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Floral scent and its correlation with AFLP data in *Sorbus*

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Abstract Comparisons between floral scent-based and DNA-molecular-based taxonomies are rare, yet such comparisons indicate that scent can provide useful taxonomic information. Here, we correlate the phytochemical differentiation in floral scent to the DNA-molecular-based differentiation in the genus *Sorbus*. Inflorescence scent patterns of the apomictic and endemic *Sorbus latifolia* microspecies *Sorbus franconica*, *Sorbus adeana*, and *Sorbus cordigastensis* originated by hybridization as well as their parental taxa *Sorbus aria* agg. and *Sorbus torminalis* were investigated with the dynamic headspace method. The scent data (presence/absence of compounds) were used to construct an UPGMA tree, to calculate a similarity matrix, and to correlate them with the published amplified fragment length polymorphism (AFLP) data of the same individuals, populations, and taxa. Flow cytometry was used to estimate the DNA-ploidy level of the taxa. Scent analyses showed a total of 68 substances, among them aromatic compounds, terpenoids, aliphatics, and nitrogen-containing compounds. The scent patterns were taxon-specific, and the number of scent components differed among taxa. The correlations with the published AFLP data on population and individual level are highly significant, indicating that the scent and AFLP data are highly congruent in the plants studied. Scent therefore provides useful taxonomic characters in *Sorbus*.

Keywords AFLP · Apomixis · Correlation analysis · Floral scent · Rosaceae · Taxonomy · *Sorbus*

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Introduction

The main function of floral scent is to attract pollinators (Plepys et al. 2002; Dötterl et al. 2006), which in turn exert selective pressures on its composition (Knudsen and Tollsten 1993; Plepys et al. 2002; Dötterl et al. 2005). Pollinator-mediated selection may lead to the evolution of pollination syndromes, meaning that plant species pollinated by the same guild of animals have similar phenotypes of their floral characteristics, including scent (Faegri and van der Pijl 1979; Fenster et al. 2004; Dobson et al. 2005). Although scent has been shown to be influenced by pollinator-mediated selection and coevolution, most studies have shown that only a limited number of substances have key functions in attracting pollinators (Dötterl et al. 2006; Svensson et al. 2010; Burger et al. 2011), whereas the occurrence of other substances may be explained by phylogeny (Steiner et al. 2011).

There are some examples showing the floral scent data support phylogenies that are morphology-based (e.g., Raguso et al. 2003; Feulner et al. 2009, 2011) and DNA-based (e.g., Levin et al. 2003). However, to the best of our knowledge, a statistical approach of the taxonomical value of scent entailing

detailed correlations between scent data and data from DNA-molecular studies using a Mantel test has so far been reported only once: In a study of *Ophrys* (Orchidaceae), no correlation was found between the scent data and DNA-molecular data (Stökl et al. 2008).

In hybrid complexes such as *Citrus*, *Ophrys*, and *Hieracium*, the scent has been shown to consist mainly of a mixture of the scent components of the parental species and only a low number of novel compounds (Gancel et al. 2002; Vereecken et al. 2010; Feulner et al. 2009, 2011). In such cases, scent analyses are a valuable tool for parental species identification (Feulner et al. 2009, 2011).

In the genus *Sorbus*, many taxa are polyploids and derived by hybridization. Such taxa are typically apomictic in that they produce seeds without the use of meiotic recombination (Nogler 1984; Jankun and Kovanda 1987; Campbel and Dickinson 1990; Talent 2009). The pollinator-mediated selection of scent patterns is of minor importance in apomicts (Feulner et al. 2009, 2011). Furthermore, the genotypic variability of apomicts is often low (cf., Talent 2009). Therefore, scent patterns may be identical among the individuals and populations of the same apomictic taxon and be of high taxonomic value (Feulner et al. 2009, 2011).

Here, we investigate the scent of the apomictic microspecies *Sorbus adeana*, *Sorbus cordigastensis*, and *Sorbus franconica*, belonging to the *Sorbus latifolia* aggregate (Rosaceae) endemic to Northern Bavaria and occupying very small parapatric distribution areas (Meyer et al. 2005; Aas and Kohles 2011). *S. latifolia* taxa originated from hybridization between *Sorbus aria* agg. (including *Sorbus pannonica*) and *Sorbus torminalis* (Düll 1961; Rich et al. 2010; Robertson et al. 2010; Feulner et al. 2013).

In a former study, the hybrid state, the intraspecific variability, and the genetic structure of taxa were investigated with amplified fragment length polymorphism (AFLP) analyses (Feulner et al. 2013). In the present study, we investigated the floral scent composition of the same type of material used in the AFLP study. We (1) investigated the scent composition of the parental species, (2) examined the distribution of compounds in the putative hybrid taxa, and (3) tested for congruence of the floral scent-based differentiation with the AFLP-based DNA-molecular differentiation (published in Feulner et al. 2013).

Material and methods

Study plants

We collected scent from *S. adeana*, *S. cordigastensis*, and *S. franconica* as well as the parental taxa *S. aria* s.str., *S. pannonica*, and *S. torminalis*. Some previously taxonomically unclassified intermediates between *S. aria* s.str. and *S. pannonica* with affinity to *S. aria* s.str. [henceforth affine (aff.) *aria*] were included in the study (see Feulner et al. 2013). For all taxa, AFLP data were available from the same populations. In 13 cases, the AFLP data and scent data were collected from the same individuals (Table 1; Feulner et al. 2013). For further information about the taxonomy, population structure, and ecology of the taxa investigated, see Feulner et al. (2013).

Volatile collection

Inflorescence scent was collected in the field using a standard dynamic headspace method as described in Feulner et al. (2009). For each taxon, three to eight individuals were sampled. The sampling was carried out on newly opened inflorescences (one inflorescence per plant and sample), between 11 a.m. and 3 p.m., the period with the most intensive scent emission (as determined by the human nose; Feulner, unpublished data). Scent samples of the leaves and the local surrounding air were collected as a control for each locality and population investigated.

Chemical analysis

The samples were analyzed on a Varian Saturn 2000 mass spectrometer and a Varian 3800 gas chromatograph with a 1079 injector that had been fitted with a ChromatoProbe kit. This kit allows the thermal desorption of small amounts of solids or liquids contained in quartz microvials (Micro-SPE; cf., Amirav and Dagan 1997; Dötterl et al. 2005). The injector split vent was opened (1/20) to flush any air from the system and closed after 2 min; the injector was heated at 40 °C for 2 min, and the temperature was then increased at a rate of 200 °C min^{-1} to 200 °C; this end temperature was held constant for 4.2 min, after which the split vent opened (1/10) and the injector cooled down.

A ZB-5 column (5 % phenyl polysiloxane) was used for the analyses (60-m long, inner diameter 0.25 mm, film thickness 0.25 μm, Phenomenex). Electronic flow control was used to maintain a constant helium carrier gas flow of 1.8 ml min^{-1}. The GC oven temperature was held constant for 7 min at 40 °C, then increased by 6 °C min^{-1} to 250 °C and held constant for 1 min. The MS interface was 260 °C and the ion trap worked at 175 °C. The mass spectra were taken at 70 eV (in EI mode) with a scanning speed of 1 scan s^{-1} from m/z 30 to 350.

The GC-MS data were processed using the Saturn Software package 5.2.1. Component identification was carried out using the NIST 08 mass spectral database or MassFinder 3, and confirmed by comparing the retention times and retention indices with published data (Adams 2007). The identification of the individual components was confirmed by comparing

Table 1 Taxon name, locality, and voucher information of the individuals analyzed

Taxon	Locality/Gauss Krüger coordinates/voucher	Number of individuals for scent sampling	Number of AFLP samples from the same population/ndividual as used for scent sampling[a]	Number of samples analyzed by flow cytometry
Sorbus aria (L.) Crantz	Grafenhäusling/4437746/5542273, *Feulner* 200–207 (UBT)	2	5/0	2
	Autobahn Rossdorf/4437013/5539920, *Feulner* 208 (UBT)	1	2/0	1
S. aff. *aria* (intermediates)	Autobahn Rossdorf/4437013/5539920, *Feulner* 209–217 (UBT)	3	4/0	1
	Grafenhäusling/4437746/5542273, *Feulner* 209 (UBT)	–	–	1
S. pannonica Kárpáti	Kordigast/4443782/5551720, *Feulner* 218–227 (UBT)	4	5/1	6 (offspring)
	Neudorf/4447194/5546549, *Feulner* 228–234 (UBT)	1	4/0	
	Brünnberg/4457310/5520910, *Feulner* 235 (UBT)	1	–	
S. adeana N. Mey.	Neudorf/4447194/5546549, *Feulner* 236 (UBT)	4	7/0	2 (offspring)
S. franconica Bornm. ex Düll	Brünnberg/4457310/5520910, *Feulner* 237–239 (UBT)	1	2/0	–
	Mugggendorf/4447515/5518390, *Feulner* 240–245 (UBT)	4	5/3	–
S. cordigastensis N. Mey.	Kordigast 4443782/5551720, *Feulner* 246–253 (UBT)	8	8/8	4 (offspring)
S. torminalis (L.) Crantz	Neudorf Bärental/4447194/5546549, *Feulner* 254 (UBT)	1	1/0	–
	Hainbach/4451892/5530457, *Feulner* 255 (UBT)	1	1/1	–
	Kordigast/4443782/5551720, *Feulner* 256–259 (UBT)	2	3/0	–

[a] Compare Feulner et al. 2013

both the mass spectrum data and GC retention data with the data of authentic standards.

Flow cytometry

The populations harvested for flow cytometry are given in Table 1. In the case of *S. aria* and *S. aff. aria*, the material was collected from adult plants. In the case of *S. pannonica*, *S. adeana*, *S. franconica*, and *S. cordigastensis*, the offspring from seeds harvested from the populations were used. The seeds were grown in the ecological botanical garden of the University of Bayreuth. DNA-ploidy levels were estimated from fresh leaf petioles using a Partec CyFlow space (Partec, Germany) fitted with a high-power UV LED (365 nm). The leaf petiole tissue of the analyzed sample and the internal standard [*Glycine max* cv. Polanka (Doležel et al. 1994) or *Lycopersicon esculentum* cv. Stupické polní tyčkové rané (Doležel et al. 1992)] were co-chopped using a razor blade in a plastic Petri-dish containing 1 ml of ice-cold Otto I buffer [0.1 M citric acid, 0.5 % Tween 20; (Doležel et al. 2007)]. The suspension was filtered through Partec CellTrics® 30 μm (Partec, Germany) in order to remove tissue debris and incubated for at least 10 min at room temperature. Isolated nuclei in filtered suspension were stained with 1 ml of Otto II buffer (0.4 M $Na_2HPO_4 \times 12H_2O$) containing the AT-specific fluorochrome 4′,6-diamidino-2-phenylindole (DAPI; 4 μg ml^{-1}) and β-mercaptoethanol (2 μl ml^{-1}). The relative fluorescence intensity was recorded for 3,000 particles. Sample/standard ratios were calculated from the means of fluorescence histograms visualized by the FloMax v2.4d software (Partec, Germany). Only histograms with coefficients of variation (CVs) <4 % for the G_0/G_1 peak of the analyzed sample were considered. The sample/standard ratios based on internal standard *L. esculentum* were adjusted to those from *Glycine max* using a coefficient based on the factor resulting from three repeats between the two standards.

Statistical analyses

Scent chemistry

Statistical analyses were performed using PRIMER v6 (Clarke and Gorley 2006). The clustering analysis (unweighted pair group method with arithmetic mean, UPGMA) of the scent samples was based on a Jaccard similarity index (calculated by coding the presence and absence of compounds as zero/one-data). The taxon specificity of the scent was tested using analysis of similarity (ANOSIM; 10,000 permutations). Function PERMDISP was used to test for differences in the interspecific scent variability (dispersion) among the taxa.

Correlation of scent and molecular AFLP data

For correlation analyses on the population level, lists of AFLP loci (see Feulner et al. 2013) and scent compounds found in the different individuals of a taxon of a specific population were generated (one list of loci and scent compounds per taxon and population). Similarity matrices (Jaccard) were calculated on the basis of these datasets and used for a Mantel test using RELATE (Spearman's rank correlation, 10,000 permutations).

To test for differences in the number of scent compounds trapped among the taxa. An ANOVA was performed with Statistica v7.0. Kolmogorov-Smirnov and Hartley were used to test for the normality and homogeneity of the variances, respectively. Unequal-N HSD was used as a post hoc test.

Results

Floral scent chemistry and taxon-specific differences

In total, 68 scent substances were identified in 35 samples of six *Sorbus* taxa (Table 2), mainly aliphatics and aromatics. The less abundant substances were terpenoids and nitrogen-containing substances. The most widespread substances were (*Z*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol acetate, benzaldehyde, 4-oxoisophoron, 4-oxoisophoron epoxide, 2-phenylethyl alcohol, and diverse lilac alcohols and aldehydes. Among the nitrogen-containing substances, methyl nicotinate, 3-pyridinecarboxaldehyde, and phenylacetonitrile were most frequently found.

DNA-ploidy

Seventeen individuals from five populations were investigated using flow cytometry. The CVs for the G_0/G_1 sample peaks ranged from 1.39 to 2.76 (mean=2.11). Three distinct classes of sample/standard fluorescence ratios were identified with means (±0.002), 0.80 (±0.004), and 1.05 (±0.013). 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) and there were several diploid counts ($2n$=34) reported for *S. torminalis* and *S. aria* (e.g., Pellicer et al. 2012), the measured DNA-ploidy levels correspond to $2x$: $3x$: $4x$ (Table 3).

Average substance numbers

The average number of scent substances (see Tables 2 and 3) differed significantly among the taxa (ANOVA: $F_{df=6,26}$=

Table 2 Presence/absence of floral scent volatiles, occurring in *n* of all individuals investigated (*n*/*n*) of 7 *Sorbus* taxa

	aria aff. *aria*	*aria* s.str.	*pann-onica*	*ade-ana*	*cordigast-ensis*	*franco-nica*	*tormi-nalis*
Aromatics							
Benzaldehyde	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Benzeneacetaldehyde	3/3	1/3	3/6	4/4	4/8	–	3/3
Methyl benzoate	3/3	3/3	6/6	4/4	8/8	5/5	–
2-phenylethyl alcohol	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Methyl phenylacetate	3/3	3/3	6/6	4/4	8/8	3/5	–
Methyl salicylate	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Anisaldehyde	1/3	2/3	2/6	–	–	–	1/4
Aliphatics							
Methyl isovalerate	3/3	3/3	5/6	4/4	–	2/5	–
2,3-butandiol	3/3	3/3	6/6	–	–	–	–
(Z)-3-hexen-1-ol	3/3	3/3	6/6	4/4	8/8	5/5	3/4
Methyl hexanoate	–	–	4/6	4/4	8/8	3/5	–
Methyl (Z)-3-hexenoate	–	–	2/6	4/4	8/8	2/5	–
Methyl 2-hydroxy-3-methylpentanoate	3/3	3/3	6/6	4/4	–	–	–
(Z)-3-hexen-1-ol acetate	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Acetic acid hexyl ester	–	–	4/6	1/4	8/8	–	3/4
(E)-2-hexen-1-ol acetate	–	–	4/6	–	–	5/5	–
Octanal	3/3	3/3	6/6	4/4	–	5/5	4/4
Homoterpenes							
(E)-4,8-Dimethyl-1,3,7-nonatriene	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Irregular monoterpenes							
4-oxoisophorone epoxide	3/3	3/3	6/6	4/4	8/8	4/5	4/4
4-oxoisophorone	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Dihydrooxoisophorone	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Monoterpenes							
α-pinene	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Camphene	3/3	2/3	6/6	4/4	8/8	–	2/4
β-pinene	3/3	3/3	6/6	4/4	8/8	5/5	3/4
(Z)-ocimene	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Limonene	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Eucalyptol	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Dihydro-5-methyl-5-vinyl-2(3H)-furanone	3/3	3/3	5/6	4/4	7/8	–	1/4
(E)-ß-ocimene	–	2/3	5/6	–	5/8	5/5	3/4
(Z)-arbusculone	3/3	3/3	6/6	1/4	–	–	1/4
(E)-arbusculone	3/3	3/3	6/6	–	–	–	1/4
(Z)-linalol-oxid furanoid	2/3	2/3	4/6	3/4	–	5/5	3/4
(E)-linalol-oxid furanoid	3/3	3/3	6/6	4/4	–	1/5	3/4
Lilac aldehyde A	3/3	3/3	5/6	–	–	–	–
Lilac aldehyd B+C	3/3	3/3	6/6	3/4	8/8	–	–
Lilac aldehyde D	3/3	3/3	6/6	3/4	3/8	–	–
Unkn MT1402	2/3	1/3	6/6	–	1/8	4/5	–
Lilac alcohol A	3/3	3/3	2/6	4/4	2/8	–	–
Lilac alcohol BC	3/3	3/3	6/6	4/4	4/8	–	–
Lilac alcohol D	3/3	3/3	4/6	4/4	2/8	2/5	–
Isomenthone	3/3	2/3	4/6	3/4	4/8	1/5	4/4
Lilac derivative	3/3	3/3	6/6	4/4	–	–	–
Linalool	3/3	3/3	6/6	4/4	–	3/5	4/4

Table 2 (continued)

	aria aff. *aria*	*aria* s.str.	*pann-onica*	*ade-ana*	*cordigast-ensis*	*franco-nica*	*tormi-nalis*
N-containing substances							
3-pyridinecarboxaldehyde	3/3	3/3	5/6	4/4	8/8	–	4/4
Amyl/isoamyl-pyrrole	–	–	–	4/4	2/8	–	3/4
Phenylacetonitrile	3/3	3/3	6/6	4/4	8/8	5/5	4/4
Methyl nicotinate	2/3	–	–	4/4	8/8	–	2/4
Unk-N1364 m/z 125, 81, 39	3/3	3/3	6/6	4/4	8/8	1/5	3/4
Unk-N1377 m/z 151, 94	–	–	–	2/4	–	–	2/4
Unk-N1498 m/z 117,91,65,50,39	3/3	3/3	6/6	3/4	8/8	2/5	–
Unk-N1530 m/z 117,91,59,50	3/3	3/3	6/6	4/4	7/8	2/5	–
Indole	3/3	3/3	6/6	3/4	–	–	–
1-nitro-2-phenylethane	3/3	3/3	6/6	4/4	8/8	3/5	–
Sesquiterpenes							
α-longipinene	1/3	1/3	6/6	–	–	–	–
Unk-ST1684 m/z 204,161, 91, 69, 55	–	1/3	2/6	–	–	–	–
Unk-ST1690 m/z 161,119, 85, 73, 58	–	–	6/6	1/4	4/8	5/5	1/4
α-copaene	2/3	1/3	4/6	–	3/8	–	–
β-bourbonene	3/3	2/3	6/6	4/4	5/8	5/5	1/4
Unk-ST1711 m/z 161,123, 81, 67, 55	2/3	–	6/6	–	4/8	–	–
Unk-ST1732 m/z 161,139, 93, 79	2/3	–	–	–	–	–	–
Longifolene	3/3	3/3	6/6	3/4	8/8	1/5	4/4
Isocomene	2/3	2/3	6/6	1/4	8/8	–	–
(E)-β-caryophyllene	3/3	3/3	6/6	3/4	6/8	5/5	4/4
α-gurjunene	3/3	3/3	6/6	3/4	5/8	3/5	–
Unk-ST1808 m/z 204,161,143,133,105	–	–	2/6	1/4	4/8	1/5	1/4
Unk-ST1831 m/z 204,189,161,133,119, 105	3/3	1/3	6/6	2/4	6/8	4/5	1/4
Unk-ST1838 m/z 161,93,41	2/3	1/3	6/6	1/4	5/8	5/5	–
Unidentified							
Unk m/z 112,140,181	3/3	3/3	5/6	4/4	–	–	–

Table 3 Sample size, average number of scent compounds, intra specific scent dispersion (mean distance to centroid, Jaccard similarity), as well as sample/standard fluorescence ratio, and DNA-ploidy level based on flow cytometry data in the *Sorbus* taxa analyzed

Sorbus taxa	*N* scent	Average number of scent substances and standard error of mean	Average dispersion and standard error of mean	*N* ploidy	Sample/standard fluorescence ratio	DNA-ploidy level based on flow cytometry and literature data
S. aria s.str.	3	49.3 (1.5)^{bcd}	5.9 (1.1)^{ae}	3	0.52 (±0.003)	2×
S. pannonica	6	56.8 (1.05)^{d}	9.6 (1.0)^{fg}	6	1.04 (±0.008)	4×
Intermediates with affinity to *S. aria* s.str.	3	54.7 (1.9)^{cd}	9.3 (2.4)^{ef}	2	0.79 (±0.006)	3×
S. adeana	4	48.3 (1.7)^{c}	10.3 (0.8)^{bg}	2	0.81 (±0.002)	3×
S. cordigastensis	8	39.4 (1.3)^{b}	14.7 (1.4)^{bce}	4	0.81 (±0.004)	3×
S. franconica	5	31.4 (1.8)^{a}	16.4 (0.8)^{c}	–	–	3×^{a}
S. torminalis	4	29.3 (1.9)^{a}	18.2 (4.3)^{abce}	–	–	2×^{b}

Different upper script letters indicate significant differences

[a] Feulner et al. 2013

[b] Pellicer et al. 2012, Meyer et al. 2014

48.06, $p<0.001$). It was lowest for *S. torminalis* (29.3) and *S. franconica* (31.4). In *S. aria* agg., the average number of scent substances increased with the ploidy level (49.3 in diploid *S. aria* s.str., 54.7 in triploid intermediates with an affinity to *S. aria* s.str., and 56.8 in tetraploid *S. pannonica*, Table 3). The scent compound number of the triploid *S. latifolia* taxa (between 31.4 and 48.3) was intermediate between those of the parental taxa *S. torminalis* and *S. aria* agg. (Table 3).

The ANOSIM (10,000 permutations) revealed that the scent is highly taxon-specific ($R_{df=6,26}=0.859$, $p<0.001$*).

The scent strongly differed (pairwise $R=1$, ANOSIM) between *Sorbus aria* agg. and *S. torminalis* (Table 2). In *S. aria* agg., several lilac alcohols, lilac aldehydes, and (*E*)- and (*Z*)-arbusculone and some nitrogen-containing compounds (i.e., indole, 1-nitro-2-phenylethane, unk-N1498, and unk-N1530) were found, which were missing in *S. torminalis* (see Table 2). *S. torminalis* possesses two specific nitrogen-compounds not found in *S. aria* agg., one unidentified (unk-N1377), and the other identified as amyl/isoamyl-pyrrole (Table 2). The unidentified nitrogen-compound (unk-N1377) was found in *S. adeana*, and amyl/isoamyl-pyrrole was found in *S. adeana* and one *S. cordigastensis* individual. Many specific lilac aldehydes and lilac alcohols occurring in *S. aria* agg. were also present in the *S. latifolia* taxa, especially in *S. adeana* and *S. cordigastensis*, whereas fewer of them occurred in *S. franconica*. We did not identify substances specific for *S. latifolia*. Some compounds shared by all parental taxa were bequeathed to none of the *S. latifolia* taxa, such as (E)-arbusculone and anisaldehyde (Table 2). A close relationship between *S. adeana* and *S. cordigastensis* is reflected in several shared substances, namely, methyl nicotinate (also occurring in *S. torminalis* and two individuals of *S. aria* aff. *aria*), lilac alcohols, lilac aldehydes, and an unidentified sesquiterpene (unk-St1750). All these substances except for lilac alcohol D were not found in *S. franconica*.

Interestingly, many sesquiterpenes (α-longipinene, unk-St1693, α-copaene, unk-St1711) occurring in the other members of *S. aria* agg. are lacking in *S. aria* s.str. The component unk-St1732 was found specifically in the intermediates *S. aria* aff. *aria*.

Scent dispersion

The scent dispersion was significantly different between the *Sorbus* taxa (100,000 permutations $F_{df=6,26}=4.92$; $p=0.0121$*, Table 3). It is highest in *S. torminalis*, followed by *S. franconica*, *S. cordigastensis*, and *S. adeana* (Table 3). It is lowest in the subgroups of *S. aria* agg., intermediates aff. *aria*, *S. pannonica*, and in *S. aria* s. str.

Scent clustering

A cluster analysis based on scent composition showed that all *S. latifolia* taxa form groups of their own (Fig. 1), whereas *S. adeana* and *S. cordigastensis* are more similar to *S. aria* agg. than to *S. torminalis*, and they are more similar to each other than to *S. franconica*. In contrast, *S. franconica* is more similar to *S. torminalis* than to *S. aria* agg.

Within *S. aria* agg., *S. aria* s.str. and *S. pannonica* are clearly separated by scent. The intermediates aff. *aria* (as identified in Feulner et al. 2013) group closer to *S. aria* s.str. than to *S. pannonica* (Fig. 1).

Correlation between AFLP data and scent data

There were significant correlations on the population level (RELATE: $R=0.791$, $p=0.004$*, 8 populations included) and on the individual level (RELATE: $R=0.823$, $p<0.001$*, 13 individuals included) between AFLP data and scent data. This indicates that genetically similar populations/taxa emitted similar scents, whereas genetically dissimilar populations/taxa emitted different scents.

Discussion

The present study revealed that parental *S. aria* agg. and *S. torminalis* are strongly differentiated by both molecular and scent data. The number of scent components identified in *S. torminalis* is significantly less than the number identified in *S. aria* agg. (see Table 3). This is mainly due to the lack of lilac alcohols, lilac aldehydes, and a reduced number of nitrogen-containing compounds in *S. torminalis* (Table 2). The qualitative differences in scent composition in these taxa are in accordance with a cpDNA-based molecular phylogeny of Pyrinae, which revealed that *S. torminalis* and *S. aria* are part of different clades and not closely related (Campbell et al. 2007).

The scent patterns of the *S. latifolia* taxa suggest the inheritance of the scent compounds from both parental species, *S. aria* agg. and *S. torminalis* (Table 2). Indeed, the scent of the *S. latifolia* taxa is a mixture of the scent components of the parental taxa. This was also found in previous studies on hybrids (Gancel et al. 2002; Feulner et al. 2009, 2011; Vereecken et al. 2010). *S. torminalis* bequeathed two specific nitrogen-containing compounds to *S. adeana* and one to *S. cordigastensis*, but none to *S. franconica* (Table 2). *S. aria* agg. bequeathed many lilac alcohols and aldehydes, especially to *S. adeana* and *S. cordigastensis*, but not to *S. franconica* (Table 2). In contrast to the results found in other studies (Gancel et al. 2002; Feulner et al. 2009, 2011; Vereecken et al. 2010), *S. latifolia* taxa was not found to emit any

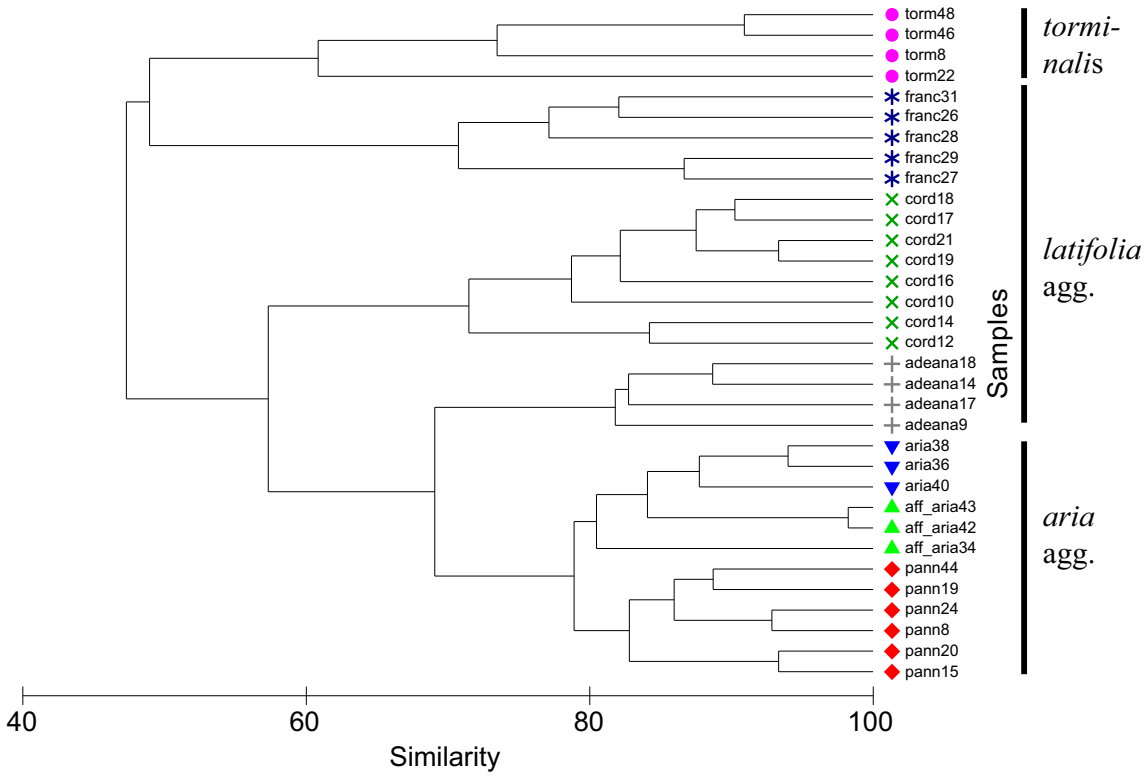

Fig. 1 Cluster analysis (UPGMA) of the scent data based on the Jaccard index of *S. latifolia* taxa *S. adeana* (*plus sign*), *S. franconica* (*asterisk*), *and S. cordigastensis* (*multiplication sign*) as well as their parental species groups *S. aria* agg., including *S. aria* s.str. (*upside down blue arrow*), *S. pannonica* (*red diamond*), intermediates aff. *aria* (*green triangle*), and *S. torminalis* (*pink circle*)

exclusively novel compounds compared to the parental taxa. However, substances such as methyl hexanoate and methyl (*Z*)-3-hexenoate were found in all *S. latifolia* taxa (*S. adeana*, *S. cordigastensis*, and *S. franconica*) and in some *S. pannonica* individuals, but not in the remaining taxa.

The close relationship of *S. adeana* and *S. cordigastensis* as revealed by AFLP data (Feulner et al. 2013) was confirmed by scent chemistry since *S. adeana* and *S. cordigastensis* share several compounds (lilac alcohols, lilac aldehydes, methyl nicotinate, and unk-St1750) which, except for lilac alcohol D, are all missing in *S. franconica*.

Although the genetic distances among the studied *S. aria* agg. taxa were small (Feulner et al. 2013), the differentiation within *S. aria* agg. (*S. aria*, *S. pannonica*, intermediates) was also confirmed by the scent data. Interestingly, the triploid intermediates of *S. aria* aff. *aria* were, as with the AFLP dataset, clustered between *S. aria* s.str. and *S. pannonica* and were closer to *S. aria* (Fig. 1). This supports the hybrid origin of these intermediates with the two taxa as parents. The samples of tetraploid *S. pannonica* had the highest compound numbers of all the investigated taxa. This is possibly related to its high ploidy level. In *S. aria* agg., the average number of scent substances increased with the ploidy level (49.3 in diploid *S. aria* s.str., 54.7 in triploid intermediates with affinity to *S. aria* s.str., and 56.8 in *S. pannonica*, Table 3). Polyploid plants often show characters which are boosted in number or size compared to their diploid relatives. The same seems to be true for the number of floral chemicals in *Sorbus*, and a similar trend was also found in the higher-ploidy cytotypes of *Gymnadenia* (Orchidaceae) (Jersáková et al. 2010). As *S. pannonica* is thought to be an intermediate between *S. aria* s.str. and *S. graeca* (Spach) Lodd. ex S. Schauer (Düll 1961), the large compound numbers in this species may also result from introgression with *S. graeca*. Having said that, scent data of the rare *S. graeca* (cf. Düll 1961; Meyer et al. 2005) have not been available so far.

Apomicts are generally considered to have lower genetic variability than sexually reproducing species (cf. Lepší et al. 2009). Yet, also in apomicts, remarkable variability can occur due to somatic recombination or rare sexual events (Capman et al. 2004; Rich et al. 2008; Paule et al. 2011; Feulner et al. 2013). Furthermore, the variability in apomicts has a time dimension and could increase with the age of the apomict due to the accumulation of mutations (cf. Rich et al. 2008). Interestingly, our AFLP study revealed a remarkably high intraspecific genetic variability for the apomictic *S. latifolia* taxa, despite their assumed clonality (Feulner et al. 2013). Similarly, the scent dispersion, especially of *S. cordigastensis* and *S. franconica*, was relatively high (Table 3). The reason for this phenomenon is not yet clear. However, in apomictic taxa such as *S. franconica*, intraspecific scent variability may be caused by the mutations of scent genes, which could have

led to reduced enzyme functionality and loss of scent substances. The reduced role of stabilizing pollinator-mediated scent selection in apomictic taxa could also contribute to an increasing variability of scent. In *Sorbus*, pollination is considered as being necessary for apomixis to occur (Jankun and Kovanda 1987). Ludwig et al. (2013) found that apomictic triploid *Sorbus* taxa with more or less clonal structure depend—because of self-incompatibility—on pollination from a di- or tetraploid *Sorbus* species. This may be true for the triploid Franconian *S. latifolia* taxa as well. To reproduce apomictically, it may be essential for these taxa to obtain pollen from members of syntopic tetraploids, such as *S. pannonica* or syntopic diploids, including *S. aria* or *S. torminalis*. The occurrence of fertile seeds in the *S. latifolia* taxa (our own observation) suggests that, despite the discovered differences in scent between the taxa, there is remarkable pollen transfer from syntopic di- and tetraploids. Therefore, scent may not play a major role for pollinator selectivity in *Sorbus*. In this context, the fact that triploids emit no novel substances compared to their parental di- or tetraploid species (as mentioned above) could play a role in facilitating cross-pollination.

Studies comparing scent and molecular data are often based on the family Orchidaceae. Nonetheless, this is a strongly contrasting system, including many deceptive plants where pollinator selection plays a key role (Salzman et al. 2007; Vereecken et al. 2010). Deceptive plants usually have a strongly increased variability of scent (Salzman et al. 2007), likely to prevent pollinators from easily learning to discriminate between the reward and the mimic (Ackerman et al. 2011). It is therefore not surprising that in deceptive *Ophrys*, no correlation of scent with genetic data was found (Stökl et al. 2008).

The *S. aria* agg. and the *S. latifolia* agg. are examples of polyploid apomictic species complexes. Such complexes exhibit important differences compared to strictly sexual groups, which might be responsible for the strong phylogenetic signal of the floral scent. Most members within such complexes are interconnected by ancient gene flow due to reticulate evolution. Therefore, the number of taxa of hybrid origin that possess a mixture of the scent of progeny taxa is high (see Gancel et al. 2002; Feulner et al. 2009, 2011). These hybrid taxa are stabilized by apomixis, and backcrossing is hindered to some degree. In sexual taxa, interpopulational differentiation of scent can occur (Dötterl et al. 2005), undermining the taxonomical signal of the scent data. But different populations of apomictic taxa often do not show scent differentiation and a population structure is often missing (i.e., Rich et al. 2008).

To conclude, our study shows that floral scent data strongly correlate with molecular genetic data in polyploid and apomictic *Sorbus* taxa and, taking other recently published work into consideration (Feulner et al. 2009, 2011), that scent is of high taxonomic value in *Sorbus* and other polyploid apomictic species complexes.

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