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Apomixis and origin of *Sorbus bohemica* (Embryological studies in *Sorbus* 2)

Apomixie a původ jeřábu českého, *Sorbus bohemica* (Embryologické studie rodu *Sorbus* 2)

Andrzej Jankun and Miloslav Kovanda

Jankun A.[1]) et Kovanda M.[2]) (1987): Apomixis and origin of *Sorbus bohemica*. (Embryological studies in *Sorbus* 2). Preslia, Praha, 59 : 97—116.

Sorbus bohemica Kovanda, a hybridogenous species of the *S. danubialis* × *S. torminalis* parentage, is shown to be an apomictic triploid ($2n = 51$). The archesporium cells or entire ovules may degenerate in the early phases of development. Apospory, diplospory and pseudogamy are present. The chromosome number of the endosperm, $5n =$ c. 130, was established. Besides the ESs with typical structure, various anomalies, e.g. reversed polarity (in 30 % of ESs) and lateral position of the egg apparatus, were observed. The synergid of an apomeiotic ES may exceptionally be fertilised, thus providing for the possibility of karyological differentiation. The results indicate that besides plants replicating the genotype of the mother plant, new genotypes may appear in the progenies. Cases of polyembryony proper were noted (with apogamy stimulated by the male gamete). The reasons for the hybrid origin of *S. bohemica* are put forth and a morphological analysis is provided. The species is a neoendemic of the České středohoří Mts., N. Bohemia, Czechoslovakia, where it is at present known from 18 localities clustered in the central part of the mountains. The total number of individuals does not exceed 200. Notes on ecology and ecobiology are given. The parent species proved to be diploid with $2n = 34$.

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INTRODUCTION

Interspecific hybridisation, accompanied by polyploidisation and apomixis, is known to be an effective means of speciation in *Sorbus*. The mechanism involved was thoroughly studied in material from Scandinavia (Liljefors 1934, 1953, 1955) and the British Isles (Warburg 1957), the home of a host of hybridogenous species, and some general conclusions from this work have been drawn. The genus in Central Europe, where it is no less rich in hybrids, has until recently remained untouched by embryological and cytogenetic research. The present authors have therefore initiated an analysis of a representative sample of Central European hybridogenous species of various parentages. In a previous study (Jankun et Kovanda 1986) we discussed the breeding system of *S. sudetica* (Tausch) Fritsch, of the *S. aria* (L.) Crantz (subg. *Aria*) × *S. chamaemespilus* (L.) Crantz (subg. *Chamaemespilus*) hybrids. The present paper deals with *S. bohemica* Kovanda, in the origin of which *S. danubialis* (Jáv.) Kárpáti (subg. *Aria*) and *S. torminalis* (L.) Crantz (subg. *Torminaria*) participated.

Tab. 1. — Chromosome numbers in *Sorbus bohemica* and the parent species, *S. danubialis* and *S. torminalis*

Species	Locality	2n	n
S. bohemica KOVANDA	České středohoří Mts., summit area of the Lovoš hill, near Lovosice, basalt, 570 m	51	25
	České středohoří Mts., W. slope of the Boreč hill, near Lovosice, phonolite, c. 400 m	51	
	České středohoří Mts., scree on the S. slope of the Plešivec hill, near Kamýk, basalt, c. 470 m	51	
	České středohoří Mts., E. slope of the Výsluní hill, near Prackovice, basalt, c. 340 m	51	
S. danubialis (JÁV.) KÁRPÁTI	České středohoří Mts., summit of the Hradiště hill, near Hlinná, basalt, 545 m	34	
S. torminalis (L.) CRANTZ	České středohoří Mts., summit area of the Lovoš hill, near Lovosice, basalt, 570 m	34	

MATERIAL AND METHODS

The sources of material were natural populations of *S. bohemica* (see Tab. 1). Also sampled was the tree on the Lovoš hill from which the type material was collected by the junior author in 1957 (KOVANDA 1961c). The methods are as described by JANKUN et KOVANDA (1986), as are the responsibilities for the various parts of the study.

RESULTS

Karyology

The chromosome number of *S. bohemica* has so far been unknown. The material for the present studies originated from four habitats in the České středohoří Mts. (Tab. 1). All investigated specimens proved to be triploid with 2n = 51 (Fig. 17). The counts were based on root-tip mitoses of young pot-grown plants and were confirmed on meiotic divisions as well as on mitoses in ovules or hypanthiums.

Embryology

Microsporogenesis

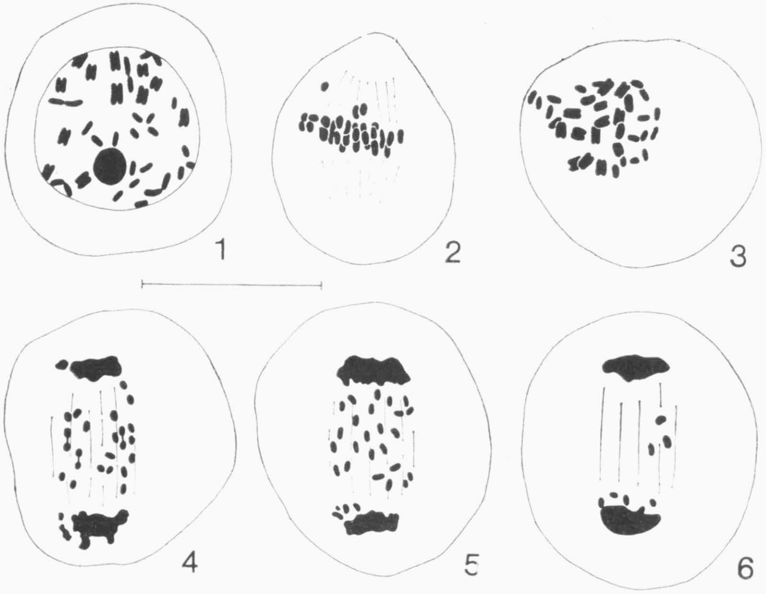
A number of disturbances characteristic of triploid hybrids occur in the course of microsporogenesis. In diakinesis (Fig. 1) as well as in the first metaphase (Fig. 3), 17 bivalents and 17 univalents were counted. The congression (see DARLINGTON 1937) of chromosome associations and chromosomes was regular in the first metaphase (Fig. 2); only a few univalents were missing from the equatorial plane of the spindle. Chromosome bridges in the cytoplasm among ana-telophase groups were sometimes present.

In the first anaphase, a number of lagging chromosomes could be seen between the two poles of the spindle. Sometimes there were also lagging bivalents still remaining in the equatorial plane of the meiotic spindle (Fig. 4).

Table 2. — A summary of the ovules examined

Stage	Number of ovules
Viable ovules	
Primordia of ovules	139
Archesporium	32
Degenerated archesporium or its separate cells	300
Synapsis	8
Leptotene	2
Metaphase I	2
Remnants of dyads	2
Degenerated tetrad	1
Initial cells of apomeiotic ES	46
1-nucleate apomeiotic ES	27
2-nucleate ES	38
2-nucleate ES (mitosis)	1
4-nucleate ES	40
4-nucleate ES (mitosis)	2
6-nucleate ES	1
8-nucleate ES (typical structure)	83
8-nucleate ES (with 3 polar nuclei)	3
8-nucleate ES (growing into micropylar canal)	6
8-nucleate ES (reaching inner integument)	5
8-nucleate ES (reversed polarity)	42
8-nucleate ES (egg apparatus and antipodals at the chalazal pole)	2
7-nucleate ES (with secondary nucleus)	1
Egg cell + 2 nuclei of endosperm	1
Egg cell (telophase) + 4 nuclei of endosperm	1
2-celled embryo + polar nuclei	9
Multicellular embryo + 2 polar nuclei	11
2-celled embryo + 2 nuclei of endosperm	1
Multicellular embryo + 4 nuclei of endosperm	2
Multicellular embryo + nuclear endosperm	2
Twin embryos	5
Nucellar embryo	2
Degenerated ovules	
Young ovules (integuments have not yet reached the top of the nucellus)	29
Older ovules	72
1-nucleate ES	1
2-nucleate ES	2
8-nucleate ES	108
2-celled embryo + nuclear endosperm	2
Total	1031

In spite of the lagging chromosomes visible between the anaphase groups, in telophase I there were as a rule only two daughter nuclei. In some pollen mother cells (PMCs), however, the chromosomes remaining in the cytoplasm (Figs. 5, 6) were eliminated or produced micronuclei (Fig. 8). The segregation of the large number of univalents is random. Various types of nuclei were observed: at one pole there was a nucleus with 24 chromosomes and 3 chromosomes remaining in the cytoplasm, and at the opposite pole a nucleus with 22 chromosomes and 2 chromosomes remaining in the cytoplasm (Fig. 7). In another case two large nuclei (one with 21, the other with 27 chromosomes) and one micronucleus consisting of 3 chromosomes were seen (Fig. 8).



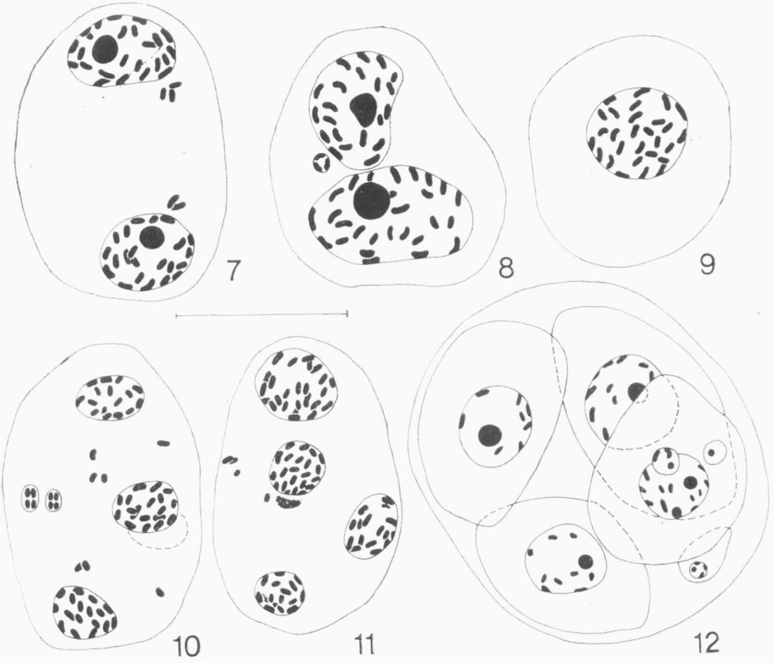
Figs. 1—6. — *S. bohemica* — microsporogenesis. 1, diakinesis $17_{II} + 17_{I}$. 2—3, metaphase I. 3, $17_{II} + 17_{I}$. 4—6, telophase I. Bar = 10 μm.

Furthermore, we observed one nucleus with 28 chromosomes (Fig. 9). The division of the PMCs is of simultaneous type. During the second metaphase the spindles were orientated either at a right angle to each other, or parallelly. The disturbances in the second meiotic division also result in the differentiation of chromosome numbers. In telophase II we observed nuclei with c. 17 and c. 18 chromosomes, micronuclei and eliminated chromosomes in the cytoplasm (Fig. 10). In another cell there were nuclei with c. 22, c. 25 and eliminated chromosomes (Fig. 11). In addition to these frequent disturbances, in the stage of tetrad, four cells were often noted. Polyads were seen exceptionally, with micronuclei forming separate cells or enclosed in one large cell (Fig. 12).

The viability of the pollen grains is poor, amounting to c. 21 % as shown by staining with acetocarmine.

Gynoecium and development of ovules

The gynoecium of *S. bohemica* is syncarpic, formed by two or exceptionally three carpels coalesced up to 1/3—2/3 of the height of the styles. The ovary is semi-inferior (Fig. 19). The course of the early stages of development of the ovary and ovules is very characteristic of *S. bohemica*. The loculi of the ovary are much larger than in any other *Sorbus* species hitherto examined. The primordium of the ovule begins to develop at the bottom of the loculi and is only later, due to the continued growth of the ovary, transposed to the middle of the inner wall of the loculus. The ovule begins to fill up the loculus only in the final phase of development. When mature, the ovule is anatropous, bitegminous and crassinucellate, as in other species of *Sorbus*. The arche-



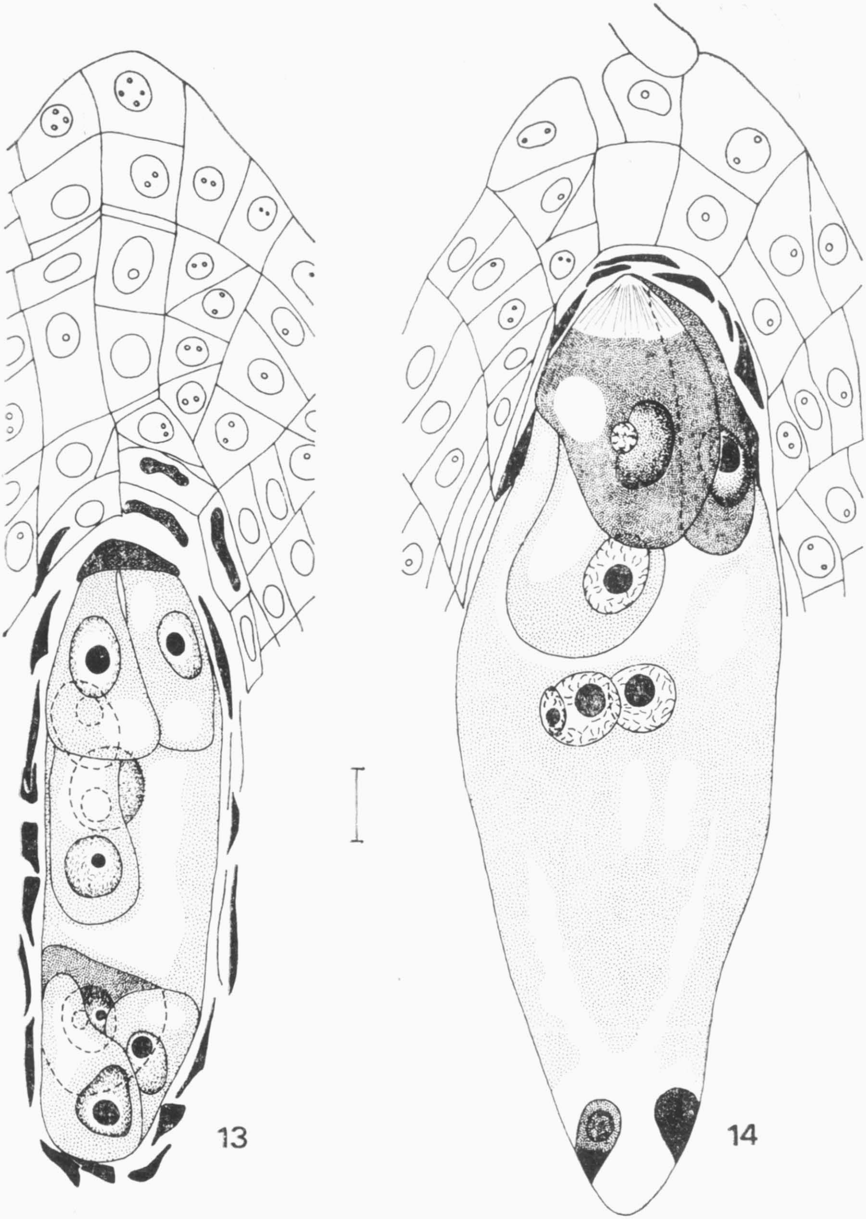
Figs. 7–12. — *S. bohemica* — microsporogenesis. 7–9, telophase I. 10–11, telophase II. 12, polyad. Bar = 10 µm.

sporium is multicellullar and cuts off the parietal cells. A nucellar cap develops on the top of the nucellus.

Phenomena of degeneration are frequent. In the majority of young ovules, decline of the cells of the primary or secondary archesporium was noted. In addition, the degeneration of entire ovules was also observed, starting from the top of the nucellus or from the funicle. In later stages of development (corresponding to the stage of 8-nucleate ES) the situation most frequently seen was an ovule in which below the nucellar cap a group of degenerated cells occurred. For a summary of the ovules examined, see Tab. 2.

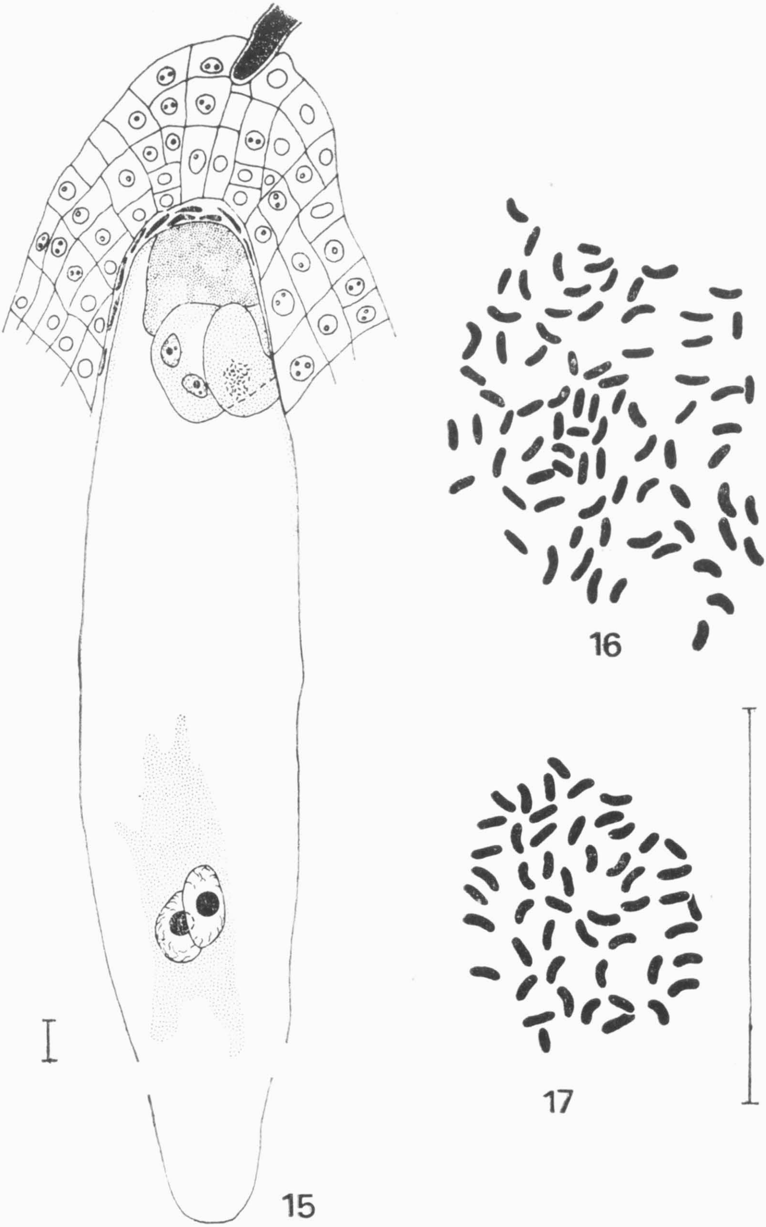
Development of embryo sac

As in other representatives of the genus *Sorbus* (see Liljefors 1953, Jankun et Kovanda 1986), in some ovules of *S. bohemica* it is very difficult to define a boundary between the archesporium cells and the vegetative cells. In some ovules secondary archesporium cells in early meiotic prophase I, metaphase I as well as remnants of degenerated dyads and tetrads were observed. In two cases typical mitotic prophases were observed in secondary archesporium cells (diplospory). It should be emphasised that only apomeiotic embryo sacs (ESs) developed in the ovules examined. As a rule the initial cells of apomeiotic ESs develop both from the cells situated in the central part of the nucellus (near the central group of degenerated cells of the archesporium) and from cells positioned immediately below the nucellar cap (apospory). These cells possessed a dense, dark-stained cytoplasm and large nuclei as well as nucleoli. The initial cells of apomeiotic ESs undergo vacuo-



Figs. 13–14. — *S. bohemica.* 13, 8-nucleate ES. 14, fertilisation of one of the two partly fused polar nuclei; the second sperm near the nucleus of the synergid. Bar = 10 μm.

lation and turn into 1-nucleate ESs. One to five apomeiotic ESs begin to differentiate within an ovule. After three mitotic divisions, 1-nucleate ESs give rise to 8-nucleate ESs. In typical mature ESs the egg apparatus con-



Figs. 15–17. — *S. bohemica*. 15, apomeiotic ES: 2-nucleate embryo, metaphase plate in synergid, remnants of the second synergid and two polar nuclei. 16, mitotic prophase plate from fertilised synergid with c. 80 chromosomes (51 from nucleus of the synergid + 29 from the sperm). 17, mitotic metaphase plate from hypanthium, 2n = 51. Bars = 10 μm.

sisting of the egg cell and two synergids is found at the micropylar pole; the central cell contains two polar nuclei and three 1-nucleate antipodals are at the chalazal pole. The synergids have a characteristic fibrillar apparatus at the micropylar pole. Antipodals are visible only in the early phases of mature ESs (Fig. 13), while later only remnants of degenerated antipodals are to be seen (Fig. 14).

In addition to the ovules in which only one ES developed we observed ten cases of parallel development of two or three ESs. As a rule they differed in the stage of their development but sometimes they were in the same stage — e.g. 1-nucleate ES + 4-nucleate ES, two 2-nucleate ESs + 4-nucleate ES, 2-nucleate ES + two 4-nucleate ESs, 4-nucleate ES + 8-nucleate ES, two 4-nucleate ESs + two 8-nucleate ESs.

A number of anomalies were found in the structure of mature 8-nucleate ESs: e.g. in 30 % of ESs reversed polarity was noted (egg apparatus at the chalazal and antipodals at the micropylar pole) (Tab. 2). Other anomalies in the structure of the ESs include the lateral position of the egg apparatus or the occurrence of both egg apparatus and atipodals at the chalazal pole. In three ESs three polar nuclei were observed. Asynchronic division of the ES nuclei may occur exceptionally, resulting in 6-nucleate ES. Also an ES with two egg apparatuses and two pairs of polar nuclei was observed. It probably arose as a result of the fusion of two mature ESs. Another type of anomaly was an unusually strong development of ESs which destroyed the nucellar cap and continued to grow in the micropylar canal. In such cases, the egg apparatus was found in the upper part of the canal. Similar disturbances were observed in ESs whose long axis was not strictly parallel to the long axis of the ovule. It was frequently directed c. 45° to the long axis of the ovule. In these cases the developing ES destroyed the nucellar cells and reached the inner integument with its apical part.

It should be added that some viable and well developed nucelli in open flowers contained only desintegrated remnants of the archesporium in the centre of the ovules.

Embryo and endosperm

Preliminary investigations concerning the development of the embryo and endosperm show that in apomeiotic ESs of *S. bohemica* the parthenogenetic development of the embryo is the rule. We observed fertilisation of partly fused polar nuclei in two instances. In one case one sperm fertilised one of the two partly fused nuclei while the other was seen next to the synergid nucleus (Fig. 14). The endosperm development is pseudogamous. Chromosome number in prophase nuclei of the endosperm was 5n = c. 130. Proof that fertilisation of the synergid may occasionally take place was a metaphase plate with c. 80 chromosomes (51 from the nucleus of the synergid + 29 from the sperm; Fig. 16). Next to the above mentioned fertilised synergid a 2-nucleate embryo was visible (Fig. 15).

Only few ESs produce viable embryos. In older stages the majority of mature 8-nucleate ESs degenerate. As a rule, the development of the embryo precedes that of the endosperm. In the majority of observed ESs the multicellular embryo was accompanied by two polar nuclei. Only in one case were the egg cell and two nuclei of the endosperm noted. In several cases polyembryony proper in conjunction with apogamy occurred. Twin embryos developed at the micropylar pole. As a rule, they differed in size. In one ovule a viable multicellular embryo was observed together with another, smaller (probably apogamous) but evidently degenerating embryo. Development of twin embryos (one being apogamous) was also observed in *S. sudetica* (JANKUN et KOVANDA 1986).

In two cases adventive (nucellar) multicellular embryos were observed. They were situated laterally, near the equatorial plane of the ESs. One of the ESs had typical polarisation but at the micropylar pole the remnants of egg apparatus were visible, while the other had reversed polarity (with the egg apparatus at the chalazal pole).

Taxonomy

The only source of information on the taxonomy and relationships of the species has so far been the brief report of "*S.* × *franconia*" by KLIKA (1937) and the original diagnosis of *S. bohemica* with some comments added (KOVANDA 1961c). The name *S. bohemica* has however been accepted by the authors of floras and enumerations (e.g. WARBURG et KÁRPÁTI 1968, HOLUB, PROCHÁZKA et ČEŘOVSKÝ 1979, DOSTÁL 1982, NEUHÄUSLOVÁ et KOLBEK 1982) and scattered records may be found in the literature. A full decription is given here.

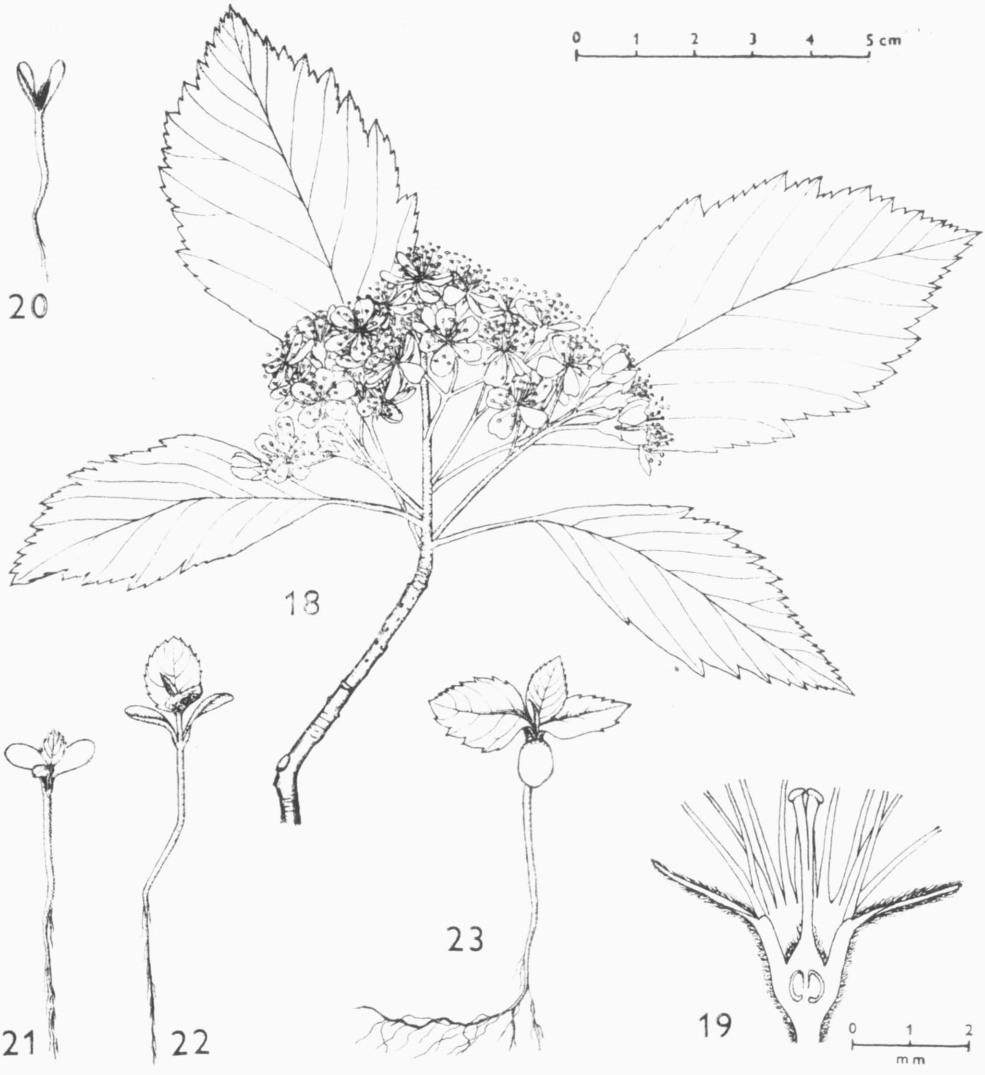
Sorbus bohemica KOVANDA Acta Univ. Carol. — Biol. 1961/1 : 77, 1961

Syn.: *S.* × *franconica* (ut „*franconia*") sensu KLIKA Sborn. Čs. Akad. Zeměd. 12 : 206, 1937, non *S. franconica* BORNM. Beih. Bot. Cbl. 36/2 : 186, 1918.

Trees or shrubs 2—4 (—6) m tall. Buds acute, almost completely glabrous. Leaf blade elliptic to ovate, in the upper part pinnatilobate and serrate, in the lower part cuneate, straight or slightly arcuate, with 2—3 (—5) remote teeth, (4.5—) 5—8 cm long and 3—4 cm wide, tomentose beneath, glabrescent on the upper side, with 8—9 (—10) pairs of veins; petiole 15—18 mm long. Inflorescence a dense, compact corymbothyrsus with tomentose, glabrescent branches. Hypanthium turbinate, tomentose; calyx-teeth triangular, acute, 2.6—3.0 mm long, erect to erecto-patent, persistent; petals obovate to elliptic, with a short claw, (5.8—) 6.0—7.8 (—8.0) mm long, hairy at the base on the adaxial side, yellowish white; stamens 20; anthers yellow; ovary semi-inferior; styles 2 (rarely 3), villose at the base, coalesced to 1/3—2/3 or rarely almost to the top; stigmas flat. Fruits broadly ellipsoid to subglobose, 8—10 mm long and 7—8 (—9) mm in diameter, orange-red, glabrous, shiny, very sparsely lenticellate; endocarp stony. Seeds chestnut brown, 3.4—4.0 mm long (Figs. 18—19, Plates III—IV).

Revised herbarium specimens: „supra petram Vrkoč", HRABĚTOVÁ 1973 BRNU; Církvice, KUBÁT 1983 LIT; Deblík, KUBÁT 1964 LIT; Milešovka, WETTSTEIN 1894 PRC, DOSTÁL et NOVÁK 1933 PRC; Kletečná, KLIKA 1935 PR; Kubačka, KLIKA 1934 PR; Výsluní („Scheinberg"), PREIS 1939 PRC, KOVANDA 1985 PR; Libochovany, PREIS 1934 PRC; Kalvárie, KUBÁT 1973 LIT; Stražiště, MISSBACH 1911 BRNU, KOVANDA 1976 PR, 1985 PR; Plešivec, KUBÁT 1969 LIT, KOVANDA 1976 PR, 1985 PR; Malíč, KUBÁT 1967 LIT; Hradiště, KOVANDA 1976 PR, 1985 PR; Lovoš, PODPĚRA 1902 BRNU, KOVANDA 1957 PRC, 1984 PR, 1985 PR (type locality); Opárenské údolí, KOVANDA 1984 PR; Boreč, KOVANDA 1978 PR, 1985 PR; Ovčín, KLIKA et SVOBODA 1934 PR; Košťál, DOSTÁL et NOVÁK 1933 PRC.

Morphologically, *S. bohemica* amalgamates characters of two distinct species, each belonging to a different subgenus: *S. danubialis* (JÁV.) KÁRPÁTI



Figs. 18–23. — *S. bohemica*. 18, Flowering twig. 19, longitudinal section of flower. 20–23, early stages of ontogenic development.

of the *S. graeca* aggregate (representing the polymorphic subg. *Aria*) and *S. torminalis* (L.) Crantz, the only representative of subg. *Torminaria*; for a survey of morphological characters, see Tab. 3. In its general appearance and leaf shape, *S. bohemica* resembles a member of subg. *Aria* rather than *S. torminalis* but is readily distinguished by the fused styles, a character completely missing in European and S.W. Asian *Aria*, but invariably present in *S. torminalis*. Other conspicuous characters aberrant for *Aria* include the colour of the fruits and their lack of indumentum, the shape of the stigma and the structure of the endocarp.

Tab. 3. — The distinguishing characters of *S. bohemica*, *S. danubialis* and *S. torminalis*

	S. bohemica	*S. danubialis*	*S. torminalis*
Underside of leaves	Tomentose	Tomentose	Glabrous or sparsely hairy
Number of veins (pairs)	8–9 (–10)	(7–) 8–10 (–11)	(5–) 6–7 (–8)
Length of petiole (mm)	(11–) 16–22 (–24)	(10–) 14–18 (–22)	(18–) 20–38 (–54)
Inflorescence	Dense, compact, branches tomentose	Dense, compact, branches tomentose	Thin, lax, branches glabrescent
Calyx teeth	Erect, 2.6–3.0 mm long, tomentose on both sides, persistent	Deflexed, 4.2–4.8 mm long, tomentose on both sides, persistent	Patent, 2–2.5 mm long, tomentose only beneath, deciduous
Petals	6–7.8 mm long, indistinctly unguiculate	6–8 mm long, unguiculate	4–5 mm long, exunguiculate
Ovary	Semi-inferior	Semi-inferior	Inferior
Styles	2 (rarely 3), coalesced to 1/3–2/3, woolly at base	2–3, free, woolly at base	2, coalesced to 1/2–2/3
Stigma	Flat	Convex	Flat
Indumentum of fruit	Absent	Present	Absent
Colour of fruit	Orange-red, shiny	Red, shiny	Brown, not shiny
Mesocarp	Heterogenous	Heterogenous	Homogenous
Endocarp	Stony	Cartillaginous	Stony
Colour of seeds	Chestnut brown	Dark brown	Rusty brown

Chemotaxonomic analysis (CHALLICE et KOVANDA 1978) revealed the presence in ***S. bohemica*** of flavone O-glycosides (luteolin 7-O-rhamnosylglucoside, luteolin 7-O-diglucoside, luteolin 4'-O-glucoside), characteristic, in Europe, of ***S. torminalis*** and ***S.*** *chamaemespilus* as well as their hybrids. Vitexin (apigenin 8-C-glucoside), characteristic only of *S. torminalis* and some of its hybrids, was found to be absent, indicating that *S. bohemica* may be the result of back-crossing of the F_1 hybrid with the *Aria* parent in which the genes for flavone C-glycosylation have become segregated from the genes for flavone O-glycosylation. A similar mode of origin has been proposed for other taxa of the *Aria* × *Torminaria* parentage, *S. bakonyensis* (JÁV.) KÁRPÁTI, ***S.*** *semiincisa* BORB. and *S. slovenica* KOVANDA (CHALLICE et KOVANDA 1978) and ***S.*** *eximia* KOVANDA (CHALLICE et KOVANDA 1986).

Concerning the *Aria* parent, it should be pointed out that *S. danubialis* (JÁV.) KÁRPÁTI, as conceived by JÁVORKA (1915) and KÁRPÁTI (1960), is in all likelihood identical with the original concept of *S. graeca* (SPACH) KOTSCHY [syn.: *S. cretica* (LINDL.) FRITSCH; see KOVANDA 1961b], Kárpáti's "*S. graeca*" corresponding to a round-leaved variety (cf. BUIA 1956). To avoid confusion, the name *S. danubialis* (JÁV.) KÁRPÁTI is retained, because it has become well established in the literature. A critical revision of *S. graeca* agg. in its entire geographical area is needed.

A conspicuous feature of *S. bohemica* is the fluctuation in the degree of coalescence of the styles which may vary even within one individual, from styles fused only at the base to styles fused almost up to the stigmas. A parallel may be found in *S. austriaca* (G. BECK) HEDL., a hybridogenous species of the *S. aria* × *S. aucuparia* parentage, in which the mode of fusion of the carpels is also subjected to considerable variation (KOVANDA 1961a). Slight variation may also be found in *S. eximia* KOVANDA, derived from *S. aria* × *S. torminalis* (KOVANDA 1984).

Another distinctive character of *S. bohemica* are the small, sparse lenticels on the fruits. In both the parent species they are much larger (in *S. torminalis* resembling tubercles) and more numerous.

It is interesting to note that intensive field research has failed to detect any traces of extant introgressive hybridisation or even F_1 hybrids between *S. danubialis* and *S. torminalis* in the distribution area of *S. bohemica*, even though the parent species often occur together in the same habitats and their flowering periods largely overlap (*S. danubialis* usually begins to flower 2 to 4 days earlier, and *S. torminalis* 3 to 5 days later than *S. bohemica*). This is surprising because elsewhere hybridisation is not uncommon; it is known to take place between *S. danubialis* and *S. torminalis* e.g. in the nearby region of Džbán, where the first parent is only sparsely distributed.

S. × *franconica* BORNM., described as a primary hybrid *S. cretica* (LINDL.) FRITSCH × *S. torminalis* (L.) CRANTZ from the vicinity of Bamberg, Federal Republic of Germany (BORNMÜLLER 1918), differs from *S. bohemica* in having smaller, round, obtuse leaves. The parentage is somewhat doubtful because *S. cretica* is not known to occur in the area mentioned and the characters given suggest rather the involvement of *S. aria* (L.) CRANTZ f. *cyclophylla* (G. BECK) JÁV. KÁRPÁTI (1960) reports *S. franconica* also from Austria and Rumania.

Geographical distribution

A number of hybridogenous species linking the members of subg. *Aria* with *S. torminalis* are known to occur in Europe (see Hedlund 1901, Warburg 1957, Kárpáti 1960, Düll 1961, Kovanda 1961c, Warburg et Kárpáti 1968). All are neoendemics, usually confined to small geographical areas. *S. bohemica* fits readily within this pattern. It was described as an endemic of the České středohoří Mts., N. Bohemia, and even after 30 years of study of the taxonomy and chorology of *Sorbus* in Czechoslovakia there are no records of its occurrence outside this area. All its localities are clustered in the central part of the mountains[1]), the maximum distance between each not exceeding 5 km (Fig. 24). The altitudinal range is from c. 280 to c. 600 m above sea level. *S. bohemica* obviously originated in these mountains and there is no reason to suppose that it originated elsewhere and then migrated into the České středohoří. Both the parent species are locally frequent in the distribution area of *S. bohemica*.

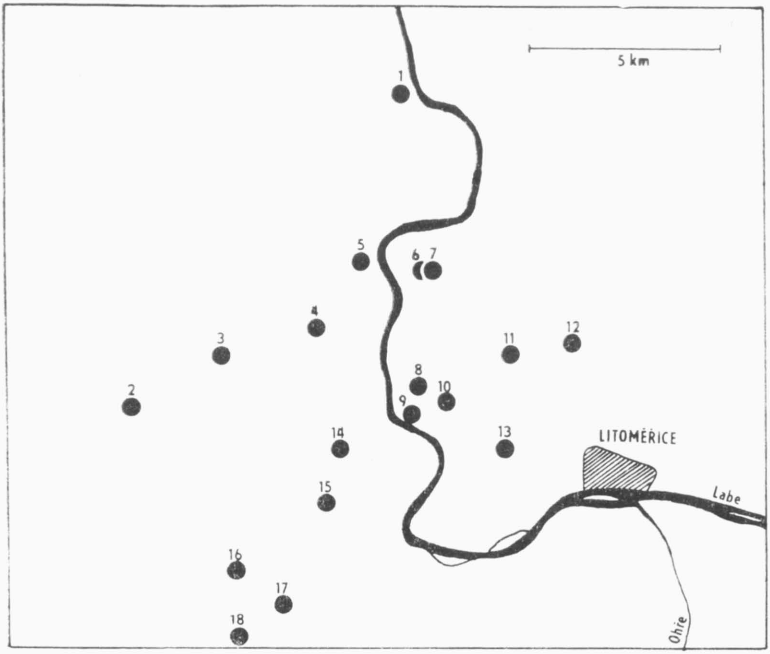


Fig. 24. — Distribution of *S. bohemica* in the České středohoří Mts. 1, below the Vrkoč rock. 2, E. slope of the Milešovka hill. 3, Kletečná hill. 4, Kubačka hill. 5, Výsluní hill. 6, Církvice, slope above the cottages. 7, Deblík hill. 8, near Libochovany. 9, Kalvárie hill. 10, Stražiště hill. 11, Plešivec hill. 12, Hradiště hill. 13, Malíč, valley S. E. of the village. 14, Opárenské údolí valley. 15, Lovoš hill (type locality). 16, Boreč hill. 17, Ovčín hill. 18, Košťál hill.

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[1]) It should be pointed out that the use of the word "mountains" in this context is misleading and is due to the absence, in the English language, of an equivalent of what is called "Mittelgebirge" in German, "středohoří" in Czech and "średniogórze" in Polish. Milešovka, the highest summit of the České středohoří, is only 837 m above sea level and much of the area is covered by thermophilous vegetation (average annual temperature, 7—8 °C).

Klika (1937) reports "*S.* × *franconia*" also from the Bezděz hill, about 40 km E. from the easternmost locality of *S. bohemica*, but the herbarium records quoted (**Podpěra** 1894 BRNM, 1897 BRNU) represent typical *S. danubialis* (**Jáv.**) **Kárpáti**. The absence of *S. bohemica* from (and the presence of *S. danubialis* on) this solitary hill (geologically a part of the České středohoří Mts.) has been confirmed by our field research.

Ecology and phytocenology

S. bohemica occurs in a variety of plant communities, being perhaps most frequent in the forest steppes and thermophilous oak woodlands of the alliance *Quercion pubescenti-petraeae* (order *Quercetalia pubescentis*) and in xerothermous scrub of the alliance *Prunion fruticosae* (order *Prunetalia*) in which *S. danubialis* also thrives. It is less common but by no means rare in the species-rich mesophilous oak-hornbeam and oak-linden woodlands (alliance *Carpinion*, order *Fagetalia sylvaticae*) and in fringe communities of the alliance *Prunion spinosae* (order *Prunetalia*) that are preferred by *S. torminalis*. Thus the hybrid combines the ecological amplitudes of both the parent species. The underlying rock is most often basalt or phonolite, or in several cases marly limestone. The aspect is most frequently south, south-western or western but eastern and even northern slopes are also successfully colonised.

Ecobiology

Flowering is in the latter half of May, sometimes extending into early June. In warm autumns, reflorescence is of frequent occurrence but tends to be restricted to certain individuals. Fruits ripe in late September and early October. Fructification is regular and prolific. All morphologically good fruits contain morphologically good seeds; those underdeveloped are usually sterile or contain stunted seeds. It will be seen from Fig. 25 that by far the most frequent type of fruit was one with a bilocular endocarp, one loculus containing one seed, the other empty, followed by a bilocular endocarp with one seed in each loculus. The other types are rare, some of them not showing up at all in the samples examined.

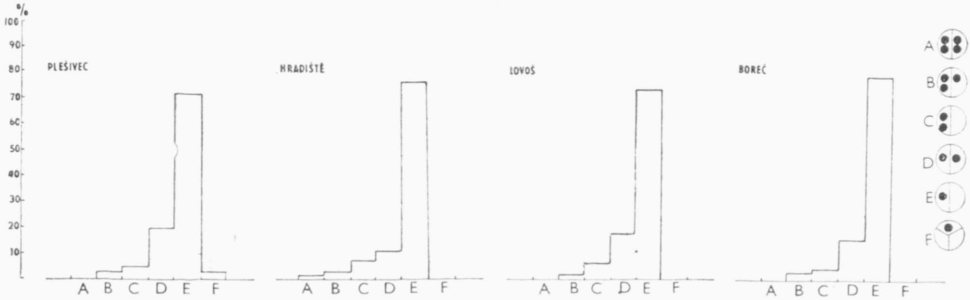

Fig. 25. — Showing the number of loculi of the endocarp and the number of seeds per loculus in four specimens of *S. bohemica* from different localities. The other possible types did not show up in the material examined.

It is important to note that following isolation of the flowers the fruit set was extremely poor. Either it did not occur at all or was arrested soon after flowering and no seeds were formed. Similar results were obtained with emasculated flowers. Of 39 emasculated flowers (20 from a specimen on the Plešivec hill, 12 and 7, respectively, from two specimens on the Lovoš hill), only two (plant from the Plešivec hill) produced fruits that reached maturity but were poorly developed and contained no vestiges of seeds.

The topodeme in the summit area of the Lovoš hill was monitored regularly in 1957—1986. The trees and shrubs flowered and fruited profusely every year except 1985 when there were virtually no flowers. This exceptional situation was obviously due to the hard winter, with temperatures falling, in January 1985, to c. —25 °C.

On germination, two cotyledons, broadly elliptic, obtuse, cuneate at the base, entire, 7—8 mm long and 5—7 mm wide, are produced. The first leaf is incise-serrate to lobate, resembling somewhat that of *S. torminalis*, while the later leaves are typical leaves of *S. bohemica* (Figs. 20—23).

In natural habitats, seedlings are not uncommon, especially on the Plešivec hill. In view of the fact that, for the reasons mentioned in the embryological part, there are relatively few seeds, this is rather surprising. In cultivation, seeds germinate only after stratification or when sown in pots embedded in soil over winter. Viability under these conditions varies from 25 to 46 %. It might be interesting to investigate the viability of seeds distributed by birds feeding on fruits. No germination was achieved with seeds in Petri dishes at room temperature.

A number of plants have been raised from seed and are kept in cultivation in two experimental gardens of the Botanical Institute of the Czechoslovak Academy of Sciences, one at Průhonice, the other at Černolice. Some of these plants will be used for a transplantation experiment, to test the possibility of re-introduction into natural habitats. The species has been classified as a threatened taxon "with a rare or dispersed occurrence requiring further study and observation" (Holub, Procházka et Čeřovský 1979). The total number of individuals known at present does not exceed 200.

DISCUSSION

Spontaneous triploid plants and triploid clones are found in a fairly large number of plant groups (Grant 1981). According to Darlington (1973), in most diploid plant species one seedling in every few hundred is a triploid. Many triploid hybrids as well as triploid varieties of cultivated plants are known.

S. bohemica is shown to be a triploid species of hybridogenous origin, with *S. danubialis* and *S. torminalis* as the parent species. In *S. torminalis* only diploids (2n = 34) are known to occur (Moffett 1931, Liljefors 1934, 1953, 1955, Tischler 1936, Baksay 1956, Jankun in Pogan, Wcisło, Jankun et al. 1980 and present studies), while *S. danubialis* has not been previously studied. Our material proved to be diploid with 2n = 34. It should be emphasised that *S. danubialis* belongs to the *S. graeca* aggregate in which two ploidy levels are present: 2n = 34 (Baksay 1956) and 2n = 68 (Jankun et Gabrieljan 1979, Zaikonnikova et Kipiani 1980).

The species and hybrids with triploid chromosome number occurring in the genus *Sorbus* are shown in Tab. 4.

Triploids are also frequent in other representatives of the *Malaceae*; e.g. Cinovskis (1971) mentioned 57 triploid species and 5 with triploid cytotypes of *Crataegus*, Bolkhovskikh et al. (1969) 7 triploid species and 22 with triploid

Tab. 4. — Triploid chromosome numbers in *Sorbus* L.

Triploid taxa (2n = 51)	Author
S. arranensis Hedl.	Liljefors 1953, 1955
S. neglecta Hedl.	Liljefors 1953, 1955
S. lancifolia Hedl.	Liljefors 1934, 1953, 1955
S. lancifolia Hedl. f. *sognensis* Hedl.	Liljefors 1953, 1955
S. subpinnata Hedl.	Liljefors 1953, 1955
S. Teodori Liljefors	Liljefors 1953, 1955
S. pinnatifida (Smith) Düll	Jankun in Pogan, Czapik, Jankun et al. 1985
S. tamamschjanae Gabr.	Jankun et Gabrieljan 1979 Žukova et Tichonova in Zaikonnikova 1982
S. bristoliensis Wilmott	Warburg in Clapham, Tutin et Warburg 1952
S. leyana Wilmott	Richards in Stace 1975
S. minima (A. Ley) Hedl.	Moffett 1931
S. bohemica Kovanda	Jankun et Kovanda (present studies)
S. aucuparia L. × *S. intermedia* (Ehrh.) Pers.	Liljefors 1953, 1955
S. aucuparia L. × *S. hybrida* L.	Liljefors 1953, 1955
Taxa with triploid (2n = 51) cytotypes	
S. Mougeotii Soy. et Godr.	Liljefors 1934
S. chamaemespilus (L.) Crantz	Liljefors 1953
S. porrigentiformis E. F. Warburg	Warburg in Clapham, Tutin et Warburg 1952

cytotypes of *Cotoneaster*, Rudienko (1978, cit. from Krylova 1981) 50 triploid varieties of *Malus* and Uhlík (1961) found 10 triploid varieties of *Pyrus* cultivated in Czechoslovakia.

A number of disturbances characteristic of triploid hybrids were found in the course of microsporogenesis in *S. bohemica*. In diakinesis and first metaphase 17 bivalents and 17 univalents were counted. The same type of conjugation of chromosomes was observed by Sax (1929) in *Sorbopyrus auricularis* (Knoop) C. K. Schneider var. *bulbiformis* (*Pyrus communis* × *Sorbus aria*). This hybridogenous taxon is regarded as a back cross of an F_1 hybrid with the *Pyrus* parent (Sax 1929). Liljefors (1955) observed the course of meiosis in several allotriploids of Scandinavian *Sorbus*. Pairing of chromosomes is very similar in all these taxa: 1—4 trivalents, the corresponding number of bivalents and 13—16 univalents were present, while in the autotriploid *S. chamaemespilus* (from the Alps) 8—10 trivalents, 8—10 bivalents and 7—5 univalents were present. In autotriploid representatives of *Malus* 1—2 quadrivalents, 14—15 trivalents and 1—5 univalents were observed by Heilborn (1935).

As a rule, the viability of the pollen grains of triploids is poor; in *S. bohemica* it amounts to c. 21 %. The pollen of the triploid *Sorbus* taxa from Scandinavia was totally degenerate or very poor. The anthers of some taxa were empty (e.g. *S. arranensis*, *S. Teodori*) or possessed only 20 % of pollen grains filled with cytoplasm (LILJEFORS 1955).

Although in the embryo sac mother cells (EMCs) of *S. bohemica* the whole cycle of meiosis may take place, only apomeiotic ESs were observed. Apospory, diplospory and pseudogamy were noted. A chromosome number for the endosperm of 5n = c. 130 was established. In the triploid taxa studied by LILJEFORS (1953) four possibilities of development may be distinguished:

(1) Meiosis in EMCs does not occur. The initial cells of apomeiotic ESs develop from the cells surrounding the remnants of degenerated EMCs (*S. arranensis*, *S. lancifolia*).

(2) Degeneration of EMCs takes place before or after meiosis. Only aposporous ESs develop (*S. neglecta*, *S. Teodori*).

(3) Meiotic, diplosporous and aposporous ESs are present (*S. aucuparia* × *S. intermedia*).

(4) Solely sexual propagation, where only reduced gametophytes are found (*S. aucuparia* × *S. hybrida*).

In *S. bohemica* a great many abnormalities in the structure of mature ESs were found. Only 59 % of ESs possess a typical structure while others show abnormalities characteristic of apomictic plants: e.g. reversed polarity (in 30 % of ESs), lateral position of the egg apparatus, occurrence of both egg apparatus and antipodals at the chalazal pole, ESs with 3 polar nuclei. On the other hand LILJEFORS (1953) emphasised that he never saw abnormal ESs in any of *Sorbus* taxa examined except the triploid hybrid *S. aucuparia* × *S. intermedia* where 6-nucleate ES and 2-nucleate ES with the nuclei in an unusual position occurred.

In most cases in *S. bohemica* two unfused polar nuclei were observed (even in ESs with multicellular embryos), and only one ES with a secondary nucleus was found. Fertilisation of partly fused nuclei was noted. In other representatives of the *Malaceae*, e.g. *Malus*, fusion with the second polar nucleus takes place as a rule after fertilisation of one polar nucleus (KRYLOVA 1981). It should be mentioned that according to CZAPIK (1985) the moment of fusion of polar nuclei varies within the family *Rosaceae* s.l. and in some taxa the secondary nucleus is not formed. CZAPIK (1983) suggested that e.g. in *Rubus* the retardation of polar nuclei fusion is connected with some stronger apomictic tendencies.

The embryo of *S. bohemica* develops parthenogenetically and the endosperm development is pseudogamous. One sperm fuses with the polar nuclei, while the other is sometimes visible within the synergid. Thus occasional fertilisation of the synergid nuclei of apomeiotic ESs takes place, leading to the formation of twin embryos, one with a higher chromosome number (chromosome summation). In one case a metaphase plate with c. 80 chromosomes was seen (51 from nucleus of synergid + c. 29 from the sperm). Sperms with aneuploid chromosomes may develop after disturbed meiosis in PMCs where nuclei with different chromosome numbers (from c. 17 to 28) were observed. These results indicate that besides plants which replicate the genotype of the mother plant, new genotypes may appear in the progenies of *S. bohemica*. LILJEFORS (1953) investigated the offspring of the triploid *S. Teodori* and discovered, in addition to the triploid maternal plants, two aberrants with 2n = c. 68 and c. 85. According to Liljefors the chromosome numbers of these plants suggest that they were hybrids and had resulted from fertilisation of the unreduced egg cells of apomeiotic ESs. Among seedlings of the solely sexual triploid hybrid *S. aucuparia* × *S. hybrida* LILJEFORS (1953) discovered two aneuploids with 2n = c. 36 and 44 which, according to him, must have resulted from the union of reduced gametes.

In the *Malaceae* chromosome summation was also observed in the genus *Malus*. Dermen (1949) reported that the triploid apomict *Malus hupehensis* (Pomp.) Rehd. occasionally gives rise to tetraploid progeny: out of 38 seedlings raised, 36 were triploid and 2 were tetraploid. A similar situation apparently exists in the triploid *M. lancifolia* Rehd., *M. coronaria* (L.) Mill. and *M. platycarpa* Rehd. Interesting results were obtained also by Olden (1953) with the tetraploid *M. Sieboldii* Rehd., a facultative pseudogamous apomict. After self-pollination of *M. Sieboldii*, plants with tetraploid and hexaploid (2n = 102) chromosome number were obtained. After crossing *M. Sieboldii* with diploid apple trees seedlings with triploid, tetraploid, pentaploid, aneuploid and mixoploid chromosome numbers as well as one dihaploid (2n = 34) were obtained. The dihaploid arose probably after parthenogenetic development of the egg cell of a meiotic ES. Haploid parthenogenesis was also noted in *Sorbus sudetica* (Jankun et Kovanda 1986). According to deWet (1979), dihaploids certainly play a significant role in maintaining variability among some apomictic tetraploids.

The preliminary observations of fructification following (1) free pollination, (2) isolation and (3) emasculation and isolation indicate that in *S. bohemica* there is a strong correlation between the development of seeds and that of fruits. The arrest of seed development brings about the arrest of fruit development. It seems reasonably certain that self-pollination is impossible. The absence of seed set following emasculation and isolation is a proof that pseudogamy is involved, thus confirming the results of embryological study.

The present studies have shown that the hybridogenous neoendemic *Sorbus bohemica* is a pseudogamous apomict. The process of chromosome summation discovered in this triploid species may give rise to karyological differentiation — increasing the number of chromosomes in the progeny. The results of morphological, embryological (conjugation of chromosomes) as well as chemotaxonomic analysis (Challice et Kovanda 1978) indicate that *S. bohemica* resulted from a back cross of the F_1 hybrid *S. danubialis* × *S. torminalis* (unreduced gamete) with the first parent.

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SOUHRN

Sorbus bohemica Kovanda, hybridogenní druh vzniklý křížením *S. danubialis* (Jáv.) Kárpáti × *S. torminalis* (L.) Crantz, je apomiktický triploid (2n = 51). Buňky archesporu nebo celá vajíčka odumírají již v časných fázích vývinu. Byla zjištěna aposporie, diplosporie a pseudogamie a stanoven chromosomový počet endospermu 5n = c. 130. V zárodečných vacích byly pozorovány různé anomálie, jako např. obrácená polarita (v 30 % zárodečných vaků), laterální pozice vaječného aparátu aj. Synergida apomeiotického zárodečného vaku může být výjimečně oplozena, což otvírá cestu ke karyologické diferenciaci. Výsledky ukazují, že kromě rostlin s geno-

typem stejným jako mateřská rostlina se v potomstvu mohou objevit i nové genotypy. Rovněž byla pozorována pravá polyembryonie (s apogamií stimulovanou samčí gametou). Jsou uvedeny důvody pro předpokládaný hybridní původ *S. bohemica* a rozbor morfologických znaků. *S. bohemica* je neoendemit Českého středohoří, kde je v současnosti znám z 18 lokalit nahloučených v centrální části pohoří. Celkový počet jedinců nepřesahuje 200. Jsou připojeny poznámky k ekologii, fytocenologii a ekobiologii. U rodičovských druhů byl stanoven diploidní počet chromosomů. 2n = 34.

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See also Plates III—IV in the Appendix.