race *Iberis saxatilis* subsp. *longistyla* from putative diploid races (explanation in the text).

cent interspecific hybridization between related diploid putative parental species in a hybrid zone, with a progressing active process of differentiation.

In perspective, taking into account the results of cytotaxonomic studies into the Bulgarian *Brassicaceae* and the distribution of a number of local cytotypes in the high mountains of SW Bulgaria, we believe it important to extend the karyological investigations of the flora in the mountains of Bulgaria and the Southern Balkans, with an emphasis on the polymorphic species, species groups and polyploid complexes in the glacial refuges of the Scardo-Pindian and Traco-Macedonian mountain systems. These surveys are an important basis for the development of molecular, phylogenetic and phylogeographic studies of the Balkan and European flora, for understanding the speciation modes analyzed in close relation with the Quaternary climatic changes, the distribution and role of autopolyploidy and allopolyploidy for the evolution of the flora. Another important aspect is to reveal the so far unknown endemic races and species and their conservation in these unique ecosystems.

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