Genetic variability and morphology of tri- and tetraploid members of the *Sorbus aria* complex in northern Bavaria

Genetická variabilita a morfologie triploidů a tetraploidů z komplexu *Sorbus aria* v severním Bavorsku

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The *Sorbus aria* complex in the Franconian Jura, northern Bavaria, consists of the sexual diploid *S. aria* s. str. and putative apomictic polyploids, among them *S. collina* and *S. danubialis*. Here, the genetic and cytotypic structure of the *S. aria* complex and the proportion of clonal versus variable genotypes with a special focus on *S. collina* are studied using nuclear microsatellite markers, flow cytometry and multivariate morphometrics. In the Franconian Jura the tetraploid *S. collina* is the most frequent taxon in the *S. aria* complex. It consists of six closely related clones and a high number of unique genotypes, which were found in 70% of the populations studied. Morphologically, most of the clones and the unique tetraploid genotypes are not significantly differentiated. Therefore, all of them could be assigned to *S. collina*. Mechanisms that could result in the high number of genotypes within *S. collina* are accumulations of mutations, remnant sexuality, multiple polyploidization events and backcrossing. The results show that *S. collina* is a genetically quite variable facultative apomictic taxon. Triploids were identified sporadically and the microsatellites pointed to an origin from repeated hybridization events between diploid *S. aria* s. str. and *S. collina*.

K e y w o r d s: apomixis, flow cytometry, hybridization, multiple origin, northern Bavaria, polyploidization, *Sorbus*, unique genotypes

Introduction

Sorbus is one of the few European woody plant genera in which polyploidization plays a crucial role in speciation (Robertson et al. 2010). The co-occurrence of different ploidy levels (diploids vs polyploids) associated with reproductive mode differentiation (sexual vs apomictic) was shown for the *S. aria* complex in Spain (Sosa et al. 2014) and Great Britain (Robertson et al. 2010, Ludwig et al. 2013), where many new polyploid taxa were recently described (Rich et al. 2010, Robertson et al. 2010).

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Northern Bavaria (Germany) is well known for its high incidence of endemism in the *Sorbus latifolia* (Lam.) Pers. and *S. hybrida* L. complexes (Düll 1961, Meyer et al. 2005). The *S. aria* complex in northern Bavaria is also strongly heterogeneous but its diversity is poorly studied.

In the study area, the Franconian Jura in northern Bavaria, there are currently three accepted taxa of the *S. aria* complex: diploid *S. aria* (L.) Crantz s. str., tetraploid *S. collina* M. Lepší, P. Lepší et N. Meyer and tetraploid *S. danubialis* (Jáv.) Kárpáti. In addition, there are intermediate forms between *S. collina* and *S. aria* s. str. (Feulner et al. 2013) and between *S. danubialis* and *S. aria* s. str., which are rare and have so far remained undescribed (Meyer et al. 2005). These plants exhibit intermediate morphology and were partly confirmed by AFLP markers in an earlier study (Feulner et al. 2013). The sexual diploid *S. aria* s. str., which is the most widespread taxon in the *S. aria* complex in Europe, is rare in the Franconian Jura and confined to the northwest of the area. *Sorbus danubialis* occurs only in the Middle and Southern Franconian Jura (Meyer et al. 2005). Both are widely replaced by *S. collina* (Lepší et al. 2015), having thicker leaves and a reduced number of lateral veins compared with *S. aria* s. str. (Meyer et al. 2005). *Sorbus collina* reproduces presumably by apomixis and occurs in the Franconian Jura as well as in parts of the Czech Republic and Austria (Lepší et al. 2015). However, the treatment of *S. collina* has greatly changed over time due to its intermediate morphological position between *S. aria* and *S. graeca* (Spach) Schauer. However, *S. graeca* is a taxon of unresolved taxonomy (Lepší et al. 2015). *Sorbus graeca* has been considered as a parental taxon for plants currently named as *S. collina* (Düll 1961, Bresinsky 1978). However, this is highly speculative. Kárpáti (1960) and others used the name for plants resembling *S. collina*. *Sorbus pannonica* was recently typified as a triploid endemic in the Transdanubian Mts in Hungary (Somlyay & Sennikov 2015). Lepší et al. (2015) describe the northern Bavarian plants as *S. collina* and discriminated it from *S. pannonica* based on differences in morphology and karyology.

Another xeromorphic taxon of the Franconian Jura is *S. danubialis*. It is mainly distributed in the Czech Republic and in Hungary and has a western outpost in the Southern and Middle Franconian Jura in Bavaria. According to the morphological species concept it is an intermediate between *S. umbellata* and *S. graeca* (Kárpáti 1960, Meyer et al. 2005). Within the *S. aria* complex it is morphologically characterized by its small rhombic shaped leaves (Meyer et al. 2005).

Whereas the distribution of the northern-Bavarian taxa is well studied (Meyer et al. 2005), less is known about their genetic structure. *Sorbus collina* was initially considered to be apomictic (Lepší et al. 2015), however, its genetic variability is higher than expected for an apomict (Feulner et al. 2013). Furthermore, we detected morphological intermediates, mostly in areas where both *S. collina* and *S. aria* s. str. occur (Feulner et al. 2013).

Here, we aim to gain insight into the morphological variability and cytological and genetic structure of the polyploid taxa in the *Sorbus aria* complex in northern Bavaria using multivariate morphometrics, flow cytometry and nuclear microsatellite markers (SSRs). We focus on *S. collina* and the so far undescribed intermediates between *S. aria* s. str. and *S. collina*. We addressed the following questions: (i) What is the genetic structure of *Sorbus collina* (clonal versus variable individuals, delimitation from other taxa in the *S. aria* complex)? (ii) Is *S. collina* morphologically uniform and separated from the

other polyploids? (iii) Are the intermediates between *S. collina* and *S. aria* derived from repeated hybridization between diploid *S. aria* s. str. and *S. collina*?

Material and methods

Study area

Fig. 1 shows the populations of the *Sorbus aria* complex studied in the Franconian Jura, a low mountain range which is formed mainly of Jurassic limestone. It is situated in northern Bavaria and is subdivided into the Northern, the Middle and the Southern Franconian Jura.

Sampled populations

For the microsatellite analysis we studied 95 individuals from 21 populations of the *S. aria* complex. The most sampled species was *S. collina,* which is the most widespread member of the *S. aria* group in the area studied. It was sampled in all three subregions of the Franconian Jura.

For morphological analyses we studied 87 individuals from the same taxa and populations with the exception of *S. collina* from population HAH and *S. danubialis* from population ZIM, for which only silicagel material was available. In Table 1, the locality of the taxa and populations investigated, the sample size and the methods of study are given. Herbarium material of the plants studied is deposited in the Herbarium of the University Bayreuth (UTB).

DNA extraction

For DNA extraction leaves were collected and stored either in ice boxes or in plastic bags with silica gel. The material stored in ice boxes was transferred into isolation tubes on the same day and kept stored at –80° C until isolation. Genomic DNA was purified from about 20 mg of leaf material as described in Feulner et al. (2013), with the following modification: the initial homogenization volume was increased to 400 µl MC1 buffer supplemented with 10 µl RNAse A and homogenization duration in the MP FastPrep-24 tissue homogenizer (MP Bio, Santa Ana, USA) was increased to two times 40 s at a speed of 6 m/s. The genomic DNA was diluted tenfold with water and used for microsatellite analysis.

Microsatellite analysis

Five microsatellite markers (MSS5, MSS13, MSS16, CH02D11, CH01F02; Robertson et al. 2010) were analysed in this study. Microsatellite fragments were labelled during PCR amplification using modified forward primers (Electronic Appendix 1) carrying 18 bp fluorescently labelled M13 extensions. The 10 µl PCR mixture included 0.04 µM forward primer carrying the universal M13 extension, 0.16 µM reverse primer, 0.16 µM universal M13 primer labelled with either BMN5, BMN6, or DY751 (biomers.net, Ulm, Germany; Electronic Appendix 1), 1x Qiagen Multiplex PCR Master Mix, 0.5x Q-Solution (Qiagen, Hilden, Germany) and 0.5 µl of diluted genomic DNA. The following PCR

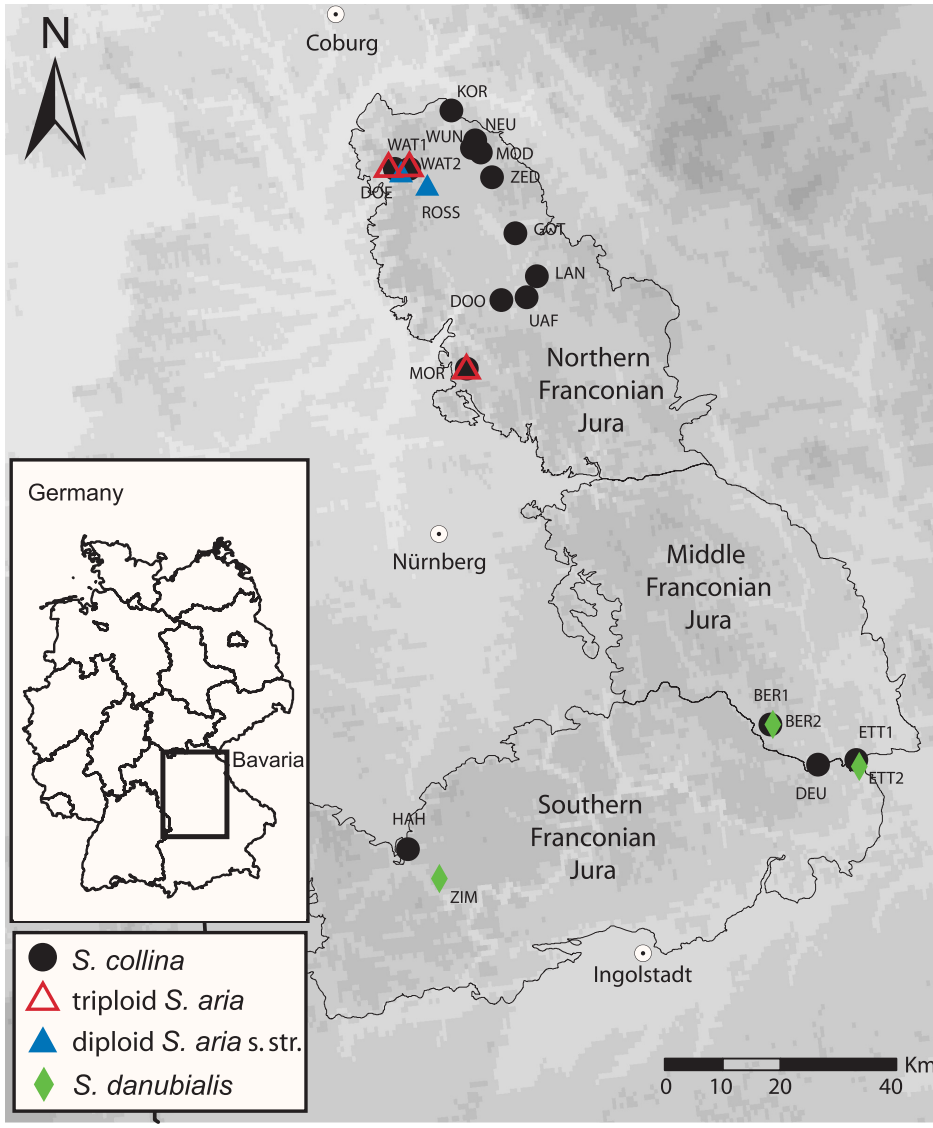

Fig. 1. – Geographical distribution of the populations and taxa of the *Sorbus aria* complex sampled in northern Bavaria, Germany (northern Bavaria is indicated by the black square in the small window on the left). On the right the geographical position of the populations in the Northern, Middle and Southern Franconian Jura are shown in detail.

cycling profile was used: Initial activation of the polymerase at 95 °C for 15 min., followed by 30 cycles at 95 °C/30 sec., 55 °C/45 sec., 72 °C/45 sec., followed by eight cycles at 95 °C/30 sec., 53 °C/45 sec., 72 °C/45 sec., followed by a final primer extension step at 60 °C for 30 min. (see Schuelke 2000; the initial activation step and the final extension step were modified as recommended in the Multiplex PCR Handbook, Qiagen, Hilden, Germany). The PCR products were combined into two multiplex sets (multiplex 1:

Table 1. – Overview and collection history of the populations of the *Sorbus aria* complex studied and the methods used: microsatellites (SSR), morphometry (MO) and cytology (FCM).

Taxon	Population	Population code	Individuals (collector's voucher no.)	n	Method used	Coordinates
S. aria s. str. (Northern Franconian Jura)	Rossdorf	ROS	FEU501–504	4	SSR, MO	49.99°N, 11.13°E
	Wattendorf	WAT1	FEU506	1	SSR, MO	50.02°N, 11.09°E
S. collina and triploids of *S. aria* complex (Northern Franconian Jura)	Unterailsfeld	UAF	FEU468, 470–472	4	SSR, MO, FCM	49.80°N, 11.34°E
	Doos	DOO	FEU473–475, 477	4	SSR, MO, FCM	49.80°N, 11.28°E
	Gottelhof	GOT	FEU435–437	3	SSR, MO, FCM	49.90°N, 11.33°E
	Zedersitz	ZED	FEU402, 486, 488–490	5	SSR, MO, FCM	49.99°N, 11.29°E
	Langeloh	LAN	FEU461, 463–467	6	SSR, MO, FCM	49.83°N, 11.37°E
	Wunkendorf	WUN	FEU410, 496–499	5	SSR, MO, FCM	50.04°N, 11.25°E
	Kordigast	KOR	FEU522–525	3	SSR, MO, FCM	50.10°N, 11.21°E
	Wattendorf2	WAT2	FEU504, 505, 507–510, 512–513	8	SSR, MO, FCM	50.02°N, 11.09°E
	Dörrnwasserlos	DOE	FEU514–518	5	SSR, MO, FCM	50.02°N, 11.06°E
	Modschiedel	MOD	FEU491–495	4	SSR, MO, FCM	50.03°N, 11.27°E
	Moritzkapelle	MOR	FEU479–485	7	SSR, MO, FCM	49.70°N, 11.18°E
	Neudorf	NEU	FEU517, 519, 520	3	SSR, MO, FCM	50.05°N, 11.26°E
S. collina (Middle Franconian Jura)	Deuerling	DEU	FEU449–453	5	SSR, MO,	49.03°N, 11.90°E
	Etterzhausen1	ETT1	FEU454, 456, 457–459	5	SSR, MO,	49.03°N, 11.99°E
	Beratshausen1	BER1	FEU438, 441, 442, 444, 446	5	SSR, MO, FCM	49.10°N, 11.80°E
	Hahnenkamm	HAH	FEU532–535	4	SSR	48.97°N, 10.92°E
S. danubialis	Zimmern	ZIM	FEU536–539	4	SSR	48.92°N, 10.98°E
	Etterzhausen2	ETT2	FEU455, 460	2	SSR, MO	49.02°N, 11.99°E
	Beratshausen2	BER2	FEU439, 440, 443, 447, 448	5	SSR, MO, FCM	49.10°N, 11.80°E

CH01F02 labelled with BMN5, CH02D11 labelled with BMN6, and MSS5 labelled with DY751; multiplex 2: MSS13 labelled with BMN5 and MSS16 labelled with BMN6) and separated on a GenomeLab GeXP Genetic Analysis System (AB Sciex, Darmstadt, Germany). The microsatellite allele lengths were obtained from electropherograms scored with GeneMarker v. 1.95 software (Softgenetics, State College, PA, USA).

The microsatellite phenotype (Becher et al. 2000) was calculated by treating the alleles as dominant loci. To try to compensate for the information loss resulting from treating the alleles as dominant, the allele distribution and common alleles in the different taxa were examined in detail and discussed. With the data matrix a Neighbor-Net network was computed using SplitsTree v. 4.11.3 (Huson & Bryant 2006) on the basis of uncorrected P as distances. We choose the Neighbor-Net network because it gives the best representation of original distances between the individuals and groups, including ambiguities. The function "weight threshold" was used to achieve an appropriate number of splits.

For each population the number of variable genotypes and the number of clones is given. A clone is defined if at least two individuals had the same genotype. The total and effective number of genotypes (1/sum of genotype frequencies) per population were calculated using the programmes Genotype v. 1.2 and Genodive v. 1.1 (Meirmans & van Tienderen 2004; Table 2).

Table 2. – Number of clones and genotypes in the populations of the *Sorbus aria* complex studied in northern Bavaria. See Table 1 for population codes.

Taxon	Population	n	Total no. of genotypes/ population	Total no. of individuals belonging to a clone	Total no. of unique genotypes/ population	Effective no. of genotypes
S. aria s. str.	ROS, WAT1	5	5	0	5	5.00
S. collina (Northern Franconian Jura)	GOT	3	1	3	0	1.00
	ZED	5	1	5	0	1.00
	MOD	4	3	2	1	2.67
	LAN	5	2	1	1	1.38
	WUN	5	2	1	1	1.47
	KOR	3	2	1	2	1.80
	UAF	4	4	1	3	4.00
	DOO	4	3	1	2	2.67
	WAT2 (only tetraploids)	5	2	2	1	2.13
	DOE (only tetraploids)	5	4	1	3	3.57
	MOR (only tetraploids)	4	4	1	4	4.00
	NEU	3	3	1	2	3.00
S. collina (Middle Franconian Jura)	DEU	5	1	5	0	1.00
	BER1	5	2	2	0	1.92
	ETT1	5	4	3	1	3.57
S. collina (Southern Franconian Jura)	HAH	4	1	1	0	1.00
S. danubialis	BER2	5	3	1	2	2.27
	ETT2	2	2	1	1	2.00
	ZIM	4	3	1	2	2.67
Triploids of the *S. aria* complex	WAT2	3	2	2	1	1.80
	DOE	1	1	0	1	1.00
	MOR	3	1	3	0	1.00

Table 3. – Percentage of common alleles in the taxa and their clones. The total number of alleles in Electronic Appendix 2 (the median in the case of the unique tetraploid genotypes of *S. collina*) of the taxa in the first column of this table was used for the calculation of allele matching. See Table 1 for population codes.

	S. aria s. str.	*S. collina*						*S. danubialis*	*S. collina* unique genotypes included	Triploids of the *S. aria* complex MOR
		North. Fr. Jura clone	Middle Fr. Jura clone 1	Middle Fr. Jura clone 2	Middle Fr. Jura clone 3	South. Fr. Jura	Fr. Jura clone			
S. collina Northern Fr. Jura clone	33	100								
S. collina Middle Fr. Jura clone 1	33	93	100							
S. collina Middle Fr. Jura clone 2	31	88	81	100						
S. collina Middle Fr. Jura clone 3	33	94	88	94	100					
S. collina Southern Fr. Jura	33	93	93	87	87	100				
S. collina Fr. Jura clone	33	93	93	94	88	87	100			
S. danubialis	27	53	53	60	60	53	53	100		
S. collina unique genotypes included	76	100	100	100	100	100	100	69	100	
Triploids of the *S. aria* compl. MOR	55	64	64	64	64	64	64	55	82	100
Triploids of the *S. aria* compl. WAT 2	50	83	83	83	83	83	83	42	92	58

DNA-ploidy level

DNA-ploidy levels were estimated by flow cytometry from fresh leaf petioles using a CyFlow space (Partec, Münster, Germany) fitted with a high-power UV LED (365 nm) as described in Meyer et al. (2014). For diploid accessions the plants already analysed in Feulner et al. (2014) were used again in this study and for calibration of the flow cytometric measurements.

For 12 individuals of the populations ETT1, DEU and HAH, we deduced the ploidy from the highest numbers of alleles occurring among five loci (Sosa et al. 2014; Electronic Appendix 2). We do not present the estimation for ETT2 and ZIM. We consider that this method is not suitable for *S. danubialis* as tetraploid samples measured with flow cytometry revealed only three alleles.

Morphometry

Leaves from the middle position on three lateral short shoots of each individual were collected and rapidly dried in a thermostatic press for six days (Widder 1970). The parameters such as width and length of the leaves, leaf shape coefficient (quotient between leaf area and perimeter multiplied by 4π), the number of teeth, their width and height, angle of leaf base and leaf top, and the position of the maximal incision on the right and left leaf blade were detected automatically after scanning using programme WinFOLIA Pro 2013a (Regent Instruments Inc., Quebec, Canada). The number of lobes and their heights and widths were recognized if the lobes fulfilled the criteria of having a leaf incision depth that is at least two times as deep as the height of a leaf tooth and a lobe width equal to the width of at least five teeth. The number of veins was counted and the specific dry weight of leaves (g/cm^2) was measured after drying the leaves for three days in a oven at 80 °C. The parameter values are given as medians or means of the three leaves measured per tree including standard deviation or minimum and maximum values. Significance of character differences between groups was tested using Statistica v. 7.0, using ANOVA, Kruskal Wallis and an unequal-N HSD test as a post-hoc test. Based on the Euclidian distances of the z-transformations of the data, different PERMANOVA analyses with PRIMER 6 (Clarke & Gorley 2006) were conducted to test for significant differences and dispersions between taxa, populations, clones and unique genotypes. PERMANOVA performs reliably when the dispersion between taxa is insignificant (Anderson & Walsh 2013). A SIMPER-test was used for testing the contribution of characters to clonal differences within *S. collina*; a principal coordinate analysis (PCoA) based on Euclidian distances of 10 standardized morphological parameters was conducted using PRIMER 6. MorphoTools was used to perform a k-nearest neighbour classification, to calculate posterior probabilities and to test for correctness of classifications (Koutecký 2015).

Results

DNA-ploidy

The sample/standard ratio of the individuals studied and the inferred DNA-ploidy are given in Electronic Appendix 3. Two distinct classes of sample/standard fluorescence ratios with means ± S.D.: 0.79±0.01, and 1.05±0.011 were detected. According to

Pellicer et al. (2012), the genome sizes within the genus *Sorbus* are relatively well conserved among different lineages, both in diploids and polyploids. As we previously calibrated the ratio of 0.52 with a diploid chromosome count (Meyer et al. 2014) the measured DNA-ploidy levels correspond to tri- and tetraploidy (Electronic Appendix 3).

Together with the diploid *Sorbus aria* accessions previously studied in Feulner et al. (2014) the *S. aria* complex in northern Bavaria consists of di-, tri- and tetraploid plants. The tetraploids *S. collina* and *S. danubialis* were the most common cytotypes. Triploids were much rarer and occurred in sympatry with tetraploids (MOR, WAT2 and DOE). Diploids were recorded only in the populations ROS and WAT1 (Electronic Appendix 2). In the populations WAT 1 and WAT 2, di-, tri and tetraploids occurred in close neighbourhood. In the remaining populations we recorded only tetraploid plants. For the populations ETT1, DEU and HAH we deduced the ploidy from the highest numbers of alleles occurring among five loci. Since the number was four, we consider the plants of these populations to be tetraploid (Electronic Appendix 2).

Microsatellite alleles

In Electronic Appendix 2 microsatellite fragments obtained using five primers developed for the plants studied are shown. For each locus between one and four alleles were retrieved. Total number of alleles ranged between seven and 10 in diploid *S. aria* s. str., 11 and 12 in triploids, 11 and 17 in *S. collina,* and in *S. danubialis* between 13 and 14. Unique alleles were recorded for taxa (allele CH02D11-161 for *S. danubialis*), triploids of the *S. aria* complex (CH02D11-174 for triploid MOR) and in two cases also for individuals (Electronic Appendix 2).

Neighbor-Net network

In the Neighbor-Net network based on the microsatellite data (Fig. 2) the main splits represent diploid *S. aria* s. str. and tetraploid *S. danubialis.* Apparent splits were also formed by three clearly separated groups of triploids of the *S. aria* complex (MOR, partly WAT2, DOE) and six different clones of *S. collina*. The clonal individuals of *S. collina* are the nearest neighbours of some slightly different tetraploid unique genotypes (i.e. 479MOR, 494MOD, 518DOE, 519 NEU, 520NEU, 505WAT2). The remaining tetraploid individuals with unique genotypes are at greater distances from the clonal members of *S. collina*.

Clonal diversity and unique genotypes

Most of the *Sorbus collina* individuals were assigned to six different clones and most of the triploids belong to two clones (Fig. 2). The clones of *S. collina* were weakly differentiated from each other, by only one allele (Electronic Appendix 2). The largest tetraploid clone (Northern Franconian Jura clone; Fig. 2) comprises 31 individuals, while in the Middle Franconian Jura three closely related clones and in the Southern Franconian Jura one clone occur. Another clone occurred in both, the Middle and Northern Franconian Jura (Franconian Jura clone; Fig. 2).

Table 2 shows that the effective number of genotypes and the number of clones are in most populations higher than one, indicating a high number of unique genotypes. They occurred in the majority of the *S. collina* populations (Table 2) and also in *S. danubialis*

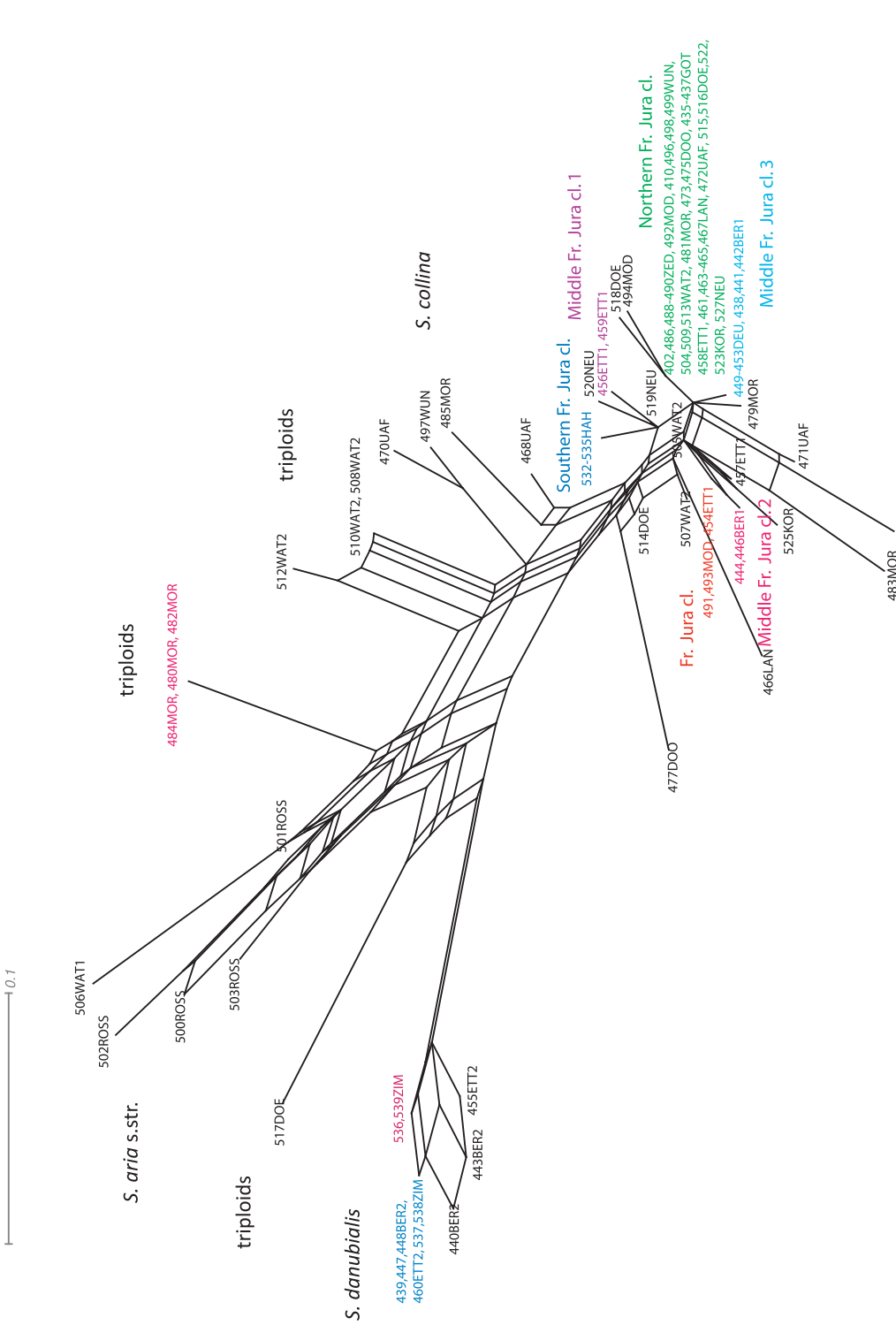

Fig. 2. – Neighbor-Net network of *Sorbus* microsatellite data based on uncorrected p-distances. Clones are indicated by different colours.

(Fig. 2; Table 2). To summarize, in *S. collina* about 27% of the individuals were unique genotypes, in triploids of the *S. aria* complex nearly 29% and in *S. danubialis* about 50%.

The unique genotypes of *S. collina* had either a lower number of alleles or, in few cases, they had more alleles than their clonal relatives. Some of them had private alleles, such as CH02D11-190 in UAF470. A conspicuous group of unique genotypes from the populations UAF, MOR and DOO share some alleles, namely MSS13-179 and MSS13-183, that were not recorded in the clonal individuals of *S. collina*. A second conspicuous characteristic of this group is that the individuals also share more alleles with *S. aria* s. str. than the clonal individuals (allele MSS13-179). Some unique genotypes of the populations UAF, LAN and the individual 497WUN could belong to parental lineages of the triploids of the population MOR (see Discussion), since two alleles (CH02D11-192 & CH01F02-213) were only recorded in triploids of MOR and these unique genotypes. Also the tri- and tetraploids of DOE share a population-specific allele (MSS16-185; Electronic Appendix 2)

Percentage of alleles shared between the taxa

Table 3 shows the percentage of alleles shared between the taxa whereas the different clones of *S. collina* were analysed with and without the group of unique genotypes. Tetraploid *S. danubialis* and *S. collina* share 27% and 33% of the alleles with diploid *S. aria* s. str., respectively. When the unique genotypes were included in *S. collina,* the level of common alleles with *S. aria* s. str. increased up to 76%.

Sorbus collina and *S. danubialis* share between 53% (Northern Franconian Jura clone), 60% (Southern Franconian Jura clone 3; Table 3) and 69% (unique genotypes included) of their alleles. Also the triploids of the populations MOR and WAT2 have a lower percentage of common alleles with the clones of *S. collina* than with *S. collina* including unique genotypes (Table 3).

Morphology

PERMANOVA revealed that the species and different populations from within species (Pseudo-$F_{18, 86} = 2.19$, $P < 0.0001$) differed in their morphology and that this species effect is reliable since it cannot be explained by differences in dispersion between the species ($F_{3, 86} = 0.18$, $P = 0.95$; Anderson & Walsh 2013). Groups were formed by diploid *S. aria* s. str., the triploids of the *S. aria* complex, and *S. danubialis* and *S. collina*.

The k-nearest neighbours classification revealed for 83 of the 87 individuals studied a match between 70% and 100% with the assigned taxon. Mismatches were two individuals of *S. aria* s. str. of ROS, assigned to *S. collina*, one individual of *S. danubialis* of BER assigned to *S. collina*, respectively, and one individual of *S. collina* of MOR assigned to *S. danubialis* (see Electronic Appendix 4).

The first two axes of the PCoA of the individuals based on leaf parameters explained 51% of the variation. The PCoA revealed four distinct morphological groups, which reflect the different taxa or ploidy levels (Fig. 3).

Morphological dispersion between the populations of *Sorbus collina* was significant ($F_{14, 53} = 3.57$, $P = 0.02$). The pairwise PERMANOVA testing the differences between the clones revealed significant differences between the Middle Franconian Jura clone 3 (plants from DEU & BER, $P < 0.05$) and the other clones except the Middle Franconian

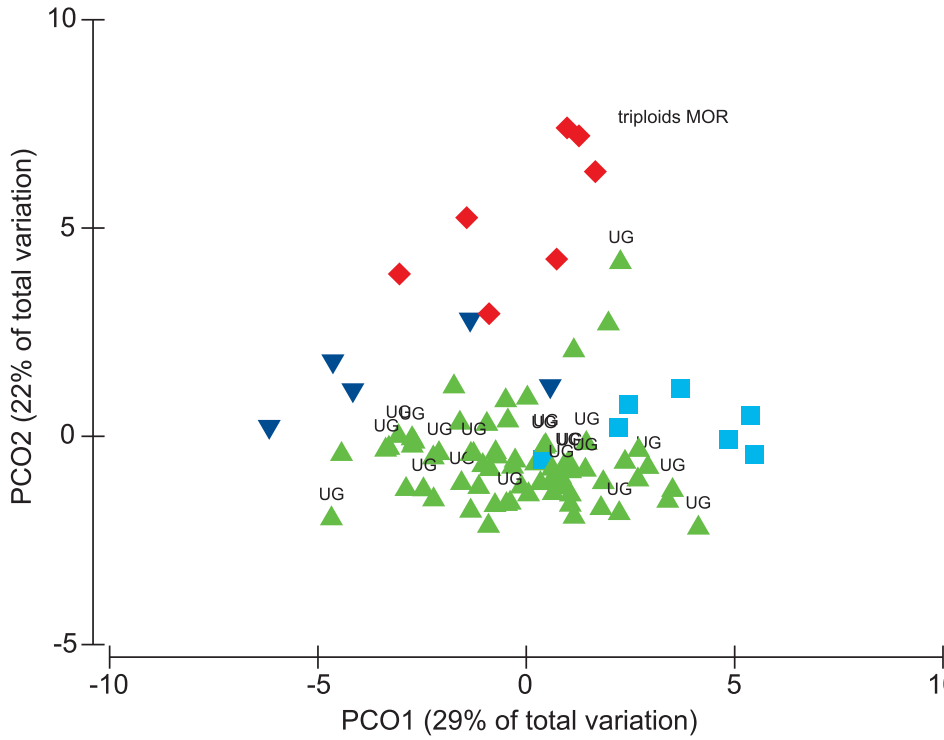

Fig. 3. – Principal coordinate analysis (PCoA) based on Euclidian distances of 10 morphological parameters of four *Sorbus* taxa. ▼ *S. aria* s. str. ▲ *S. collina*, ■ *S. danubialis*, ◆ triploids of the *S. aria* complex. UG = unique genotypes.

Jura clone 2. The SIMPER-test revealed that the specific dry mass contributed most to the differences between clone 3 and the remaining clones (average square distance contribution between 22.9% and 33.7%). The dispersion between the different clones and the unique genotypes within *S. collina* was not significant ($F_{5, 82} = 1.36$, $P = 0.12$) meaning that the unique genotypes could not be distinguished from the clonal plants.

Unlike in *Sorbus collina*, the morphological dispersion between the populations of the triploids was not significant ($F_{2, 4} = 8.84$, $P = 0.14$). Nevertheless, the triploids of MOR are morphologically more similar than the other triploids (Fig. 3).

The morphometric values of the taxa are given in Table 4. The triploid individuals were in general separated from *S. collina* and *S. aria* by the feature "lobed leaves" (see Table 4). Moreover, the triploids are significantly different from *S. collina* in the feature "angle of leaf tip" since they have more acute tips and in the percentage position of maximal leaf incision (see Table 4, unequal-N HSD post-hoc test). These features are the most important for differentiating between the triploids of the *S. aria* complex and *S. collina* in the area studied.

Table 4. – Morphological character values (median or mean) of the taxa of the *Sorbus aria* complex studied. Significant differences according to unequal-N HSD are indicated with different upper script letters.

	S. aria s. str.	Triploids of the *S. aria* complex	*S. collina*	*S. danubialis*
Character	Mean±SD or median (min–max)	Mean±SD or median (min–max)	Mean±SD or median (min–max)	Mean±SD or median (min–max)
Leaf perimeter [cm]	38.3±4.9^{a}	32.9±3.6^{a}	33.7±5.0^{a}	25.7±3.6^{b}
Leaf area [cm^2]	65. 7±15.4^{a}	48.5±11.7^{b}	48.9±9.1^{b}	30.5±7.2^{c}
Max. leaf length [cm]	12.4±1.6^{a}	9.9±1.3^{b}	9.7±0.8^{b}	7.5±0.9^{c}
Max. leaf width [cm]	7.7±1.0^{a}	7.0±0.7^{ab}	7.2±0.8^{ab}	6.0±0.8^{b}
Position max. leaf width [%]	48.6±2.9$^{n.s.}$	51.3±4.6$^{n.s.}$	52.7±3.0$^{n.s.}$	49.4±2.6$^{n.s.}$
Ratio max. BL/max. BB	1.6±0.1^{a}	1.4±0.1^{b}	1.4±1.0^{bc}	1.3±0.06^{c}
Form coefficient	0.554±0.016$^{n.s.}$	0.559±0.039$^{n.s.}$	0.550±0.077$^{n.s.}$	0.579±0.038$^{n.s.}$
Angle of leaf base [°]	49.7±4.4$^{n.s.}$	53.6±3.9$^{n.s.}$	51.0±3.8$^{n.s.}$	53.7±3.3$^{n.s.}$
Angle of leaf tip [°]	7.7 (5.3–9.3)^{a}	9.3 (7.3–14.3)^{a}	11.3 (8.3–13. 7)^{b}	10.0 (8.3–11.0)^{a}
Pos. max. cut (left) [%]	78.3±7.7^{abc}	75.1±9.4^{b}	82.6±5.2^{c}	74.3±9.7^{abc}
Pos. max. cut (right) [%]	79.3 (66.2– 85.8)^{ab}	75.9 (60.9–84.4)^{b}	85.3 (64.7–91.6)^{a}	82.4 (65.1–87.5)^{ab}
Number of teeth	67.9±11.3^{a}	52.3±7.8^{b}	52.2±7.8^{b}	43.6±3.4^{b}
Tooth height[cm]	0.089±0.009^{a}	0.098±0.008^{b}	0.109±0.011^{ab}	0.102±0.013^{ab}
Tooth width [cm]	0.215 (0.196–0.228)$^{n.s.}$	0.212 (0.196–0.273)$^{n.s.}$	0.223 (0.189–0.308)$^{n.s.}$	0.204 (0.171–0.219)$^{n.s.}$
Number of lobes	0 (0)	6.3 (4.3–7.3)	0 (0)	0 (0)
Lobe height [cm]	0 (0)	0.39 (0.28–0.46)	0 (0)	0 (0)
Lobe width [cm]	0 (0)	1.4 (1.2–1.6)	0 (0)	0 (0)
Number of leaf veins	23.5±1.4^{a}	20.3±0.9^{b}	19.8±1.2^{bc}	18.5±1.0^{c}
Specific dry weight [g/cm^2]	0.005±0.001^{a}	0.007±0.001^{ab}	0.007±0.002^{ab}	0.009±0.002^{b}

Discussion

The *Sorbus aria* complex in northern Bavaria consists mainly of tetraploid individuals and a low number of di- and triploid plants. *Sorbus aria* s. str. is very rare in the Franconian Jura, most likely because the tetraploid taxa are better adapted to drier soil conditions due to their relationship with south-eastern European and Mediterranean *Sorbus* taxa.

Sorbus collina is considered to be apomictic (Lepší et al. 2015), although no data was presented about its genetic variability. Here we found that it consists of several clones and a high number of unique genotypes. Interestingly, the Middle Franconian Jura clone 3 differs in the feature "specific dry mass" from most of the other clones of *S. collina,* but not the Middle Franconian Jura clone 2. This clone may be somewhat closer to *S. danubialis*, as discussed below. However, this character may be ecologically unstable and unsuitable for taxonomic differentiation. Genetic differences between the other clones are also small. Therefore, clone 3 should be included in *S. collina*.

One third of the individuals of *Sorbus collina* studied were unique genotypes (Fig. 2; Table 2, Electronic Appendix 2). They did not differ morphologically and therefore should be treated as *S. collina*. The reasons for this high degree of unique genotypes in Bavarian *S. collina* are accumulations of mutations, different polyploidization events (multiple origin) and backcrossing and remnant sexuality.

Accumulations of mutations may be responsible for slight differences between the unique genotypes in northern-Bavarian *Sorbus* (i.e. 519NEU & 505WAT2). Strongly differentiated microsatellite phenotypes may have arisen by independent polyploidization events (from the same parental taxa), backcrossing or presumably the ability to reproduce sexually. A closer look at the distribution in the allele patterns point towards different phenomena, which may coincide especially in the populations UAF, MOR, DOO and WUN.

The unique genotypes 470UAF, 471UAF, 483MOR, 474DOO and 477DOO have alleles that are not present in the other genotypes studied (MSS13-183, CH02D11-190). They have more alleles in common with *S. aria* s. str. than the clones of *S. collina* (MSS13-179 & CH02D11-196). This could be either the result of different independent polyploidization events between different individuals of the same taxa (multiple origin, Nelson-Jones et al. 2002), or due to backcrossing with *S. aria* s. str. Backcrossing yields mainly triploid individuals; tetraploids could arise by the fertilization of unreduced triploid egg cells with haploid pollen (triploid bridge; Talent 2009, Rich et al. 2010). The Northern Franconian unique tetraploid genotypes do not have specific and indicative alleles of the Franconian triploids (e.g. the tetraploids of MOR do not share any specific alleles with the triploids in this population; Electronic Appendix 2). Therefore, participation by the recent Northern Franconian triploids is unlikely. If the unique tetraploid genotypes have originated from the backcrossing of sexual *S. collina* and *S. aria* s. str. with unreduced gametes, we would expect them to have more alleles in common with *S. aria* s. str. (Electronic Appendix 2). So independent polyploidization events are possible, however, we could not rule out backcrossing since we could not exclude that we investigated too few *S. aria* s. str. due to its rarity in the area studied.

A sign of sexual reproduction in some unique genotypes of *Sorbus collina* may be the occurrence of individual-specific alleles, which are present in the conspicuous populations UAF, DOO, WUN and MOR (Individual 470UAF, 474DOO, 477DOO). Remnant sexuality was recently reported in some tetraploids of the *S. aria* complex in Great Britain (Ludwig et al. 2013) and Bosnia-Herzegovina (Hajrudinović et al. 2015). In an earlier study we report that the progeny of some northern Bavarian *S. collina* are genetically more variable than the adults, which indicates facultative sexual reproduction (Feulner et al. 2013).

A greater loss of microsatellite alleles compared to the alleles in clonal *Sorbus collina* was recorded in the unique tetraploid genotypes 468UAF, 494MOD and 485MOR (see Electronic Appendix 2). The reason for this loss could be autogamy (Ludwig et al. 2013) or cross pollination causing meiotic loss of alleles, when compared to the parental plants.

Half of the individuals of *S. danubialis* have unique genotypes, but the differences between the clones were much lower than in *S. collina*. Hence we have no indication of remnant sexuality in *S. danubialis* in the area studied and accumulation of mutations alone may be responsible for the recorded allelic variability. If not influenced by the low number of samples, the low variability in *S. danubialis* may be caused by genetic drift or a border effect, since this species is at its westernmost distribution in the area studied.

In *S. collina* it is obvious, that the clonal individuals are much more abundant than the unique genotypes (see Fig. 2). This could be simply an effect of the dominance of apomictic reproduction compared to sexuality in amphimictic tetraploids. However, an unresolved question is whether sexuality sporadically occurs or whether there are even

obligatory sexual tetraploid lineages in *S. collina.* Alternatively, selection could also play a role. Especially in open landscapes clonal individuals are thought to have a higher colonization potential than sexual individuals (Tomlinson 1966, Krahulec & Krahulcová 2011). However, Sailer et al. (2014) have shown that in *Hieracium pilosella* sexual members are better inter-specific competitors than clonal individuals, which would be an argument against the clonal plants having a competitive advantage.

Sorbus aria s. str. is considered to be a contributor to the evolution of both *S. collina* and *S. danubialis.* Although only a few individuals of *S. aria* s. str. were studied, these taxa share five alleles, this is a third of the total number of alleles in polyploid taxa (Table 2, Electronic Appendix 2). This finding is in accordance with the classical concept (Düll 1961, Bresinsky 1978) that indicates that the Franconian plants described as *S. collina* are intermediates derived from *S. aria*.

Sorbus danubialis and the northern-Bavarian *S. collina* share 10 alleles (five more than they share with diploid *S. aria* s. str.). Based on our data we could not rule out that *S. danubialis* was involved in the origin of *S. collina* or vice a versa (Electronic Appendix 2). In the Middle Franconian Jura, the populations BER1 and DEU and *S. danubialis* occur together and form mixed populations (Fig. 1). The Middle Franconian Jura clones 2 and 3 have a closer relationship with *S. danubialis*, since they have a *S. danubialis* specific allele (MSS5-147, see Table 2, Electronic Appendix 2). This may be a sign of gene flow between *S. danubialis* and *S. collina*. Hence, in order to fully confirm the origin of *S. collina,* a comprehensive analysis including *S. graeca* and *S. umbellata* is necessary.

Triploids in the Sorbus aria complex

The triploid individuals of MOR, WAT2 and DOE are examples of multiple origins of morphologically similar but genetically different types (Fig. 2, Electronic Appendix 2). As the three triploid populations are genetically distinct they must have been derived by independent crossings between *S. aria* s. str. and *S. collina*. The triploids of population MOR have alleles (CH01F02-213 & CH02D11-192) typical of some of the tetraploid unique genotypes (466LAN, 497WUN & 470UAF; Electronic Appendix 2). These tetraploids most probably belong to their ancestral lineages, as the opposite, that they functioned as a bridge for the evolution of these tetraploids, is unlikely. Crosses between di- and tetraploids are the typical origin for triploids (Ludwig et al. 2013, Hajrudinović et al. 2015). The triploids of MOR are peculiar; it is the only population at present without *Sorbus aria* s. str. in its vicinity. These triploids must have been formed in the past.

Interestingly, the lobed leaves typical of the northern-Bavarian triploids are also found in triploid taxa in Great Britain (i.e. *Sorbus wilmotiana*; Rich et al. 2010) and the Czech Republic (*S. moravica*, *S. cucullifera* and others; Lepší et al. 2015). Therefore, lobed leaves may be a sign of triploidy, not only in the area studied. On the other hand, *S. aria* s. str. can also sometimes have lobed morphotypes (forma *incisa*; Kovanda 1961).

As currently understood the triploids in the Northern Franconian Jura are not genetically uniform and occur in small numbers. They occur in sympatry with tetra- or/and diploids. Due to self-incompatibility their generative propagation depends to a great extent on accompanying di- or tetraploid *Sorbus* taxa (Ludwig et al. 2013). Valuable descriptions of novel taxa are hampered by the small population sizes and distribution ranges and

depend on the possibility of clearly delimitating them from triploids already described from other areas.

Conclusion

Surprisingly, in *Sorbus collina* unique genotypes that do not morphologically deviate occur in most populations. The reasons for this variability are most likely the accumulation of mutations, facultative sexual reproduction and processes such as multiple origins and backcrosses. Moreover, *S. aria* was involved in the origin of *S. collina* as a parental taxon. *Sorbus collina* is characterized as a genetically variable taxon and a facultative apomict. The triploid types are the result of a secondary contact between tetraploids and *S. aria* s. str.

See www.preslia.cz for Electronic Appendices 1–4

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Souhrn

Komplex *Sorbus aria* v pohoří Francký Jura v severním Bavorsku zahrnuje sexuální diploidy *S. aria* s. str. a předpokládané apomiktické polyploidy, jako jsou *S. collina* and *S. danubialis*. Studovali jsme genetickou a cytotypovou strukturu komplexu *S. aria* a zastoupení klonálních a geneticky variabilních genotypů pomocí jaderných mikrosatelitních markerů, průtokové cytometrie a mnohorozměrné morfometriky; soustředili jsme se zejména na taxon *S. collina*. V pohoří Francký Jura je nejčastějším taxonem komplexu *S. aria* tetraploidní *S. collina*, tvořený šesti blízce příbuznými klony a velkým počtem jedinečných genotypů, které byly nalezeny v 70 % studovaných populací. Většina klonů a unikátních tetraploidních genotypů není morfologicky diferencována, takže je možno všechny přiřadit k *S. collina*. Mechanismy, jež mohou generovat velký počet genotypů v rámci taxonu *S. collina*, zahrnují kumulaci mutací, zbytkovou sexualitu, vícenásobnou polyploidizaci a zpětné křížení. Naše výsledky ukazují, že *S. collina* je geneticky relativně variabilní, fakultativně apomiktický taxon. Triploidi se vyskytují sporadicky a mikrosatelity ukazují, že vznikají opakovanou hybridizací mezi diploidním *S. aria* s. str. a tetraploidním *S. collina*.

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