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Low rates of apomixis and polyploidy in progeny of Thuringian *Sorbus* subgenus *Tormaria*

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Abstract

Triassic limestone areas of Thuringia are well-known for their richness of hybrids between *S. aria* and *S. torminalis* (subgenus *Tormaria*). Those have been originally described as endemic taxa (i.e. *Sorbus heilingensis, S. isenacensis*). However, the former endemics have been recently synonymized with *S. × decipiens* (*Karpatiosorbus × hybrida* (Bechst.) Sennikov & Kurtto) due to diploidy and putative sexuality. Generally, crosses between diploid and sexual parents should segregate and intermediates as well as morphotypes close to the parents should be the outcome. To test for clonality versus segregation, we investigated progeny of 10 *Tormaria* mother trees, derived from open pollination. Three to ten seedlings of each mother tree were cultivated, and six leaf characters were measured being indicative for subgenus *Aria*, *Tormaria* and *Torminalis*. For genetic characterization, we used three ISSR microsatellite markers and one chloroplast marker. The progeny of nine mother trees segregated; the *S. aria* genome prevailed in pollen clouds. One polyploid mother tree, however, produced partly clonal progeny and proved to be polyploid and facultative apomictic. This means that in Thuringian *Tormaria* polyploidization occurs at low rates starting from diploid hybrids. Exact comparisons between geno- and phenotypes of the progeny proved a frequent uncoupling between genetic markers and genes responsible for leaf morphology, pointing to excessive genomic rearrangement within *Tormaria* mother trees. Only 1/3 of *Tormaria*-genotypes possessed the *Tormaria*-phenotype. Most of them belong to *S. aria* and to *S. aria* forma *incisa*, a *S. aria* type with slightly incise leaves. This means that plasticity of parental species may be explained by excessive introgression between *S. aria* and *S. torminalis*.

Keywords Distorted segregation · Environmental selection · *Sorbus* · Spontaneous polyploidization · *Tormaria*

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Introduction

Sorbus is one of the taxonomically most challenging tree genera in Central Europe, hybridization between the basic taxa *Sorbus aria, S. torminals* and *S. aucuparia*, polyploidization and apomixis lead to an immense variety of taxa of hybrid origin (Nelson-Jones et al. 2002; Robertson et al. 2010). In most cases those hybrid taxa are apomicts and have been described as endemic microspecies, being of high importance for the local biodiversity (comp. Meyer et al. 2005; Lepší et al. 2015). *Sorbus aria* as an aggregate of several diploid and polyploid taxa has mandatory to be involved in those hybridization processes (comp. Rich et al. 2010). Here we investigate hybrids derived from crosses between *Sorbus aria* agg. and *Sorbus torminalis* in Thuringia, named here as *Sorbus* subgenus *Tormaria* following Májovský and Bernátová (2001). In Thuringia series of microspecies of the subgenus *Tormaria* have been described as endemics (i.e.,

S. heilingensis, S. isenacensis and others) by Düll (1961). Recently it became obvious that those taxa are diploid and sexual (Leinemann et al. 2010, 2013; Meyer et al. 2014). Therefore, they have been synonymized with *S.×decipiens* (*Karpatiosorbus×hybrida* (Bechst.) Sennikov & Kurtto), the primary diploid hybrid between *S. torminalis* and *S. aria*. Those diploids, even when they form large populations such as in Thuringia, do actually not play the same role as polyploid endemics that are fixed by apomixis due to outcrossing and segregation. Diploid primary crosses between *S. aria* and *S. torminalis* are known to be of low fertility and rare (Düll 1961). However, this was not the case for the former Thuringian S. subgenus *Tormaria* species. The Thuringian diploid *Tormaria* were originally described as species by Düll (1961) because they show a high fertility and produce a lot of viable progenies (Meyer et al. 2014).

Triploid endemics of the subgenus *Tormaria* have been described for Great Britain, Czechia and Germany and other countries (Robertson et al. 2010; Ludwig et al. 2013; Feulner et al. 2017; Hajrudinović et al. 2015; Lepší et al. 2015; Kurtto et al. 2018).

An important role for the occurrence of polyploidy in *S.* subgenus *Tormaria* putatively plays the *S. aria* parent. The ploidy of the *S. aria* aggregate (*Sorbus* subgenus *Aria*, *Aria* acc. to Kurtto et al. 2018) can vary (comp. Robertson et al. 2010; Rich et al. 2010; Lepší et al. 2015; Feulner et al. 2019), however it is obvious that the occurrence of apomictic hybrid taxa in areas like in the Franconian Jura, in Great Britain or in parts of Czechia often corresponds with the occurrence of tetraploid taxa of the *Sorbus aria* aggregate such as *S. collina, S. danubialis* or *S. porrigentiformis* (Meyer et al. 2014; Rich et al. 2010).

In the study area those polyploid *S. aria* agg. are missing and only *S. aria* s.str. occurs. Since the Thuringian diploid trees of *Sorbus* subgenus *Tormaria* are putatively not able to form genetically fixed progeny by apomixis, they should segregate in the F2 generation according to the Mendelian rules.

Here, we analyze the progeny of *Tormaria* hybrids between *S. aria* and *S. torminalis* of Thuringia originally known as *Sorbus heilingensis* Düll (Düll 1961), now affiliated to *S.×decipiens* (Meyer et al. 2014). The investigated individuals occur in the surrounding of Heilingen near Orlamünde, on a triassic calcareous mountain range („Hohen Straße", an in the "Reinstädter Grund"). About 260 plants are known so far (Kahlert et al. 2011), growing in a mixed forest, being of high vitality and show a high stem size. Inventories of the adult *Sorbus* species pattern in the study area showed that *S.* subgenus *Tormaria* trees make up a proportion of about 30% of the *Sorbus* trees. The parent *S. torminalis* dominates with approx. 60%, *S. aria* s. str. reaches only 10%.

The Thuringian *S.* subgenus *Tormaria* are believed to be sexual and segregate according to Mendelian rules, nevertheless low rates of polyploidization and apomixis could not be excluded. Studies with single tree progenies are missing so far, testing this assumption. Moreover, even if the progeny segregates, it is not clear, to which taxa they segregate and whether backcrosses play a role. Therefore, we investigate here their genetic and morphological constancy. It is known from other studies that F2 generations of hybrids show high rates of segregation distortion meaning that genetic markers do not segregate as could be expected from the Mendelian rules (Singh et al. 2012; Kianian and Quiros 1992). That means that high rates of rearrangements that leads to strong inconsistencies between geno- and phenotypes in progeny of *Tormaria* mother trees can be expected. Furthermore, segregation studies on progenies may elucidate introgressive gene transfer from *S. aria* to *S. torminalis*. Such a transfer can be highly selective in hybrid zones. Martinsen et al. (2001) showed for *Populus* hybrids that only some of the studied markers were transmitted via the hybrid state from one *Populus* taxon to another.

Introgression could be responsible also for morphological plasticity of the parental species. An example is *S. aria* "forma (f.) *incisa*", which has—similar to *Tormaria*— slightly deep lobes at the leaf margins. A detailed description of *Sorbus aria* f. *incisa*, including leaf plates is given by Düll (1961) who emphasizes that those *S. aria* forms are not rare in the study range. The phenological most promising difference between *S. aria* f. *incisa* is that members of *Tormaria* have acute instead of rounded leaves at the top and deeper lobes, making it possible to distinguish them accordingly (comp. Düll 1961). A reason for the existence of those morphological types being intermediate between *S. aria* and S. subgenus *Tormaria* could be excessive introgression or backcrossing between diploid *Tormaria* and their parents (Meyer et al. 2005).

In a case study, we analyze the progeny of *Tormaria* hybrids between *S. aria* and *S. torminalis* of Thuringia originally known as *Sorbus heilingensis* Düll (Düll 1961), now affiliated to *S.×decipiens* (Meyer et al. 2014). Open questions are the intraspecific genetic and phenotypic variation of the progeny, rates of intra- and interspecific crosses including backcrosses and rates of clonal reproduction. We use genetic microsatellite and chloroplast markers that are species specific to verify the intermediate state of the parental *Tormaria* mother trees (Leinemann et al. 2013). With those genetic markers and leaf morphological measurements we investigate the variability of up to ten progeny trees of 10 mother trees arisen by open pollination.

The following research questions were addressed:

1. How genetically and morphologically variable are the progenies of the *Tormaria* mother trees?
2. Are there cases of polyploidization and apomixis among the progenies?

Material and methods

Data sampling

Seeds from 10 *S. × decipiens* trees, derived from open pollination, were collected in the study area (Table 1). After seed germination between 3, 4, 6, 9 (in four cases) and 10 (in three cases) progeny plants were retrieved and cultivated for two years in the ecological botanical garden Bayreuth. The vitality of the seedlings was in most cases high to moderate and only in 2 cases low. The progeny trees were used for genetic studies and phenological leaf investigations (Table 1).

Morphological investigation

Based on commonly used botanical characteristics for *S. aria*, *S. aria* f. *incisa*, *S. torminalis* and *S. × decipiens* (Müller et al. 2021; Düll 1961), we determined the taxonomy of the progeny and affiliated them to either *S. aria*, subgenus *Tormaria* or *S. torminalis*. We mostly relied our decision on the leaf shape, incision (weak in *S. aria*, intermediate in *S.* subgenus *Tormaria* and strong in *S. torminalis*) and number of veins. When we found a weakly incise leaf those individuals were affiliated to *S. aria* f. *incisa*, a relatively wide distributed but rare form of *S. aria* having clear but short incisions on the leaf margins (Düll 1961). With this method we built a table where we showed the relative percentage of progeny of each mother tree belonging to either *S. aria* (inclusively f. *incisa*), *S. torminalis* or *S.* subgenus *Tormaria*.

Do rely not only on a somewhat subjective determination, we measured the leaf morphology of every progeny individual with WinFOLIA (Reagent Instruments Inc., Quebec, Canada), harvested from 2 years old seedlings cultivated at the ecological-botanical garden Bayreuth. It has to be mentioned that in that age no standardized leaf material could be used since short shoots usually used are not derived at this state. We tried to use three relatively well-developed leaves of each progeny and measured the following characters and used them to form the following quotients for statistics:

1. leaf width/leaf blade length × 100
2. max. incision length/max leaf width × 100
3. leaf length/petiole length × 100
4. position max. leaf width in %
5. angle at lobe tip above max. incision/180*100
6. mean number of veins/max. mean number of veins*100

The leaf series of the progeny of three mother trees are exemplary given in the electronic supplement to show their variation (comp. electronical supplement Fig. S1–S3).

For the measurements with WinFOLIA, the leaf was scanned and a pixel classification was performed with the program WinFOLIA to separate the pixels of the leaf from those of the surroundings. The maximum width of the leaf is automatically detected by the program. The length of the horizontal line at the widest point of the leaf is determined. The length of the maximum incision on the left or right side, respectively, is determined using a tangent line crossing the incision gap at the leaf margin and the maximum incision length is measured starting from the center of this tangent. Left and right measurement are then averaged. Starting from the deepest incision, the angle at the end of the lateral lobe above is measured on both sides. The values are averaged afterwards. The petiole length is determined along the petiole. In the case of a curved petiole, several lines are formed, the lengths of which are finally added together (Fig. 1).

The position of the maximum width of the blade is given relative to the leaf length. The number of lateral nerves is counted on both the left and right sides and averaged afterwards. A lateral vein is considered to be one if it extends from the midrib to the leaf margin.

Kruskal–Wallis-Anova in R (R Core Team 2019) and Dunn'sTest with the R-package Dunntest (Dinno 2018) were used to test for significant differences between the taxa and their characters. Nonlinear multidimensional scaling

Table 1 Geographic coordinates of the *Tormaria* mother trees harvested for progeny analyses, number of seedlings analysed morphologically and genetically and estimated vitality of the seedlings according to their growth behaviour

Mother tree	UTM coordinates		Seedlings	Vitality
A 01	673554,272079	5629127,983	9	Moderate
A 02	673520,016281	5629234,99	10	High
A 03	673297,88148	5629185,859	10	High
A 04	672794,558727	5629601,909	9	Moderate
A 05	672795,251494	5629570,396836	9	High
A 06	672684,411907	5629455,79002	10	High
A 07	672762,458277	5629601,957465	9	Moderate
A 08	671608,715916	5629572,161805	3	Moderate
A 09	672355,487796	5630118,082488	6	Low
A 10	672273,95518	5629579,65455	3	Low

Fig. 1 Leaf phenological measurements, from left to right: leaf width, leaf blade length, max. incision length, angle of the lobe tip above max. incision, petiole length and position max. leaf width
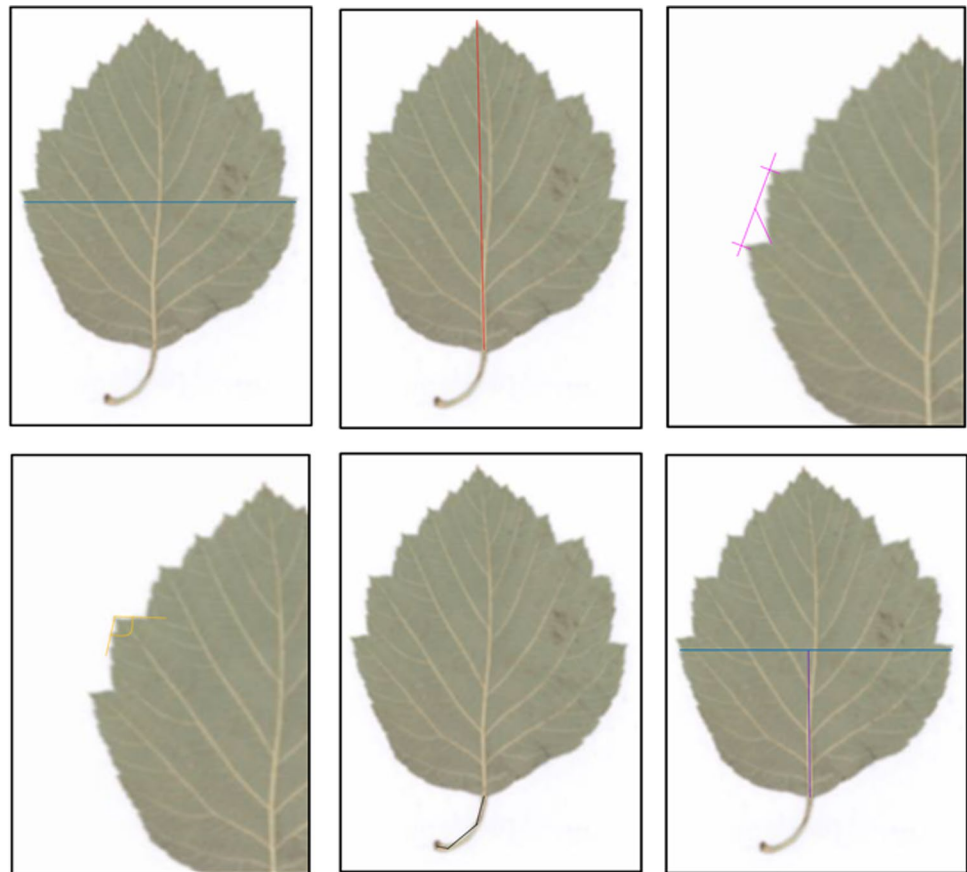
(NMMS) with Bray–Curtis distance in Past 4 (Hammer et al. 2001) using the characters which were significantly different, was used visualizing the differences between the taxa.

DNA-marker

DNA isolation was performed according to standard procedures using the extraction kit DNeasy® from Qiagen (Qiagen, Hilden, Germany). PCR was done using three nuclear microsatellite primers (MSS5, MSS13 and MSS16) and two chloroplast markers (ccmp3 and ccmp4) as described in (Leinemann et al. 2010, 2013; Oddou-Muratorio et al. 2005; Weising and Gardner 1999). The fragment size of the amplified DNA sequences was determined using an ABI 3100 sequencer. Results were analysed using GeneMapper v4.1 software from Applied Biosystems. Analysis of genetic data was done with GenAlEx 6.4 (Peakall and Smouse 2006). The gene locus MSS 16 was used as a nuclear-encoded species-differentiating gene marker, since fragments of 144, 146 and 148 bp are generally found only in *S. aria*, and larger fragments are found exclusively in *S. torminalis* (see Leinemann et al. 2013). Individuals with only Aria or Torminalis fragments in the genotype were assigned as "homologous" to the Aria or Torminalis group, respectively. Individuals with

one fragment each of the Aria and Torminalis groups were identified as "heterologous" i.e., hybrids.

Here, the microsatellite markers were used also for differentiation between diploids and polyploids, since polyploids have regularly more than two alleles per locus. This was proven also in Feulner et al. (2019) for *Sorbus*.

Pollen clouds

For disordered genotypes from open pollination, i.e., genotypes with unidentifiable maternal contribution, determination of the pollen contribution (a or t) in a given genotype is only possible for homozygous or, in our case, homologous mating. Therefore, to obtain a rough estimate of the proportion of a or t pollen contributions, the number of aa and tt genotypes in each single tree progeny (STP) was counted.

Results

The Kruskal–Wallis Anova analysis was significant for three measurements: maximal incision length per leaf width, angle of the first lobe tip above the largest incision and the vein numbers significantly differentiate the *Tormaria* group from either the *Torminalis* and *Aria* group. *S. aria* f. *incisa*

however was not separated from *S. aria*, however possessing larger mean leaf incisions and lower angles than *S. aria*.

As the Dunn's test revealed, the morphological intermediary of the Tormaria individuals were clearly supported by these characters (Fig. 2).

Based on the significant characters (leaf incision/width, the angle of the first lobe tip above the largest incision and the vein numbers) the NMDS (Fig. 3) revealed three groups that rather well correspond to the *S. torminalis* (triangle symbol), *S. aria* including *S. aria* f. *incisa* (point symbols) and *S. × decipiens* (diamond symbol) phenotypes that were affiliated to the progeny by field botany criteria as described above (Fig. 3). Whereas the Tormaria mother trees cluster closely together, the Tormaria progenies show a rather loose grouping. There are two individuals of the *Tormaria* group that cluster intermingled between *S. aria* and *S. aria* f. *incisa*. The rest of the *Tormaria* individuals clustered close to the mother trees (pink diamonds in Fig. 3) or between *S. aria/S. aria* f *incisa* and *S. torminalis*.

Genetic results

Since DNA fragments (alleles) with a length of less than 150 bp are only found in *S. aria*, whereas alleles of more than 150 bp are only found in *S. torminalis* (Leinemann et al. 2013), the MSS16 locus allows to assign the individual seedlings as homologous or heterologous with respect to their origin from the pure species. According to the microsatellite allele pattern which are typical for either the one or the other parent, the genetic type of the progeny (homologous AA, TT, or heterologous AT) is given in supplementary data Table 1 combined with the maternal chloroplast type indicating the mother taxon.

Based on nuclear SSR markers the single tree progenies (STP) are significantly differentiated with an overall genetic differentiation of $F_{ST} = 22.6\%$ ($p = 0.001$). STPs 1, 3, 5, 8, 10 have maternally a *S. torminalis* parent (haplotype S14 or S22) and the STPs 2, 4, 6, 7, 9 have a maternally inherited aria haplotype (S9). The haplotypes of the corresponding STPs are uniform and identical to the respective maternal haplotype.

The MSS16 genotypes confirm the early hybrid state of the mother trees in 9 cases, here the marker MSS16 showed alleles of both parents, *S. aria* and *S. torminalis* (see Table S1). The mother tree A4 was a surprising exception as it possesses the aa-genotype and the *Tormaria*-phenotype. Moreover, the occurrence of three fragment in two of the analysed microsatellite markers indicated polyploidy (see

Fig. 2 Boxplots of significant character differences (*A S. aria*, *Ai S. aria* f. *incisa*, *TA S*. subgenus *Tormaria*, *T S. torminalis*). Lower letters indicate significant differences according to Dunn's test on the 0.1 significance level
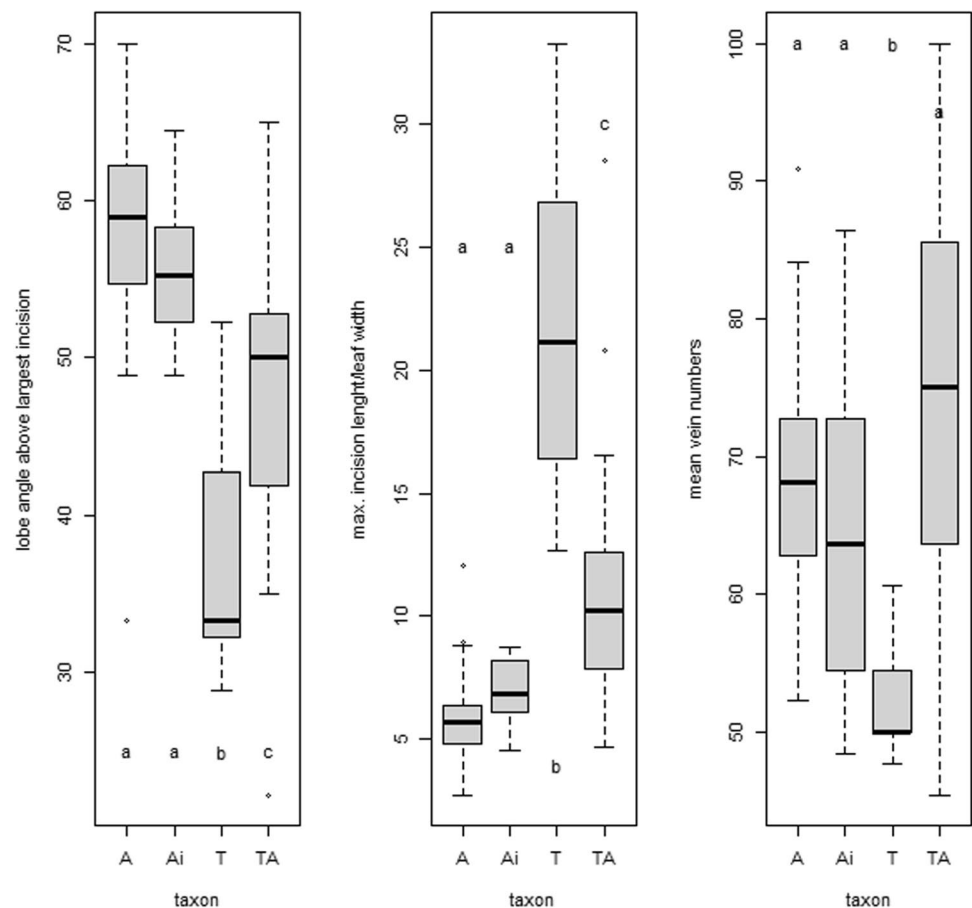
Fig. 3 Morphological groups of the progeny revealed by NMDS (Bray–Curtis distance) based on leaf incision/width, the angle of the first lobe tip above the largest incision and the vein numbers: (*filled triangle S. torminalis* phenotype, *point S. aria*, *green point S. aria* f. *incisa*, *diamond Tormaria*, *pink diamond Tormaria* mother trees)
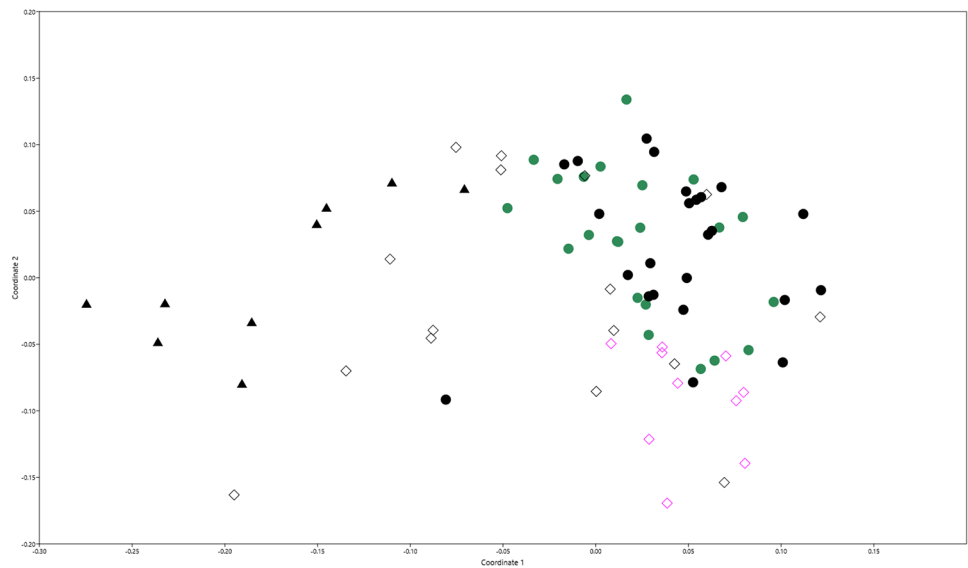

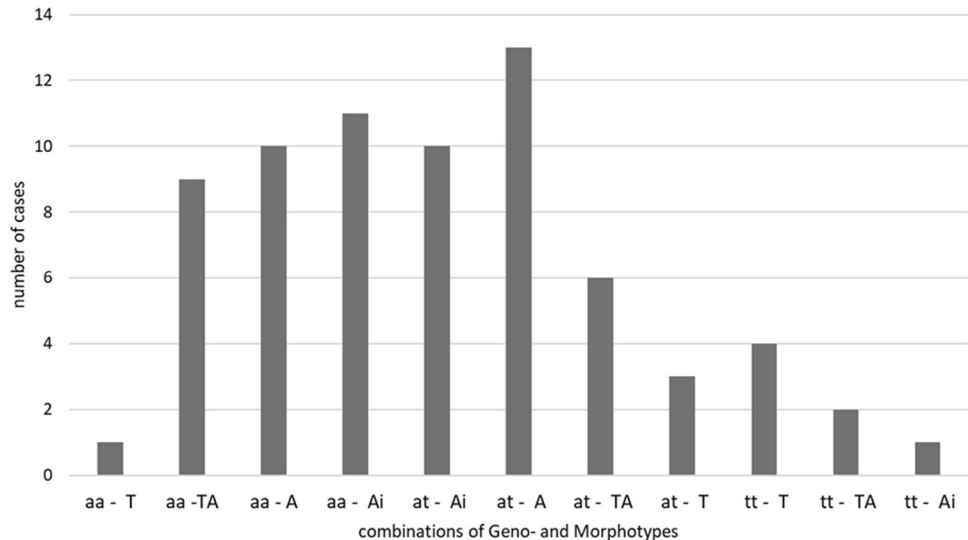**Fig. 4** Combinations of Geno— (lowercase letters) and phenotypes and their frequencies in *Sorbus* progeny derived from 10 F1 *Tormaria* mother trees

Table S1). Two seedlings of the mother tree A4 share identical genotypes with the mother and are therefore of potentially apomictic origin (Table S1, S3).

Inconsistency between genetics and morphology

Taxon affiliation based on morphotypes (MT) and the genotypes (GT) of the progeny was partly consistent, partly inconsistent (Fig. 4, supplementary Table. S1, Table S2).

Altogether, 45% of the progeny had the GT aa, 41% the GT at and 11% the GT tt, whereas the MT A was represented with 32%, the MT Ai with 31%, the MT TA with 24% and the *S. torminalis* MT with 13%. Figure 4 shows the cases for different combinations of GTs- and MTs in *Sorbus* progenies. Only 68% of genotype aa revealed the MT A or Ai. Half of the GT tt revealed the *torminalis*-MT and only

25% of GT at had a *Tormaria* MT. Mostly, progeny with GT at had the A or Ai MT. This shows that phenotype and morphotype were strongly inconsistent, pointing to uncoupling of the genes responsible for the one or the other due to segregation distortion (see Kianian and Quiros 1992). The most frequent combination between geno- and phenotype among the progeny was GT at—MT A. The second frequent combination was GT aa—MT ai (Fig. 4). The *S. aria f. incisa* MT had in many cases the homozygote gt aa. The third most combination was GT at with MT Ai. Interestingly, the combination GT aa—MT TA was the fourth frequent combination, which is rather unexpected. The expectable combination GT at—MT at (i.e., A02-6) was only the fifth frequent combination. The GT at occurred in MT T slightly more often than the combination GT tt and MT AT (i.e. in progeny A3-1). The strange combinations GT aa- MT T

and GT tt, MT Ai were completely against each expectation and may be again explained by excessive recombination and uncoupling of genetic marker genes and phenogenes.

All those inconsistencies could be explained when assuming, that crossing over in the F1 mother tree already uncoupled the genetic markers from the morphogenes responsible for the phenotype.

Pollen clouds

The pollen clouds of the single tree progenies (STPs) show clear differences (Fig. 5). Despite a large proportion of unspecified pollen, the percentage of a- and t-type pollen contributions varies strongly. Between 10 and 100% of a-type alleles and 0% and about 20% of t-type alleles. Overall, the contribution of the identified a-pollen is four times higher than that of the t-pollen.

The occurrence of different morphotypes in the progenies varies between 2 (A6, A9, A8) and 4 (A2) (Fig. 5). There is no clear tendency that the portion of morphotypes A and AI is effected by the amount of paternally inherited a-alleles. However, most of the T and TA morphotypes are observed in the progenies (A2, A3, A5) that were pollinated by pollen clouds with the lowest proportion of A alleles and vice versa showed the highest proportion of T alleles. Overall, the number of A- and AI-morphotypes is as twice as high as the number of T- and TA-morphotypes.

Polyploidization and clonality

The mother tree A4 has a *Tormaria* phenotype but possesses the homozygote aa genotype. Despite the "wrong" genotype, which is explainable by the uncoupling of

morpho- and phenogenes in the F2 generation. It shows very acute lobes typical for *Tormaria* and not for *S. aria* f. *incisa*, that would have rounded lobes. Therefore, the mother tree A4 may be most likely originated from interbreeding between *Tormaria* individuals or backcrossing of *Tormaria* with *S. aria*. Most interesting, among the progeny of A4 there are 2 individuals evidential for apomictic seed production possessing an allele-pattern identical with the seed mother. Since the other seedlings of mother tree A4 are genetically different and mostly diploid, this counts for facultative apomixis and a variable system (depending on the pollen type?) resulting probably in aneuploid progeny which was shown by Keller et al. (2015) in a sexual reproductive triploid *Tormaria* population.

Discussion

As could be expected we could confirm for nine of ten cases that the progeny of *S*. subgen. *Tormaria* is variable and originated from sexual reproduction. Similar results were found for diploid members of section *Tormaria* in Bavaria by Keller et al. (2015) and by Leinemann et al. (2013) for Thuringia.

The mother tree A4 is an exceptional and a rare example of spontaneous polyploidisation and facultative apomixis producing clonal progeny in two cases. So far, it was suggested to be very unlikely that in areas, where no polyploid *S. aria* parents are distributed, polyploid *Tormaria* can be formed (see Meyer et al. 2014). This study shows that in low rates such polyploidisation could occur starting from diploid *Tormaria*. The polyploid mother tree A4 produces partly clonal seeds, partly variable progeny. Mixed pathways of

Fig. 5 Frequencies of a-, t- pollen cloud contributions in the progeny sorted in ascending order of frequency of a-types together with the respective proportion of morphotypes in the progenies
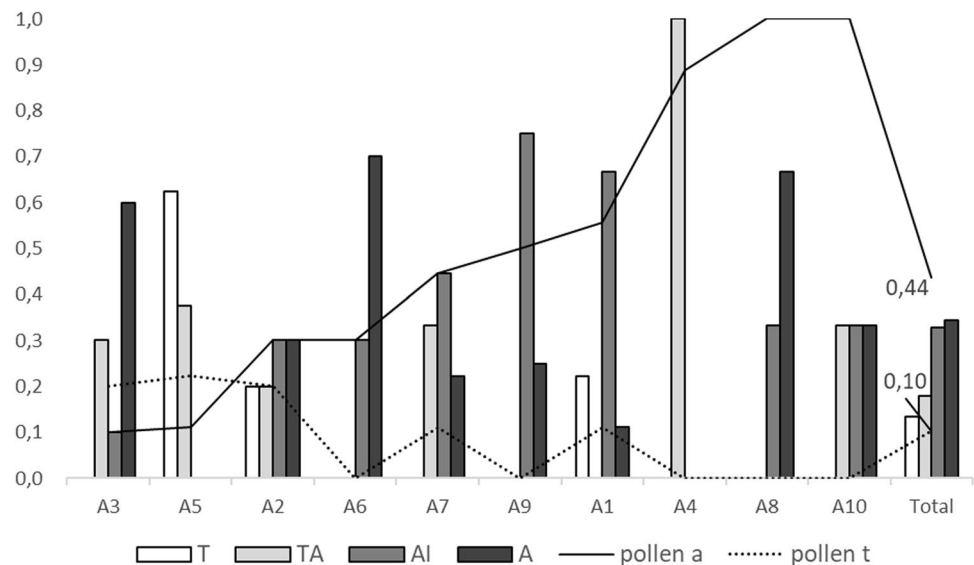
reproduction are typical for polyploid *Sorbus* subgenus *Aria* which are often facultative apomictic and partly sexual (see Feulner et al. 2017) but have yet not been described from subgenus *Tormaria*. The mother tree A4 could be clearly assigned to *Tormaria* due to its incisions with clearly acute lobes (comp. Fig. S2) but it has an aa genome. Frequent decoupling of pheno- and genotye may occurs in early hybrid generations (see below), which could explain the inconsistencies between geno- and phenotype in this plant. After the identification of those polyploids revealed by this study it is possible to look for those types in the investigated Thuringian population and their exact frequency could be determined.

As could be expected the progeny of the sexual mother trees segregated. Almost half of the progenies had the GTs aa (44%) and at (45%), respectively, and only 10% possessed the GT tt, whereas the MT AA was represented with 32%, the MT Ai with 31%, the MT TA with 24% and the T MT with 13%. The inconsistencies between morphology and genetic data reflect the strong rearrangement of the genome in the parental plants. This leads to the uncoupling of morphology and taxon markers, which was—to our best knowledge—not detected before in *Sorbus*. The excessive recombination due to hybridisation is also the reason for a related phenomenon, known as segregation distortion, it means that not all genetic markers were transmitted to the next generation (i.e., in *Brassica* taxa; Singh et al. 2012). Segregation distortion due to excessive genomic rearrangement is reported to increase when the parental genomes of the F1 mother tree is strongly different (Kianian and Quiros 1992) and hampers an exact genetic identification of the progeny, as became obvious here.

This study showed a prevalence of a-genomes in the pollen cloud (Fig. 5). Backcrosses with *S. aria* could be a likely explanation for this observation; the dominance of unidirectional introgression towards *S. aria* was reported before in Aas et al. (1994) and Zieliński and Vladimirov (2013). However, the prevalence of *S. aria*-pollen is not true for all progeny (see Fig. 3, 4). Genotype frequencies and ratios such as 11% aa, 77% at and 22% tt in mother tree A3 or 12,5% aa, 66% at and 25% tt in mother tree A5 overbeat the ratios predicted by the 2. Mendelian rule or caused by backcrosses in equal rates with both parents. The low rates of homozygotes and the higher fractions of heterozygous genotypes with 77%, respectively, 66% could indicate selection against homozygotes or abortion of homozygotic gametes. Therefore, it seems doubtful whether the prevalence of backcrossing occurs in general. Martinsen et al. (2001) shed light on the phenomenon that hybrids can serve as gene filters. Following Martinsen et al. (2001) the *Tormaria* individuals may be a filter for introgression and transmit not all markers equally and transmitting primarily *S. aria* alleles e. g. due to a higher fertility of these pollen grains (gametic selection, see Kianian and Quiros 1992).

Interestingly, we found that the Ai-MT is overrepresented in the progeny (28%) and most of the GTs ta belong to *S. aria* and to *S. aria* f. *incisa,* a *S. aria* type with slightly incise leaves. This means that the plasticity of parental species may be explained by excessive introgression between *S. aria* and *S. torminalis.* Even the genetic identification of the progeny could fail due to segregation distortion, the phenotype may be a result of geneflow between *S. aria* and *S. torminalis* via the high numbers of sexual *Tormaria* hybrids. The Thuringian *Tormaria* behave different to most homoploid hybrids studied since those are known to be rare and have a very low fertility (Düll 1961; Ungerer et al. 1998). An explanation for their high fertility could be that in the study area the parental plants *S. torminalis* and *S. aria* may have undergone a long introgressive history and their genomes may be not as different as their species rank indicates.

Maybe overlap or non-overlap of flowering time of *S. aria*, respectively, *S. torminalis* and *S. × decipiens* may be responsible for explain the prevalence of *S. aria* pollen. Other factors could be selection of pollen on the stigma, different pollen germination or easily the spatial pattern of the trees. But despite this, *S. aria* is the rarest *Sorbus* taxon in the natural population. Inventories of the adult *Sorbus* species pattern in the study area showed that *S. torminalis* dominates among the *Sorbus* taxa (approx. 60%), and *S.* subgen. *Tormaria* reaches 30%, but *S. aria* only 10%. This counts for environmental selection against *S. aria*. Moreover, due to our best knowledge, a removal of *S. aria* due to forest management could be excluded. It seems that individual rich diploid *Tormaria* populations are formed at sides more suited for *S. torminalis*. This was found by Aas et al. (1994) before in a similar taxon scenario in the Swiss Jura and may give rise to look after shared ecological, physiological or phenological similarities between *S. torminalis* and *Tormaria* (i.e. shade tolerance, higher stem than *S. aria*).

If *Tormaria* interbreed, the Thuringian populations could also be the starting point for recombinant speciation leading to diploid *Tormaria* populations (Ungerer et al. 1998; Buerkle et al. 2000). This mechanism, however, seems not to play a big role in *Sorbus* since than we should find more morphologically stable populations of diploid sexual *Tormaria*. It seems to be more likely that this excessive recombination may facilitate polyploidization as an escape from hybrid sterility as could be observed here.

Conclusion

This study revealed that low rates of polyploidization starting from hybridization between diploids occur as escape from segregation and that polyploidization occurs even when

there are only diploid parents in the hybrid zone. This diploid based polyploidization mechanisms may have played a so far underestimated but very important role in hybrid speciation in *Sorbus*, probably also in areas where polyploid parents occur. Although there may be a genetic drive towards *S. aria* genotypes in the F2 generation those types may be less viable under the ecological conditions of the study area. Therefore, strong ecological selection favouring only a part of the taxa of a hybrid zone is another very important finding of this study.

Declarations

Conflict of interest The authors declare no competing interests.

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