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Relationship between *Pinguicula bohemica* Krajina and *Pinguicula vulgaris* L. (*Lentibulariaceae*) from the karyological point of view

Vztah mezi *Pinguicula bohemica* Krajina a *Pinguicula vulgaris* L. (*Lentibulariaceae*) z hlediska karyologie

Anna Krahulcová and Vlasta Jarolímová

Botanical Institute, Czechoslovak Academy of Science, 252 43 Průhonice, Czechoslovakia

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The somatic chromosome number of 2n = 64 is reported in the Bohemian endemic *Pinguicula bohemica* and in the spontaneous hybrid between *P. bohemica* and *P. vulgaris* (= *P.* × *dostalii*). The same chromosome number of 2n = 64 is confirmed in *P. vulgaris*. Thus, both the species were found to be octoploids. Different theories about the origin of *P. bohemica* are discussed in detail.

Introductory remarks

Pinguicula bohemica Krajina represents one of the endemic critically endangered species of Bohemia (Bělohlávková 1989, Kubát 1986, Studnička 1989). Recently its occurrence is limited only to one locality near Jestřebí in N Bohemia (Bělohlávková 1989, Studnička 1989). The other extincted localities known in the past (Bělohlávková 1989, Studnička 1989) were situated in the central part of the Elbe river valley. The much more extensive area of *P. vulgaris* surrounds the small territory, where *P. bohemica* occurs (Studnička 1989). Nevertheless, the conspicuous distinguishing characters exist between the genetically pure populations of the both species (Bělohlávková 1989, Studnička 1989). Some of the recent authors recognize these mostly morphological characters as sufficient to consider *P. bohemica* as the separate species (Kubát 1986, Bělohlávková 1989, Studnička 1989). Some other authors evaluate this taxon on the lower taxonomical level, i.e. as subspecies (Hadač 1977, Kubát 1981).

P. bohemica and *P. vulgaris* becoming in contact have produced the hybrid *P.* × *dostalii* described by Bárta (1944). It is necessary to point out, that Dostál (1989) *P.* × *dostlii* Bárta incorrectly as the hybrid between *P. alpina* and *P. bohemica*. The hybrids *P.* × *dostalii* show intermediate characters between the parental species *P. bohemica* and *P. vulgaris* (Bělohlávková 1989, Studnička 1989). The hybrid individuals, possessing the various degree of these intermediate characters, were found among others in the protected area Polabská černava near Mělnická Vrutice in C. Bohemia (Bárta 1944, Bělohlávková

Table 1. List of plants studied with their localities

Taxon	Locality	Chromosome number (2n)
P. bohemica Krajina (Fig. 1)	N Bohemia, distr. Česká Lípa, Jestřebí village, in the wet meadow below the hill of "Konvalinkový vrch", 2 km E of the village, 250 m alt. Coll. R. Bělohlávková 1981, 1982, 1986.	64 (total of 7 individuals)
	N Bohemia, distr. Česká Lípa, Jestřebí village, in the "Baronský rybník", ca 1.5 km N of Staré Splavy village, 260 m alt. Coll. R. Bělohlávková 1989.	64 (2 individuals)
P. dostalii (= *P. bohemica* × *P. vulgaris*) (Fig. 2. c,d)	C Bohemia, distr. Mělník, Mělnická Vrutice village, in the protected area "Polabská černava", 190 m alt. Coll. R. Bělohlávková 1985, 1989.	64 (3 individuals)
P. vulgaris L. (Fig. 2. a,b)	C bohemia, distr. Rakovník, Třtice village, in the wet meadow in the protected area "V bahnách", 400 m alt. Coll. R. Bělohlávková 1985.	64 (1 individual)

1989, Studnička 1989). *Pinguicula bohemica* had been collected in this locality in the past as well (the survey see Studnička 1989). Recently any "pure" individuals of *P. bohemica* were no longer found there (Studnička 1989, Bělohlávková 1989, Bělohlávková pers. commun.).

Hypotheses on the evolution of *P. bohemica*

Three hypotheses on the evolutionary relationship between *P. bohemica* and *P. vulgaris* have been published: 1. According to Casper (1962), *P. bohemica* belongs to the group of the local variants, which can be included into *P. vulgaris*. The special characters of *P. bohemica* as well as the intermediate characters of the hybrids described previously, occur in the populations of the "standard" *P. vulgaris*, too. Thus, there is no reason to recognize *P. bohemica* as a separate taxonomic unit (Casper 1962). 2. Hadač (1977), evaluating *P. bohemica* as subspecies, presumes that it evolved within the relict, isolated populations of *P. vulgaris* in the course of the late-glacial period. 3. Studnička (1989) based his hypothesis on the karyological data. According to his statement, the chromosome number of *P. bohemica* is 2n = 32, so that *P. bohemica* would be tetraploid. On the other hand,

the chromosome number of *P. vulgaris* had been many times reported as 2n = 64, i.e. it is based on the octoploid level (Löve et Löve 1974). Studnička (1989) considers this difference between the ploidy level of *P. bohemica* and that of *P. vulgaris* stated by himself as the primary fact, on which the hypothesis on the evolution of *P. bohemica* can be based. On the contrary to Hadač (1977), he does not classify *P. bohemica* as the species related to *P. vulgaris*, which is considered as the evolutionary youngest European Pinguicula species (Casper 1962). With regard to the supposed tetraploid level, Studnička (1989) puts *P. bohemica* close by the other European tetraploid species of *Pinguicula*, showing some more archaic characters than *P. vulgaris* has (*P. grandiflora* Lam., *P. leptoceras* Reichenb., *P. longifolia* Ramond ex DC., *P. balcanica* Casper, *P. vallisneriifolia* Webb).

Results

Our results (Tab. 1, Fig. 1, Fig. 2.) obtained in the course of the karyological study of *P. bohemica*, have confirmed recurrently the chromosome number of 2n = 64 (Bělohlávková 1989). Our statements are thus different compared to the number published by Studnička (1984, 1989, Studnička in Kubát 1986). Therefore we present more details here.

The chromosomes were counted in the root-tip meristems from living plants. They were pretreated by the colchicine solution, by paradichlorbenzene or by 1-bromonaphtalene for 2 hours, fixed in acetic-ethanol, macerated in 1N HCl at 60 °C for 5 min and squashed in lacto-propionic orcein. The plants were collected either in the natural habitats or they came from the vegetative as well as the generative offspring of plants formerly transferred and grown in the experimental garden. The vauchers were not made, because the characteristical differences between the both species are recognizable especially in the living plants (Bělohlávková 1989). All the plants studied are still cultivated in the glasshouse of the experimental garden of the Botanical Institute, Průhonice.

Discussion

According to our results, the chromosome number of *P. bohemica, P. vulgaris* and of their hybrid is identical, i.e. 2n = 64. Both the taxa are octoploids corresponding to the basic chromosome number of x = 8 known in the genus *Pinguicula* (Casper 1962).

The theories explaining the relationship between *P. bohemica* and *P. vulgaris* cannot be based on the comparison of their chromosome numbers. However, our results do not contradict the hypothesis by Hadač (1977), suggesting the differentiation of *P. bohemica* inside the relict populations of *P. vulgaris*. From the view of our results, the hypothesis by Casper (1962) cannot also be entirely rejected. The formation of (probably fertile) hybrids suggests the close relationship between the both taxa. *P. bohemica* could thus be one of the predominantly autogamic stabilized lines of *P. vulgaris*, occupying the restricted area in Bohemia. This hypothesis can be supported by the information about the ways of reproduction in both species. According to the flower morphology, the autogamy is possible in *P. vulgaris* (Casper 1962). The autogamy is probably the main way of the generative reproduction in *P. bohemica* as well. Further, the particular genetic lines can be stabilized by means of the abundant vegetative reproduction (Studnička 1989). As can be seen, both these hypotheses (Casper 1962, Hadač 1977) differ in the evaluation of characters and of their reflection in classification, but they need not necessary rule out each other.

On the other hand, the hypothesis by Studnička (1989) should be rejected to our data and his interpretation of the evolution of *P. bohemica* is not justified. The chromosome

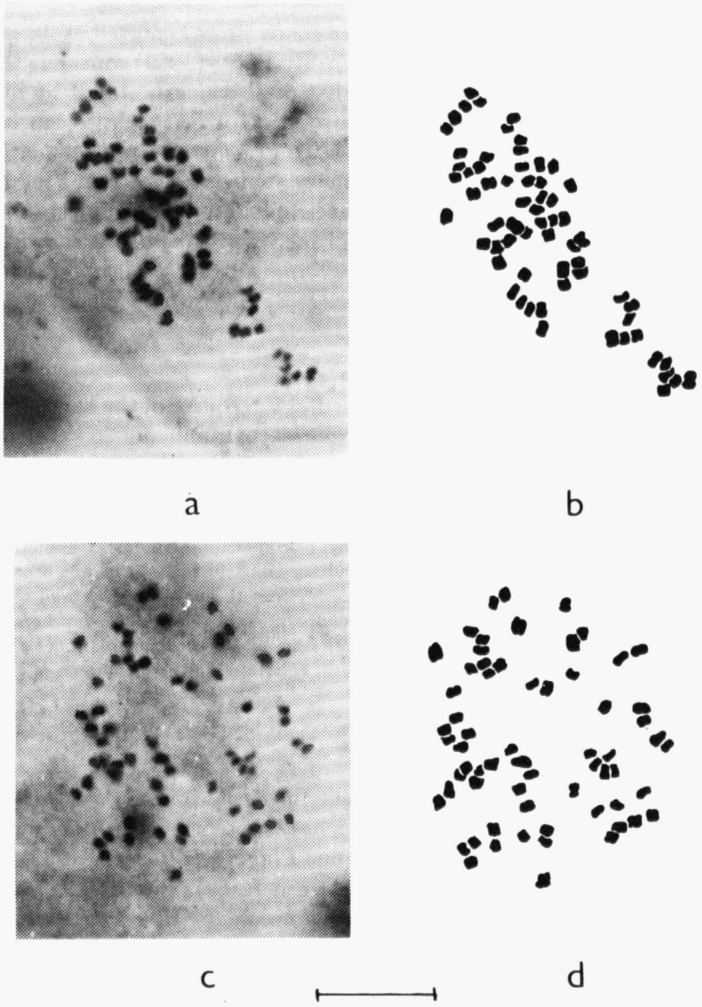

Fig. 1. Photographs (a,b) and explanatory diagrams (b,d) of root tip metaphases in *Pinguicula bohemica*, locality Jestřebí - Baronský rybník, 2n = 64. Scale = 10 μm.

number of 2n = 32, reported by him in *P. bohemica* (Studnička 1984, 1989, Studnička in Kubát 1986), should be considered as incorrect.

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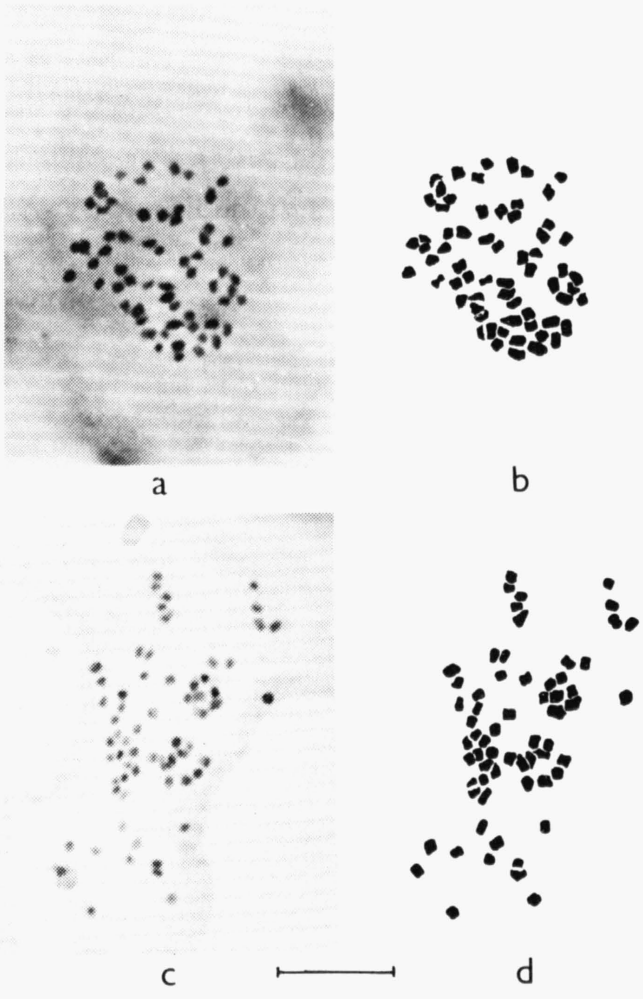

Fig. 2. Photographs (a,b) and explanatory diagrams (b,d,) of root tip metaphases: in *Pinguicula vulgaris*, locality Třtice, 2n = 64 (a,b); in the hybris *Pinguicula bohemica* × *Pinguicula vulgaris*, locality Mělnická Vrutice, 2n = 64 (c,d). Scale = 10 μm.

Souhrn

Autoři předkládají výsledky karyologického studia českého endemického druhu *Pinguicula bohemica* Krajina, dále příbuzného druhu *P. vulgaris* L. a jejich spontánního hybrida *P.* × *dostalii*. Zjištěný chromozómový počet je u obou druhů i jejich hybrida totožný (2n = 64) a odpovídá oktoploidní úrovni. Tento shodný chromozómový počet nevyvrací hypotézu o diferenciaci *P. bohemica* v rámci reliktních izolovaných populací *P. vulgaris* (Hadač 1977) a tím i těsný vývojový vztah obou taxonů. Chromozómový počet 2n = 32, dříve publikovaný Studničkou (1984, 1989, Studnička in Kubát 1986), je s největší pravděpodobností chybný. Teorii o vývojové příbuznosti domněle tetraploidní *P. bohemica* s jinými evropskými tetraploidními tučnicemi (Studnička 1989) nelze proto považovat za dostatečně podloženou.

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