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## **Karyological investigations in Bulgaria during the last decade (1983-1993)**

### **Abstract**

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A great many studies by Bulgarian botanists were published during the last decade. A brief survey of their subjects and main results is provided, together with a full bibliography. Apart from providing new data on chromosome numbers and karyograms relating to Bulgarian plant material, many of these studies uncover basic mechanisms and patterns of chromosomal evolution in the investigated groups. Polyploidy, dysploidy and aneuploidy are a frequent corollary of evolutionary processes in various species and species groups of the Bulgarian flora.

The beginning of the karyological investigations in Bulgaria is marked by the year 1933 when Hristov & Popov's work on the karyology of the genus *Hieracium* was published. These authors, however, used non-Bulgarian material for their investigation.

For the first time Genčev (1937), investigating the chromosomes of *Dianthus* species, studied materials of Bulgarian origin. If we accept this later date as the real start of Bulgarian karyology, we should then accept 1964 as the beginning of the intensive and systematic phase of such investigations, when the laboratory of cytotaxonomy in the Institute of Botany of the Bulgarian Academy of Sciences was founded. Even now it remains the basic Bulgarian research centre for the karyological investigation of the country's wild flora, but not any more the only one. Karyological studies are also performed at the Department of Botany of the University of Agriculture in Plovdiv and at the Department of Botany of the Biological Faculty of Sofia University. All three units are working mainly in this field.

Since the beginning of karyological studies, about 1700 species of Bulgarian higher plants have been investigated, out of a total of 3560 species known from the country. It should be noted that karyological studies so far concentrate almost exclusively on vascular plants and mainly on herbaceous species, trees being investigated only to a small extent.

During the last decade the chromosomes of Bulgarian populations of about 780 species of 42 families (mainly *Asteraceae*, *Brassicaceae*, *Cyperaceae*, *Fabaceae*, *Poaceae*, *Lamiaceae*, *Ranunculaceae*, and *Scrophulariaceae*) have been counted. Many of the

reported numbers are first counts on Bulgarian material, others are first counts for the taxon as a whole. The karyotypes of taxa have been described and analysed.

The data and results are included in c. 100 papers published during this period, all from Bulgarian authors. The foreign contribution has been almost negligible: Nijš (1983) reported chromosome numbers for 38 Bulgarian populations of the *Rumex acetosella* complex ( $2n = 28, 32, 36, 40, 42$ ); Greilhuber & Speta (1985), for *Scilla bulgarica* Speta ( $2n = 18$ ) and *Scilla drunensis* (Speta) Speta ( $2n = 36$ ); Teppner (1991) for 3 populations of *Onosma visianii* Clementi ( $2n = 18$ ). One should add the contribution of foreign botanists working in collaboration with Bulgarian colleagues: Stepánková (Stoeva & Stepánková 1990) and Kovanda (Kovanda & Ančev 1989) from the Czech Republic, Sopova and Sekovski (Sopova & al. 1992) from the F.Y.R. Makedonija, and Hart (Hart & Češmedžiev 1985) from the Netherlands.

Apart from concrete data, the past decade is also characterized by general results, e.g. the uncovering of basic mechanisms and regular patterns of karyotype evolution in the investigated groups. Karyological data have been successfully used in solving many taxonomic problems. It is not possible to mention all such studies in the following survey, but I will try to present the most important ones, which by their results, analysis and conclusions have helped elucidate or solve various taxonomic problems.

In a series of papers, Kuzmanov (1985), Kuzmanov & Georgieva (1983, 1987, 1990), Kuzmanov & al. (1983, 1986, 1988, 1989, 1990, 1991, 1993a, 1993b), and Peneva & al. (1988), reported new or summarized previous data for a considerable part of the *Astera-ceae*. In agreement literature data, the question of the primary basic chromosome number in this family was discussed (Kuzmanov & Jurukova-Gránčarova 1983). The basic chromosome number  $x = 10$  is met only in *Scolymus* species (*Scolyminae*), which are considered by many authors to be the most primitive. The number  $x = 10$  is supposed to derive through increasing dispoloidy from  $x = 9$ . The number  $x = 9$  was found in two subtribes, *Cichoriinae* and *Crepidinae*, where the assumption by Stebbins & al. (1953, see Kuzmanov & Jurukova-Gránčarova 1983), that the more primitive genera and species have higher and the evolutionarily specialized species lower basic chromosome numbers, was confirmed. Decreasing dispoloidy reaches  $x = 3$  in some species of *Hypochaeris* and *Crepis*. The lowest diploid chromosome number determined on a Bulgarian population pertains to *Hypochaeris cretensis* (L.) Bory & Chaub., with  $2n = 6$  (Kuzmanov & al. 1993a).

In Bulgarian species of *Leucanthemum*, Kuzmanov & al (1988) found three ploidy levels:  $2x$  (*L. praecox* (Horvatić) Horvatić),  $4x$  (*L. vulgare* Lam.) and  $6x$  (*L. pallens* (Gay) DC.). They noted that variation in the group seems smaller in S.E. Europe and Bulgaria in comparison with Central Europe. The diploid species are evidently most widespread, the hexaploid ones most restricted in distribution. Several cases of aneuploidy were observed.

In the *Cichorioideae* and *Asteroideae*, Kuzmanov & al. (1993a, 1993b) published a first count for *Chondrilla urumovii* Deg. ( $2n = 10$ ), a sexual diploid and one of the rare diploid species in the genus. Its distribution in Bulgaria confirms the importance of the Rhodopes and Slavjanka Mts as refugia for palaeoendemic species of the Bulgarian and Balkan flora. Other species counted are *Inula aschersoniana* Janka ( $2n = 16$ ), *Senecio macedonicus* Griseb. ( $2n = 40$ ), *Achillea lingulata* Waldst. & Kit ( $2n = 36$ ), *Achillea*

*thracica* Velen. ( $2n = 18$ ), and *Jurinea stoechadifolia* (M. Bieb.) DC. ( $2n = 32$ ). Two aneuploid cytotypes in *Scorzonera hispanica* L. ( $2n = 12$ ) and *Scorzonera purpurea* subsp. *rosea* (Waldst. & Kit.) Nyman ( $2n = 12$ ) were reported by the same authors. In *Asteroideae* the polyploidy is much more common, e.g. in *Filago*, *Inula*, *Senecio*, and *Tripleurospermum*, than in *Cichorioideae*.

The largest *Asteraceae* tribe in the Bulgarian flora is *Cardueae*. Kuzmanov & al. (1991) found that in the karyotypes of the species studied symmetrical (metacentric and submetacentric) chromosomes predominate. Satellite chromosomes were present in all karyotypes studied. The species in a general way evolve toward polyploidy (mainly tetraploidy), karyotype asymmetry, and in some cases aneuploidy. *Centaurea* is the largest *Asteraceae* genus in our flora. Kuzmanov & Georgieva (1990) mentioned that the primary basic number is  $x = 15$ , and that the values of  $x = 12$ ,  $x = 11$  and  $x = 10$  found in Bulgarian species result from decreasing dispoloidy correlated with increasing specialization during the evolution of the genus. They concluded that the different  $x$  values demonstrate the significance of dispoloidy in the evolution of the genus on the whole continent.

Some interesting results have been published on *Brassicaceae* species (Ančev 1983b, 1984b, 1991; Ančev & Hardalova 1989; Ančev & Peneva 1984; Ančev & al. 1987a; Delipavlov & Češmedžiev 1983b). They mainly concern *Alyssum* (Ančev, 1984b, 1991) and *Erysimum* (Ančev & al. 1987a). The chromosome numbers and karyotypes of 21 *Alyssum* species have been studied by Ančev (1991), where diploid ( $2n = 16$ ) and tetraploid chromosome numbers ( $2n = 32$ ) were established. The karyotype is comparatively symmetrical, with meta- and submetacentric chromosomes with manifest centromeres in the diploids. Satellite chromosomes are characteristic for both diploid and polyploids. The basic chromosome number in the genus is  $x = 8$ . Aneuploids also were found. The Bulgarian species of the genus show three basic trends of karyotype evolution: divergent differentiation on the diploid level as a result of chromosome mutations; polyploidization, the tetraploid number  $2n = 32$  being most frequent; and aneuploid changes, found only in annual species.

The chromosome number of some species of this family was counted for the first time: *Erysimum drenovskyi* Deg. is tetraploid with  $2n = 28$  (Ančev & al. 1987a), and *Andrzeiowskia cardamine* Rchb. has  $2n = 16$  (Delipavlov & Češmedžiev 1983b). Ančev (1983b) reported chromosome numbers for 22 synanthropic species of *Brassicaceae*.

Intensive study on Bulgarian *Cyperaceae* were performed by Stoeva (1984a, 1985, 1987, 1989, 1992a-c), Stoeva & Popova (1990, 1991, 1992, 1993), Stoeva & Stepánková (1990), and Stoeva & al. (1992). First chromosome counts on Bulgarian material were reported for many species, and some species were studied for the first time at all, e.g., *Carex pirinensis* Aht. ( $2n = 54$ ). The highest chromosome number counted in this family is  $2n = 186$  for *Cyperus glaber* L. (Stoeva 1992c).

The chromosome number varies in populations of *Carex caryophyllea* Latourr. Stoeva & Popova (1990) found four chromosome numbers for this species:  $2n = 66, 67, 68, 69$ , the first being predominant. The karyotype consists of very short, holocentric chromosomes. *C. sect. Digitatae* and *sect. Acrocystis* were also studied for their chromosome characters (Stoeva & Popova 1991, 1993). Aneuploid series were registered in some of

the species:  $2n = 48, 50, 52, 54, 56$  in *Carex digitata* L., and  $2n = 52, 53, 54$  in *C. ornithopoda* Willd. The chromosome number in the former species varies mainly between populations, but in the second, within populations. In *C. sect. Glaucae* (Stoeva & Popova 1992) a hexaploid chromosome number was found for *Carex cuspidata* Host:  $2n = 114$ , which is higher than was previously known from the literature. The presence of three ploidy levels in *C. sect. Glaucae*,  $2x, 4x$  and  $6x$ , is interesting since different ploidy levels are rather rare in *Carex*. Moreover, polyploidy appears to be the main mode of karyotype evolution and in speciation in *C. sect. Glaucae*, while in most other *Carex* sections agmatoploidy prevails. Karyological data for *Blysmus* and *Eriophorum* have been published (Stoeva 1992b) along with others for more than 30 species of the same family (Stoeva 1985, 1992c). For some of them, the karyotypes are described.

In the *Ranunculaceae*, Kuzmanov (1986) and Kuzmanov & al. (1987b) studied karyologically a large number of native populations of all Bulgarian *Thalictrum* species and confirmed the major role of polyploidy (from tetraploid to dodecaploid) in the evolution of that genus. Since the chromosomes are relatively small it is difficult to analyse the karyotype.

Some other genera of *Ranunculaceae* have been studied by Koeva-Todorovska (1987, 1988a, 1992). All investigated species of *Nigella* (Koeva-Todorovska 1987) are diploids with  $2n = 12$ . Differences were found with respect to the number and position of the satellites. *Nigella* belongs to the *Helleboreae*, whose basic chromosome number is  $x = 8$ . The majority of investigated species of *Delphinium* (Koeva-Todorovska 1988a) are diploids too ( $2n = 16$ ). On the basis of her karyological results on *Aconitum*, *Delphinium* and *Consolida*, Koeva-Todorovska (1992) tried to elucidate phylogenetic relationships within the *Delphinieae*. The basic number is  $x = 8$ , and other numbers ( $x = 6, 7$  and  $9$ ) are considered to be derived from it. *Anemone* was also studied (Sopova & al. 1992). The chromosome numbers range from diploid to hexaploid. *A. nemorosa* L. ( $2n = 30$ ) was found to be an aneuploid species. This chromosome number is of special interest, since it is uncertain whether the species is a tetraploid with reduced chromosome number or derives from the cross between two closely related species with  $2n = 14$  and  $2n = 16$ . The basic chromosome numbers in this genus are  $x = 7$  and (prevalently)  $8$ . Satellite chromosomes were observed in all karyotypes, they are usually subacrocentric or acrocentric. Some degree of karyological differentiation was discovered within species, due to chromosome rearrangement.

Many species of *Poaceae* were studied karyologically by Petrova & Kožuharov (1983, 1987, 1989), Petrova & al. (1984), Kožuharov & al. (1983, 1988), Stoeva (1983a-b, 1984b, 1985, 1986, 1988) Kožuharov & Stoeva (1983), Kožuharov & Petrova (1991), and Stoeva & Kožuharov (1983a-b, 1985). For many species, Bulgarian populations were counted for the first time. For some, mainly of *Aegilops*, *Agropyron*, *Deschampsia*, *Poa*, *Hordeum*, *Festuca*, *Secale*, and *Koeleria*, karyotype morphology was analysed and discussed. The prevalence of diploids among lowland species of *Koeleria* (Kožuharov & al. 1988) shows that the Balkan Peninsula is one of the centres of diversification of the genus; higher ploidy levels belong to endemic taxa. Speciation within this genus is recent, resulting from contacts in new, man-made ecological niches that displace indigenous plant communities.

particular (Markova 1987), some of which are Balkan or Bulgarian endemics: e.g. *T. stojanovii* Deg. ( $2n = 28$ ) and *T. atticus* Čelak. ( $2n = 26$ ).

Terzijski (1987a-b) carried out an in-depth karyological study of *Vicia*. He found that the most common basic chromosome number is  $x = 7$ . Diploids are dominant, and reduction in number and chromosome rearrangements, not polyploidy, play a major role in the evolution of the genus. The karyotype of *V. meyeri* Boiss. was analysed by Terzijski & Dimitrov (1983). Chromosome numbers of other species of *Fabaceae* were reported by Pavlova & Kožuharov (1993) for *Astragalus* and by Kruševa (1986, 1988) for *Astragalus*, *Genista*, etc.

Stoeva (1991) and Stoeva & Ivanova (1989) studied different Bulgarian *Papaver* populations for their chromosome numbers and karyotypes. The taxa investigated are diploid and tetraploid ( $2n = 14, 28$ ), and the karyotypes consist of submetacentric and, predominantly, acrocentric chromosomes. The species studied show intermediate features between E. Mediterranean and W. to Central European ones.

Markova (1985) made a detailed karyotaxonomic study of the *Potentilla taurica* group. She counted diploid and tetraploid chromosome numbers for the species investigated, and described their karyotypes.

Chromosome numbers for many other species of different families were counted by Markova (1983a), Markova & Goranova (1987, 1989), Markova & Černeva (1984), Češmedžiev (1983a-b), Hart & Češmedžiev (1985), Češmedžiev & Petkova (1988), Nikolov (1991: more than 70 species from the reserve "Bajuvi dupki – Džindžirica", Kuzmanov & al. (1987a, 1992), Delipavlov & Češmedžiev (1983a), Stefanova-Gateva (1987), Ančev & al. (1987b), and others.

In conclusion the last decade was characterized by intensive karyological studies by Bulgarian botanists, revealing basic mechanisms and patterns of chromosomal evolution in the investigated groups. Polyploidy, dysploidy and aneuploidy characterize the evolutionary pattern of various species and cytodemes of the Bulgarian flora.

In concluding I would like to thank all my colleagues who have contributed by their work to a better karyological knowledge of the Bulgarian flora. Everyone acquainted with this kind of work will know that it requires great persistence, constancy, and continuous improvement. It is sad that some colleagues are not with us any more, among them Bogdan Kuzmanov, whom we painfully miss. He was one of the enthusiastic founders of the cytotaxonomic laboratory at the Institute of Botany of the Bulgarian Academy of Sciences, and will be remembered as the main investigator of the karyology of Bulgarian *Asteraceae*.

I like to hope that the young who come after us will be even more persistent, more enthusiastic than we have been; that they will continue our work until the very last species of the Bulgarian flora shall be karyologically investigated. In this I firmly believe!

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