Taxonomical use of floral scent data in apomictic taxa of *Hieracium* and *Sorbus* derived from hybridization

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List of Publications

1) Feulner M., Schuhwerk F., Dötterl S. 2009: Floral scent analysis in *Hieracium* subgenus *Pilosella* and its taxonomical implications. Flora 204: 495–505.

2) Feulner M., Schuhwerk F., Dötterl S. 2011: Taxonomical value of inflorescence scent in *Hieracium* s. str. Biochemical Systematics and Evolution 39: 732–743.

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3) Feulner M., Liede-Schumann, S., Meve, U., Weig A., Aas, G.: Genetic structure of *Sorbus latifolia* (Lam.) Pers. taxa endemic to Northern Bavaria. Submitted to Plant systematics and evolution**,** PLSY-D-12-00168.

In preparation for submission to Organisms Diversity & Evolution:

4) Feulner, M., Pointner, S., Heuss, L., Aas, G., Dötterl, S.: Floral scent and its correlation with genetic data in *Sorbus* taxa.

Publications not included into this thesis:

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6) Feulner, M., Konnert, M. 2007: Autochthone Weißtannenvorkommen in den Schluchten Fränkischer Keupergebiete. Diskussionsbeitrag zu deren genetischer Struktur, Artenausstattung, waldbaulicher Behandlung und Kartierung in Natura 2000, Waldoekologie online 4: 91–110.

Declaration of contribution to publications

The thesis contains four research articles. Most of the research work presented in this thesis was carried out by myself at the University of Bayreuth including all sample collections, most analytic work and the most statistics under profound support of Prof. Dr. Stefan Dötterl, PD Dr. Ulrich Meve, Dr. Alfons Weig, PD Dr. Gregor Aas and Prof. Dr. Sigrid Liede-Schumann. I prepared the manuscripts under consideration of the comments of all coauthors.

1st publication

Feulner M., Schuhwerk F., Dötterl S. (2009): **Floral scent analysis in *Hieracium* subgenus *Pilosella* and its taxonomical implications.** Flora 204: 495–505.

The field work was done by myself, data analysis was done by myself under the profound support of PD Dr. Stefan Dötterl. Norbert Meyer and Dr. Franz Schuhwerk contributed to species selection, gave profound advice about localities of endemic *Hieracium* taxa and helped with species identification in the field and of herbarium specimens. I prepared the manuscript by recognizing the profound comments of my co-authors.

2nd publication

Feulner M., Schuhwerk F., Dötterl S. (2011): **Taxonomical value of inflorescence scent in *Hieracium* s. str.** Biochemical Systematics and Ecology 39: 732–743.

The field work was conducted by myself, data analysis was done by myself under the profound support of PD Dr. Stefan Dötterl. Dr. Jochen Müller, Norbert Meyer and Dr. Franz Schuhwerk gave profound advice about localities of endemic *Hieracium* taxa and helped with species identification in the field and of herbarium specimens. I prepared the manuscript by recognizing the comments of my co-authors.

3rd publication

Feulner, M., Liede-Schumann, S., Meve, U., Weig A., Aas G.: **Origin and genetic structure of three *Sorbus latifolia* (Lam.) Pers. taxa endemic to Northern Bavaria.** Submitted to Plant systematics and evolution, PLSY-D-12-00168-1.

The plant material collection was conducted by myself under the support of Dr. Gregor Aas (EBG Bayreuth) who had the idea for this research. AFLP Laboratory work was conducted by Michaela Hochholzer and Dr. Alfons Weig (both DANECO Bayreuth). Chromosome counts were conducted under supervision of PD Dr. Ulrich Meve. Data analysis was done by myself under the support of Dr. Alfons Weig and Prof. Dr. Sigrid Liede-Schumann. I prepared the manuscript by recognizing the comments of my co-authors.

4th publication

Feulner, M. Pointner S., Heuss L., Aas G., Dötterl S.: **Correlation between taxonomic groupings of *Sorbus* microspecies based on floral scent and genetic data.** In preparation for submission to Organisms Diversity & Evolution.

The scent collection was done by the bachelor candidates Lisa Heuss, Stefan Pointner and myself. Data analysis was done by myself under the profound support of PD Dr. Stefan Dötterl. I prepared the manuscript by recognizing the comments of my co-authors.

Content

Summary

Scent investigations are mainly carried out in the context of the ecological function of scent components for pollinator attraction, to study their geographical variability or their evolution. In contrast, profiles of inflorescence scent compounds were rarely used for phylogenetic analyses and taxonomy. So far no investigations are available that focus on scent of apomictic plant groups and its value for the taxonomy of these groups. Apomicts produce fertile seeds without pollination, either from somatic cells of the nucellus or unreduced embryo sac cells. Overall, apomixis occurs scattered over the whole Angiosperm tree in early as well as late branching families. Rosaceae and Asteraceae are particularly rich in apomictic taxa. In these families genera such as *Hieracium* and *Sorbus* contribute a lot to local species biodiversity in Central Europe because of their high number of apomictic species and taxa, which are often endemic. Most members of these genera built up polyploid microspecies swarms initiated by hybridization events. The reticulate structure of these taxon complexes leads to taxonomic difficulties that can not be solved by morphology alone. Therefore, molecular or chemical markers are needed to investigate the parentage of such taxa and their collective species membership.

An important starting point of this research was the finding that artificial hybrids of *Citrus* produce a combination of the leaf and peel volatiles of their parents. This led to the hypothesis that natural hybrids might likewise produce scent patterns combined of the volatiles of their parents. Inflorescence scents were investigated from 64 mainly Bavarian taxa of the genus *Hieracium* (Subgenus *Pilosella* and Subgenus *Hieracium*). In *Sorbus* (Rosaceae) we focussed on three taxa of the *S. latifolia* agg., endemic to Northern Bavaria, *S. adeana*, *S. cordigastensis* and *S. franconica* and their parental species from the *S. aria* aggregate (agg.), as well as *S. torminalis.* Samples were collected with dynamic headspace method. Substances were identified by gas chromatography coupled to mass spectrometry (GC-MS). Scent data were evaluated by using various cluster methods. In *Hieracium*, additional reticulation analyses were applied that trace conflicting signals in a phylogram which can be interpreted as hybridizations between the taxa involved.

In *Sorbus* additional AFLP (Amplified Fragment Length Polymorphism) analyses were carried out from the populations and individuals that were studied for scent and genetic and scent data were correlated.

In *Hieracium,* the inflorescense scent consisted mainly of sesquiterpenes, monoterpenes, aliphatics and aromatics. In the flower scent of *Sorbus* besides these substances also nitrogene-containing substances were found.

In *Hieracium* (both subgenera) as well as in *Sorbus,* taxa of hybrid origin showed a mixed scent pattern compared with the parental taxa. In many cases, the parental taxa that had been suggested by morphological investigations or revealed by genetic investigations could be confirmed by scent. In *Hieracium* subgenus *Pilosella* however, based on scent data, some critical subspecies could be shown to belong to another collective species (e.g. *H. bauhini* ssp. *hispidissimum*) than the one that had been proposed for morphological reasons.

In general, scent patterns correlate well with morphological or genetical groupings, both in *Hieracium* and in *Sorbus*. Reticulate scent analyses in *Hieracium* showed that some taxa are probably derived from more than two ancestors. Scent data reflected even taxonomic patterns on a higher level, i.e. the sectional level. In *Hieracium* s.str. two main groups were found, the high growing and late flowering taxa such as *H. umbellatum* and *H. laevigatum* on the one hand, and the low growing earlier flowering taxa such as *H. murorum* and *H. bifidum* on the other hand.

The AFLP study revealed that the selected members of the *Sorbus latifolia* agg. are genetically clearly differentiated and mostly of clonal structure. They are more closely related to *S. aria* than to *S. torminalis*. The *S. aria* agg. has a complex structure. Besides *S. aria* s.str. and *S. pannonica* there are also intermediate plants with affinity (aff.) to either the one or the other of these taxa. *S. cordigastensis* was derived from intermediates aff. *S. aria* s.str., and both *S. adeana* and *S. franconica* derived from intermediates aff. *S. pannonica* or *S. pannonica* itself.

Floral scent of the same *Sorbus* taxa was investigated and compared with AFLP data on individual as well as on population level. Correlation analysis revealed a very high correlation between scent and AFLP data on individual and population level.

Overall, this work shows that in two unrelated plant complexes, *Hieracium* and *Sorbus,* which both harbour a high number of apomictic species derived by hybridization, scent is of high taxonomical value. The main reason for this correlation may be that most taxa in the investigated groups possess mixed scent patterns from their parents or progenitors because they are of hybrid origin. In addition, intraspecific variability of scent patterns is low within apomictic taxa due to their clonality, simplifying the taxonomic use of scent data. Furthermore, the role of pollinator mediated selection of scent is reduced because of apomixis.

Zusammenfassung

Schwerpunkt der Untersuchungen von Blüten- und Infloreszenzdüften ist ihre ökologische Funktion bei der Anlockung von Bestäubern, ihre geografische Variabilität, oder es werden evolutive Aspekte des Duftes untersucht. Nur wenige Studien beschäftigen sich dagegen mit Düften und ihrem Potential zur Aufklärung taxonomischer Fragestellungen. Insbesondere bei apomiktischen Pflanzengruppen gibt es außer den hier vorgelegten Untersuchungen bei *Hieracium* und *Sorbus* bisher keine weiteren Untersuchungen zu diesem Thema. Bei Apomikten erfolgt die Embryobildung ohne Befruchtung aus einer somatischen Nucelluszelle oder einer unbefruchteten, unreduzierten Embryosackzelle. Apomixis findet man in Europa gehäuft bei Asteraceen und Rosaceen und hier insbesondere bei den Gattungen *Hieracium* (Asteraceae) und *Sorbus* (Rosaceae). Viele apomiktische Taxa dieser Gattungen gehören zu polyploiden Kleinartenschwärmen, die durch Hybridisierung entstanden sind. Aufgrund retikulater Merkmalsverteilung sind bei solchen Pflanzenarten die Ausgangssippen sowie die Zugehörigkeit von Kleinarten zu größeren Einheiten oft aus der Morphologie allein nicht zuverlässig abzuleiten.

An künstlich erzeugten *Citrus*-Hybriden konnte nachgewiesen werden, dass sie neben wenigen neuen Düften eine Mischung aus den jeweiligen elterlichen Düften besitzen. Daher erschien es interessant, bei Pflanzenkomplexen, die zu großen Teilen auf natürliche Hybridisierung zurückgehen, die Zusammensetzung der Düfte zu untersuchen und deren Nutzen für systematische Fragestellungen zu erforschen. In der vorliegenden Arbeit wurden Blüten- und Infloreszenzdüfte von 64 vielfach endemischen Taxa der Gattung *Hieracium* (Subgenus *Pilosella* und Subgenus *Hieracium*) untersucht. Bei *Sorbus* wurden drei in Nordbayern endemische Vertreter der *S. latifolia*-Gruppe (*Sorbus adeana*, *S. cordigastensis*, *S. franconica*) untersucht, die durch Hybridisierung zwischen *S. aria* und *S. torminalis* entstanden sind. Duft wurde im Gelände mittels der „Dynamic Headspace" Methode abgesaugt und mit Gaschromatographie gekoppelt mit Massenspektrometrie (GC-MS) analysiert. Die Düfte wurden aufgrund von Ähnlichkeiten in Beziehung gebracht, dabei kamen bei *Hieracium* auch Retikulationsanalysen, welche Hybridisierungs-Ereignisse aufdecken können, zum Einsatz. Bei *Sorbus* wurden bisher fehlende genetische Untersuchungen mittels AFLP (amplified fragment length polymorphism) Analysen durchgeführt. Dies ermöglichte eine direkte Korrelation von Duft- und genetischen Daten auf Populations- und teilweise auch Individuenebene.

Bei *Hieracium* wurde der Infloreszenzduft vor allem von Sesquiterpenen, Monoterpenen, Fettsäurederivaten, und einigen Aromaten bestimmt. Im Duft von *Sorbus*

fanden sich neben den genannten Substanzklassen zusätzlich Stickstoffverbindungen. Sowohl bei *Hieracium* als auch bei *Sorbus* ließ sich bei hybridogen entstandenen apomiktischen Arten eine Kombination der Blütendüfte aus den Ausgangssippen nachweisen. Daher konnten aufgrund der Duftmuster vielfach die Elternarten von Hybrid-Arten identifiziert bzw. eingegrenzt werden. Bei beiden untersuchten Gattungen waren Gruppierungen auf Basis von Blütendüften sehr kongruent mit bisherigen Taxonomien. Bei *Hieracium* subgenus *Pilosella* allerdings zeigten die Duftdaten, dass in einigen Fällen kritische Taxa wie *H. bauhini* ssp. *hispidissimum* besser einer nächst verwandten Kollektivart zugeordnet werden sollten, in diesem Fall *H. densiflorum*. Retikulationsanalysen zeigten, dass bei einigen Sippen Beziehungen zu mehr als zwei Arten bestehen, was auf Introgression hindeutet.

Duftbasierende Gruppierungen bestätigten bei *Hieracium* s.str. die durch genetische Studien gefundene Differenzierung der Gattung in hochwüchsige, spätblühende Sippen wie *H. umbellatum* und *H. laevigatum* und niederwüchsigen und früher blühende Sippen wie *H. murorum* und *H. bifidum*. AFLP-Analysen bei in Nordbayern endemischen Arten der *Sorbus latifolia*-Gruppe belegten, dass die hybridogenen Arten genetisch deutlich differenziert sind und weitgehend klonale genetische Struktur aufweisen. Sie stehen dem *Sorbus aria* Aggregat näher als *S. torminalis*. Als wahrscheinlichste Elternsippen für *S. franconica* und *S. adeana* wurden Zwischenformen, die *S. pannonica* genähert sind oder *S. pannonica* s.str. selbst identifiziert. Dagegen stammt *S. cordigastensis* von Zwischenformen ab, die *S. aria* s.str. genähert sind. Gruppierungen auf Grundlage der Blütendüfte bestätigen die hybridogene Entstehung des *S. latifolia* Aggregates und seine größere Nähe zu *S. aria* agg. als zu *S. torminalis*. Eine korrelative Analyse zwischen den Duft- und den AFLP- basierten Gruppierungen zeigte, dass in *Sorbus* die Korrelationen der jeweiligen Gruppierungen sehr hoch sind. Daher ergibt sich aus dieser Arbeit, dass Blütendüfte bei Pflanzengruppen ganz unterschiedlicher Familien (Asteraceae und Rosaceae), aber ähnlicher hybridogener Sippenstruktur und apomiktischer Fortpflanzungsweise hoch aussagekräftige Marker für taxonomische Fragestellungen sind. Ausschlaggebend dafür sind die Konstanz der Düfte aufgrund der klonalen Struktur der apomiktischen Arten sowie der hohe Anteil von Hybrid-Sippen, deren Duft aus dem der Elternarten zusammengesetzt ist. Auch die bei Apomikten reduzierte Rolle von bestäuberbasierter Selektion von Düften trägt zur taxonomischen Aussagekraft der Düfte bei.

1. General Introduction

Floral scent has several functions. Scent compounds can conserve the flower by their antibacterial effects (Junker et al. 2011). Compounds can constitute also repellents against feeding insects (Kessler and Baldwin 2007, Junker and Blüthgen 2008), or some compounds may even be a byproduct of metabolism (Levin et al. 2003). However, the main function of floral scent is the attraction of pollinators such as insects or other animals. Most scent studies deal with its role as chemical signals for pollinators, its variability or with evolutionary aspects (Plepys et al. 2002, Dobson et al. 2005; Dötterl et al. 2005, Whitehead and Peakall 2009). Studies on the use of floral sent in taxonomy and phylogeny are rare. Nevertheless, there are some studies, in which scent was successfully used for phylogenetic analyses, all dealing with sexually reproducing plant species. In these studies scent data were congruent with genomic or morphological taxonomy, i.e. in the genera *Nicotiana* (Raguso et al. 2006), *Cypripedium* (Barkman 2001), *Ophrys* (Gögler et al. 2009) and the family Nyctaginaceae (Levin et al. 2003). An objection against the use of floral scent for taxonomy may be seen in convergent evolution of floral scents due to scent preferences of shared pollinators (i.e. Knudsen and Tollsten 1995) and therefore a lack of correlation between genetic markers and scent composition. Pollinator mediated selection is suggested to lead to pollination syndromes which means that plant species pollinated by the same guilde of animals have similar floral phenotypes (Faegri and van der Pijl 1979, Fenster et al., 2004, Dobson et al. 2005, Schiestl and Dötterl 2012). Although floral scent can be influenced by pollinator selection and (diffuse) coevolution, in many cases investigated so far, only some substances have a key function in attracting pollinators (Füssel et al. 2007, Burger et al. 2010, 2011, Schäffler et al. 2012), whereas other scent substances of scent may be determined more by phylogeny than by pollinator selection (Steiner et al. 2011, Schäffler et al. 2012).

The situation is different in apomictic plants, which can be found in early and late branching lineages (Hörandl and Hojsgaard 2012). In the Central European flora apomixis is found mainly in *Crataegus, Sorbus* (both Rosaceae), and *Hieracium* (Asteraceae) (Koltunow et al. 2011, Talent 2009), and members of Poaceae, Brassicaceae and Ranunculaceae. Here, seed formation occurs without pollination (Koltunow et al. 2011, Talent 2009, Bicknell et al. 2000, Nogler 1984); a special case is pseudogamy (see below). Therefore, apomicts most likely behave differently to sexually reproducing species and scent and genetic data may be more congruent. In contrast to sexually reproducing species apomicts mostly do not rely on pollination and therefore, the role of selection on

phenotypic floral characters by pollinators may be less important than in sexually reproducing plants. However, mechanisms of apomixis are manifold and complicated (Talent 2009) and pollination and related activities are not completely absent in apomicts. In the pseudogamous apomixis type, which occurs in *Sorbus* (Jankun and Kovanda 1987), pollination is still necessary to induce fruit set (Campbell and Dickinson 1990). Pollination activities are described also in studies about remnant sexuality in *Hieracium* (Rosenbaumová et al. 2012, Krahulcová et al. 2000, Krahulcová and Krahulec 2000, Fehrer et al. 2007). Pollen of apomictic *Hieracium* is partly fertile and therefore, apomicts can still cross with sexual *Hieracium* taxa, however, such crosses are usually rare (Krahulcová et al. 2000, Krahulcová and Krahulec 2000, Mráz et al. 2005).

In apomicts, their clonality may be more determinant of the scent patterns than selective pollinator pressure. Apomictic populations typically consist of genetically highly similar individuals; therefore scent is conserved and identical between the individuals of one taxon. However, mutations, somatic recombination and very low rates of sexuality still could lead to individual differences in apomictic taxa as well (Campbell and Dickinson 1990). Overall, it can be argued that scent in apomicts is only determined by the first two of the three factors that Raguso (2001) mentioned: "floral scent is a mosaic product of biosynthetic pathway dynamics, phylogenetic constraints, and balancing selection due to pollinator and ovivore attraction".

Apomixis, polyploidy and a high degree of hybridization can often be found coupled in plants (e. g. Campbell and Dickinson 1990, Talent 2009, Hörandl and Hojsgaard 2012). In Central Europe, most species of *Hieracium* (Asteraceae) and *Sorbus* (Rosaceae) resulted from hybridization (cf. Fehrer et al. 2009, Rich et al. 2010). They typically built up polyploid complexes that contribute considerably to Central European species richness (Schuhwerk 2002, Meyer et al. 2005, Rich et al. 2010). Thus, an important starting point of floral scent research in such groups is the study of inheritage of volatiles in hybrids. Gancel et al. (2002) studied volatiles of leaves and peels on somatic (artificial) hybrids of *Citrus*, and showed that the *Citrus* hybrids produce, besides a low number of novel substances, a combination of the volatiles of their parents (Gancel et al. 2002). Therefore, in *Hieracium* and *Sorbus,* where many species are derived from natural hybridization, it should be possible to identify the parental taxa of hybrids on the basis of scent patterns and test whether morphology-based analyses of parentage are plausible. In *Hieracium* "basic species" and "intermediate species" are distinguished (Nägeli and Peter 1885, Zahn 1921-1923, 1930-35, Schuhwerk 2002), referring to non-hybrid species and

species putatively derived from hybridization showing intermediate characters, respectively (Nägeli and Peter 1885). Furthermore, in *Hieracium*, there is a vast amount of subspecies and microspecies that were pooled to so-called ”collective species” (Nägeli and Peter 1885). However, not in every case the morphology-based assignment of subspecies to collective species is convincing, because differences between taxa are too poor or characters show a reticulate distribution. Furthermore, many subspecies and microspecies are possibly of polytopic origin. It is an important question whether all subspecies identified by morphology really belong to a collective species and to which extent these collective species are natural monophyletic units (cf. Schuhwerk 2002).

The genus *Sorbus* also comprises a large variety of intermediate taxa emerged from hybridization between common, widely distributed diploid taxa (e.g., *Sorbus torminalis, S. aucuparia*, *S. aria* s.str.). In some areas of Europe often locally or regionally endemic hybrids developed and were subsequently stabilisized by apomixis (Kárpáti 1960, Düll 1961, Meyer et al. 2005, Lepší et al. 2009, Rich et al. 2010, Robertson et al. 2010). Comparatively well investigated examples are *Sorbus adeana*, *S. cordigastensis,* and *S. franconica,* which are endemic to Northern Bavaria (Meyer et al. 2005, Aas and Kohles 2011). Similar to *Hieracium*, important questions in *Sorbus* concern relationships between hybrid taxa, possible polytopic origins and parent identification. In *Sorbus latifolia* agg. investigated here, it is of fundamental importance to uncover the second parental lineage of the complex *S. aria* agg. It consists of several facultative or obligatory sexual taxa, i.e. *S. aria* s.str. and *S. graeca*, but also of apomictic lineages such as *S. pannonica* Kárpáti (Kutzelnigg 1995, Meyer et al. 2005). It is not clear so far which of these may be parental to *S. latifolia* taxa.

2. Aims of research

In this work I investigate whether floral scent is useful to uncover taxonomic relationships in natural species complexes where hybridization plays a prominent role.
I focus on *Hieracium* subgenus *Pilosella* and *Hieracium* s. str. as well as on the genus *Sorbus* (Rosaceae).

Special attention is adressed to the following questions:

- Are there congruencies between the clusterings based on floral scent data and morphological-based taxonomy in hybrid polyploid complexes?

- Is there a correlation between floral scent and genomic-based taxonomy in hybrid polyploid complexes?

3. Synopsis

3.1 Material and Methods

Study plants

Hieracium is one of the most species-rich genera in the world. Depending on which taxonomy is applied either the classical ranking with a species/subspecies system preferred by Nägeli and Peter (1885), and later by Zahn (1921–23,1930–35) or the additionally applied microspecies system (in Russia, Scandinavia in Central Europe; Bräutigam and Greuter 2007) the number of taxa strongly varies between 500 and 7000. Traditionally, the genus is subdivided into three subgenera, the holarctic subgen. *Hieracium,* subgen. *Pilosella* in Europe and America, and subgen. *Chionoracium* in America. Especially in Great Britain, Russia and Scandinavia, *Pilosella* is treated as a genus of its own. This view was recently adopted by Bräutigam and Greuter (2007) for Central European treatments as well. The nature of apomixis differs between subgenus *Hieracium* and subgenus *Pilosella*. In the former diplospory is found, meaning that that embryos develop from embryosac mother cells without meiosis and double fertilization (Koltunow and Grossniklaus 2003, Koltunow et al. 2011). In the latter, in contrast, apospory is the apomixis type, in which a somatic cell of the nucellus becomes the embryosac (Gadella 1984, Koltunow et al. 2011).

The taxonomy of *Hieracium* is complicate. Morphological taxonomy comes to its limits, because in *Hieracium* usually the characters are reticulatedly scattered over the whole complex. Usually, the large number of species is organized in basic (non-hybrid) and intermediate (hybrid) taxa (Nägeli and Peter 1885).

Here, mainly Bavarian material of *Hieracium* is investigated (Plate 1). In subgenus *Pilosella* intermediate species such as *H. densiflorum, H. zizianum, H. calodon, H. schneidii* and basic species such as *H. piloselloides* or *H. cymosum* were investigated. In subgenus *Hieracium* intermediate species such as *H. glaucinum, H. wiesbaurianum* and *H. lachenalii* and basic species such as *H. bifidum, H. murorum* or *H. bupleuroides* were included. Most species are rare and threatened and occur at open sites, e. g. limestone rocks. Also rare species endemic to Bavaria and therefore of particular interest such as *H. schneidii, H. franconicum* and *H. harzianum* were included into this study. In *Hieracium* subgen. *Pilosella* taxa were bound together to so-called “collective species” (Nägeli and Peter 1885). Some of these collective species are weakly differentiated against each other, i.e. *H. bauhini* and *H. densiflorum* or *H. zizianum* and *H. piloselloides*.

Hieracium harzianum ssp. *pseudofranconicum* Harz et Zahn, endemic to Northern Franconia on limestone rocks, Walberla.

Hieracium caesium Fr., a rare relict species of the Southern Franconian Jura on limestone rocks, Essing.

Hieracium schneidii x pilosella, a rare spontaneous hybrid between *H. schneidii* Schack et Zahn and *H. pilosella* L.

H. schneidii Schack et Zahn, endemic to the Northern Frankonian Alb.

Plate 1: Examples of investigated *Hieracium* taxa.

Furthermore, subspecies attribution to the one or the other collective species sometimes seems to be subjective (i.e. *H. bauhini* ssp. *hispidissimum*). Such units are doubtful because it is not clear yet whether they constitute natural units or are of polytopic origin (cf. Schuhwerk 2002). Therefore, it is important to investigate to which collective species some doubtful subspecies belong.

The holarctic genus *Sorbus* (Plate 2) comprises a large variety of intermediate species evolved from hybridization between common and widely distributed taxa such as *Sorbus torminalis, Sorbus aucuparia* and *Sorbus aria*. In some areas of Europe endemic hybrids have been developed that are stabilized by apomixis (Kárpáti 1960, Düll 1961, Meyer et al. 2005, Lepší et al. 2009, Rich et al. 2010, Robertson et al. 2010). These hybrid species are distributed mostly in calcareous areas of Europe. In *Sorbus,* besides diplospory and apospory (Jankun and Kovanda 1987), another apomixis type, pseudogamy occurs, in which pollination is necessary to induce fruit set (Jankun and Kovanda 1987, Campbell and Dickinson 1990). According to Meyer et al. (2005) selfing is sufficient to induce fruit set in pseudogamous *Sorbus*.

Here, *Sorbus adeana*, *S. cordigastensis* and *S. franconica* were investigated which are endemic in Northern Bavaria (Meyer et al. 2005, Aas and Kohles 2011). They have a small distribution area and typically occur along forest margins or in very open forest stages. Their parental lineages are *S. torminalis* and *S. aria* agg. The latter has a complicated phylogenetic structure. It comprises besides obligatory sexual taxa such as *S. aria* s.str., also several facultative or obligate apomictic lineages such as *S. pannonica* and *S. graeca*. *Sorbus pannonica* is a xeromorphic member of *S. aria* agg. and more widespread than *S. aria* s.str. in the northern Franconian Alb (Kutzelnigg 1995, Meyer et al. 2005). It is a non-lectotypified taxon which comprises presumably apomictic morphotypes filling the morphological gap between *S. aria* s.str. and *S. graeca* (Spach) Loddiges ex Schauer (Kárpáti 1960, Kutzelnigg 2005, Meyer et al. 2005). *Sorbus graeca* is another xeromorphic member of the *S. aria* agg., mainly distributed in the Mediterranean floral region. It reproduces sexually or is a facultative apomict (Kutzelnigg 1995). It is uncertain whether *S. graeca* occurs in the study area at all (Düll 1961, Kutzelnigg 1995, 2005), but individuals that can be attributed morphologically to *S. graeca* were found in the Northern Franconian Alb (own obs.), yet, it is difficult to delimitate this element against *S. pannonica.*

Sorbus pannonica Kárpáti, on the edge of pine forests in the Northern Franconian Alb, Oberailsfeld.

Sorbus fanconica Bornm. ex Düll, on the edge of pine forests, endemic to Northern Franconia, Oberailsfeld.

Fruits of *Sorbus pannonica* Kárpáti, in the Southern Franconian Jura, Deining.

Sorbus adeana N. Mey., endemic to the Northern Franconian Alb, Modschiedel.

Plate 2: Examples of investigated *Sorbus* taxa.

In the present thesis three main methods were applied: Scent collection and analysis using dynamic headspace and gas chromatography coupled to mass spectrometry (GC-MS), amplified fragment length polymorphism (AFLP) analyses and morphological analyses.

Volatile collection and chemical analyzes of floral/inflorescence scent (publication 1, 2 and 4)

Floral scent was collected in the field using the dynamic headspace method described by Dötterl and Jürgens (2005), and Dötterl et al. (2005a,b). Capitula (*Hieracium*) or inflorescences (*Sorbus*) were enclosed within a polyester oven bag (Toppits) and the emitted volatiles were trapped in an adsorbent tube through the use of a membrane pump (ASF Thomas, Inc.). As absorbent tube, we used ChromatoProbe quartz microvials of Varian Inc. (length: 15 mm; inner diameter: 2 mm), cut the closed end, filled them with a mixture (1:1) of 3 mg Tenax–TA (mesh 60–80) and Carbotrap (mesh 20–40), and fixed the adsorbent mixture in the vial with glass wool. Simultaneous collections of both the flower scent and surrounding air are used to distinguish between floral compounds and ambient contaminants. In *Sorbus* we used green leaf samples as blank, so we could reveal the floral scent by substracting these substances, whereas in *Hieracium* we collected the inflorescence scent because we used the surrounding air as blank.

For each taxon, one sample of two to six individuals was collected. In *Hieracium*, sampling was carried out on fresh and newly opened inflorescences (the capitula of *Hieracium* are composed of many florets with most (90%) of them open at time), 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). In *Sorbus*, the 100–150 flowers of the pseudo-umbels bloom in parallel. Here, headspaces covered single inflorescences, each. In both study plants, scent was collected for 3 to 5 minutes after a time of 3 to 10 minutes, where the scent accumulated in the closed bag.

Headspace samples were analyzed on a Varian Saturn 2000 mass spectrometer coupled to a Varian 3800 gas chromatograph equipped with a 1079 injector (GC-MS) as described earlier (Dötterl and Jürgens 2005, Dötterl et al. 2005). The GC-MS data were processed using the Saturn Software package 5.2.1. Component identification was carried out using the NIST 02 mass spectral database, or MassFinder 2.3, and confirmed by the comparison of retention times with published data (Adams 1995). Identification of individual components could be confirmed by the comparison of both mass spectrum and GC retention data with those of authentic standards.

Scent data analysis (Publication 1,2 and 4)

For both *Hieracium* and *Sorbus* data sets, pairwise qualitative similarities were calculated using the Jaccard similarity index. The significance of differences in scent profiles among taxa was assessed by ANOSIM with 10,000 random permutations based on these similarity matrices using Primer Version 5 and 6 (Clarke and Gorley 2001, 2006).

In *Hieracium*, a reticulation network analysis was conducted with the program t-rex, version 4.0a1 (Makarenkov 2001) to analyse the relationships among the taxa. This method allows to visualize relationships of species interconnected with more than one ancestor (Legendre and Makarenkov 2002), which is important for analysing groups, such as *Hieracium*, with many taxa of hybrid origin (see Feulner et al. 2009). In this approach, a neighbour joining tree was constructed using a dissimilarity matrix (1-Jaccard), and homoplasies were made visible by so-called reticulation lines. Those homoplasies point towards hybridization or introgression (Legendre and Makarenkov 2002). In *Hieracium*, in addition to the presence and absence of compounds, we also calculated the average relative (percentage of total) amount of scent compounds of the single taxa.

In *Sorbus*, the Jaccard matrix was used to cluster the scent data with UPGMA using Primer Version 5 and 6 (Clarke and Gorley 2006). Additionally intraspecific variability of scent data was compared among species using PERMDISP in PRIMER Version 6 (Clarke and Gorley 2006).

Molecular methods, DNA marker

Sample collection and DNA extraction (Publication 3)

Leaf samples of *Sorbus* were taken in May and June 2010. Immediately after harvesting they were placed in plastic bags and put in a box with ice for transportation. At the same day, leaves were washed with ethanol in the laboratory and frozen in an extraction tube at –80 ° C until extraction. Frozen leaf samples (40 – 70 mg, 1 – 2 cm^2) were extracted using widespread extraction systems and plant kits (NucleoMag 96 Plant kit; Machery-Nagel, Düren, Germany, FastPrep®-24 Tissue Homogenizer (MP Biomedicals Europe, Illkirch, France). The purified genomic DNA was diluted tenfold and used for all subsequent PCR reactions.

AFLP (Publication 3)

AFLP is a fingerprinting method that allows discrimination between individuals (Vos 1995). It is helpful for closely related plant groups and can detect clonal structures derived i.e. by apomictic reproduction (Vos 1995).

For AFLP is of fundamental importance to find appropriate specific primers for the second specific PCR step. For the preliminary primer search 24 primer combinations were tested, and the following six combinations were then selected for this study because they yielded the best results in species differentiation: MCAA/E-ACG, M-CAC/E-ACG, M-CAC/E-ACA, M-CAT/E-ACG, M-CTC/E-ACG, MCTT/E-ACG.

AFLP Data analysis (Publication 3)

The reactions were separated on a vertical electrophoresis system (4200 Sequence Analysis System, Li-Cor Biosciences, Bad Homburg) together with DNA size markers (50–700 bp Sizing Standard, Li-Cor Biosciences, bad Homburg). AFLP banding patterns were evaluated using GeneMarker1-95 software (SoftGenetics). Band classes were calculated with a tolerance factor of 0.1 %. A neighbour joining (NJ) analysis of the presence and absence matrix was conducted (Nei-Li distance), followed by a bootstrap (BS) analysis after internode rooting with 1000 replicates using the program TREECON (Van de Peer and De Wachter 1994). For data of *S. aria* agg., we additionally applied model-based clustering (Pritchard et al. 2000) using the program STRUCTURE (http://pritch.bsd.uchicago.edu/structure.html) in order to retrieve the most likely number of groups within the *S. aria* agg. In order to investigate genetic variability, the number of polymorphic loci and Nei´s gene diversity "NGD" (Nei 1972) were calculated with Popgene (Yeh and Yang 1999). As a measure for the genetic distance between taxa we calculated Nei´s standard genetic distance (Ds) using the program POPGENE (Yeh and Yang 1999).

Chromosome counts (Publication 3)

In *Sorbus*, chromosome numbers were counted from root tip meristems of cultivated progeny of *S. cordigastensis, S. adeana, S. franconica*, and *S. pannonica* (one seedling per taxon), grown in the Ecological Botanical Garden and harvested in May 2010. Some of the seedlings were also included in the AFLP analysis. The fresh root tips were pretreated in 0.002 hydroxychinoline (4hrs), fixed in CARNOY´s solution and stained in carmine

following Snow (1963). From the stained root tips we prepared squash preparations in 45% acetic acid, and observed somatic metaphase in the microscope.

Correlation analyses between scent and AFLP data (Publication 4)

Correlation analyses between in scent and AFLP data were made on individual level and population level. For correlation analyses on population level presence-absence data occurring at least in one individual from both data sets - AFLP (see Feulner et al. 2013, submitted) and scent - were used. Similarity matrices (Jaccard) were calculated and these were the input for the RELATE correlation analysis (Spearman Rank correlation, 10,000 permutations) in PRIMER Version 6 (Clarke and Gorley 2006).

3.2 Results and Discussion

Floral scent analysis in *Hieracium* subgenus *Pilosella* and its taxonomical implications (Publication 1).

In *Hieracium* subgen. *Pilosella* floral scents of 27 predominantly Bavarian intermediate species, mostly of the collective species *Hieracium calodon*, *H. zizianum* and *H. densiflorum* were investigated with dynamic headspace method. Reticulate analyses were applied to depict hybrid speciation by visualizing relations between samples placed far from each other in a tree (Makarenkov 2001).

Altogether, 56 floral scent compounds were identified, mainly aromatics, fatty acid derivatives and mono-, homo- and sesquiterpenes. The chemical patterns were found to be taxon-specific and are thus of taxonomical value. The result that the basic (non-hybrid) species of *Hieracium* subgen. *Pilosella* such as *H. piloselloides*, *H. echiodes* or *H. cymosum* were well separated by scent underlined the utility of scent for taxonomical investigations in *Hieracium*. For many species of presumed hybrid origin, reticulation analyses of scent data allowed insights into their parentage. One example is *H. fallax* ssp. *durisetum*. This taxon is an intermediate between *H. cymosum* and *H. echioides*. In the scent tree it clusters close to *H. cymosum,* but the reticulation analysis connected it with *H. echioides*. The reason is that it has scent compounds of both taxa, on this basis it is possible to detect parental taxa (see above). In the scent tree, different subspecies of one taxon (e.g. *H. zizianum*) often clustered together. Exceptions have to be evaluated and can have taxonomical implications. *Hieracium bauhini* ssp. *hispidissimum* did not cluster with other members of *H. bauhini* but with *H. densiflorum.* In consequence it should be assigned to *H. densiflorum* and not to *H. bauhini.* There are morphological characters that support this placement, suggesting that the evaluation of some morphological characters needs to be reconsidered in the classification of *Hieracium* (in this case the cymose inflorescence structure). Another interesting result is that in the cluster analysis some subspecies of *H. densiflorum* do not group together with the other members of this group, but rather with the *H. echioides* derivates, such as *H. calodon* and *H. fallax.* They also show some morphological affinities to section *Echioides* such as dense, thick and curved bristle-like hairs on the stem. The subspecies *H. densiflorum* ssp. *cymosiforme* and *H. densiflorum* ssp. *psammotrophicum* may have been derived from taxa of section *Echioides*, as could been deviated from their strong thick and curved bristle-like hairs, too.

Hieracium subgen. *Pilosella* taxa strongly deviated in their numbers of scent compounds (12-30 compounds). In hybrids this may lead to an overestimation of the

influence of one of the parents. Nevertheless, this can make influences of species detectable which are not apparent in morphology. An example of this aspect is the primary hybrid *H. schneidii* x *pilosella*. This species is hard to differentiate against *H. piloselliflorum* by morphology alone. This taxon was found and identified here by floral scent and unpublished RAPD-markers (Gebauer and Feulner 2008, unpub.) for the first time. It grows as a spontaneous hybrid among its parents.

Overall, scent patterns implicate that only a low number of taxa may be the ancestors of most of the hybrid taxa. Interestingly, this has been suggested already by Zahn (1921-1923, 1930-35), and the presumably parental species involved have been named as basic species by him (cf. Zahn 1921–1923, 1930–35). The placement of *H. caespitosum* in the scent tree close to *H. zizianum* (an intermediate species) speaks against its status as "basic species". It shares also morphological traits with *H. zizianum* such as the straight hairs on the stem. Also Tichomirov (2000) considered *H. caespitosum* as hybrid between *H. onegense* (syn: *H. caespitosum* ssp. *brevipilum*, an eastern distributed species) and *H. lactucella*. It would be interesting in further studies to investigate the scent of *H. onegense* to confirm this hypothesis.

Taxonomical value of inflorescence scent in *Hieracium* s. str. (Publication 2)

Publication 2 deals with the taxonomical value of inflorescence scent in *Hieracium* s. str. in Central Europe. *Hieracium* s. str. comprises a vast number of mostly apomictic taxa presumably originated from hybridizations in the past. Inflorescence scents of 37 taxa from seven sections of *Hieracium* subgen. *Hieracium* were investigated by headspace analyses. Overall, 58 different scent compounds belonging to aromatics, sesquiterpenes, homoterpenes, monoterpenes and fatty acid derivatives were found. As in *H.* subgen. *Pilosella* (publication 1) inflorescence scent was found to be highly taxon-specific in *Hieracium* subgen. *Hieracium*. Taxonomy suggested by scent patterns was compared with results from genetic studies (Fehrer et al. 2009) that include many taxa investigated here by scent. Fehrer et al. (2009) identified two main groups, termed "western clade" and "eastern clade" in *Hieracium* s.str. using sequence data of chloroplast and mitochondrial markers (Fehrer et al. 2009). This differentiation was explained by different glacial refugia (Fehrer et al. 2009). The scent study identified the same main groups as in Fehrer et al. (2009). However, we found that these groups are identical with two distinct morpho- and flowertypes, the high-growing and late-flowering-one, such as *H. umbellatum* and *H. laevigatum* and the low-growing and earlier flowering morphotype, such as *H. murorum*

and *H. bifidum*. Some substances such as linalool and linalool oxide were found dominantly in species with high growth, whereas monoterpenes such as terpinolene were rather typical for the low-growing morphotypes. The low-growing scent group comprises sections such as *Oreadea*, *Hieracium* and *Bifida*, the high-growing group comprises the sections *Drepanoidea*, *Tridentata* and *Hieracioides*. Members of the low-growing species groups such as *H. wiesbaurianum* or *H. glaucinum* may be of polytopic origin since their taxa often clustered intermingled in the scent cluster. In contrast, in the scent tree most subspecies of *H. murorum* are placed next to each other and therefore are most likely monophyletic. Also *H. bifidum* is mainly placed in a group of its own despite some morphologically deviating members (i.e. *Hieracium bifidum* ssp. *stenolepis* var. *valdefloccosum*). Interestingly, the investigated *H. bifidum* taxa of *H. bifidum* grex *bifidum* and grex *subcaesium* are nested between *H. murorum* (low growing) and *H. glaucum* (high growing) in the scent tree. This intermediate position for a whole species group was proposed already by Koch (1838) and Zahn (1906) due to its morphological intermediacy and is confirmed here by scent data. This finding shows that floral scent can confirm the taxonomical position of species even at higher rank (i.e. sectional level).

In *H. franconicum* (intermediate between *H. murorum* and *H. bupleuroides*) interpopulation differences could be found. Populations from Baden-Württemberg were closer to *H. bupleuroides* whereas populations from Franconia were closer to *H. murorum*. The same results were revealed by AFLP studies (Feulner, unpublished data). This result was unexpected and shows that hybrid taxa in *Hieracium* s.str. could be influenced by introgression.

The scent study reveals an intermediate position for some taxa hitherto considered as basic species such as *H. lachenalii* and *H. laevigatum*, supporting the results of Fehrer et al. (2009) based on molecular data. The scent study shows that the intermediate taxa such as *H. lachenalii, H. saxifragum, H. caesium* were derived by multiple hybridization events between a very restricted number of members of the two morphological groups (high-growing and low-growing). This imitates a clinal variation derived by stepwise evolution as was described by so-called reduction lines (i.e. *Hieracium umbellatum - H. laevigatum - H. lachenalii - H. murorum*, comp. Zahn 1921–1923, 1930–1935). Although the species of *Hieracium* subgen. *Hieracium* are older hybrids, their phylogeny is not concealed by potential mutations. Thus, floral scent composition is a highly conserved trait in *Hieracium* s.str.

Origin and genetic structure of three *Sorbus latifolia* (Lam.) Pers. taxa endemic to Northern Bavaria (Publication 3)

In publication 3 the genetic structure of apomictic *Sorbus latifolia* taxa of hybrid origin and their parental taxa (*S. aria* agg. and *S. torminalis*) was studied. Within *Sorbus latifolia* agg., focus was on *Sorbus franconica*, *S. adeana*, *S. cordigastensis*, all endemic to Northern Bavaria. Within *S. aria* agg. we investigated *S. aria* s.str., *S. pannonica* and intermediates with either affinity (aff.) to the one or to the other. Results from AFLP studies, Neighbour-Joining and Bayesian clustering confirmed the hybrid origin of the *S. latifolia* taxa, which were 1.3 to 1.5 times closer related to *S. aria* agg. than to *S. torminalis*. The three Franconian *S. latifolia* taxa were remarkably different between each other, confirming their microspecies status. The differentiation was higher than within the *S. aria* aggregation (e.g. between *S. aria* and *S. pannonica*). Responsible for the strong genetic differentiation among *S. latifolia* agg. is their independent origin from different subgroups of the *S. aria* group that were involved as ancestors. Whereas *S. adeana* and *S. franconica* were derived from crosses between *S. torminalis* and *S. pannonica* or intermediates between *S. aria* and *S. pannonica* aff. *S. pannonica*, for *S. cordigastensis* intermediates aff. *aria* were proven to be parental. Another reason for pronounced genetic distances among *S. latifolia* members could lie in different times of origin involving different ancestors that had changed genetically over time. This explanation corresponds to the one of Düll (1961), who explained the differences in area size of the *S. latifolia* taxa with different dates of origin.

The genetic structure of *S. aria* agg. is shown to be complicated. Besides diploid and sexual *S. aria* s.str. and triploid apomictic *S. pannonica* we identified intermediates with affinity to the one or the other that partly consist of presumably facultative apomictic lineages. These types may be interconnected by gene transfer and go back to a polyploidy cycle as described by Talent (2009) or Rich et al. (2010). According to these authors, facultative apomictic triploids can arise from crosses between sexual diploids after formation of unreduced gametes in one of the crossing partners. Apomictic and sexual tetraploids can also be formed by fertilisation of unreduced eggs of triploids by pollen from diploids. And last but not least, tetraploids can cross with diploids and form - again - triploids. According to Talent (2009) in those complexes selection for apomixis takes place because sexual reproduction is hampered in triploids and favors apomictic lineages. Such events could lead to the coexistence of genetically variable and clonal *Sorbus aria* agg. populations, as found in this study.

It was an unexpected result of our study that intermediates between *S. aria* and *S. pannonica* were identified to be parental for *S. latifolia* taxa in Bavaria instead of *S. aria* and *S. pannonica* themselves. However, this result is confirmed by geographical range patterns of the species because most *S. latifolia* taxa in Bavaria occur in areas from where intermediates of *S. aria* and *S. pannonica* have been described. This is true for the study area, the western Albtrauf of the northern Franconian Alb (i.e. *S. adeana, S. cordigastensis*), the western Albtrauf of the southern Franconian Alb (i.e. *S. schuwerkiorum* N. Mey., *S. fischeri* N. Mey.,) or parts of lower Franconia (i.e. *S. badensis* N. Mey., *S. herbipolitana* N. Mey., comp. area maps in Meyer et al. 2005). Intermediate plants may be more suited as parental taxa than the sexual S. *aria* s.str. or the apomictic *S. pannonica* since they may be able to reproduce in both ways, either apomictic (inherited from *S. pannonica*) or sexual (inherited from *S. aria* s.str). As facultatively apomicts these intermediates could have bequeathed apomixis to the *S. latifolia* taxa.

Floral scent and its correlation with genetic data in *Sorbus* taxa (Publication 4).

In publication 4 the floral scent of *Sorbus latifolia* taxa was investigated, derived from hybridization between parental *S. aria* agg. and *S. torminalis*. Focus was on *Sorbus franconica, S. adeana* and *S. cordigastensis* endemic to Northern Bavaria (Meyer et al. 2005). The same populations and, mostly individuals were used as in the previous study that used AFLP markers. The scent data (presence/absence of compounds) were used to construct an UPGMA tree, and to calculate a similarity matrix to correlate them, both on individual as on population level, with AFLP data (publication 3). To the best of our knowledge, such a statistical approach to test for a correlation between scent and genetic data was performed only once previously, in a study about *Ophrys* (Orchidaceae, Stökl et al 2008). In that study no significant correlation was found.

A total of 68 chemical substances was identified, among them aromatic compounds, mono- and sesquiterpenes, aliphatics, and nitrogen containing compounds. Scent patterns were found to be taxon-specific, and the number of scent compounds differed significantly among most taxa. Correlations with AFLP data on population and individual level were highly significant, indicating that scent and genetic data are highly congruent in the plants studied. Scent clusters and AFLP trees revealed identical information about systematic relationships among the taxa and intraspecific variability.

Scent clusters were very similar with the AFLP tree of the same taxa on individual and population level. The *S. latifolia* taxa clustered between *S. aria* and *S. torminalis*, they

clustered closer to *S. aria* agg. than to *S. torminalis*. The closer similarity to *S. aria* is caused by the *S. latifolia* taxa sharing many more substances with *S. aria* than with *S. torminalis*. *Sorbus cordigastensis* and *S. adeana* are most closely related to each other, corresponding to the AFLP study. They had more scent compounds in common than either of them with *S. franconica*.

The scent of the parental taxa (*S. aria* agg. and *S. torminalis*) was extremely distinct. In *S. aria* agg., 50 to 56 compounds were identified, whereas *S. torminalis* harboured only 29 compounds. This reduced number was mainly due to a lack of lilac derivatives, which were characteristic for *S. aria* agg. In *S. torminalis,* but not in the *S. aria* agg. two nitrogen-containing substances were found, amyl/isoamyl-pyrrole, and a still unidentified compound. With exception of *S. franconica*, the *S. latifolia* s.l. showed an intermediate number of scent compounds, compared to the compound numbers of the parents. *Sorbus franconica* showed very low numbers (not significantly different from those of *S. torminalis*). The *S. latifolia* taxa inherited from the *S. aria* parent most lilac derivatives. *Sorbus cordigastensis* and *S. adeana* inherited from *S. torminalis* amyl/isoamyl-pyrrole and the unidentified n-bearing compound. In contrast to other examples, where hybrid taxa contained also novel substances besides a mixture of scent of the parents, we did not identify such novel compounds in *S. latifolia*. However, methyl hexanoate and methyl (3Z)-hex-3-enoate are present in all *S. latifolia* taxa (*S. adeana*, *S. cordigastensis* and *S. franconica*) but only in some *S. pannonica* individuals. On the other hand, some substances of parental taxa were not bequeathed to the hybrids (e.g. (E)-arbusculone and anisaldehyde).

3.3 Conclusion and perspectives

In the present work it was shown that scent data in *Hieracium* and *Sorbus* are of high taxonomical value. In publication 1 and 2 strong congruencies between taxonomies derived from scent and genetic data could be found for *Hieracium* (subgen. *Pilosella* and subgen. *Hieracium*). In *Sorbus* (publication 4) a correlative analysis was conducted to test the correlation between scent and genetic AFLP marker data. The correlation was found to be very high indicating that also in *Sorbus* scent data are very informative for taxonomy. In the following I describe the most important reasons that are suggested to be responsible for the high reliability of scent clusters in apomictic groups.

First, complexes, such as *Hieracium* and *Sorbus latifolia* agg., are relatively young with most of the taxa derived after the last ice age through hybridization (Fehrer et al. 2009, Campbell et al. 2007). Because of reticulate evolution, ancient geneflow occurred between most taxa of the complex. The mixed scent patterns of taxa of hybrid origin allow identifying parental taxa or introgression and therefore are responsible for the high reliability of scent taxonomy.

The second important reason for the high taxonomic value of scent is apomixis. In apomicts, every individual of one taxon is genetically highly identical and also scent is highly identical between different populations of one taxon. In contrast, in sexual species scent differentiation could be found between populations of one taxon i.e. in *Silene* L. (Caryophyllaceae, see Dötterl and Jürgens 2005) and the differences may increase the lower the rates of gene flow between populations are because of genetic drift.

In *Hieracium* and *Sorbus*, even the role of scent mutations that can reduce the taxonomical information of scent may be relatively low, since it was shown on the example of *Hieracium* s.str., that even in ancient hybrids the track of phylogeny is not concealed by mutations. Furthermore, apomictic plants do not rely on pollination for seed set. In apomicts, scent is not that strong under pollinator-mediated selection influencing scent patterns than in sexually reproducing taxa (comp. Faegri and van der Pijl, 1979, Fenster et al., 2004, Dobson et al., 2005, Schiestl and Dötterl 2012). However, even in apomicts the influence of pollinator mediated selection may not totally be absent. I.e. in the apomixis type pseudogamy, pollination is still necessary to induce fruit set (Jankun and Kovanda 1987). Moreover, in *Hieracium* subgen. *Pilosella*, the number of facultative apomicts is high (Fehrer et al. 2007). And last but not least, in *Hieracium* and *Sorbus* there are also some obligate sexually reproducing species. It has to be investigated whether convergences of scent toward the olfactory preferences of pollinators can be found in those

sexually reproducing taxa and to which extend they influence scent patterns of sexual taxa. Interestingly, in *Sorbus* the sexually reproducing *S. aria* s.str., *S. torminalis* and *S. aucuparia* are not very closely related (comp. Campbell et al. 2007) and therefore scent comparisons between these species may be interesting regarding phenomena such as convergence. Even less is known concerning shared pollinators, however, first investigations have shown that strong differences in flower visitors occur between the sexually reproducing *Sorbus torminalis, S. aria* and *S. aucuparia* (Heuss, L. 2010, Bachelor thesis).

So far, scent studies have been carried out only for a minority of middle European *Hieracium* species. It would be important to include species distributed in the Alps, such as *H. villosum, H. incisum* as well as *H. alpinum* into the study. These elements played an important role for hybrid speciation of taxa growing in southern Germany such as some *H. bifidum* members. However, scent collection in the mountains is more difficult than in the lowlands, since dry and sunny conditions, which are necessary for scent collections, are rare. Under wet and rainy weather conditions, capitula of *Hieracium* are closed. Therefore, only few *Hieracium* plants could be sampled from higher altitudes (over 2000 m) so far. However, the first results also point towards high constancy among individuals of the same taxa and remarkable differences between taxa; further studies will be conducted to include those in our investigation. In *Hieracium* subgen. *Pilosella* it would be very interesting to increase the number of species investigated in the future and to include species from southern, eastern and even western Europe that may have played an important role as parental for intermediate taxa.

Summarizing, scent-based taxonomies have been proven to be highly useful in plant groups were many members were derived by hybridization and are fixed by apomixis. They can be an appropriate alternative to genetic markers and promise useful results in taxonomy.

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Publications

Publication 1

1. Floral scent analysis in *Hieracium* subgenus *Pilosella* and its taxonomical implications.

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Floral scent analysis in *Hieracium* subgenus *Pilosella* and its taxonomical implications

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Abstract

Species-rich *Hieracium* subgen. *Pilosella* is well-known for a high degree of endemism and infra-specific differentiation including many subspecies (“microspecies”) of very restricted distribution. In *Hieracium* subgen. *Pilosella* floral scents of 27 predominantly Bavarian species, mostly of *Hieracium calodon, H. zizianum* and *H. densiflorum*, are investigated here. Floral scent compositions were studied by GC-MS analysis of dynamic headspace samples. Altogether, 56 floral scent compounds were identified, mainly benzenoids, fatty acid derivatives, monoterpenes, homoterpenes and sesquiterpenes. The chemical patterns were found to be taxon-specific and are thus of taxonomical value. The data support some rearrangements at subspecific level, such as the inclusion of *H. bauhini* subsp. *hispidissimum* in *H. densiflorum*. These rearrangements are supported by morphological data. The traditional species concepts, however, are mostly corroborated by our scent data.

Keywords: Plant terpenes; Chemotaxonomy; Microspecies; Reticulation; Apomictic plants

Introduction

The main question of floral scent investigation regards interactions of flowering plants and their pollinators. Floral scent components are well-known for playing an important role as attractants for pollinators (e.g., Dobson et al., 2005; Dötterl et al., 2006; Plepys et al., 2002). Floral scent compounds can also function as repellent for herbivores or pathogens. However, Levin et al. (2003) point out that these compounds may not be functional in every case, because they are often by-products of the metabolism. Floral scents can be species-specific, helping to maintain species integrity via their effects for pollinator preselection. Differences among species in floral scent composition were detected in a large range of plant families (Knudsen et al., 2006). However, profiles of floral scent compounds were rarely used for phylogenetic analyses and taxonomy. An objection to the use of floral scent patterns in systematics is that convergence of floral scents may play an important role in plants in general, caused by similar pollinator pressures acting on plants of independent origin (Dobson et al., 2005). Nevertheless, comparative studies of floral scent and DNA data in orchids (Barkman, 2001; Williams and Whitten, 1998) as well as in Nyctaginaceae (Levin et al., 2003) revealed that the tree topologies generated by

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Table 1. Voucher and locality information for plant material used in this study.

Taxon	Voucher	Number of samples per population
H. lactucella Wallr.	Neustädtlein, 49°58′N, 11°25′; 15.7.2006, *Feulner* 1 (UBT)	2
H. pilosella L.	Neustädtlein, 49°58′N, 11°25′; 30.6.2004, *Feulner* 2 (UBT)	1
	Oberailsfeld, 49°49′N, 11°21′O; 21.6.2005, *Feulner* 3 (UBT)	2
	Ökol. Bot. Garten Bayreuth, 49°56′N, 11°35′O; 25.6.2004, *Feulner* 4 (UBT)	3
H. bauhini Schult. subsp. *bauhini*	Bindlach, 50°00′N, 11°37′O; 12.6.2006, *Breitfeld* and *Feulner* 5 (UBT)	2
H. bauhini Schult. subsp. *hispidissimum* (Rehm.) Zahn	Gößweinstein, 49°46′N, 11°19′O; 10.6.2005, *Feulner* and *Bolze* 6 (Herbar Bolze 2)	3
H. piloselloides Zahn subsp. *praealtum* (Vill. ex Gochnat)	Pottenstein, 49°46′N, 11°25′O; 25.6.2005, *Feulner* 7 (UBT)	2
	Velburg, 49°13′N, 11°40′O; 23.6.2005, *Feulner* 8 (UBT)	1
H. cymosum L. subsp. *cymosum*	Etterzhausen, 49°02′N, 11°59′O; 1.7.2006, *Feulner* 9	1
	Ökol. Bot. Garten Bayreuth, 49°56′N, 11°35′O; 10.6.2005, *Feulner* 10 (UBT)	2
H. caespitosum Dumort. subsp. *caespitosum*	Ökol. Bot. Garten Bayreuth, 49°56′N, 11°35′O; 15.6.2006, *Feulner* 11 (UBT)	2
	Weiden, 49°40′N, 12°10′O; 1.7.2006, *Feulner* 12 (UBT)	1
	Zeil, 50°01′N, 10°35′O; 25.6.2006, *Feulner* 13 (UBT)	1
H. echioides Lumn. subsp. *echioides*	Brandenburg, Brodowin, 52°55′N, 13°56′O; 15.7.2006, *Feulner* 15 (UBT)	3
H. densiflorum Tausch subsp. *psammotrophicum* (Schack and Zahn)	Altdorf, 49°33′N, 11°35′O; 10.6.2006, *Feulner* 16 (UBT)	2
H. densiflorum Tausch subsp. *bauhinifolium* (NP.)	Velburg, 49°13′N, 11°40′O; 11.6.2005, *Feulner* and *Meyer* (Herbar Meyer 1)	2
Hieracium densiflorum Tausch subsp. *umbelliferum* (Nägeli & Peter) Gottschl.	Pottenstein, 49°46′N, 11°25′O; 1.7.2005, *Bolze* (Herbar Bolze 5)	3
H. densiflorum Tausch subsp. *ochrocephaloides* (Harz and Zahn)	Lochau, 49°58′N, 11°23′O; 15.7.2006, *Feulner* 18 (UTB)	2
H. densiflorum Tausch subsp. *cymosiforme (NP.)*	Velburg, 49°13′N, 11°40′O; 7.2005, *Meyer99-13.3a* (M)	2
H. calodon phyllophorum NP.	Gößweinstein, 49°46′N, 11°19′O; 1.7.2005, *Feulner* and *Bolze* (Herbar Bolze 1)	3
H. calodon Tausch ex Peter subsp. *pseudofallax* Touton	Neudorf, 50°04′N, 11°16′O; 15.7.2006, *Feulner* 19 (UTB)	3
H. calodon "Ravensburg"	Thüngersheim, 49°52′N, 9°50′O; *Schuhwerk* 95/27 & *Meierott* (M)	3
H. schneidii Schack and Zahn	Oberailsfeld, 49°49′N, 11°21′O; 8.6.2005, *Feulner* 21 (UTB)	3
	Waischenfeld, 49°50′N, 11°21′O; 8.6.2005, *Bolze* (Herbar Bolze 4)	2
	Zauppenberg, 49°49′N, 11°23′O; 9.6.2006, *Bolze* (Herbar Bolze 5)	1
H. fallax Froel. subsp. *durisetum* NP.	Regensburg, 49°01′N, 12°07′O; 2005, *Schuhwerk s. nr. (Merxmüller 33371)* (M)	2
H. zizianum Tausch subsp. *pachyphyes* Zahn	Etterzhausen, 49°02′N, 11°59′O; 1.7.2006, *Feulner* 24 (UTB)	2
H. zizianum Tausch subsp. *zizianum*	Steifling, 49°49′N, 11°24′O; 29.5.2005, *Feulner* 25 (UTB)	2
	Pegnitz-Bahnhof, 49°45′N, 11°32′O; 17.7.2006, *Feulner* 26 (UTB)	1
H. zizianum Tausch subsp. *adenocymigerum* Gerstl. and Zahn	Kirchahorn, 49°50′N, 11°23′O; 15.7.2007, *Feulner* 28 (UTB)	3
H. spurium Chaix subsp. *tubulatum* (Vollm.) Zahn	Weltenburg, 48°53′N, 11°49′O; 3.6.2006, *Schuhwerk 87/36 and Lippert* (M)	3
H. fallacinum F. W. Schultz subsp. *fallacinum*	Üttingen, 49°47′N, 9°43′O; 20.5.2005, *Feulner and Meyer* 29 (UTB)	4
H. fallacinum F. W. Schultz	Steifling, 49°49′N, 11°24′O; 15.6.2005, *Bolze and Feulner* (Herbar Bolze 3)	3
H. schneidii x H. pilosella	Oberailsfeld, 49°49′N, 11°21′O; 7.2005, *Feulner* 31 (UTB)	2
	Pottenstein, 49°46′N, 11°25′O; 7.2005, *Feulner* 32 (UTB)	1
H. glomeratum Froel. subsp. *glomeratum*	Hof ,50°18′N, 11°54′O; 2006, *Feulner* 33 (UTB)	1
	Coburg, 50°15′N, 10°57′O; 2006, *Feulner* 34 (UTB)	1
H. aurantiacum L. subsp. *aurantiacum*	Oberstdorf , 47°24′N, 10°16′O; 2006, *Feulner* 35, (UTB)	2
	Ökol. Bot. Garten Bayreuth, 49°56′N, 11°35′O; 2005, *Feulner* 36 (UTB)	3

non-coding DNA markers are in some cases congruent with those based on floral scent data.

The methods to analyse scent patterns for taxonomical questions are manifold (Barkman, 2001; Levin et al., 2003). The simplest method is the use of presence/absence data of different flower volatiles. For taxonomical questions, differentiation between compounds of floral and green parts of the plants seems to be negligible (cf. Levin et al., 2003).

Considerations on the taxonomic value of floral fragrance analysis concentrated so far on outbreeding groups, while inbreeding groups and groups with reticulate relationships have not been considered.

Reticulation in Scandinavian species of *Hieracium* subgen. *Pilosella* was proven by Tyler's (2005) investigations using isoenzyme markers. He found no specific patterns of isoenzymes useful for species discrimination, and concluded that gene flow must be common among the species. Reticulation in *Hieracium* subgen. *Pilosella* comprises a high degree of putative hybrid species with intermediate characters often genetically isolated by apomixis. Further, different reproductive modes, such as allogamy, autogamy or apomixis can occur together in the same capitula (Krahulcová et al., 2000). Apomictic elements even can introgress as Krahulcová and Krahulec (2000) have found in artificial crossing experiments involving pentaploid apomictic *Hieracium* species. In this case, the apomictic pentaploids just serve as pollen donors, whereas the tetra- and diploids act as recipients.

A large number of species in *Hieracium* subgen. *Pilosella* are poorly characterized morphologically. Difficulties to identify the elements of the complex have stimulated *Hieracium* taxonomists to describe "collective species"–binding together formal subspecies or microspecies–although many of these elements behave in nature as fixed apomictic species (cf. Schuhwerk, 2002). Nägeli and Peter (1885) proposed the first taxonomic concept for *Hieracium* which is still in use in Central Europe, whereas in other parts of Europe (e.g., Scandinavia and Russia) alternative concepts are applied (cf. Schuhwerk, 2002). The concept of Nägeli and Peter (1885) is based on the idea that there are some well distinguishable species, the so called "basic species", with unique morphological characters. However, most of the elements in *Hieracium* subgen. *Pilosella* show intermediate characters, therefore, they are treated as "intermediate species" by Nägeli and Peter (1885). The authors considered some of them to be of hybrid origin.

In this study, we investigate 27 taxa mostly of Bavarian origin (Table 1), representing about one third of the Bavarian species of subgen. *Pilosella*. In the taxa studied, both spontaneous hybrids growing together with at least one parental species only, and putative hybrids showing independent traits in morphology, ecology or distribution are included. Here, we focus especially on *H. densiflorum, H. zizianum, H. fallax* and *H. calodon*, all intermediates between the main species *H. cymosum, H. echioides, H. piloselloides* and *H. bauhini*. Some taxa, e.g., *H. densiflorum* subsp. *cymosiforme, H. densiflorum* subsp. *bauhinifolium* or *H. bauhini* subsp. *hispidissimum* display morphological characters questioning their membership in the described collective species. Furthermore these taxa are very rare or even endemic to Bavaria. Members of the "Echinina" species group (*H. fallax, H. calodon* and *H. schneidii*) are well-known glacial relicts (Merxmüller, 1982) and, therefore, of special interest.

In *Hieracium* subgen. *Pilosella* many species are expected to be of hybrid origin. Against this background, it will be tested whether floral scent substances are useful markers for detection of hybrids and their origin.

Material and methods

Study plants

This paper deals with taxa of *Hieracium* subgen. *Pilosella* in Germany with special attention to species-rich Bavaria. All accessions, authors of taxa, voucher specimens and localities are given in Table 1. Taxa of a subspecies level were given when their state was confirmed by previous studies, i.e. Schuhwerk (2002); Schuhwerk and Lippert (1997, 2002) and Gottschlich (1996).

Volatile collection

Floral scent was collected in the field using the dynamic headspace method described by Dötterl and Jürgens (2005), and Dötterl et al. (2005). Capitula were enclosed within a polyester oven bag (Toppits®) and the emitted volatiles were trapped in an adsorbent tube through the use of a membrane pump (ASF Thomas, Inc.) for eight minutes. As absorbent tube, we took ChromatoProbe quartz microvials of Varian Inc. (length: 15 mm; inner diameter: 2 mm), cut the closed end, filled them with a mixture (1:1) of 3 mg Tenax-TA (mesh 60–80) and Carbotrap (mesh 20–40), and fixed the adsorbent mixture in the vial with glass wool. Simultaneous collections of both the flower scent and surrounding air were used to distinguish between floral compounds and ambient contaminants.

Sampling was carried out on fresh and newly opened capitula, between 11 a.m. and 3 p.m., the period with the most intensive scent emission (Feulner, unpublished data). In multi-headed synflorescences more than one open capitulum was sampled.

Chemical analysis

The samples were analysed using a Varian Saturn 2000 mass spectrometer, and a Varian 3800 gas chromatograph with a 1079 injector, that had been fitted with the 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). The injector split vent is opened (1/20) to flush all air from the system and closed after 2 min; simultaneously, the injector is heated from 40 °C (temperature during the first 2 min) with a rate of 200 °C/min to 200 °C; this temperature is held for 4.2 min, after which the split vent opens (1/10) and the injector cools 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 for 7 min at 40 °C, then increased by 6 °C/min to 250 °C and held for 1 min. The MS interface was 260 °C and the ion trap worked at 175 °C. The mass spectra are taken at 70 eV (in EI mode), with a scanning speed of 1 scan s^{-1} from m/z 30 to 350.

Data analysis

The GC-MS data were processed using the Saturn Software package 5.2.1. Component identification was carried out using the NIST 02 mass spectral database, or MassFinder 2.3, and confirmed by the comparison of retention times with published data (Adams, 1995; Davies, 1990). Identification of individual components could be confirmed by the comparison of both mass spectrum and GC retention data with those of authentic standards.

Statistical analysis

For statistical analyses, a similarity matrix (Sörensen similarities) was constructed using a presence/absence matrix of scent data. The significance of differences in scent profiles among taxa was assessed by ANOSIM (Clarke and Gorley, 2001), with 10,000 random permutations.

For further analyses, the taxa samples (mostly 2–5 individuals per taxon) were merged by counting a single substance, if it was represented in at least half of the samples. To analyse the relationships among the taxa, we conducted a reticulation network analysis with the program t-rex Version 4.0a1 (Makarenkov, 2001). For analysis of groups with many species of hybrid origin, normal tree models are not suitable, because they cannot depict relationships of species interconnected with more than one ancestor (Legendre and Makarenkov, 2002). Therefore, these authors developed the software t-rex Version 4.0a1 (Makarenkov, 2001; Legendre and Makarenkov, 2002), which is applied for this study.

The latter program is calculating a distance reduction between the preliminary joined arrangements by a special algorithm made visible by adding reticulation lines. The dashed lines are symbols for homoplasy in the data set (Makarenkov, 2001; Legendre and Makarenkov, 2002). T-rex cannot distinguish between the reasons of homoplasy, be it gene flow via hybridisation events or convergence.

Results

A total of 56 different floral scent compounds were found in the 27 investigated *Hieracium* taxa (Table 2). There are remarkable differences among the taxa concerning the number of scent components ranging from 19 components in *H. pilosella* to 31 in *H. bauhini* subsp. *bauhini*.

The identified compounds belong to benzenoids, sesquiterpenes, monoterpenes and fatty acid derivatives. The most commonly occurring compounds were (Z)-3-Hexen-1-ol (found in all investigated species), D-Limonene, (E)-β-Ocimene, Ylangene (all found in 27 of 28 taxa), (Z)-Ocimene (26 taxa), (E)-4,8-Dimethyl-1,3,7-nonatriene and Methylsalicylate (in 25 taxa). Most scent samples were dominated by (E)-β-Ocimene (average amount 20%), (Z)-3-Hexen-1-ol (15%) and (E)-4,8 Dimethyl-1,3,7-nonatriene (11%). The relative amount of (Z)-3-Hexen-1-ol was highest in *H. zizianum* subsp. *pachyphyes* with 67%; (E)-4,8-Dimethyl-1,3,7-nonatriene reached the highest relative amount in *H. calodon x H. fallacinum* (36%), and (E)-β-Ocimene in *H. densiflorum* subsp. *psammotrophicum* (49%).

Members of *H. densiflorum* emitted a broader range of monoterpenes, and -Phellandrene, l-Fenchone and Linalool reached high relative amounts in these taxa. For *H. cymosum* subsp. *cymosum* or *H. zizianum* subsp. *zizianum* high values of sesquiterpenes as Copaene and Ylangene were found. In *H. zizianum* subsp. *zizianum, H. piloselloides* subsp. *praealtum* and *H. fallacinum* subsp. *fallacinum* higher amounts of acids (Hexanoic acid and Octanoic acid) were identified. (Table 2)

The present-absent data of scent are highly specific in the investigated *Hieracium* taxa (ANOSIM R-value = 0.769, $p < 0.01$), allowing the identification of taxa by floral scent data. Therefore, the samples of one taxon were be merged for the reticulation analysis. The result of the reticulation analysis is shown in Fig. 2.

The tree consists of three main groups, in which the taxa are arranged in most cases as expected by morphology. However, some differences to the concept

of Zahn (1930–35) occur. In particular, some taxa of e.g., *H. densiflorum*, *H. bauhini* and *H. fallacinum,* considered closely related by Zahn, fail to cluster together. In Fig. 2, H. *bauhini* subsp. *hispidissimum* groups with *H. densiflorum* subsp. *umbelliferum* and not with *H. bauhini* subsp. *bauhini*. Both taxa are placed together with *H. cymosum* subsp. *cymosum,* neighbouring most of the other *H. densiflorum* subspecies, with the exception of *H. densiflorum* subsp. *cymosiforme* (see below).

Species of section *Echinina* (*H. schneidii, H. calodon* and *H. fallax*) are arranged together, in the group neighbouring the *H. densiflorum*/*H. cymosum* species. The endemic *H. schneidii* clusters together with the spontane hybrid *H. schneidii x H. pilosella* and *H. calodon* subsp. *phyllophorum. H. fallax* subsp. *durisetum* and *H. calodon* subsp. *pseudofallax* are nearest neighbours.

The three investigated subspecies of *H. zizianum* build up a complex of their own showing the smallest overall distances in the analysis. There are no reticulations to the putative parental species *H. cymosum* and *H. piloselloides*. The closest neighbours are *H. caespitosum* subsp. *caespitosum* and *H. glomeratum* subsp. *glomeratum*.

Hybrids of *H. pilosella,* such as *H. spurium* subsp. *tubulatum* and *H. fallacinum* cluster together with *H. pilosella*, while they are morphologically more similar to their second parent, *H. cymosum*, *H. densiflorum* or *H. zizianum,* respectively. In contrast, *H. schneidii x H. pilosella*, which is morphologically more similar to *H. pilosella,* clusters with its second parent, *H. schneidii*. The two investigated subspecies of *H. fallacinum* occupy distant positions in Fig. 2.

Reticulation lines indicating homoplasy are found between a wide range of species, for example, *H. densiflorum* subsp. *cymosiforme* and *H. fallax* subsp. *durisetum* or *H. echioides* and *H. fallax* subsp. *durisetum*.

Discussion

The floral scent of most species is dominated by (E)-β-Ocimene, a substance known as floral scent from many different plant species (Knudsen et al., 2006). Other dominating substances such as (Z)-3-Hexen-1-ol or (E)-4,8-Dimethyl-1,3,7-nonatriene are known as floral, but primarily as green leaf volatiles. The high amount and variety of acids in floral scent of *Hieracium* subgen. *Pilosella*, such as Heptanoic and Nonanoic acid is remarkable, however, these compounds are also found in other Asteraceae species, e.g., in *Leontopodium alpinum* (Erhardt, 1993). These compounds may be responsible for the unpleasant note in the floral scent of studied *Hieracium* species, and may, as in other plant species, play a role as attractant of flies (Erhardt, 1993), which are prominent flower visitors of *Hieracium* (Feulner, unpublished data). Another acid, namely Hexanoic acid, has been found in one species of sapromyophilic stapeliads as main component; it smells like urine and also may be an important fly attractant (Jürgens et al., 2006).

Besides flies, honey bees and other short-tongued bees are known as pollinators of yellow-flowering Asteraceae species (Feulner, unpublished Data; c.f. Westrich, 1989). Further, other insects, such as bugs, butterflies and beetles were observed as flower visitors (Feulner, unpublished data). It is unclear which flower visitors contribute to the reproductive success of these plant species/subspecies, and to what extent.

The high *R*-value of the ANOSIM analysis demonstrates that there are distinct scent profiles which are taxon-specific (see chapter Results). This is the essential presupposition for their use in taxonomy. Supported by the scent profile circumscriptions, the taxonomical status of the misfits has to be considered, as, for example, in the case of *H. bauhini* subsp. *hispidissimum* which is more similar to *H. densiflorum* subsp. *umbelliferum* as to *H. bauhini* subsp. *bauhini*.

The reticulation method after Makarenkov (2001) has been rarely used in taxonomy so far, and is used here for the first time in *Hieracium*. The method has been especially conceived for analysis of homoplasy, e.g., revealed by hybridisation events. According to Legendre and Makarenkov (2002), it is possible to detect unique patterns in species not clustering together in the first step of the analysis, here neighbour joining, and make them visible by adding dashed lines to the tree.

If a hybrid origin is rather probable on the base of further investigations, reticulation lines can be interpreted as consequence of hybridisation or introgression. In many cases reticulation lines are in accordance with unique morphological characters of taxa belonging to different collective species. In our study, e.g., reticulation lines between *H. bauhini* subsp. *bauhini* and *H. densiflorum* subsp. *bauhinifolium* go along with morphological similarities, such as the lack of stellate hairs on the leaves, as well as a late starting flowering time. Considering a hybrid origin of *H. densiflorum* taxa, reticulation lines may indicate that *H. densiflorum* subsp. *bauhinifolium* is more similar with the putative parental species *H. bauhini* as other taxa of *H. densiflorum*.

In the case of *H. fallax,* which is morphologically intermediate between *H. echioides* and *H. cymosum,* the presence of a reticulation line to *H. echioides* together which the topology of the neighbour joining tree confirms the assumption of a proposed hybrid origin (see Fig. 2).

Another example is *H. densiflorum* subsp. *cymosiforme,* which is connected to *H. fallax*. Morphological data such as the shape and density of bristled hairs demonstrate the close relationship of these taxa.

Table 2. Average relative amounts (%) of floral scent volatiles in 27 *Hieracium* subgen. *Pilosella* taxa.

	Bb	Pp	Cc	Dp	Do	Du	Bh	Db	Cp	Cps	CxF	Fd	Ee	Dc	Cy	Gg	La	Si	SxP	P	Zz	Zp	Za	Fl	Ff	St	Aa
Total number of compounds	31	28	32	28	30	19	18	28	26	21	27	26	23	25	28	22	18	27	23	19	29	29	29	17	23	22	28
Number of compounds used for analysis	31	24	31	28	30	17	18	28	20	21	27	26	23	25	21	22	18	22	19	10	28	29	29	12	23	18	22
Number of samples/populations collected	2/1	3/2	4/3	2/1	2/1	3/1	2/1	2/1	3/2	2/1	2/1	2/1	2/1	2/1	3/2	2/1	2/1	4/3	3/2	5/3	3/2	2/1	2/1	3/1	2/1	3/1	4/2
Benzenoids																											
Benzyl alcohol		tr	3				5		tr			tr	9		16						tr*	tr			4		5
Benzeneacetaldehyde									tr*								1										
Methyl salicylate	tr	6	9	4	tr	tr	5	1	7	7	3	tr		3	2	tr		4	tr	3	10	3	18	2	tr		5
4-Methoxybenzaldehyde			5												tr*	2				tr*		tr	4				4
Fatty acid derivatives																											
(Z)-3-Hexen-1-ol	tr	18	7	12	29	4	22	20	17	6	21	3	19	4	16	tr	4	8	3	27	3	67	5	13	24	6	53
(E)-3-Hexen-1-ol	tr	1*		5	tr		6		3	tr	tr	2	1	tr	2*			3	4					6	7		
Pentanoic acid	tr																				tr						
Hexanoic acid	1		tr	6								2	10	tr		47		2	7	4*	7	3	5	6	tr	11	1
(Z)-3-Hexenyl acetate	tr	tr	tr	tr				tr	tr	tr		tr	1	1				tr			tr	4	tr	28	1	2	tr
Heptanoic acid	1		2	tr							tr		tr		1*	7				5	tr		2				2
Octanoic acid	3	12	tr*						2		5	tr	8	tr		13		tr	30		15		10	10	6		3
(Z)-3-methyl butyrate	tr	1	1		13			tr	tr	tr	9	8	tr	12	tr*	tr		tr	2		2	tr	tr			2	tr
Hexyl methyl butyrate												tr		tr													
Nonanoic acid	tr	4	3		tr			9			3		3	4		3	tr	tr*	3*	10*	1	2	5	tr	tr	2	2
Geranyl isovalerat																	tr										
Decanoic acid		4													tr*		tr			9			3		tr	3	tr
Ionone		tr*	tr	tr	tr				tr*		tr			2		tr	tr										2
Irregular terpenes																											
(E)-4,8 Dimethyl-1,3,7-nonatriene	25	4	18	10	3	5	9	20	24	14	36	16	2	12	12	1		25	6		14	7	11		10	6	2
Monoterpenes																											
α-Phellandrene		19			19	23	5	tr							3		7							5		10	
β-Pinene	tr				tr	17	17	tr	3*	3	tr				1		20	3			tr	tr					
Limonene	7	18	1	tr	tr	tr	2	tr	1	33	3	tr	4		tr	8	65	tr	8	5	tr	tr	9	2	tr	30	2
(Z)-β-Ocimene	3	1	5	5	7	9	1	3	3	4	4	4	tr	5		tr		4	2	5	4	3	2	4	5	7	3
Eucalyptol																	tr		1								
(E)-β-Ocimene	48	4	29	49	17	19	16	14	18	22	4	54	5	22	39	13		35	6	6	36	5	14	9	24	3	7
MT 39,71,121				2	tr	tr	3	1					2				2								2	2	tr*
L-Fenchone	tr			tr	tr	5	tr	10																		3*	

Linalool				tr	1	tr*	tr	tr	tr*	tr		tr		3												
MT 107, 43, 79, 133											tr												tr*			
Myrthenal	tr						2			tr																
Myrtenol																	tr									
D-Verbenone													tr				tr	tr			tr	tr		tr		3	tr
Sesquiterpenes																											
Ylangene	tr	tr	tr	tr	tr	tr	3	tr	11	tr	tr	3		13	tr	tr	tr	3	10	10	tr	3	3	13	9	7	3
α-Copaene	4	3	6		3	12	2	13	4*	9	9	2	10		5	2	tr	4	10	3*	3	tr	4				tr
β-Bourbonene	tr	1	1			tr		tr							tr												
(Z)-Jasmone	tr		1					tr	tr	tr	tr	tr		7	tr			tr	tr	8*					1		tr
ST 119, 91, 141, 161													5														
ST 39,93,119,161,204	tr	tr	tr								tr				tr		tr				tr	tr	tr	1	2	tr	tr*
ST 43,55,71,91,107,120,161	tr											tr	7			tr											
ST 39,91,133,161,189		tr	tr	tr	tr			1		tr	tr		4	tr			tr		tr		tr	tr	tr	tr	tr	tr*	2
(E)-β-Caryophyllene	tr	tr	tr	tr	tr	tr		tr		tr	tr	tr	1	4	tr	tr	tr	3*		3	tr	tr			tr	tr	tr
(E)-α-Bergamotene			tr										2			tr											tr*
α-Gurjunene	tr	2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr		tr	tr	tr		tr	2	tr	tr		3	tr	tr	tr	
Aromadendrene				tr							tr	tr						tr*									
γ-Muurolene	tr	tr	tr	tr	tr	tr		1	tr	tr		tr			tr			tr	2	tr	tr	tr	tr	tr			tr*
Germacrene D	tr	tr						tr						1							tr						
Guaiene											tr				tr*			tr*	tr*		tr	tr	tr			tr*	
α-Selinene		tr*	2	tr	1	tr*		tr	tr	tr					tr	tr		tr								tr*	
Amorphene	tr	tr*	tr	tr	tr	tr		tr	tr		tr	tr	2	tr	tr	tr		tr	tr*	tr	tr	tr	tr				tr*
β-Selinene	tr		1	tr	tr	tr		tr			tr			tr	tr	tr		tr			tr	tr	tr				
δ-Cadinene					tr																						
γ-Cadinene		tr	tr	tr	tr														1	tr					tr		
ST 41,105,161,189	tr	tr	tr	tr	tr		2	tr	tr					tr	tr			tr*	tr	tr	tr	tr	tr		tr	1	
ST 93,105,122,145,161,204	tr		tr	tr	tr			tr	tr*		tr			tr	tr										tr		
Unidentified substances																											
39,74,112												tr															
81,105,138				3					1	tr		tr		3	tr												
133,109,95,81,67			tr	tr	tr													tr	tr*		tr	tr	tr	tr			tr*

The compounds are listed according to compound class and retention time. In unknowns the most characteristic mass fragments are listed. The compounds found in the minority of individuals and, therefore, not used for taxonomic analyses are marked by an asterisk (*).

(Bb) H. bauhini bauhini; (Pp) H. piloselloides praealtum; (Cc) H. caespitosum caespitosum; (Dp) H. densiflorum psammotrophicum; (Do) H. densiflorum ochrocephaloides; (Du) H. densiflorum umbelliferum; (Bh) H. bauhini hispidissimum; (Db) H. densiflorum bauhinifolium; (Cp) H. calodon phyllophorum; (Cps) H. calodon pseudofallax; (CxF) H. calodon x fallacinum; (Fd) H. fallax durisetum; (Dc) H. densiflorum cymosiforme; (Cy) H. cymosum cymosum; (Gg) H. glomeratum glomeratum; (Ia) H. lactucella; (si) H. schneidii; (Sxp) H. schneidii x pilosella; (P) H. pilosella; (Zz) H. zizianum zizianum; (Zp) H. zizianum pachyphyes; (Za) zizianum adenocymigerum; (Fl) fallacinum; (Ff) H. fallacinum fallacinum; (St) H. spurium tubulatum; (Aa) H. aurantiacum aurantiacum; (Ee) H. echioides echioides; tr, trace amounts (<0,5%).

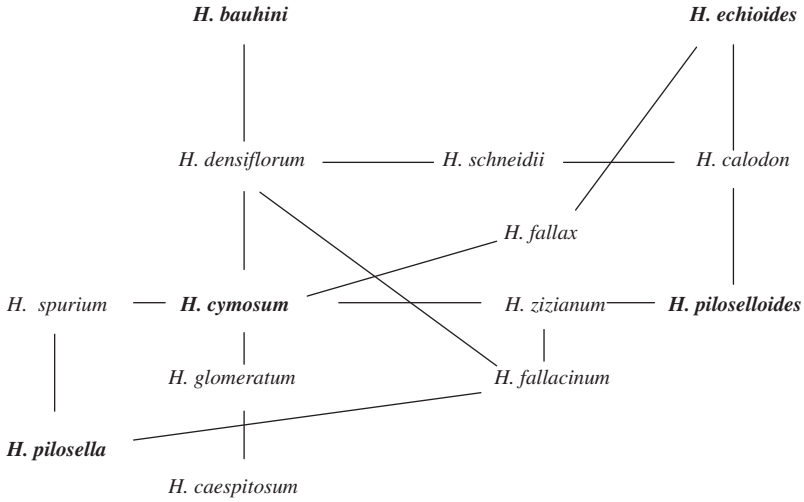

Fig. 1. Relationships among the ''basic species'' and ''intermediate species'' of investigated *Hieracium* subgen. *Pilosella* according to Zahn (1930–35). Basic species are printed in bold.

Scent patterns can aid in hybrid detection if scent profiles are inherited more or less completely from the putative parents to the hybrid offspring. An important result for this research revealed by Gancel et al. (2002). There, the inheritage of volatiles was studied on somatic hybrids of *Citrus* (Gancel et al., 2002), using volatiles of leaves and peels. It was shown that the *Citrus* hybrids produce a combination of the volatiles of their parents, but some individual compounds are usually added.

To our knowledge, the heredity mode of floral scents in *Hieracium* has not yet been investigated in detail. Considering our scent analyses (Fig. 2), it is obvious that *Hieracium* hybrids do not cluster between the potential parental species as ''intermediate hybrids'', but closer to one parent indicating that floral scent is not equally inherited. This could be the reason why, e.g., *H. densiflorum* subsp. *umbelliferum* and *bauhini* subsp. *hispidissimum*, both morphological intermediates between *H. bauhini* and *H. cymosum* (c.f. Zahn, 1930–35, own observations) are arranged here close to *H. cymosum* (Fig. 2). The primary hybrid *H. schneidii x H. pilosella* shares eight scent compounds exclusively with *H. schneidii* and one exclusively with *H. pilosella*, revealing again an unbalanced relationship closer to *H. schneidii* than to *H. pilosella* (Tab. 2). Its status as a primary hybrid of *H. schneidii* and *H. pilosella*, however, was confirmed by a RAPD analysis (Feulner, unpublished data).

H. glomeratum and *H. aurantiacum* are placed in a group neighbouring the *H. caespitosum* group as could be expected from morphological data. *H. glomeratum* is known as morphological intermediate between *H. caespitosum* and *H. cymosum* (Zahn, 1930–35, see Fig. 1). A close relationship between *H. glomeratum* and *H. caespitosum* was found also by Fehrer et al. (2005) and is discussed in detail there. Also, *H. aurantiacum* is morphologically close to *H. caespitosum*, showing a high degree of morphological similarity.

H. echioides, in contrast, is a member of another section of subgen. *Pilosella* and similarities of scent patterns with *H. glomeratum* and *H. aurantiacum* cannot be explained by morphology.

H. zizianum taxa form a group of their own, and are neighbouring *H. caespitosum* s. str. and *H. glomeratum* s. str. (Fig. 2). *H. zizianum* is regarded to be a morphological intermediate between *H. cymosum* and *H. piloselloides*. According to Gottschlich (1996), *H. zizianum* occurs mostly independently from its putative parental species. According to floral scent patterns, it is doubtful whether *H. zizianum* taxa are spontaneous hybrids, since no close similarities of floral scent patterns or reticulations to the putative parental species *H. cymosum* and *H. piloselloides* could be retrieved (Table 2). The close similarity between the scent patterns of *H. zizianum* and *H. caespitosum* is surprising, but there are morphological traits as the identical type of straight bristled hairs with a dark base, which seem to corroborate this result. Nevertheless, the phylogenetic value of the similarity in scent patterns of *H. zizianum* and *H. caespitosum* has to be proven by further investigations.

Hieracium fallacinum is presumably not monophyletic. In the field, *H. fallacinum* occurs together with its parents *H. pilosella* and *H. zizianum* or *H. densiflorum*, whereas the subspecies *H. fallacinum* subsp. *fallacinum* grows independently restricted to a relatively large area in Franconia and seems to be a fixed apomictic hybrid (acc. Schuhwerk and Lippert, 1997).

Species as *H. schneidii* and taxa of *H. calodon* and *H. fallax*, all known as *H. echioides* derivatives (see Zahn, 1930–35), cluster together (Fig. 2). The plants of *H. calodon* from the Ravensburg mountain

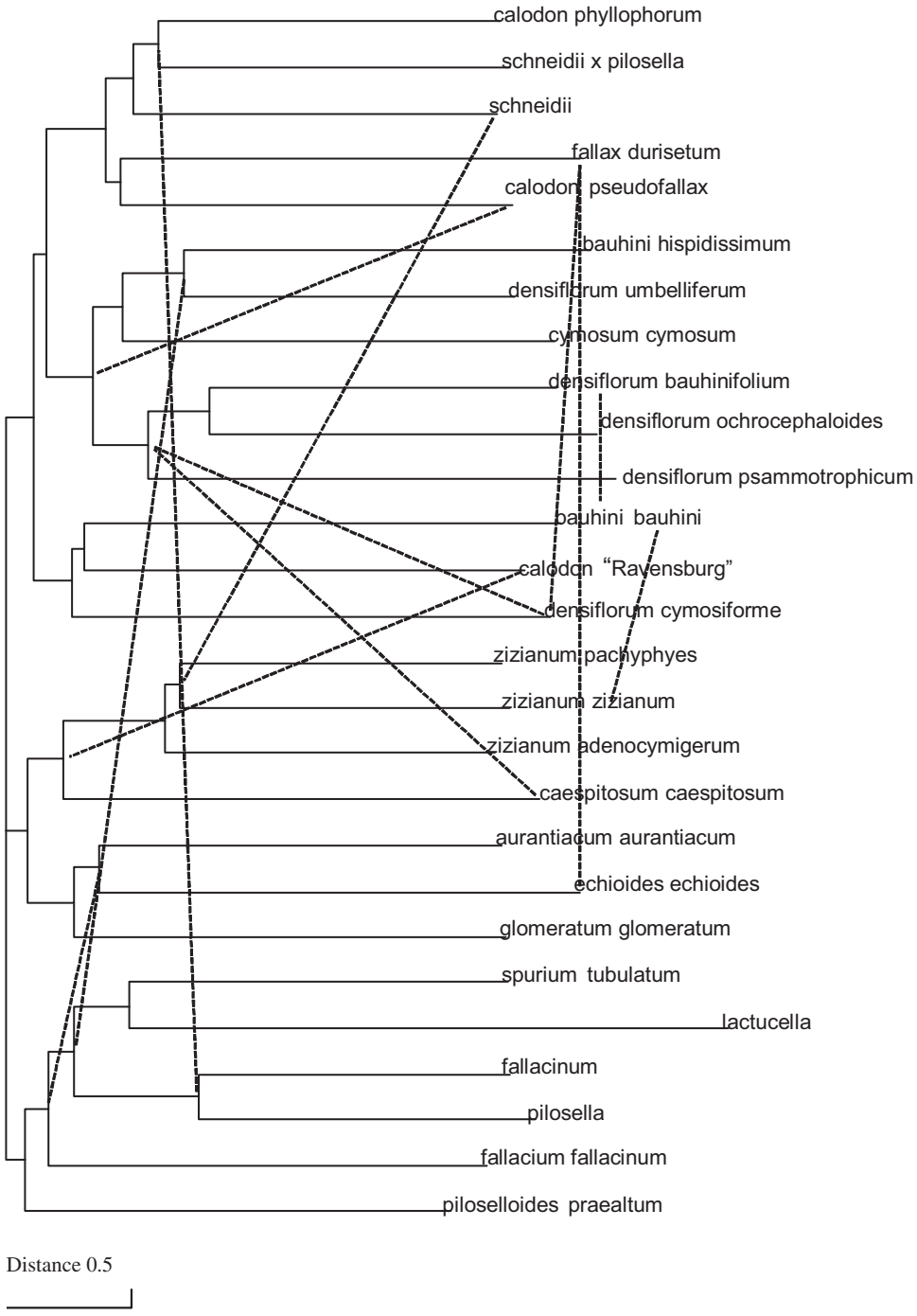

Fig. 2. Neighbour Joining tree with 12 reticulations added to the basic additive tree, number of objects $n = 27$ (by t-rex, Makarenkov, 2001).

slope (Lower Franconia), do not cluster together with other *H. calodon* taxa, but in our analysis they group with *H. bauhini* subsp. *bauhini* and *H. densiflorum* subsp. *cymosiforme* being connected by reticulation lines with the *H. zizianum*/*H. caespitosum* group (see Fig. 2). This species deviates in morphology from other *H. calodon* species by the deeply branched inflorescence and a more hairy stem (cf. Schuhwerk and Lippert, 1997).

H. calodon subsp. *pseudofallax* is connected to the base of the *H. densiflorum* clade, probably due to introgression. This taxon together with *H. densiflorum* subsp. *cymosiforme* indicates a close relationship between the *H. echioides* derivatives such as *H. fallax, H. schneidii* and *H. calodon* to *H. densiflorum* taxa, corroborated by identical morphological characters such as the large and curved bristled hairs.

Concerning *H. schneidii,* endemic to the northern Franconian Jura, regarded to be morphologically intermediate between *H. calodon* and *H. densiflorum* (Zahn, 1921–23; Merxmüller, 1982), our data partly corroborate such an idea as it is placed together with the putative parent *H. calodon* (Fig. 2). However, a reticulation line links the taxon with the morphologically very close *H. zizianum* group, and thus the homoplasy in floral scent is probably pointing to introgression.

The role and the appearance of floral odour in apomicts are not fully understood. In the apomictic *H. schneidii* (cf. Schuhwerk and Lippert, 1997) neither reduction in the number of scent compounds nor reduction of the total amount of floral scent is found. It has to be considered, that even apomicts may act as pollen donators and, therefore, scent does not loose its function in attraction of pollinators (see Krahulcová et al., 2000).

Floral scents predominantly have their function in pollination biology and are, therefore, subjected to selection pressure. Unrelated plants, which are known to be pollinated by similar pollinators have often evolved a similar spectrum of compounds (Dobson et al., 2005). According to Levin et al. (2003), this is the most critical point why scent components might be unsuitable as markers for phylogenetic considerations. The finding that some of the ''basic'' species cluster rather closely together shows that floral scent has not undergone as much change during evolution as could be expected from the deviating morphology. On the other hand, in plant groups where a high number of hybrid species exist, the variability of floral scent should increase within hybrid speciation processes by an accumulation of differences of the parental species additionally supplied by introgression. Therefore, floral scent data should be helpful for detection of hybrid origin and introgression in *Hieracium* subgen. *Pilosella*.

The results of our study support–besides some exceptions–the taxonomical concept of Zahn, which is based on a strict discrimination of the vast majority of forms into subspecies. Such ranking is not in conflict with our study, because even subspecific floral scent profiles could be retrieved for most of Zahn's taxa.

To conclude, the scent data show that most subspecies belonging to the proposed collective species of Zahn cluster together. Exceptions in clustering, like the case of *H. bauhini* subsp. *hispidissimum,* can improve interpretations of morphological characters.

The results presented here can just give a first framework, because only a restricted number of taxa could be included. Also, results may be significant for the investigation area only. Complexes such as *Hieracium* are difficult to investigate, because the frequency of sexually reproductive species and apomicts may depend on the area. The more sexually reproductive species the higher are the opportunities for infra- and interspecific gene flow.

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Taxonomical value of inflorescence scent in *Hieracium* s. str.

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ABSTRACT

In Central Europe *Hieracium* s. str. comprises a vast number of mostly apomictic taxa presumably originated from hybridization in the past. Inflorescence scents of 37 taxa from 7 sections of *Hieracium* subgenus *Hieracium* were investigated by headspace analysis. Overall, 58 different scent compounds belonging to benzenoids, sesquiterpenes, homoterpenes, monoterpenes and fatty acid derivatives were found. The scent patterns were used to perform a neighbour joining and reticulation analysis and the results are discussed against the background of current taxonomy. The scent clustering revealed a clear segregation between sections *Drepanoidea, Tridentata* and *Hieracioides* against members of the sections *Hieracium, Oreadea* and *Bifida*. The scent tree reflected distinct morphological and phenological groups in *Hieracium* and were congruent with actual genetic groupings. Actual section circumscriptions were supported with some exceptions concerning the sections *Oreadea* and *Vulgata*. Reticulation analyses of scent data reflected the hybrid status of intermediates, such as *Hieracium franconicum*, *Hieracium caesium*, as well as taxa of *Hieracium wiesbaurianum* and *Hieracium glaucinum*. The data also pointed towards a hybrid origin of members of some putative non-hybrid taxa such as *Hieracium lachenalii*, which is in accordance with recent molecular studies. The taxonomical usefulness of scent data in dominantly apomictic taxa is discussed.

1. Introduction

Investigations of floral scent deal mainly with questions concerning the interaction between flowers and pollinators, and many studies were conducted in order to identify substances attractive for pollinators (e.g. Plepys et al., 2002; Dötterl et al., 2006). In contrast to this field of research, there are only few studies dealing with the use of scent data for taxonomy (Williams and Whitten, 1998; Barkman, 2001; Levin et al., 2003). Feulner et al. (2009) demonstrated for the first time that such analyses are useful for investigating reticulate complexes as those of *Hieracium* subgen. *Pilosella.* In this species group, where recent hybridization occurs very often, inflorescence scent patterns were found to be taxon-specific and useful for taxonomic considerations (Feulner et al., 2009). Furthermore, parental taxa of hybrids could be identified on the basis of scent compounds (Feulner et al., 2009).

Subgenus *Hieracium* is distributed in temperate areas of North America, Asia and Europe (Bräutigam, 1992), however, it is most diverse in Europe (Zahn, 1922–1938).

It is a reticulate complex, but in contrast to *Hieracium* subgen. *Pilosella*, recent hybridization is rare and was only reported from Southern and Eastern Europe so far (Mráz et al., 2005; Mráz and Paule, 2006; Fehrer et al., 2007). In Central

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Europe, most taxa are triploid apomicts and seem to have arisen from ancient hybridization (Schuhwerk, 2002; Fehrer et al., 2009).

Here, we tested whether scent patterns are useful for taxonomic considerations in a presumable ancient hybrid complex using *Hieracium* s. str. as a model. In Middle Europe nearly all *Hieracium* taxa are polyploid apomicts (tri- and tetraploids), which produce seeds without pollination (Bräutigam and Greuter, 2007). In sexual plants pollinator mediated selection of floral scent appears to be important (Salzmann et al., 2007), such processes should become redundant in apomicts, where floral scents have no function in pollinator attraction and therefore may be under relaxed selection. Therefore it is likely that mutations could lead to a loss or gain of scent compounds, except those scent substances which have functions others than pollinator attraction (e.g. repellents against florivores, anti-pathogenes). Other floral features may also be under relaxed selection in *Hieracium*, and indeed, many apomicts are known to be male sterile and don't produce pollen at all (Štorchová et al., 2002, own investigations). Such changes may especially be evident in taxa that are ancient hybrids and apomicts for many generations. Therefore, it may be more difficult to identify parental taxa in *Hieracium* s. str. by scent data than in groups such as *Hieracium* subgen. *Pilosella* where recent hybridization still occurs (comp. Feulner et al., 2009).

Taxonomy in *Hieracium* s. str. is complicated. Many authors separate the two subgenera of *Hieracium* into two genera (Bräutigam and Greuter, 2007). The taxonomic concept of "basic species" und "intermediate species" is still used for *Hieracium*, which according to Zahn (1922–1938), helps in interpreting the large number of taxa with characters intermediate among two or more species. It further assigns the high number of subspecies or microspecies to so-called "collective species". However, collective species are only a theoretical construction, because the microspecies or subspecies are the real taxa (Schuhwerk, 2002).

Taxa investigated here belong to "basic" species of *Hieracium* such as *H. schmidtii*, *H. bupleuroides*, *H. murorum* and *Hieracium bifidum* and "intermediate" taxa such as *H. franconicum*, *Hieracium glaucinum* and *H. wiesbaurianum*. We investigated whether scent patterns reflect the current taxonomic concept and the interpretation of taxa as intermediate, and can the composition of volatiles contribute to understanding a putative hybrid origin. Furthermore, we ask whether scent similarities are in accordance with actual genetic investigations (Fehrer et al., 2009) and current section delimitation.

2. Method

2.1. Study plants

All accessions, authors of taxa, voucher specimens and localities of the investigated *Hieracium* s. str. taxa are given in Table 1. Taxon identification follows Zahn (1922–1938). As additional information growth form and hair types of the involucrum (bristled, stellate and glandular) are given in Table 1. *H. wiesbaurianum* taxa of Thuringia were determined by Jochen Müller, Jena. The recent nomenclature changes in this subgenus (Greuter, 2007) were not applied, since not all taxa used in this study were published following the new nomenclature.

Section circumscription (Table 2) follows Gottschlich (2009), for taxa not included in the latter study, we used the concept of Sell and West (1976).

2.2. 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 two to six individuals were collected. Sampling was carried out on fresh and newly opened capitula (one capitulum per plant and sample), between 11 a.m. and 3 p.m, the period with the most intensive scent emission (Feulner, unpublished data).

2.3. Chemical analysis

The samples were analysed on a Varian Saturn 2000 mass spectrometer, and a Varian 3800 gas chromatograph with a 1079 injector, that had been fitted with the 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). The injector split vent was opened (1/20) to flush any air from the system and closed after 2 min; the injector was heated with 40 °C for 2 min, and temperature was then increased with a rate of 200 °C/min to 200 °C; this temperature was held 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 for 7 min at 40 °C, then increased by 6 °C per min to 250 °C and held for 1 min. The MS interface was 260 °C and the ion trap worked at 175 °C. The mass spectra are taken at 70 eV (in EI mode) with a scanning speed of 1 scan s^{-1} from m/z 30 to 350.

Table 1
Voucher and locality information for plant material used in this study. All accessions originate from Germany. Additional information about growth form and hair cover of the involucrum is given.

Taxon	Species code	Voucher	Number of scent samples per population	Growth form and flowering time	Hair types of involucrum
H. bupleuroides C.C. Gmel. ssp. *bupleuroides*	bb	Bavaria, Gößweinstein, 49°46′N, 11°20′E, 30.7.2008, *Feulner* 95 (UBT)	2	tall-growing and late flowering	stellate, slightly bristled
		Streitberg, 49° 48′N, 11°11′E, 30.7.2008, *Feulner* 40 (UBT)	2		
H. glaucum ssp. *isaricum* (Nägeli ex J. Hofm.) Nägeli et Peter	gli	Bavaria, Ruhpolding, Fischbachtal, 47°42′N, 12°39′E, 10.8.2007, *Feulner* 41 (UBT)	3	tall-growing and late flowering	stellate
H. franconicum (Griseb.) Zahn	fw	Bavaria, Forchheim, Walberla, 49°42′N, 11°09′E, 30.7.2008, *Feulner* 101 (UBT)	3	tall-growing and late flowering	stellate, bristled and glandular
	fh	Eggloffstein, Hardt, 49°42′N, 11°15′E, 30.7.2008, *Feulner* 42 (UBT),	2		
	fb	Beuren, 48°32′N, 9°15′E, 20.7.2008, *Feulner* 43 (UBT)	3		
H. harzianum Zahn	h	Bavaria, Forchheim, Walberla, 49°42′N, 11°09′E, 30.7.2008, *Feulner* 43 (UBT)	3	tall-growing and late flowering	stellate, bristled and glandular
H. schmidtii ssp. *kalmutinum* (Zahn) Gottschlich	sk	Bavaria, Karlstadt, Kalbenstein, 49°58′N, 9°46′E, 29.5.2008, *Schuhwerk* 86/181 (M).	3	low-growing and early flowering	stellate, bristled and sparsely glandular
H. schmidtii ssp. *comatulum* (Jord. ex Boreau) Gottschlich	sc	Bavaria, Naila, Höllental, 50°20′N, 11°41′E, 21.6.2007, *Feulner* 45 (UBT)	4	low-growing and early flowering	slightly stellate, bristled and glandular
H. glaucinum ssp. *medium* (Jord.) O. Bolòs & Vigo	gm	Bavaria, Karlstadt, Kalbenstein, 49°58′N, 9°46′E, 29.5.2008, *Feulner* 46 (UBT)	2	low-growing and early flowering	glandular
H. glaucinum ssp. *similatum* (Jord. ex Boreau) Zahn	gs	Bavaria, Knetzgau, 49°59′N, 10°37′E, 29.5.2008, *Feulner* 100 (UBT)	1	low-growing and early flowering	bristled and glandular
		Üttingen, Steinbruch, 49°46′N, 9°43′E, 29.5.2008, *Feulner* 47 (UBT)	1		
H. glaucinum ssp. *oegocladum* (Jord. ex Boreau) Soó	go	Bavaria, Schmidmühlen, Aichaer Berg 49°16′N, 11°56′E, 5. 6. 2009, *Feulner* 72	2	low-growing and early flowering	glandular
H. glaucinum Jord.	gpo	Bavaria, Pottenstein, Felsenbad 49°46′N, 11° 24′E, *Feulner* 48 (UBT)	2	low-growing and early flowering	bristled and glandular
H. glaucinum grex cinerascens (Jord.) Zahn	gci dol	Bavaria, Döhlau, 49°57′N, 11°39′E, 20.5.2008, *Feulner* 49 (UBT)	2	low-growing and early flowering	glandular
H. glaucinum grex *cinerascens* (Jord.) Zahn	gci mun	Bavaria, Münnerstadt, 50°15′N, 10°11′E, 15.5.2008, *Feulner* 50 (UBT)	2	low-growing and early flowering	glandular
Hieracium glaucinum ssp. *prasiophaeum* (Arv.-Touv. et Gautier) Greuter	gp	Bavaria, Forchheim, Ehrenbürg, 49°42′N, 11°09′E, *Feulner* 73 (UBT)	3	low-growing and early flowering	sparsely bristled and glandular
		Weismain, Neudorf, 50°34′N, 11°15′E, *Feulner* 77 (UBT)	3		
H. onosmoides Fr.	o	Bavaria, Karlstadt, Kalbenstein, 49°58′N, 9°46′E, 15.5.2008, *Feulner* 51 (UBT)	2	tall-growing and late flowering	bristled, sparsely glandular and stellate
H. wiesbaurianum ssp. *semicinerascens* Bornm. et Zahn	ws	Bavaria, Münnerstadt, 50°15′N, 10°11′E, 29.5.2008, *Feulner* 52 (UBT)	2	low-growing and early flowering	stellate, bristled and glandular
Hieracium [wiesbaurianum] parvimaculatum Jochen Müll.	wp	Thuringia, Jena, Haselberg, 50°55′N, 11°32′E, 5.6.2008, *Feulner* 53 (UBT)	2	low-growing and early flowering	stellate, bristled and glandular
H. [wiesbaurianum ssp. jenzigense var.] euwiesbaurianiforme (Schack et Zahn) Jochen Müll.	we	Thuringia, Jena, Haselberg, 50°55′N, 11°32′E, 5.6.2008, *Feulner* 54 (UBT)	2	low-growing and early flowering	stellate, bristled and glandular

Table 1 (*continued*)

Taxon	Species code	Voucher	Number of scent samples per population	Growth form and flowering time	Hair types of involucrum
H. wiesbaurianum ssp. *jenzigense* Bornm. et Zahn	wj	Thuringia, Jena, Haselberg, 50°55′N, 11°32′E, 5.6.2008, *Feulner* 55 (UBT)	2	low-growing and early flowering	stellate, bristled and glandular
H. wiesbaurianum ssp. *niphanthodes* Bornm. et Zahn	wn	Thuringia, Schirnewitz, 50°51′N, 11°32′E, 5.6.2008, *Feulner 56* (UBT)	2	low-growing and early flowering	stellate, bristled and glandular
H. [sommerfeltii] crinicaesium (Schack et Zahn) Jochen Müll.	wc	Thuringia, Schirnewitz, 50°51′N, 11°32′E, 5.6.2008, *Feulner* 57 (UBT)	2	low-growing and early flowering	stellate, bristled and glandular
H. wiesbaurianum ssp. *apertorum* Bornm. et Schack ex Zahn	wap	Thuringia, Leutratal, 50°52′N, 11°34′E, 5.6.2008, *Müller* 9794	2	low-growing and early flowering	stellate, bristled and glandular
H. wiesbaurianum ssp. *arnoldianum* Zahn	war	Bavaria, Eichstätt, Steinbruch, 48°54′N, 11°10′E, 19.7.2008, in cult. Meyer, 20.7.2008	1	low-growing and early flowering	stellate, bristled and glandular
		Altdorf Titting, 48°59′N, 11°17′E, in cult. *Meyer*, 20.7.2008	1		
		Mühlheim Gailachtal, 48°51′N, 10°59′E, in cult. *Meyer*, 20.7.2008	1		
H. wiesbaurianum s l. Uechtr. ex Baenitz	wsch	Bavaria, Arnsberg, Schambachtal, 48°54′N, 11°22′E, 5.6.2009, in cult. *Meyer*	2	low-growing and early flowering	stellate, bristled and glandular
Hieracium bifidum ssp.*stenolepis* var.*valdefloccosum* (Vollm.) Zahn	bsv	Bavaria, Heitzenhofen, Öder Grainberg, 49° 08′N, 11°56′E, 5.6.2009, *Feulner* 74 (UBT)	1	low-growing and early flowering	stellate, bristled and sporadic glandular
		Deuerling, 49°02′, 11° 54′, 5.6.09, Schuhwerk 09/100	2		
H. bifidum Kit. ex Hornem.grex *bifidum*	bbf	Bavaria, Ruhpolding, Fischbachtal, 47°41′N, 12°39′E, 10.8.2007, *Feulner* 61 (UBT)	2	low-growing and early flowering	stellate, bristled
H. bifidum Kit. ex Hornem.grex *bifidum*	bbk	Bavaria, Krögelstein, 49°58′N, 11°16′E, 6.6.2007, *Feulner* 62 (UBT)	2	low-growing and early flowering	stellate, bristled
H. bifidum ssp. *basicuneatum* Zahn	bsc	Bavaria, Forchheim, Ehrenbürg, 49°42′N, 11°09′E, *Feulner* 63 (UBT)	3	low-growing and early flowering	stellate, bristled and glandular
H. murorum ssp. *silvularum* (Jord.) Zahn	msi	Bavaria, Betzenstein, Burgruine 49° 41′N, 11°25′E, 20.6.2008, *Feulner* 64 (UBT)	2	low-growing and early flowering	glandular
H. murorum L.	mk	Bavaria, Kupferberg, 50°08′N, 11°35′E, 15.6.2007, *Feulner* 65 (UBT)	2	low-growing and early flowering	glandular
H. murorum L.	mw	Bavaria, Forchheim, Ehrenbürg, 49°42′N, 11°09′E, *Feulner* 80 (UBT)	2	low-growing and early flowering	glandular
H. murorum L.	mne	Bavaria, Neustädtlein, Horlache, 49°58′N, 11°25′E, 10.6.2008, *Feulner* 66 (UBT)	2	low-growing and early flowering	glandular
H. murorum L.	mb	Bavaria, Neudorf, Bärental, 50°34′N, 11°15′E, 20.6.2006, *Feulner* 90 (UBT)	1	low-growing and early flowering	glandular
H. saxifragum Fr. ssp. *dufftii* Zahn	sd	Bavaria, Steinbruch Guttenberg, 50°09′N, 11°34′E, 15.6.2007, *Feulner* 67 (UBT)	2	tall-growing and late flowering	slightly bristled and glandular
Hieracium caesium Fr.	ca	Bavaria, Gräfenberg, Almos 49° 40′N, 11°21′E, 20.6.2008, 15. 6. 1996, *Wagenknecht* s. *n.* (M)	2	tall-growing and late flowering	stellate, bristled, slightly glandular
Hieracium caesium Fr. ssp. *caesium*	cc	Bavaria, Essing, 48°56′N, 11° 47′E, 20.8.2008, *Feulner* 68 (UBT)	2	tall-growing and late flowering	stellate, bristled

(*continued on next page*)

Table 1 (*continued*)

Taxon	Species code	Voucher	Number of scent samples per population	Growth form and flowering time	Hair types of involucrum
					(*continued on next page*)
Hieracium lachenalii C. C. Gmel.	l	Bavaria, Neustädtlein, Horlache 49°58′N, 11°25′E, 20.6.2007, *Feulner* 69 (UBT)	2	tall-growing and late flowering	glandular
		Mistelbach, Buchstein, 49°55′N, 11°32′E, 12.6.08, *Feulner* 70 (UBT)	2		
		Naila Höllental 50°20′N, 11°41′E, 9.6.08, *Feulner* 71 (UBT)	2		
Hieracium laevigatum Willd.	lae	Bavaria, Neustädtlein, Horlache, 49°58′N, 11°25′E, 14.7.2008, *Feulner 72* (UBT)	2	tall-growing and late flowering	slightly short glandular
		Rehauer Forst, 50°16′N, 12°40′E, *Feulner* 73 (UBT)	1		
Hieracium umbellatum L.	u	Bavaria, Neustädtlein, Horlache 49°58′N, 11°25′E, 20.8.2007, *Feulner* 78 (UBT)	2	tall-growing and late flowering	glabrous

2.4. Data analysis

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 data base, or MassFinder 3, and confirmed by comparison of retention times with published data (Adams, 2007). Identification of individual components was confirmed by comparison of both mass spectrum and GC retention data with those of authentic standards.

2.5. Statistical analysis

A similarity matrix (Sörensen similarities) was constructed based on the presence/absence of compounds. The significance of differences in scent profiles among taxa was assessed by ANOSIM with 10,000 random permutations using Primer (Clarke and Gorley, 2001).

For further analyses, the samples of a specific species (2–6 individuals per taxon) were merged. A single substance was treated as present in a taxon if it occurred in at least half of the individual samples. To analyse the relationships among the taxa we conducted a reticulation network analysis with the program t-rex, version 4.0a1 (Makarenkov, 2001). This method allows to visualize relationships of species interconnected with more than one ancestor (Legendre and Makarenkov, 2002), which is important for analysing groups, such as *Hieracium*, with many taxa of hybrid origin (see Feulner et al., 2009). In this approach, a neighbour joining tree was constructed using a dissimilarity matrix (1-Jaccard), and homoplasies are made visible by so-called reticulation lines. Those homoplasies point towards hybridization or introgression (Legendre and Makarenkov, 2002).

In addition to the presence and absence of compounds, we also calculated the average relative (percentage of total) amount of scent compounds of the single taxa (see Table A.1).

Table 2
Sections of *Hieracium* s. str., according to Gottschlich (2009) and Sell and West (1976) for the investigated species. Basic species printed in bold. For intermediate species the formula of the taxa to which they are morphologically intermediate (comp. Zahn, 1922–1938) are given in brackets.

Section	taxa
Hieracium sensu Gottschlich (2009)	***H. murorum***
Oreadea (Fr.) Arv.-Touv. sensu Gottschlich (2009)	***H. schmidtii***
	H. glaucinum (schmidtii-murorum)
Oreadea (Fr.) Arv.-Touv. sensu Sell and West (1976)	*H. saxifragum* (lachenalii > schmidtii)
	H. onosmoides (lachenalii < schmidtii)
Bifida (Arv.-Touv.) Clapham sensu Gottschlich (2009)	***H. bifidum***
	H. wiesbaurianum (bifidum-schmidtii/glaucinum)
Vulgata (Griseb.) Willk. & Lange sensu Sell and West (1976)	***H. lachenalii***
	H. caesium (lachenalii-bifidum)
Drepanoidea Monnier sensu Sell and West (1976)	***H. glaucum***
	H. bupleuroides
	H. franconicum (bupleuroides-murorum)
	H. harzianum (laevigatum-franconicum)
Hieracioides Dumort. sensu Sell and West (1976)	***H. umbellatum***
Tridentata (Fr.) Arv.-Touv. sensu Sell and West (1976)	***H. laevigatum***

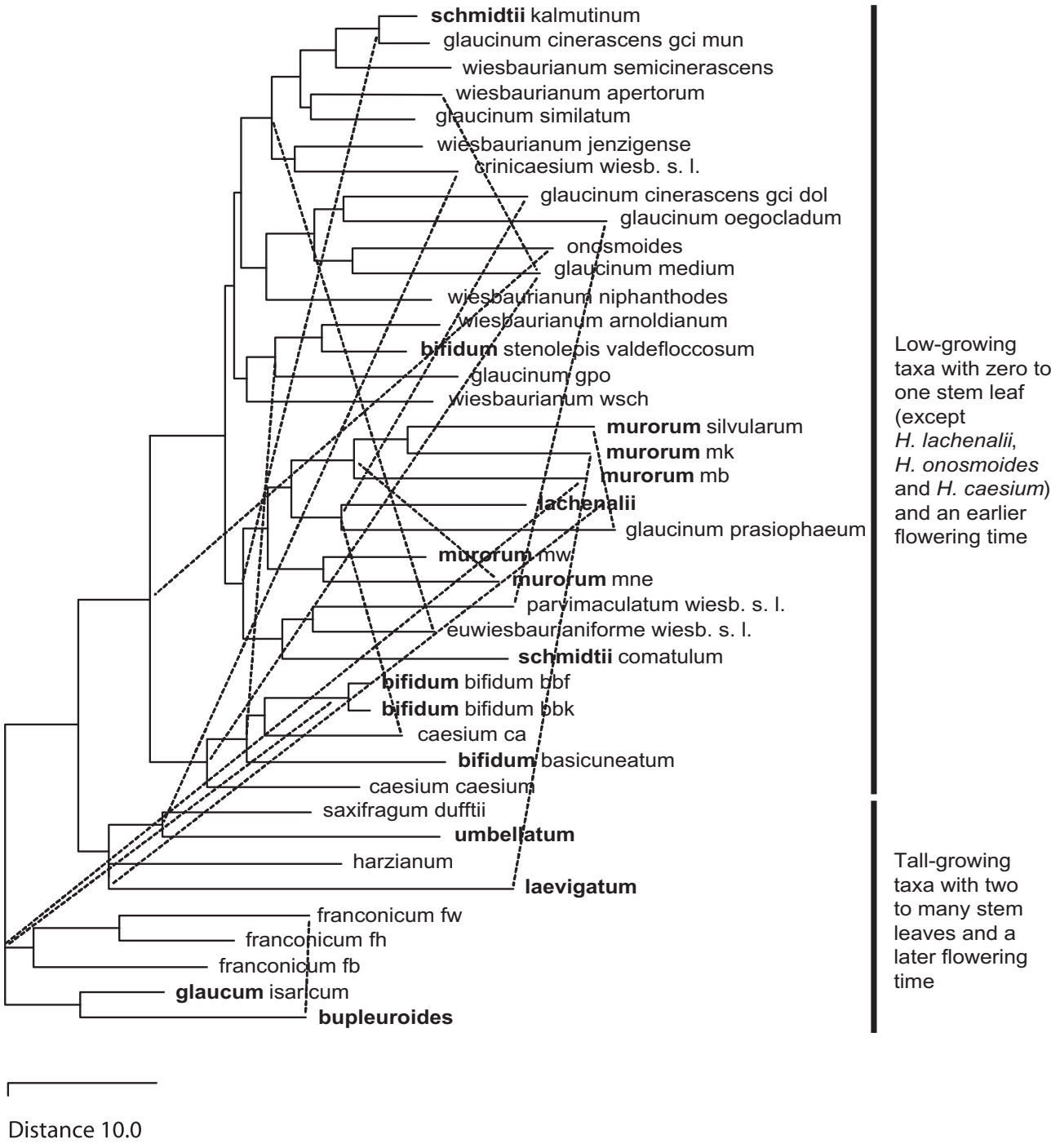

Fig. 1. Neighbour joining tree (1-Jaccard) with reticulations added to the basic additive tree limited to 17, number of objects $n = 40$ (by t-rex V. 4.1, Makarenkov, 2001). Basic species are printed in bold.

3. Results

3.1. Scent compounds and patterns

A total of 58 different inflorescence scent compounds were present in the *Hieracium* taxa (Table A.1). The compounds belong to benzenoids, sesquiterpenes, homoterpenes, monoterpenes and fatty acid derivatives. Most numerous substances were sesquiterpenes with nearly half of the number of identified substances. There were remarkable differences among the taxa concerning the number of scent components, ranging from 22 components in *H. glaucinum* ssp. *prasiophaeum* to 40 in *Hieracium umbellatum*. The scent patterns (presence/absence of compounds) were highly taxa specific (ANOSIM: $R = 0.86$; $p = 0.01$), allowing the identification of taxa by scent data.

The most abundant and commonly occurring compounds were (Z)-3-hexen-1-yl acetate (average relative amount 27%), (Z)-3-hexen-1-ol (19%), methyl salicylate (10%), (E)-4,8-dimethyl-1,3,7-nonatriene (6%), (E)-β-ocimene (4.6%), α-copaene (4.4%), 2-phenylethyl alcohol (3.4%), linalool, limonene, and phenylacetaldehyde (Table A.1).

3.2. Grouping of taxa in the neighbour joining tree on the basis of scent similarity

In the neighbour joining tree (Fig. 1) the investigated 37 taxa clustered in four main- and several subgroups, based on the presence and absence of compounds. Overall, scent data were – despite some exceptions (see below) – congruent with the growth form and flowering time (tall growth, many stem leaves and later flowering time versus low growth, zero to one stem leaf and earlier flowering time, see Table 1) of the taxa studied. We did not find exclusive compounds for the tall-growing or for the low-growing taxa group as a whole. However, (Z)-Linalooloxid furanoid occurred in most of the tall-growing taxa and only in a few of the other morphotypes. p-Methylanisole occurred only in the tall-growing taxa of section *Drepanoidea* (*H. franconicum, H. bupleuroides* and *H. glaucum* ssp. *isaricum*; comp. Table A.1). In the majority of the low-growing taxa terpinolene was present, but missing in most of the tall-growing morphotypes.

The tall-growing group comprised members of four sections, among them *Drepanoidea* (e.g., *H. bupleuroides*, *H. franconicum*), *Tridentata* and *Hieracioides* (see Fig. 1, Table 2).

A well segregated second group was built by low-growing taxa (e.g., *H. bifidum*, *H. schmidtii, H. murorum*, *H. glaucinum* and *H. wiesbaurianum*), in which members of *H. bifidum* and *Hieracium caesium* were sister to the remaining taxa. However, this second group also contains a few high-growing morphotypes, i.e., *Hieacium lachenalii, H. caesium*, and *H. onosmoides* (see Fig. 1).

H. lachenalii clustered with *H. murorum*, whereas the putative derivatives of *H. lachenalii* (i.e., *H. onosmoides*, *H. caesium*) clustered with *H. glaucinum* and *H. bifidum*, respectively.

3.3. Identification of scent homoplasies and reticulate relationships of taxa

Reticulations linked in most cases morphologically intermediate hybrid taxa with their probable parents even if they did not built a group in the neighbour joining tree. As an example, *H. lachenalii* groups together with *H. murorum*, but is connected by a reticulation line to *Hieracium laevigatum*. Indeed, *H. lachenalii* shares its involucrum-indument (dense glandular hairs) with *H. murorum* while its taller growth form resembles *H. laevigatum* (see Table 1). *H. caesium* ca (see Table 1) clustered with *H. bifidum* but a reticulation line linked it to *H. lachenalii*, indicating scent homoplasies in accordance with its morphological intermediary between *H. bifidum* and *H. lachenalii*. It possesses stellate hairs on the involucrum like *H. bifidum,* but two stem leaves, as it is often found also in *H. lachenalii* (Table 1). The putative origin of the endemic species *H. franconicum* by hybridization between *H. bupleuroides* and *H. murorum* (see Table 2) was partly confirmed by our analysis, because a reticulation line linked the group with one taxon of *H. murorum* but additionally also with some members of the *H. bifidum*/*H. caesium* group.

Taxa of a specific section (e.g. *Bifida, Oreadea* or *Hieracia*) did not cluster together in all cases, instead, sectional subgroups occurred in different places of the tree (Fig. 1). However, if reticulations between taxa (e.g. between *H. wiesbaurianum* and *H. bifidum* or *H. glaucinum* and *H. murorum*) are taken into consideration, scent results fit better with the section concept according to Sell and West (1976) and Gottschlich (2009).

4. Discussion

4.1. Subgenus specific components

In *Hieracium* subgen. *Hieracium* many components were identified which were already found in our previous study of subgenus *Pilosella* (Feulner et al., 2009). Substances such as (Z)-3-hexen-1-yl acetate or (Z)-3-hexen-1-ol dominate the scent in both subgenera (conf. Feulner et al., 2009), monoterpenes, such as fenchone could be found only in subgenus *Pilosella* (see Feulner et al., 2009). Substances that could be found so far only in subgenus *Hieracium* are among others p-methylanisole, carvone, pyroids and furanoids of linalool oxid and γ-terpinene. Some substances are much more widespread in *Hieracium* s. str. than in *H.* subgen. *Pilosella* (e.g. benzeneacetaldehyde, linalool).

4.2. Conformity between scent grouping and current taxonomy

Members of the section *Drepanoidea* cluster closely together (Fig. 1) which is in strong accordance with current sectional classification (cf. Zahn, 1922–1938, Stace, 1998; Gottschlich, 2009) as well as molecular investigations (Fehrer et al., 2009). Only the intermediate *H. harzianum* clustered closer to its putative second parental taxon *H. laevigatum*.

The close grouping of the low-growing taxa, including putative derivatives of *H. lachenalii*, is in accordance with high morphological and genetic similarity among these types (Fehrer et al., 2009). Furthermore, the weak correlation of scent groups and sectional groups within the low-growing taxa points towards a complex and presumably polyphyletic evolution. Nevertheless, within this group, scent data revealed a clear segregation of *H. murorum* against members of *H. bifidum*. Therefore, our data confirm the actual taxonomical treatment of Gottschlich (2009), assigning *H. bifidum* and *H. murorum* to different sections, and not combining it in one, as proposed by Sell and West (1976). Scent data confirm the result of Fehrer

et al. (2009), suggesting that *H. lachenalii* is not a true "basic" species but a hybrid between a member of the "Western clade" (*H. murorum*, *H. bifidum*, etc.) and the *H. umbellatum* group (*H. umbellatum*, *H. laevigatum* and others). Similarly, our data point towards a hybrid origin of *H. lachenalii* with participation of *H. murorum* and – fitting with the genomic data of Fehrer et al. (2009) - the *H. umbellatum/H. laevigatum* group (Fig. 1). From scent data, *H. lachenalii* could not retrieved as parent to taxa like *H. onosmoides, H. saxifragum* ssp. *dufftii* or *H. caesium* ssp. *caesium*, which had previously been considered as intermediates of *H. lachenalii*. *H. saxifragum* ssp. *dufftii* according to scent data has a closer relationship with *H. umbellatum/H. laevigatum*, which is supported by its relatively poor-hairy involucrum cover, corresponding more to *H. laevigatum* than to *H. lachenalii*.

Other examples for the conformity between scent-grouping and molecular sequence data are the vicinity of *H. bupleuroides*/*H. glaucum* and *H. umbellatum*/*H. laevigatum*, all belonging to the "Eastern clade" sensu Fehrer et al. (2009) as well as the close grouping of *H. schmidtii, H. murorum* and *H. bifidum*, which all are members of the "Western clade" (Fehrer et al., 2009). Overall, the conformities of our results with results based on genetic analyses give strong support for the taxonomic reliability of scent data in *Hieracium*.

The distant placement of *H. schmidtii* ssp. *comatulum* and ssp. *kalmutinum* indicates divergent evolution of both subspecies (see Fig. 1), in accordance with their considerably deviating morphology. *H. schmidtii* ssp. *kalmutinum,* which clusters together with *H. wiesbaurianum* and *H. glaucinum* taxa, has indeed more common morphological features with *H. wiesbaurianum* taxa (e.g. presence of bristled hairs on the upper leaf side and densely stellate hairs on the involucrum) than with *H. schmidtii* ssp. *comatulum* (e.g. glabrous upper leaf side and slightly stellate hairs on the involucrum), making this clustering reliable. Furthermore, the two *H. schmidtii* subspecies also behave strongly different according their ecology: *H. schmidtii* ssp. *kalmutinum* grows on calcareous and *H. schmidtii* ssp. *comatulum* only on acidic soil (e. g. Schuhwerk, 1990). As a consequence *H. schmidtii* ssp. *kalmutinum* will be keyed out as *H. wiesbaurianum* by Bräutigam and Schuhwerk (in press).

All investigated *H. glaucinum* taxa which are morphologically either closer to *H. murorum* (e.g. ssp. *oegocladum*, ssp. *medium*) or to *H. schmidtii* (ssp. *prasiophaeum*, ssp. *similatum)* group next to *H. murorum* and *H. schmidtii* ssp. *comatulum.* Therefore, scent clustering does not favour any sectional delimitation, neither that of Sell and West (1976), uniting *H. murorum* and *H. glaucinum* in section *Hieracium,* nor that of Gottschlich (2009), affiliating it to sect. *Oreadea* (Table 2).

H. glaucinum and most *H. wiesbaurianum* taxa are close neighbours or cluster partly intermingled. This might be due to their common parent, *H. schmidtii* (Table 2). Some authors (e.g. Zahn, 1922–1938), however, discuss *H. glaucinum* instead of *H. schmidtii* as parental taxon of *H. wiesbaurianum*. Both ideas are supported by our data, since some of the *H. wiesbaurianum* taxa (e.g. *H. parvimaculatum*) neighbour *H. schmidtii* ssp. *comatulum*, others (e.g., *H. wiesbaurianum* ssp. *semicinerascens,* ssp. *niphanthodes*) *H. glaucinum.* One obvious example for the latter scenario is *H. wiesbaurianum* ssp. *semicinerascens* which neighbours *H. glaucinum* ssp. *cinerascens* mun (Fig. 1). Both taxa share unique morphological features, such as the multi-headed inflorescences and the stem leaf morphology.

The other putative parent of *H. wiesbaurianum* is *H. bifidum* (Table 2). With exception of *H. bifidum* ssp. *stenolepis* var. *valdefloccosum* (*bsv*), both taxa groups are well separated by scent: *H. wiesbaurianum* taxa and *bsv* emit (E)-β-ocimene, which is absent from the other investigated *H. bifidum* taxa, whereas benzeneacetaldehyde is only emitted by *H. bifidum* ssp. *bifidum* and ssp. *basicuneaum* (Table A.1). Interestingly, also in AFLP analyses (Reisch/Meyer, ined.) *H. bifidum* ssp. *stenolepis* var. *valdefloccosum* groups closer to taxa of *H. wiesbaurianum* than to taxa of *H. bifidum.*

In conclusion, our data confirm the hybrid origin of morphologically intermediate taxa such as *H. glaucinum, H. wiesbaurianum, H. franconicum* and also suggest a hybrid origin of *H. lachenalii* so far considered as "basic" taxon. One common assumption is that many *Hieracium* s. str. taxa originated during and shortly after the last ice age, a time when hybridization between sexual "basic" species appeared to be frequent (Zahn, 1922–1938, Fehrer et al., 2009). Most of the species studied are apomicts in which inflorescence/flower scent does not longer have a function as pollinator attractant. Therefore, scent profiles may be faced with mutational chances that do not underlay conserving pollinator mediated selection (see chapter 1). However, scent mutations did not seem to occur in such rates that they conceal the tracks of phylogeny. Otherwise it would not have been possible to identify intermediates by scent or to find a correlation with taxonomy. Therefore, scent profiles as well as other floral features (e.g. inflorescence morphology) are rather conserved in apomictic *Hieracium*. This may reflect the relatively young age of many apomictic taxa. Also Fehrer et al. (2009) concluded that, because of an extremely low level of ITS variation, most species evolved in the Quaternary, a time of rapid speciation in *Hieracium* s. str.

Another reason for scent conservation may be that a large range of substances may have functions others than pollinator attraction (e.g. repellents against florivores, anti-pathogenes; comp. Pichersky and Gershenzon, 2002). Nevertheless, there are some rare reports about recent gene flow in putative apomictic *Hieracium* (see Chapman et al., 2004; Tyler and Jönsson, 2009), which can only be mediated by insect pollinators. In such cases, pollinator mediated selection of inflorescence scent may still be of some importance.

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Appendix

Table A1

Average relative amounts (%) of floral scent volatiles* in 37 ***Hieracium*** s. str. taxa

taxon code**	gpo	go	gp	gci_dol	gci_mun	gm	gs	u	lae	h	fb	fh	fw	bb	gli	l	msi	mne	mk	mb	mw	wap	war	wp
Number of samples/populations	2/1	2/1	6/2	2/1	2/1	2/1	4/2	2/1	3/2	2/1	3/1	2/1	3/1	4/2	3/1	6/3	2/1	2/1	2/1	2/1	2/1	2/1	3/2	2/1
Benzenoids																								
p-Methylanisole											tr	tr	tr	10.0	0.1									
Benzeneacetaldehyde	tr					5.6	1.5*			0.8	2.3*	7.8	0.3			tr*								
Phenylethyl alcohol	tr		0.3*	0.3	tr	tr	0.6	3.7	0.1	47.2	3.6	24.3	13.2	0.3	1.6	4.9		tr	tr	5.1	3.2	2.5	14.6	
Methyl salicylate	3.7	tr	5.3	tr	32.3	tr	tr	5.4	10.5	12.7	0.4	0.6	17.7	8.5	33.3	7.3	tr	6.9	32.7	10.3	12.3	tr	tr	2.2
4-Methoxybenzaldehyde	tr	tr	0.4	tr	0.1	1.7	1.2	tr	tr*	0.3	0.2	0.8	0.3	0.4	1.0	tr	tr		tr	1.8	0.7	tr	0.9	
Fatty acid derivatives																								
cis-3-Hexen-1-ol	52.0	3.1	51.2	14.4	8.7	30.9	32.1	30.4	12.2	12.6	27.5	16.4	26.2	6.4	12.7	15.8	25.4	11.2	11.1	5.4	5.8	48.6	13.7	38.2
Hexanoic acid	tr	tr	tr*	0.1	tr	tr	tr		0.9	0.2	0.5	1.2	tr	1.1	4.1		tr	10.4	tr	5.1	tr	5.8	tr	tr
(Z)-3-Hexen-1-yl acetate	24.0	17.6	18.4	46.6	35.9	39.1	27.9	50.6	30.6	14.3	34.1	10.1	20.1	5.7	15.4	33.4	68.5	12.9	32.0	8.7	43.3	27.9	35.4	41.0
Hexyl-acetate											tr*					21.8*								
Heptanoic acid							tr		tr*	0.6	tr	tr	tr	tr	tr	tr*		1.8	1.0	10.7				
Octanoic acid							5.0	0.5	0.7	0.8			0.5*	0.1*				2.5	5.9		1.0	0.3		
Nonanoic acid			tr*		0.3		3.2	0.4	0.6	1.5	0.8	0.5	0.7	0.2	0.2		tr	0.4	tr	5.3	0.8	tr		
Ionone	0.2	tr	tr	0.1	tr	tr	0.1	tr			0.6	0.4	0.4	0.3	0.1	tr	tr	tr	tr	0.4	1.2	0.2	0.1	tr
Homoterpenes																								
(E)-4,8-Dimethyl-1,3,7-nonatriene	4.1	1.2	3.0	14.9	6.8	1.9	6.5	0.1	15.6	0.1	4.7	0.3	0.1	5.9	1.2	5.8	0.5	28.4	2.4	11.6	5.4	2.5	0.7	1.0
Monoterpenes																								
β-Pinene	0.4	3.8	0.6	tr	4.0	tr	tr	tr	1.5	2.5	4.7	tr	9.3	8.5	tr	tr	tr	5.6	0.3	2.5	tr	tr	tr	0.1
Limonene	4.6	68.5	3.5	0.7	1.3	1.4	1.1	1.8	2.4	0.1	1.9	3.1	5.0	4.9	0.6	0.7	1.0	0.6	1.4	10.1	0.7	1.6	0.8	5.3
(Z)-β-Ocimene						tr			3.7*				tr*	tr		tr*						tr		
(E)-β-Ocimene	tr	1.0	3.5	11.6	3.7		5.5		9.5		5.6	tr		6.8	3.8	1.1	3.3	0.6	2.5	6.6	4.2	tr	4.9	tr
Y-Terpinene	1.9		1.3		1.0		tr		tr	tr	0.1	tr	tr	0.3	0.4	tr	tr		0.5	tr	tr	tr	tr	tr
(Z)-Linalooloxid furanoid								0.8			tr*			2.7	1.9	tr								
Terpinolene	tr		tr		tr					tr							tr	tr	tr	0.3	0.2	tr	tr	
(E)-Linalooloxid furanoid	1.0		tr*					0.6	0.7	0.1	0.3*	1.9		22.3	2.6	0.3					1.3		3.2	
Linalool	1.0		3.3	3.2	0.7	tr	2.1	1.4	2.3	2.6	2.9	7.0	1.1	8.5	3.9	3.0	tr	0.1	8.2	0.3	9.8	0.9	4.9	1.5
Verbenone											tr	tr	0.3	0.4	tr*					tr				
(Z)-Linalool oxid pyranoid								0.4	tr					1.3	1.2	tr								
(E)-Linalool oxid pyranoid								tr	1.2	tr				1.7	1.1									
α-Terpineol								tr																
Myrthenal	0.1		tr*					tr						0.3		tr*	tr						tr	
Carvone		0.7	tr*			0.7						tr	0.3	0.3	tr	tr*				1.6		tr	0.3	
Sesquiterpenes																								
St-unkn. 204, 175, 133, 40								tr																
St-unkn. 204,189, 161,119,40							tr*	tr		tr	tr	tr	tr											tr
St-unkn. 204, 161, 105, 43	tr			tr				tr		tr	tr					tr						tr	tr	
Silphin-1-ene	tr	tr			tr		1.0	0.5	tr*	tr	0.3	4.0	0.3*		0.9			0.3			1.3	tr	tr	0.8
St-unk. 204, 161, 105, 39	0.4	tr	0.8	0.8	0.3	tr	0.2	0.4	0.3	0.9	1.1	0.6	1.0	1.0	0.9	0.3	tr	2.9	0.5		0.3	0.3	1.1	1.1
α-Copaene	2.0	2.3	7.0	5.8	1.9	2.5	2.2	0.9	5.0	1.1	1.6	4.3	1.3	0.5	4.3	4.1	0.9	11.6	0.6	1.0	4.4	4.7	14.6	1.0
St-unkn. 204, 189, 161, 147, 133, 119	0.2	tr	tr	tr	0.6	tr	0.2	tr		0.9	tr	tr	tr	0.3	tr	tr	tr	1.4		10.4	tr	tr	tr	2.7
St-unkn. 189, 161,119, 147,105,91	tr	tr	tr	tr	tr	tr	1.8	0.4	0.4	tr	0.6	4.0	tr	tr	0.8	tr		tr		tr	tr	0.6	0.1	0.6
α-Isocomene	tr	0.2	tr*	0.2	tr	0.9	3.2	0.5	0.1*	tr	0.6	7.9	0.3	tr	4.2			tr			1.5	0.5	tr	1.9
St-unkn. 204, 189, 161, 133,79, 39		tr	tr*					tr		tr	0.1	tr	0.2	tr										

St-unkn. 189, 161, 133, 91, 67, 39								tr															
St-unkn. 189, 161, 147, 91, 66, 43	1.4	0.5	1.0	0.4	0.8	tr	0.3	0.2	tr*	tr	1.3	2.4	1.1	0.7	0.6	0.3	tr	1.0	tr	1.3	1.6	0.5	1.0	2.0
α-Cedrene			tr*					tr	0.1		tr					tr*								
(E)-β-Caryophyllene	0.3	tr	tr*	0.2	0.1	tr	1.5	0.3	0.3	0.2	0.8			0.3	2.3	tr	tr	0.7	tr	0.8	tr	0.4	1.5	0.3
γ-Amorphene	0.2	tr	0.3	0.3	tr	tr	tr	0.1	0.2	tr	0.6		0.1	tr	0.3	0.2	tr	0.4	tr	0.9	0.3	tr	0.2	tr
Thujopsene		tr				tr	tr		tr		1.9											tr	tr	tr
Aromadendrene					tr																			
allo-Aromadendrene	tr			tr	tr	tr	tr															0.7		
γ-Gurjunene	tr	0.5		0.2	0.1	7.7	1.4	0.2	0.4	tr					tr	0.3	tr	0.1	0.5	tr		0.7	1.8	tr
St-unkn. 189, 161, 105, 81, 55																								
Germacrene d		tr		tr							0.3													
β-Selinene					tr	5.3	0.7	0.2	0.1							tr*						tr		
α-Muurolene	0.2		tr	tr	tr	2.0	0.3	0.3	0.3	tr	tr	tr	tr	0.2	tr	tr					tr	0.1	0.3	
α-Selinene	tr				tr	tr	tr	tr	tr	tr	tr	tr			tr	tr			tr			tr	tr	
γ-Cadinene	0.9	tr	tr	tr	0.3	tr	tr	tr			tr*					tr	tr	tr			tr	0.3	tr*	
Lilial	0.8	tr																						
δ-Cadinene		tr	tr	tr	0.3	tr	0.1	tr			0.2	tr	tr*	tr*	tr	tr	tr	tr	tr		tr	0.4	tr	tr
St-unkn. 204, 189, 107, 39		tr		tr		tr																	tr	
St-oxide 220, 205, 177, 149, 121	tr				tr		tr	tr	tr*	tr	0.4*	2.4	tr*		0.2*	tr*		tr		tr	0.2			0.2

Taxon code**	**wsch**	**bsv**	**wc**	**wj**	**we**	**wn**	**ws**	**sc**	**sk**	**sd**	**o**	**bsc**	**bbk**	**bbf**	**cc**	**ca**
Number of samples/populations	2/1	3/1	2/1	2/2	2/1	2/1	2/1	4/1	3/1	2/1	2/1	3/1	2/1	2/1	2/1	2/1
Benzenoids																
p-Methylanisole																
Benzeneacetaldehyde								22.6	tr*		0.9	14.7	2.6	7.0	5.1	24.0
Phenylethyl alcohol	0.3	1.2	tr	tr		1.0	tr		tr	tr	tr	0.3*	tr	1.1	0.7	5.7
Methyl salicylate	30.6	tr	tr	tr	tr	1.2	16.8	0.5	34.0	2.2	35.3	1.5	65.2	12.5	3.8	12.9
4-Methoxybenzaldehyde	0.3	0.8	tr	1.1	0.3	0.5	0.2	0.1	1.3	0.4	0.8	tr	1.4	tr	0.2	0.6
Fatty acid derivatives																
cis-3-Hexen-1-ol	4.9	4.2	37.2	17.9	4.2	20.6	14.1	5.6	18.2	10.3	20.5	10.8	2.0	29.0	11.8	19.6
Hexanoic acid	4.9	tr	0.3	tr	tr	tr	tr	tr	tr		0.2		0.2	tr	0.1	
(Z)-3-Hexen-1-yl acetate	8.9	12.6	17.1	27.7	66.7	31.8	30.7	4.8	18.8	35.4	23.6	54.0	7.3	22.5	23.8	15.2
Hexyl-acetate																
Heptanoic acid	tr							tr*	tr*	tr	tr		tr	tr		
Octanoic acid			2.2						0.2*	0.2					0.2	
Nonanoic acid	tr		0.3		tr			tr	0.4		0.5	tr*			tr	
Ionone	tr	0.2	0.2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.3
Homoterpenes																
(E)-4,8-Dimethyl-1,3,7-nonatriene	21.3	2.5	14.4	2.4	5.9	7.4	3.3	2.1	9.5	20.7	1.1	0.3	1.1	1.1	1.8	2.2
Monoterpenes																
β-Pinene	0.8	tr	tr	0.3	tr	12.8	0.1	0.2	tr	tr	4.2	0.7	0.2	0.2	17.2	tr
Limonene	4.2	0.2	0.5	tr	3.1	0.7	0.9	2.9	0.4	0.4	2.2	8.3	1.3	0.8	18.8	0.3
(Z)-β-Ocimene				tr	2.1											
(E)-β-Ocimene	9.8	29.0	6.6	28.3	tr	tr	9.2		9.2	7.0						
γ-Terpinene	0.5	0.2		0.6	1.1		tr	tr	0.4	tr		tr	tr	tr	0.1	tr
(Z)-Linalooloxid furanoid			tr													
Terpinolene	tr	tr	tr	0.2	tr		tr	tr	tr			tr*				
(E)-Linalooloxid furanoid	1.5	0.3	tr			1.7				0.6	0.2	2.1	1.5	0.4	2.2	2.4
Linalool	2.5	6.1	0.7	6.0	1.4	1.2	0.9	2.6	tr	2.0		1.2	3.6	3.7	tr	3.7
Verbenone			tr													
(Z)-Linalool oxid pyranoid										0.5			1.3	0.4	tr	tr

(*continued on next page*)

Table A1 (*continued*)

Taxon code**	wsch	bsv	wc	wj	we	wn	ws	sc	sk	sd	o	bsc	bbk	bbf	cc	ca
(E)-Linalool oxid pyranoid																
α-Terpineol																
Myrthenal																
Carvone	0.2	tr	tr	tr		tr						tr*	tr	tr	0.3	tr
Sesquiterpenes																
St-unkn. 204, 175, 133, 40																
St-unkn. 204,189, 161,119,40		tr	tr	tr	tr	tr	0.2	tr		tr				0.2	tr	
St-unkn. 204, 161, 105, 43		tr	tr		tr	tr	tr			tr						
Silphin-1-ene	0.3	1.9	2.5	2.4	2.7	1.6	6.0	8.0	0.9	0.6	0.2	tr	0.4	4.6	2.2	0.4
St-unk. 204, 161, 105, 39	0.2	1.1	0.6	0.3	0.8	0.1	0.3	0.5	0.4	1.1	0.4	0.5	0.5	0.2	0.1	0.7
α-Copaene	3.0	24.2	3.8	2.6	tr	3.9	3.3	2.2	2.7	11.9	2.3	3.5	3.2	2.5	4.4	6.9
St-unkn. 204, 189, 161, 147, 133, 119	tr	0.9	tr	tr	tr	2.1	1.7	28.0	tr	tr	tr	1.0	2.2	tr	tr	tr
St-unkn. 189, 161,119, 147,105,91	0.4	1.4	2.7	3.7	5.1	1.5	1.0	tr	0.9	0.2	0.6	tr	0.9	4.3	1.8	tr
α-Isocomene	3.2	5.2	4.0	3.5	4.5	3.9	2.3	tr	1.4	1.7	0.1	tr	2.2	6.2	2.9	0.8
St-unkn. 204, 189, 161, 133,79, 39	tr		0.5	0.8						0.9	tr	tr				
St-unkn. 189, 161, 133, 91, 67, 39																
St-unkn. 189, 161, 147, 91, 66, 43	0.4	4.7	0.3	0.8	0.6	2.6	1.5	19.0	0.2	0.5	0.2	0.6	1.9	0.9	0.1	1.3
α-Cedrene								tr		tr	0.1				0.2	
(E)-β-Caryophyllene	tr	tr	2.1	0.7	0.9	1.2	0.8	tr	0.1	0.5	0.4	tr	tr	0.2	0.3	
γ-Amorphene	tr	0.6	tr	0.1	tr	tr	0.2	tr	tr	0.5	0.2	tr	0.6	tr	0.1	0.3
Thujopsene	tr	tr	tr	tr	tr	tr	tr		tr	tr	tr				tr	
Aromadendrene							tr		tr							
allo-Aromadendrene	tr					tr	tr		tr*		0.2	tr			tr	
γ-Gurjunene	tr	1.5	0.6	tr	tr	1.2	0.3	tr	tr	0.6	2.2	tr	tr	tr	0.7	2.1
St-unkn. 189, 161, 105, 81, 55									tr*	tr		tr				
Germacrene d							tr				0.4	tr	0.1	tr		
α-Selinene			tr	tr			tr		tr		0.2					
α-Muurolene	tr	tr	0.1	0.1	tr	0.1	0.2	0.2	tr	0.2	0.3	tr	0.2	0.2	tr	0.2
α-Selinene		tr	tr	tr			tr		tr	tr	tr	tr	tr	tr	tr	tr
γ-Cadinene	0.3		0.3	tr	tr	1.2	0.1	tr	0.1	tr	0.7					tr
Lilial	tr	tr						0.3								
δ-Cadinene		tr	0.4	tr	tr	tr	tr		0.1	0.2	0.4	tr	tr	tr	tr	tr
St-unkn. 204, 189, 107, 39											1.5					
St-oxide 220, 205, 177, 149, 121	1.2	0.8	2.1	tr	0.3	1.1	5.7	tr	0.4	1.0		tr	tr	1.6	0.9	tr

*The compounds are listed according to compound class and retention time. In unknowns the most characteristic mass fragments are listed. The compounds found in the minority of individuals and therefore not used for analyses are marked with an asterisk (*). tr stands for trace amount which is given by relative amounts of less than 0.1 %.
**For taxon code see Table 2 in the manuscript.

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Publication 3

3. Genetic structure of *Sorbus latifolia* (Lam.) Pers. taxa endemic to Northern Bavaria.

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Genetic structure of three *Sorbus latifolia* (Lam.) Pers. taxa endemic to Northern Bavaria

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Abstract

The Franconian Alb (Bavaria, Germany) is rich in endemic *Sorbus* taxa, considered as apomictic microspecies and derived by hybridization between *S. aria* agg. and *S. torminalis* (*Sorbus latifolia* agg.). Molecular studies using the AFLP technique, Neighbour joining, Bayesian clustering, Principal Coordinate analysis and voucher studies were used to investigate genetic structure and origin of adult plants and cultivated offspring of three endemic *S. latifolia* taxa, namely *S. cordigastensis, S. franconica* and *S. adeana* and probable parental species from the *S. aria* aggregate and *S. torminalis*. The *S. latifolia* taxa, adults and progeny, showed low genetic variability and a more or less clonal structure, confirming an apomictic mode of reproduction. The investigated *S. latifolia* taxa were remarkable different among each other, confirming their status as microspecies. The AFLP

data confirmed the hybrid origin of the *S. latifolia* taxa, they were 1.3 to 1.5 times more closely related to *S. aria* agg. than to *S. torminalis*. The *S. aria* agg. showed a complicate genetic structure and fell into four main groups, two intermediate groups besides *S. pannonica* and *S. aria* s.str. Some progeny of *S. pannonica* was more variable than expected and clustered partly with other groups indicating gene flow within *S. aria* agg. Different subgroups of the *S. aria* aggregate may be parental for the *S. latifolia* taxa, contributing to the remarkable genetic distances between them.

Key words

Sorbus, Northern Bavaria, genetic structure, parental species identification, AFLP

Introduction

In Central Europe, the genus *Sorbus* L. (Rosaceae) includes several widespread, diploid (2n = 34; Liljefors 1955, Düll 1959) and sexually reproducing species, namely *S. aria* (L.) Crantz, *S. torminalis* (L.) Crantz, and *S. aucuparia* L. Spontaneously sexually reproducing and out-crossing hybrids can be found between *S. aria* and *S. torminalis* and between *S. aria* and *S. aucuparia*; however, these hybrids are rare (comp. Aas et al. 1994, Meyer et al. 2005). Besides such unstable hybrids (i.e. *S.* x *tomentella* Gand., *S.* x *pinnatifida* (Sm.) Düll), an impressive number of stable hybrids have been described as endemic apomictic microspecies from many areas of Europe. A high diversity of endemic *Sorbus* microspecies has been reported especially from Britain (Rich et al. 2010, Robertson et al. 2010), the Czech Republic (Karpati 1960, Lepší et al. 2009), and from parts of southern Germany, in particular Thuringia and Northern Bavaria (Düll 1961, Meyer et al. 2005). Most of these microspecies show a limited distribution, but contribute considerably to the local species diversity and therefore attract increasing notice of species protection efforts (Meyer et al. 2005). The *S. latifolia* (Lam.) Pers. aggregate comprises microspecies derived by hybridization between *S. aria* agg. and *S. torminalis* (Düll 1961, Challice and Kovanda 1978, Aas et al. 1994). Members of this aggregate presumably originated polytopically in the postglacial period (Düll 1961). The origin of the Northern Bavarian *S. latifolia* taxa was not yet investigated in detail (see Meyer et al. 2005). Parental species identification is complicated by the fact that in the study area the *S. aria* aggregate (agg.) consists of a wider range of taxonomically not sufficiently investigated forms (see below). In Bavaria, so far 17 microspecies of *S. latifolia* agg. have been recognized (cf. Meyer et al. 2005). Here, we focus on three microspecies endemic to the northern Franconian Jura (Germany,

Bavaria), namely *Sorbus franconica* Bornm. ex Düll, *S. cordigastensis* N. Mey. and *Sorbus adeana* N. Mey. These taxa are very similar, they differ to some extent in leaf and fruit morphology, mainly in the size and shape of the leaves, the number of lateral veins and the color and shape of the fruit (for details see Meyer et al. 2005). Morphologically, they resemble *Sorbus aria* agg. more than *Sorbus torminalis* (Meyer et al. 2005). All three investigated *S. latifolia*-taxa are distributed parapatrically in the study area (see Meyer et al. 2005). The distribution areas of *S. adeana* and *S. cordigastensis* are situated close to each other (distance about 10 km) and are restricted to a few square kilometers only (Meyer et al. 2005, Aas and Kohles 2011), whereas *S. franconica* has a much wider range in the Franconian Alb more southward and distant from the two other taxa. The taxa regularly grow sympatrically with species from the *Sorbus hybrida* group (hybrid taxa between *S. aria* and *S. aucuparia*), *S. torminalis* or with members of *S. aria* agg., such as *S. pannonica* Kárpáti. *Sorbus pannonica* is a xeromorphic member of *S. aria* agg., and is more widespread in the northern Franconian Alb than *S. aria* s.str. (Kutzelnigg 1995, Meyer et al. 2005). It is a non-typified taxon, which comprises presumably apomictic morphotypes filling the morphological gap between *S. aria* s.str. and *S. graeca* (Spach) Loddiges ex Schauer (Kárpáti 1960, Kutzelnigg 2005, Meyer et al. 2005). *Sorbus graeca,* another xeromorphic member of *S. aria* agg., is mainly distributed in southern, southeastern and eastern Europe. It can reproduce sexually or facultatively apomictic (Kutzelnigg 1995). It is uncertain whether *S. graeca* occurs in the study area (Düll 1961, Kutzelnigg 1995, 2005), but individuals that are very similar to *S. graeca* have been found in the northern Franconian Alb (own obs.); yet, it is difficult to delimitate *S. graeca* against *S. pannonica.*

In this paper the AFLP technique is used to investigate the genetic structure of *S. latifolia* taxa and its probable parents in northern Franconia. Questions addressed include (1) how wide is the genetic distance between the *S. latifolia* taxa; (2) do *S. latifolia* taxa have a clonal structure and do they reproduce as apomicts; (3) which member of *S. aria* agg. - besides *S. torminalis* - is most likely parental for these *S. latifolia* taxa? Additional chromosome counts give insights in the cytology of the taxa.

Material and Methods

Plant material

For AFLP-analyses we collected leaf material from *Sorbus cordigastensis* (one of one locality) and *S. adeana* (at one of the three known localities, comp. Meyer et al. (2005)) and from two populations of *S. franconica.* Sampled individuals were chosen randomly. Leaf material was also collected from four to seven populations of parental species co-occurring or coming close to the microspecies distribution area. Seven plants of *S. torminalis* from different localities were harvested. From the *S. aria* agg. we collected samples at two populations of *S. pannonica.* Plants from one site (Neudorf) are similar to *S. graeca* (roundish leaves as broad as long, serration as long as broad, comp. Düll 1961, Kutzelnigg 1995) and plants from the locality "Kordigast" are affiliated to the typical form of *S. pannonica* termed “tennis racket” by local botanists because it has obovate oblong leaves. Furthermore, we collected plants from four populations in the contact area of *S. aria* and *S. pannonica* in the north-western part of the Franconian Alb. There, populations included plants that could be clearly affiliated to *S. aria* s.str., but also plants with morphological similarity to *S. pannonica*. Such intermediates were also found within the range of *S. cordigastensis*. These intermediates were morphologically deviating from the thin-leaved *S. aria* s.str. by rough leaves and variable leaf shapes ranging from ovate to orbicular. From *S. pannonica* they differed in usually having more veins on their leaves.

Additionally, material from seedlings of the investigated taxa were included into this study (Table 1). To this purpose, seeds of three mother trees of *S. adeana, S. cordigastensis, S. franconica, S. pannonica* and *S. aria-S. pannonica* intermediates were harvested in autumn 2009 from the same populations chosen for the investigation of adults. Seeds were germinated and plants were grown in the Ecological-Botanical Gardens (University of Bayreuth, EBG). Four seedlings from each mother tree were analyzed.

Table 1 Taxa, site locality and voucher information of the individuals analysed.

Taxon	**Locality // Gauss Krüger coordinates**	**Individuals**	**Taxon code, Voucher number**
Sorbus aria (L.) Crantz	Autobahn Rossdorf // 4437013/ 5539920	5	aria70-74
	Wattendorf // 4434614/ 5543670	5	aria75-79
	Grafenhäusling // 4437746/ 5542273	5	aria80-84
	Rossdorf // 4437884/ 5540559	5	aria85-89

S. pannonica Kárpáti	Kordigast // 4443782/ 5551720	5	panK8-panK10, panK13, panK34
	Neudorf // 4447194/5546549	4	panK22, panK24, panK28, panK29
Offspring *S. pannonica*	Kordigast // 4443782/ 5551720	12	(MT 7) panOff154-157 (MT 8) panOff150-153 (MT 9) panOff146-149
S. adeana N. Mey.	Neudorf // 4447194/5546549	7	ade004, ade005, ade006, ade023, ade025, ade027, ade030
Offspring *S. adeana*	Neudorf // 4447194/5546549	11	(MT 3) adeOff162-adeOff165 (MT 4) adeOff166, adeOff167, adeOff 169 (MT 6) adeOff090-adeOff093
S. franconica Bornm. ex Düll	Brünnberg // 4457310/ 5520910	2	franc067, franc069
	Muggendorf // 4447515/ 5518390	5	franc044, franc047, franc 051, franc053, franc064
Offspring *S. franconica*	Muggendorf // 4447515/ 5518390	12	(MT 2) francOff102-francOff 105 (MT 3) francOff122- franc Off125 (MT 4) francOff126-francOff 129
S. cordigastensis N. Mey.	Kordigast // 4443782/ 5551720	8	cord003, cord011, cord014, cord017, cord018, cord020, cord031, cord039
Offspring *S. cordigastensis*	Kordigast // 4443782/ 5551720	12	(MT 1) cordOff110-cordOff113 (MT 2) cordOff106-cordOff109 (MT 7) cordOff130-cordOff133
S. torminalis (L.) Crantz	Gottelhof // 4451879/5530456	1	tor001
	Neudorf // 4447194/5546549	1	tor021
	Hainbach // 4451892/ 5530457	1	tor002
	Muggendorf // 4447515/ 5518390	2	tor042, tor046
	Kordigast // 4443782/ 5551720	2	tor012, tor033, tor043

Molecular methods

DNA extraction

Leaf samples were taken in May and June 2010. Immediately after harvesting they were placed in plastic bags and put in a box with ice for transportation. At the same day, leaves were washed with ethanol in the laboratory and frozen in an extraction tube at –80 ° C until extraction. Frozen leaf samples (40 – 70 mg, 1 – 2 cm^2) were blended in 200 µl extraction buffer (NucleoMag 96 Plant kit; Machery-Nagel, Düren, Germany, containing 5 µl RNAse A) with a FastPrep®-24 Tissue Homogenizer (MP Biomedicals Europe, Illkirch, France) for 40s at a speed of 6m/s. Insolubles were pelleted at 15,000 x g for 5 min at room temperature. Genomic DNA was prepared from the supernatant using the NucleoMag 96 Plant kit adapted to the KingFisher automated purification system (Thermo Scientific, Langenselbold, Germany). Details of the nucleic acid purification procedure are presented in Table A1 of the supporting information. The purified genomic DNA was diluted tenfold and used for all subsequent PCR reactions.

AFLP analysis

AFLP analysis was conducted following the method of Vos (1995) using the IRDye Fluorescent AFLP Kit for Large Plant Genome Analysis (Li-Cor-Biosciences, Bad Homburg, Germany). All reactions were conducted as described in the Li-Cor application manual, but adapted in the following manner: a) 200 ng genomic DNA were used for the EcoRI/MseI restriction digestion, which was extended to 16 hours; b) a 1:4 (instead a 1:10) dilution of the adaptor-DNA ligation mixture was used for preamplification reactions; c) a 1:40 dilution of the preamplification reaction was used for selective amplification. For a preliminary primer search 24 primer combinations were tested, and the following six combinations were then selected for this study because they yielded the best results in species differentiation: M-CAA/E-ACG, M-CAC/E-ACG, M-CAC/E-ACA, M-CAT/E-ACG, M-CTC/E-ACG, M-CTT/E-ACG.

Image collection and analysis

The reactions were separated on a vertical electrophoresis system (4200 Sequence Analysis System, Li-Cor Biosciences, Bad Homburg) together with DNA size markers (50–700 bp Sizing Standard, Li-Cor Biosciences, bad Homburg). AFLP banding patterns were evaluated using GeneMarker1-95 software (SoftGenetics) and a presence-absence matrix

was constructed. Also, unique bands were scored and were not left out from analysis. Band classes were calculated with a tolerance factor of 0.1 %.

Chromosome counts

Chromosome numbers were counted from root tip meristems of one seedling, respectively from three to four mother trees (MT) of *S. cordigastensis* (MT 2,5,6,7), *S. adeana* (Neudorf MT 3,4,6), *S. franconica* (MT 2,3,4, s.n.) and *S. pannonica* (locality Neudorf, MT 2,5, locality Kordigast MT 8,9), grown in the EBG and harvested in May 2010. Some of the seedlings were also included in the AFLP analysis (Table 1). The fresh root tips were pretreated in 0.002 hydroxychinoline (4hrs), fixed in CARNOY´s solution and stained in carmine after Snow (1963). From the stained root tips we prepared squash preparations in 45% acetic acid, and observed somatic metaphase plates in the microscope.

Statistical analyses

A neighbour joining (NJ) analysis of the presence - absence matrix was conducted (Nei Li distance), followed by bootstrap (BS) analysis after internode rooting with 1000 replicates using the program TREECON (Van de Peer and De Wachter 1994). The tree was rooted with a *S. torminalis* individual. The taxon clades or subgroups (in case of *S. aria* agg.) revealed were used for all further calculations (Fig. 1).

For data of *S. aria* agg., we additionally applied model-based clustering (Pritchard et al. 2000) using the program STRUCTURE (http://pritch.bsd.uchicago.edu/structure.html) in order to retrieve the most likely number of groups within the *S. aria* aggregate. For AFLP data the recessive allele criterion was used and set to 1. Data were analysed as diploid because we did not know the exact ploidy levels of the plants, which varies between di- and tetraploid. A total of 10 independent runs with K set to 2-10 using the admixture model option with correlated frequencies (prior mean FST ¼ 0.1 equal for all populations) were performed. The most likely number of groups is characterized by a maximum posterior probability Ln P(D) and the highest stability of results revealed from each of ten runs (comp. Pritchard et al. 2000; Falush et al. 2003, Gugerli et al. 2008). A burn-in of 50,000 steps followed by 50,000 iterations gave stable results after testing different burn-in periods and iterations. STRUCTURE calculates also the proportion of an individual genotype originating from each of the K groups (= q). The individuals were assigned to each of the K groups using a threshold of q of 0.3-0.8 or higher.

A Principal Coordinate analysis (PCo) of the data was conducted with PRIMER (Jaccard Index) (Clarke and Gorley 2001).

In order to investigate genetic variability, the number of polymorphic loci and Nei´s gene diversity "NGD" (Nei 1972) were calculated with POPGENE (Yeh and Yang 1999) setting the program routines for a diploid, dominant marker data set. It was assumed that the NGD of probable apomictic taxa should be clearly lower than the one of sexual taxa (comp. Nybom and Bartish 2000), and the NGD of seedlings of apomicts should not exceed the NGD of the adults.

As a measure for the genetic distance between taxa we calculated Nei´s standard genetic distance (Ds) using the program POPGENE (Yeh and Yang 1999). For the *S. latifolia* taxa the proportions between the genetic distances to *S. aria* agg. and to *S. torminalis* were calculated to find out to which parent they are genetically more closely related. For the *S. aria* agg. we calculated the distances to the four subgroups revealed in the NJ tree (see Fig. 1). We also tested the calculation using more subgroups as indicated by Bayesian clustering, however, the results did not deviate and therefore we not show them here.

Voucher study

Vouchers (lateral shoots), that were simultaneously collected with the material for AFLP analyses from individuals of each *S. aria* agg. subgroup were deposited in the herbarium UBT, and morphologically analysed regarding shape of broadest leaves, number of veins and serration (Fig. 3).

Results

AFLP analyses

578 markers were identified from six primer combinations. The percentage of polymorphic loci of the *S. latifolia* taxa varied between 6.75 % and 22.32 % (Table 3). With 52.42 % the intermediate *S. aria - pannonica* group aff. *S. aria* s.str. reached the highest percentage of polymorphic loci. *S. aria* s.str. reached 43.25 %, *S. torminalis* 26.99 % (Table 2).

Neighbour joining-tree and Bayesian clustering for *S. aria* agg.

In the neighbour joining tree (Fig. 1) the *S. latifolia* taxa grouped between *S. aria* agg. and *S. torminalis*, but closer to *S. aria* agg. The individuals of *S. aria* agg. and *S. torminalis* were much more strongly dissimilar than the individuals of the putative microspecies *S. adeana, S. cordigastensis* and *S. franconica* (Fig. 1). All *S. latifolia* accessions constituted

highly supported groups of their own (BS 100 for *S. adeana*, 84 for *S. cordigastensis,* and 97 for *S. franconica*), and were clearly separate from each other. The offspring of the *S. latifolia* taxa clustered with the adults and the progeny of a single mother tree clustered together.

The *Sorbus aria* agg. clade was strongly supported (BS 99) and could be divided into four groups, the *S. pannonica* group, the *S. aria s.str.* group and two groups of intermediates (Fig. 1). One group of intermediates (aria76, aria77, aria79, panOff150, panOff 151, panOff153, panOff156) was retrieved more closely to the *S. pannonica* group and is therefore called "intermediate group affine (aff.) *pannonica"* (Fig. 1). Another group was retrieved more closely to *S. aria* s.str., it is therefore referred to as "intermediate group aff. *S. aria"*. This group consists of two subclades (the one with aria074, aria075, aria082, aria086 and the other one with aria070, aria073, aria078) (Fig. 1). The offspring of two mother trees of *S. pannonica* is partly grouped within *S. pannonica* and partly within the intermediate group aff. *pannonica* (Fig. 1).

In the Bayesian clustering of the *S. aria* agg. the Ln P(D) was highest and standard deviation of posterior probabilities was lowest for seven groups, (Ln P(D) for K = 7 between -7915 and -8085; 10 runs). The assignment of individuals to seven groups was very constant and the groups were in general in good accordance with the groups and subgroups revealed by the NJ tree. Bayesian groups were identic to the NJ tree subgroups for aria074, aria075, aria082 (see above), aria087, aria088, aria089 or aria076, aria077, aria079, pannoff150, pannOff151, pannOff153. Individual aria086 formed a group of its own.

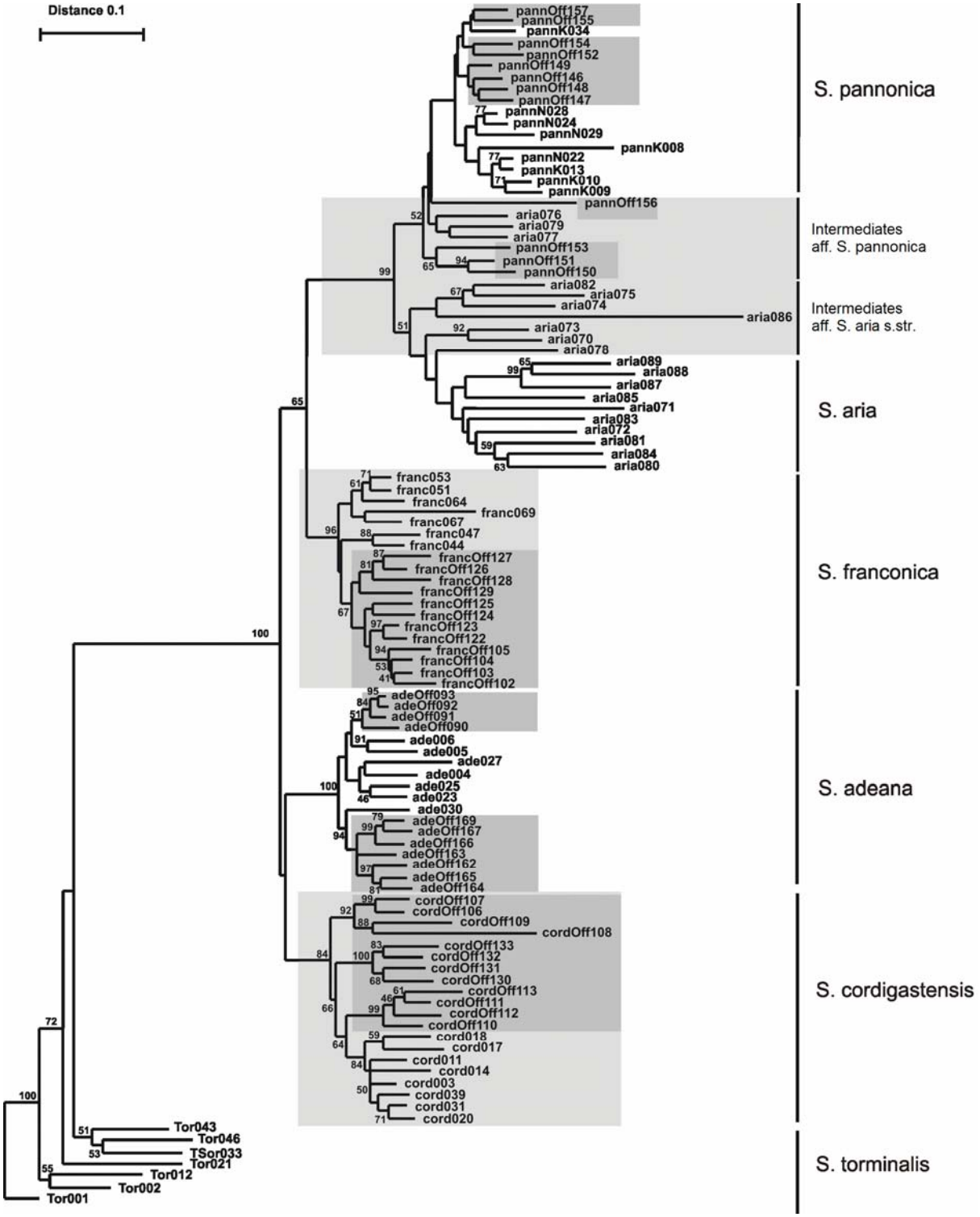

Fig. 1 Neighbour joining tree with Nei Li distance and bootstrap values with 1000 replicates using TREECON. Offspring individuals are shadowed dark-gray.

Voucher study

Voucher studies of *S. aria* agg. revealed that the intermediates aff. *S. aria* s.str. deviated from *S. aria* s.str. (Fig. 3a) by a thicker leaf texture. Their leaf shape was variable, ovate or obovate (Fig. 3c). The intermediates aff. *S. pannonica* (Fig. 3d) had more leaf veins (about 10–11) than *S. pannonica* (8 veins, Fig. 3b) and the serration of the leaf margins extended to the leaf base; furthermore, the leaf shape was roundish instead of obovate and the leaf base was rounded and not cuneate as in *S. pannonica*.

Nei´s gene diversity

Nei´s gene diversity (NGD) of the taxa and subgroups of *S. aria* agg. is shown in Table 2. Gene diversity values of the *S. latifolia* group (adults and seedlings) were rather similar. They varied for *S. adeana* between 0.029 and 0.083, for *S. cordigastensis* between 0.051 and 0.088, and for *S. franconica* between 0.041 and 0.088 (see Table 2). The genetic diversity in the *S. aria* aggregate differs remarkably. It was high for the plants identified as *S. aria s.str.* (0.129) and for plants of the intermediate group with affinity to *S. aria* s.str. (0.177). It was lower for the intermediate *S. aria-S. pannonica* group with affinity to *S. pannonica* (0.098) and very low for *S. pannonica* (between 0.027 and 0.070). Genetic diversity was also relatively low in the second putative parental taxon *S. torminalis* (NGD 0.089).

None of the progeny was found to be identical – possibly a consequence of band reproducibility, because also unique bands were scored (cf. Material and Methods). However, the genetic diversity of the progeny of *S. franconica* and *S. adeana* did not exceed the values of the adults. NGD exceeded somewhat the values of the adults in case of *S. pannonica* progeny of mother tree 8, (NGD 0.070) and *S. cordigastensis* progeny of mother tree 2 (NGD 0.088) (Table 2).

Table 2 Percentage of polymorphic loci and Nei´s Gene diversity of *Sorbus* taxa for diploid dominant AFLP marker. (*For taxon groups of *S. aria* agg. see NJ tree in Fig 1).

	n	Percentage of polymorphic loci	Neis gene diversity
S. aria s.str. *	10	43.25 %	0.129
Intermediate *S. aria* - *pannonica* group aff. *S. aria* s.str.*	7	52.42 %	0.177
Intermediate *S. aria* - *pannonica* group aff. *S. pannonica*.*	7	29.24 %	0.098
S. torminalis	7	26.99 %	0.089
S. pannonica Kordigast	5	16.78 %	0.060
S. pannonica Kordigast offspring of mother tree (MT) 7	4	15.40 %	0.056
S. pannonica Kordigast offspring MT8	4	16.96 %	0.070
S. pannonica Kordigast offspring MT9	4	7.44 %	0.027
S. pannonica Neudorf adults	4	7.27 %	0.028
S. adeana adults	7	22.32 %	0.083
S. adeana offspring MT3	4	11.07 %	0.042
S. adeana offspring MT4	3	6.92 %	0.029
S. adeana offspring MT6	4	6.75 %	0.029
S. cordigastensis adults	8	17.13 %	0.060
S. cordigastensis offspring MT1	4	12.63 %	0.053
S. cordigastensis offspring MT2	4	21.28 %	0.088
S. cordigastensis offspring MT7	4	12.98 %	0.051
S. franconica adults	7	21.97 %	0.088
S. franconica offspring MT2	4	10.73 %	0.041
S. franconica offspring MT3	4	11.59 %	0.046
S. f ranconica offspring MT4	4	14.71 %	0.059

Nei's genetic distance between *Sorbus latifolia* taxa and the *S. aria* aggregate

Nei's genetic distance (Ds) between the *S. latifolia* taxa varied between 0.093 and 0.102 (Table 3). The lowest genetic distance was found between *S. adeana* and *S. cordigastensis* (0.093), distances were wider between *S. franconica* and *S. cordigastensis* (0.101), and widest between *S. franconica* and *S. adeana* (0.102, Table 3). In comparison, the genetic distances between the *S. latifolia* taxa were higher than between *S. aria* agg. taxa, such as *S. aria* s.str. and *S. pannonica* (Ds = 0.085).

Comparing the genetic distances between *S. latifolia* agg. and the different subgroups of *S. aria* agg., lowest Nei´s genetic distances (Ds) were found between the *S. latifolia* taxa and the *S. pannonica – S. aria* intermediate groups. For *S. franconica* and *S. adeana* distances were lowest to the intermediates aff. S. *pannonica* (Ds = 0.128, 0.145), the distances of *S. franconica* and *S. adeana* to *S. pannonica* were only insignificantly higher (Ds = 0.129, 0.149). Instead, for *S. cordigastensis* the genetic distance was clearly lowest to the intermediates aff. *S. aria* s.str. (Ds = 0.109, Table 3). The second lowest distance was found between *S. cordigastensis* (Table 3) and *S. aria* s.str. (Ds = 0.122, Table 3).

The proportion of Ds values to both parental taxa or taxa groups revealed that the *S. latifolia* taxa were about 1.3 to 1.5 times more distant to *S. torminalis* than to *S. aria* agg. The genetic distance to *S. torminalis* was lowest for *S. adeana* (proportion of Ds = 1.3) meaning that *S. adeana* is more closely related to *S. torminalis* than it is to *S. cordigastensis* and *S. franconica*.

Table 3 Nei's standard genetic distance between the taxa and groups.

Sorbus	*torminalis*	*pannonica*	*aria s.str.*	*Intermediates aff. pannonica*	*Intermediates aff. aria s.str.*	*cordigastensis*	*franconica*
torminalis	-						
pannonica	0.293	-					
aria s.str.	0.243	0.085	-				
Intermediates aff. pannonica	0.308	0.030	0.090	-			
Intermediates aff. aria	0.253	0.067	0.046	0.060	-		
cordigastensis	0.170	0.124	0.122	0.124	0.109	-	
franconica	0.193	0.129	0.154	0.128	0.134	0.101	-
adeana	0.201	0.149	0.179	0.145	0.151	0.093	0.102

Principal ordination

In the Principal Coordinate analysis (Fig. 2) the *S. latifolia* taxa were placed closer to the *S. aria* group than to *S. torminalis*. Their position was, however, not exactly intermediate between the parental taxa, on the second axis (PCo2) they were somewhat shifted towards the back (see discussion). *S. franconica* was clearly placed lower on the third axis (PCo3) than the other investigated *S. latifolia* taxa. The three PCo axes explained about 50 % of total variation.

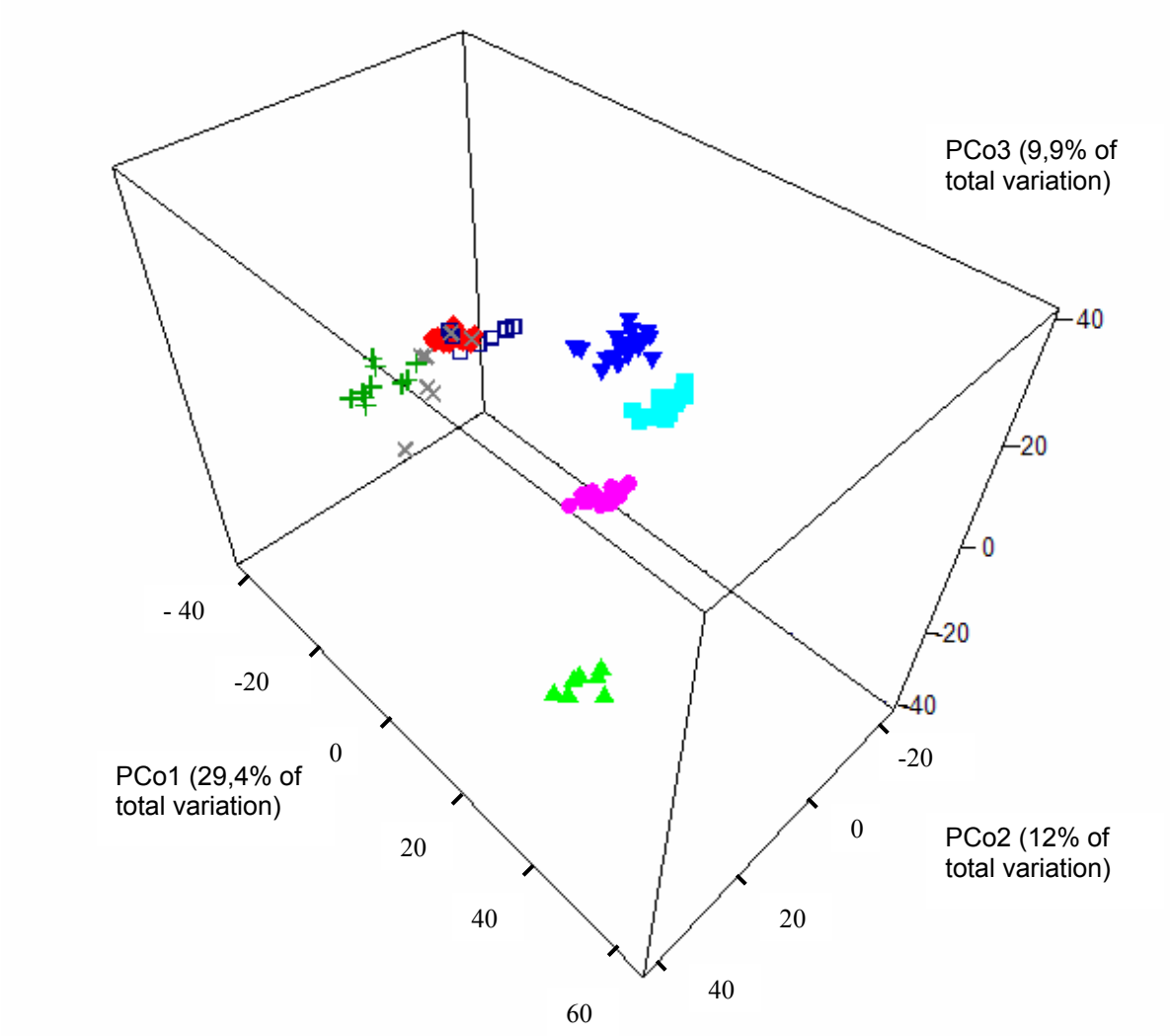

Fig. 2 Plot of the first three axes of a principal coordinates analysis using Jaccard similarity of *Sorbus* AFLP data. The three axes explain about 50 % of total variation within the data set. Legend ▲ *S. torminalis*, ■ *S. adeana*, ● *S. franconica*, ▼ *S. cordigastensis* □ aff. *S. pannonica*, + *S. aria* s.str., x aff. *S. aria* s.str.

Chromosome counts

The preparation of the chromosomes proved to be difficult. In most cases the chromosome numbers could only be estimated, counting was only possible in one to two of three (four) seedlings investigated, i.e. *S. adeana* MT 3, *S. cordigastensis* MT 7, *S. franconica* MT 2, MT 3. These countings and additional estimates revealed a triploid chromosome number of 2n = 51 for *S. adeana*, *S. cordigastensis*, *S. franconica* and *S. pannonica.*

Discussion

As expected, all *Sorbus latifolia* taxa clustered between *S. aria agg.* and *S. torminalis,* but closer to *S. aria* agg. (see below). *S. adeana, S. cordigastensis* and *S. franconica* form distinct groups with robust statistical support and without any outliers in different subclades. Nei's Gene Diversity (NGD) of the putative apomictic taxa *S. adeana, S. cordigastensis, S. franconica* and *S. pannonica* was clearly lower compared to the NGD of *S. aria* s.str. and the *S. aria-S. pannonica* intermediate groups. Therefore, the low levels of *S. latifolia* taxa and *S. pannonica* support that these taxa represent apomicts of a more or less clonal structure, as suggested before by Düll (1961) and Meyer et al. (2005). Therefore, their treatment as microspecies is supported here. The low NGD (0.089) of the sexually reproducing *S. torminalis* can be attributed to the low number of individuals analysed. Gene diversity of *S. adeana* offspring and *S. franconica* offspring was clearly smaller than the NGD of the adults as it could be expected for progeny of apomictically reproducing plants (the small differences between the progeny is due to the scoring method, see above). But this was not the case for progeny of *S. cordigastensis* from mother tree 2 (MT 2) and two mother trees collected as *S. pannonica* (MT 7, MT 8, Table 2). Therefore it is likely that some sexual processes in *S. cordigastensis* and *S. pannonica* occur rarely, resulting in a higher variability of the progeny (see below).

Interestingly, Nei´s genetic distances between the *S. latifolia* taxa are higher compared to those between the two members of *S. aria* agg., *S. pannonica* and *S. aria* s.str. (Table 3). An important reason for the large genetic distance of *S. latifolia* members may be that for them members of different subgroups of the *S. aria* agg. were parental. According to our findings, *S. adeana* and *S. franconica* showed lowest genetic distances to the intermediates aff. *pannonica,* respectively to *S. pannonica* (Table 3). In contrast, for *S. cordigastensis* clearly the lowest genetic distance was found to the intermediates aff. *S. aria* s.str. (Table 3). This is also in accordance with morphological features (comp. Düll 1961, Meyer et al. 2005), since *S. adeana* and *S. franconica* show typical characters for *S. pannonica* or intermediates aff. *pannonica* (thick texture, roundish fruits), whereas *S. cordigastensis* shows typical traits of *S. aria* s.str. or intermediates aff. *aria* s.str. (i.e. long leaves and a rather thin leaf texture). However, although *S. cordigastensis* and *S. adeana* are derived from different members of the *S. aria* agg., genetic distances between them were lowest compared i.e. to *S. franconica* (comp. above, Table 3). This could be explained by a common *S. torminalis* ancestor which most likely was member of the same population considering the closely neighbouring distribution range of *S. adeana* and *S. cordigastensis*.

In the PCo analysis (Fig. 2) *S. franconica* is placed in a distance from the other *S. latifolia* taxa on the third axis (Fig. 2), indicating that for this taxon more strongly different populations were ancestral than for *S. adeana* and *S. cordigastensis*. The reason for this PCo placement may be that *S. franconica* is a much older taxon than the other *S. latifolia* taxa, reflected by its larger distribution area compared to *S. adeana* or *S. cordigastensis*. Our results thus correspond to the results of Düll (1961), who was the first to explain the differences in area size of the *S. latifolia* taxa with their different dates of origin.

The *S. aria* agg. has a complicate structure in the study area, which was not investigated hitherto in detail (see introduction). According to our results it consists of four main groups with several subgroups (see Fig. 1) that were overall identical to the Bayesian groupings. Our results indicate that most *S. pannonica* taxa have a clonal structure and therefore reproduce as apomicts, as hypothesized by Düll (1961) and Meyer et al. (2005). However, some *S. pannonica* individuals do not seem to be strictly apomictic (see below).

In the NJ tree two main intermediate groups could be identified, either with morphological and genetic affinity to *S. pannonica* or with affinity to *S. aria* s. str. (see Fig. 1). Within the intermediates aff. *S. aria* s.str. subgroups could be identified (in accordance with Bayesian clustering), the one consisting of individual aria070 and aria073, the other one of aria074, aria075 and aria082 (see Fig. 1). Some subgroups (e.g. aria074, aria075, aria082 or aria070, aria073) consist of individuals with identical leaf shape and may belong to constant lineages (supported by a high BS value, comp. Fig. 1), presumably stabilized by apomixis. Some other subgroups may be permeable due to facultative apomixis, since two mother trees collected as *S. pannonica* produced offspring that either clustered with the *S. pannonica* group or with the *S. aria – S. pannonica* intermediate group aff. *S. pannonica* (individual panOff150, panOff151, panOff153, pannOff156, see Fig. 1). However, we could not include the parentals of these offspring into our study, because they were not marked in the field when we collected the seeds.

For the complex structuring of the *S. aria* aggregate a cycle between ploidy levels and alternating reproduction modes may be responsible, similar to those described by Rich et al. (2010) for *Sorbus* or by Talent (2009) for *Crataegus*. According to these authors, facultative apomictic triploids can arise from crosses between sexual diploids after formation of unreduced gametes in one of the crossing partners. Apomictic and sexual tetraploids can be formed by fertilisation of unreduced eggs of triploids by pollen from diploids. And last but not least, tetraploids can cross with diploids and form - again - triploids. According to Talent (2009) in such complexes selection for apomixis takes place

and favors apomictic lineages, possibly leading to the coexistence of genetically variable and clonal *Sorbus aria* agg. populations, as found in this study.

Following Rich et al. (2010) and Talent (2009), the triploid *S. latifolia* taxa could have been derived either from crosses between diploids after formation of unreduced gametes, most probably in the *S. aria* parent (see below). Alternatively, tetraploid facultative apomicts from the *S. aria* agg. could be parental for the triploid *S. latifolia* taxa. Both possibilities would fit with the finding that *S. latifolia* taxa were about 1.3 to 1.5 times higher distant to *S. torminalis* than to *S. aria* agg., meaning that their triploid genomes may consist of two sets of *S. aria* genome and one set of *S. torminalis* genome. However, determination of the ploidy levels in the *S. aria* agg. could not be performed in this study, we would expect tetraploidy, though a flow cytometry study of *S. aria* agg. including intermediates of *S. aria* agg. is therefore needed to confirm our hypothesis.

As mentioned above, *S. adeana* and *S. franconica* showed comparable low genetic distance to the intermediates aff. *pannonica* and to *S. pannonica*, whereas *S. cordigastensis* showed the lowest genetic distance to the intermediates aff. *aria* s.str. (Table 3). Possibly the intermediates are well suited as parents because they might be able to reproduce in both ways, either apomictically or sexually. As facultative apomicts these intermediates could have bequeathed apomixis to the *S. latifolia* taxa. Furthermore, the majority of the *S. latifolia* taxa in Bavaria is distributed in, or very close to areas in which intermediates of *S. pannonica*, *S. aria* and even *S. danubialis* (Jáv.) Kárpáti have been described. This is the case for the study area, the western Albtrauf of the northern Franconian Alb (i.e. *S. adeana, S. cordigastensis*), the western Albtrauf of the southern Franconian Alb (i.e. *S. schuwerkiorum* N. Mey., *S. fischeri* N. Mey.), or parts of lower Franconia (i.e. *S. badensis* N. Mey., *S. herbipolitana* N. Mey., comp. area maps in Meyer et al. 2005). Those range patterns support our finding that intermediates of *S. aria* agg. could have served as parents for *S. latifolia* taxa. The revealed important role of - so far - unclassified members of *S. aria* agg. should lead to an integration of those populations into plant protection efforts.

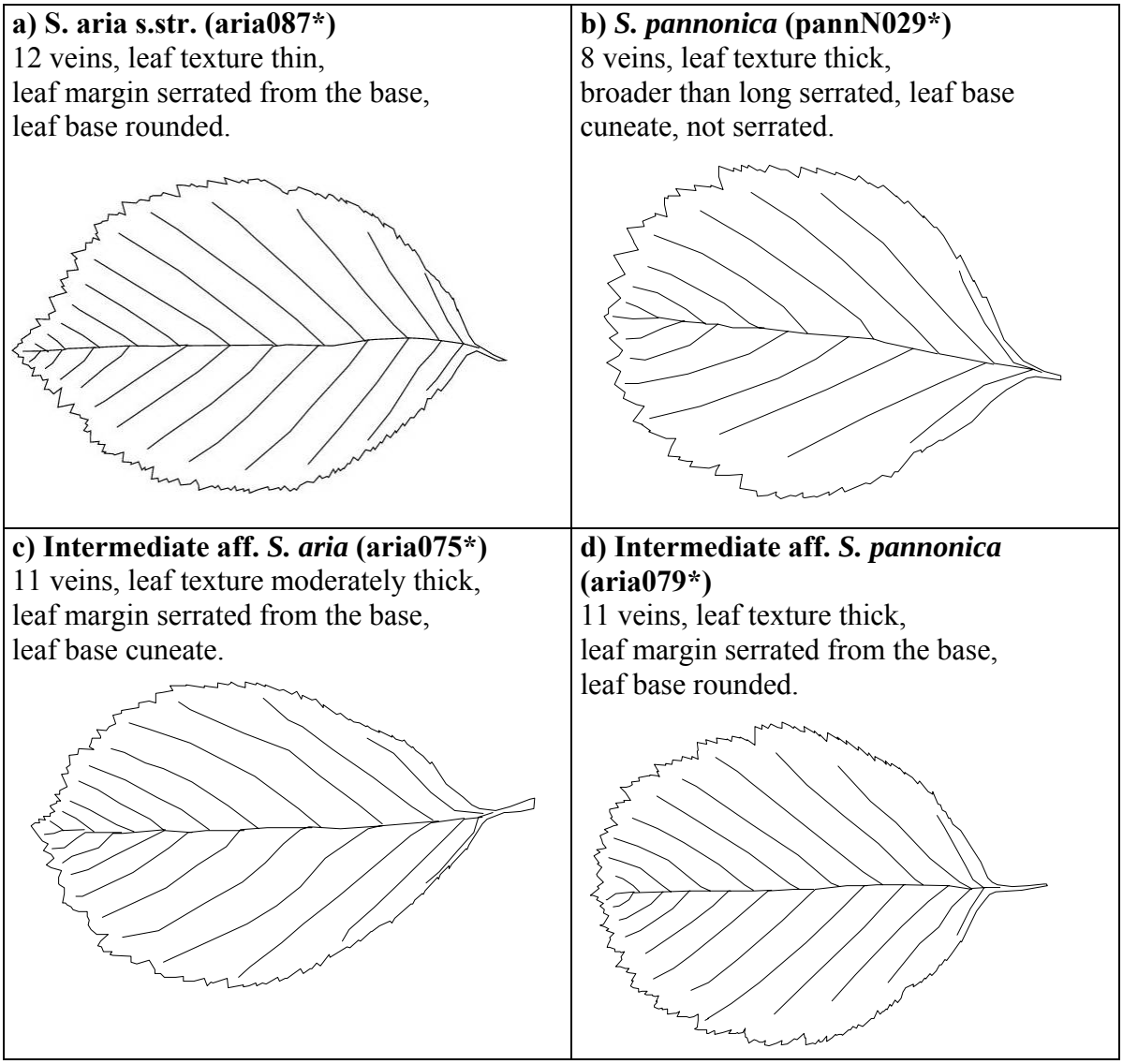

Fig. 3 Leaf shape of the broadest leaf of lateral shoots from selected vouchers of *S. aria* agg. investigated in this study incl. additional information about leaf characters and group affiliation (*for abbreviations see Table 1).

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Supporting information

Table S1: Plate layout and instrument settings for DNA purification via BindIT 3.1 KingFisher software. After step 7, eluted DNA was transferred to plastic cups and kept at -20°C.

Settings			Step 1 Bind MC2	Step 2 Wash MC3	Step 3 Wash MC4	Step 4 Wash Ethanol	Step 5 Wash MC5	Step 6 Elution MC6	Step 7 Disposal of magnetic beads
		Plate	A	B	C	D	E	F	G
	Layout		92 µl supernatant 8 µl magnetic beads 100 µl buffer	200 µl buffer	200 µl buffer	200 µl 80% Ethanol	200 µl buffer	20 µl buffer	
Beginning	Precollect		no	no	no	no	no	no	
	Release time [mm:ss]		no release	00:30	00:30	00:30	no release	00:15	00:20
	Release speed		-	fast	fast	fast	-	fast	fast
Mixing/ pause	Pause for manual handling		no	no	no	no	no	no	
	Mixing time [mm:ss]		05:00	01:00	01:00	01:00	01:00	10:00	
	Mixing speed		medium	fast	fast	fast	medium	medium	
End	Postmix		no	no	no	no	no	no	
	Collect count		3	3	3	3	3	6	
	Collect time [s]		1.5	1.5	1.5	1.5	1.5	1.5	

Publication 4

4. Floral scent and its correlation with genetic data in *Sorbus* taxa.

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Floral scent and its correlation with genetic data in *Sorbus* taxa

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Abstract

Statistical analyses between floral scent and genetic marker data for testing their taxonomical correlation are rare. We investigated inflorescence scent patterns of apomictic *Sorbus latifolia* microspecies, *Sorbus franconica, S. adeana* and *S. cordigastensis* endemic to northern Bavaria, originated by hybridization and their parental taxa with dynamic headspace method. The scent data (presence-absence of compounds) were used to construct an UPGMA tree, and to calculate a similarity matrix to correlate them, both on individual as well as population level, with AFLP data published in an earlier study. Scent analyses showed a total of 68 chemical substances, among them aromatic compounds, mono- and sesquiterpenes, aliphatics, and nitrogen containing compounds. Scent patterns were taxon specific, and the number of scent components differed among taxa. Correlations with AFLP data on population and individual level are highly significant, indicating that scent and genetic data are highly congruent in the plants studied. Scent therefore is a useful marker for taxonomical questions in *Sorbus*.

Keywords

Floral scent, apomixis, AFLP, *Sorbus*, taxonomy, correlative analysis;

Introduction

Studies of sexual (i.e. Levin et al. 2003; Raguso et al. 2006), but also of apomictic species complexes (Feulner et al. 2009; 2011) revealed that taxonomic conclusions based on scent can be highly congruent with those based on genetic markers.

The main function of floral scent is the attraction of pollinators (Dötterl et al. 2006; Plepys et al. 2002). Therefore, scent data may be influenced by pollinator mediated selection (Knudsen and Tollsten 1993; Plepys et al. 2002; Dötterl et al. 2005). One consequence of this is the evolution of pollination syndromes which means 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 is influenced by pollinator-mediated selection and coevolution, in most studies it was 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. 2012), whereas other substances may be more determined by phylogeny than by pollinator-mediated selection (Steiner et al. 2011; Schäffler et al. 2012). There are some examples in which scent data supported taxonomy and revealed a good conformity with DNA data (i.e. Levin et al. 2003; Raguso et al. 2006). However, to our best knowledge, a statistical approach of the taxonomical value of scent by detailed correlations between scent data and data from genetic markers was so far only presented once in a study dealing with *Ophrys* (Orchidaceae, Stökl et al. 2008). Here, however, significant correlation between both data sets was not found (Stökl et al. 2008).

Scent data in apomicts may behave differently compared to sexual species, because apomictic plants produce seeds without fertilisation and do not rely on pollination (Nogler 1984; Jankun and Kovanda 1987; Talent 2009). Therefore, pollinator-mediated selection influencing scent patterns is of minor importance in apomicts (comp. Feulner et al. 2009; 2011). Furthermore, the intra-individual genetic variability of apomicts is extremely low, therefore, scent patterns also may be strongly identical between individuals and populations of the same apomictic taxon. Apomixis is often coupled with hybrid speciation (Talent 2009) as it is the case in the *Sorbus latifolia* group (Rosaceae). *Sorbus latifolia* taxa originated from hybridization between *S. aria* agg. and *S. torminalis* (Düll 1961; Rich et al. 2010, Feulner et al. 2013; submitted). Among members of the *Sorbus latifolia* agg. are many taxa endemic to restricted regions in i.e. Great Britain, Czech Republic, and Germany (Düll 1961; Lepší et al. 2009, Meyer et al. 2005; Robertson et al. 2010; Rich et al. 2010).

Interestingly, it has been shown in other hybrid complexes such as *Citrus, Ophrys* and *Hieracium,* that the scent consists mainly of a mixture of scent components of the parental species (Gancel et al. 2002; Vereecken et al. 2010; Feulner et al. 2009; 2011), and only a low number of new compounds. Therefore, scent analyses may be a valuable tool for parental species identification, and indeed, the taxonomic reliability of scent patterns was shown to be high in groups originated by hybridization (Feulner et al. 2009; 2011).

Here, we investigate the scent of the apomictic microspecies *S. adeana, S. cordigastensis*, and *S. franconica* belonging to the *S. latifolia* aggregate endemic to Northern Bavaria and occupying very small parapatric distribution areas. In a former study the hybrid state, the intraspecific variability and the genetic structure of parental taxa was investigated with AFLP analyses (Feulner et al. 2013; submitted). In the present study, based on the same material investigated in the AFLP study (Feulner et al. 2013; submitted), we investigated floral scent composition and correlated scent clustering with the AFLP tree, to estimate the correlation between both data sets.

Material and method

Study plants

We collected scent from *S. adeana* (one of one known population, comp. Meyer et al. 2005), *S. cordigastensis* (one of approximately three known populations), *S. franconica* (two of > 50 known populations) as well from parental taxa such as *S. aria* s.str., *S. pannonica* and *S. torminalis* (comp. Feulner et al. 2013; submitted). From the *S. aria* agg. also intermediates between *S. aria* s.str. and *S. pannonica* with affinity to *S. aria* s.str. (comp. Feulner et al. 2013; submitted) were included into the study. These plants are appelated as aff. *aria*. For all taxa, AFLP data were available from the same populations (Feulner et al. 2013, submitted) and in 11 cases AFLP data and scent data were collected from the same individuals (Table 1, Feulner et al. 2013, submitted). For further information about taxonomy, population structure and ecology of the taxa investigated, see Feulner et al. (2013, submitted).

Table 1: Taxa, locality and voucher information of the individuals analysed.
(* compare Feulner et al. 2013, submitted).

Taxon	**Locality / Gauss Krueger coordinates / Voucher**	**number of individuals for scent sampling**	**number of AFLP samples from the same population/individual as used for scent sampling***
Sorbus aria (L.) Crantz	Grafenhäusling 4437746/ 5542273, *Feulner* 200–207 (UBT)	2	5/0
Intermediates aff. aria s.str.	Autobahn Rossdorf 4437013/ 5539920, *Feulner* 208–217 (UBT)	4	5/0
S. pannonica Kárpáti	Kordigast 4443782/ 5551720, *Feulner* 218–227 (UBT)	4	5/1
	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
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	7/8
S. torminalis (L.) Crantz	Neudorf Bärental 4447194/5546549, *Feulner* 254 (UBT)	1	1/0
	Hainbach // 4451892/ 5530457, *Feulner* 255 (UBT)	1	0/1
	Kordigast 4443782/ 5551720, *Feulner* 256–259 (UBT)	2	3/0

Volatile collection

Inflorescence scent was collected in the field using a standard dynamic head-space method as described in Feulner et al. (2009). For each taxon, two to six individuals were sampled.

Sampling was carried out on fresh and 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 leaves and surrounding air were collected as control for each locality and population investigated.

Chemical analysis

The samples were analysed on a Varian Saturn 2000 mass spectrometer, and a Varian 3800 gas chromatograph with a 1079 injector, that had been fitted with the 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 minutes; the injector was heated with 40 °C for 2 min, and the temperature was then increased with a rate of 200 °C/min to 200 °C; this end temperature was held 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 for 7 min at 40 °C, then increased by 6 °C per min to 250 °C and held for 1 min. The MS interface was 260 °C and the ion trap worked at 175 °C. The mass spectra are taken at 70 eV (in EI mode) with a scanning speed of 1 scan s^{-1} from m/z 30 to 350.

Data analysis

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 data base or MassFinder 3, and confirmed by comparison of retention times with published data (Adams 2007). Identification of individual components was confirmed by comparison of both mass spectrum and GC retention data with those of authentic standards.

Statistical analysis

A cluster analysis (UPGMA) based on Jaccard similarity index (calculated using the presence-absence of compounds) was constructed in PRIMER Vers. 6 (Clarke and Gorley 2006). The taxon specificy of scent was tested with ANOSIM (10.000 pemutations) in

PRIMER Vers. 6. PERMDISP in PRIMER Vers. 6 was used to test the significance of the interspecific dispersion of scent data among taxa.
For correlation analyses on population level all AFLP bands (see Feulner et al. 2013; submitted) and scent compounds found in the different individuals of a population were counted. Similarity matrixes (Jaccard index) were calculated and used for the RELATE correlation analyses (Spearman Rank correlation, 10.000 permutations) in PRIMER Vers. 6 (Clarke and Gorley 2006).

To test for differences in the number of scent compounds trapped among taxa, ANOVA was performed. KOLMOGOROV-SMIRNOV and HARTLEY were used to test for normality and homogeneity of variances, respectively. Unequal-N HSD was used as post-hoc test.

Results

Floral scent chemistry

In total, 68 substances were identified in 35 samples of six *Sorbus* taxa (Table 3), mainly aliphatics and aromatics. Less abundant were monoterpenes, irregular monoterpenoids and nitrogen-containing substances**.** Most widespread were i.e. (*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 nitrogen-containing substances, methyl nicotinate, 3-pyridinecarboxaldehyde, and phenylacetonitrile were occurring in most samples.

The ANOSIM (10.000 permutations) revealed that the scent is highly taxon specific ($R_{df=6, 26}= 0.859$, $p<0.001$).
Scent substances are extremely different (pairwise R= 1, ANOSIM) between *Sorbus aria* agg. and *S. torminalis*. *Sorbus aria* agg. is differentiated against *S. torminalis* by the appearance of several lilac alcohols, lilac aldehydes and (*E*)- and (*Z*)-arbusculone and by the presence of some unidentified n-containing compounds, which were missing in *S. torminalis* (i.e. unk-N1498, unk-N1530, indole or 1-nitro-2-phenylethane, see table 3). The *Sorbus latifolia* taxa inherited most lilac aldehydes, lilac alcohols and nitrogen-containing compounds from the *S. aria* parent. *S. torminalis* possesses two specific nitrogen-compounds not found in *S. aria* agg., one unidentified (unk-N1377), the other identified as

amyl/isoamyl-pyrrole. The unidentified nitrogen-compound was found in *S. adeana*, amyl/isoamyl-pyrrole was found in *S. adeana* and one *S. cordigastensis* individual.

We did not identify substances that occurred in *S. latifolia* alone. However, methyl hexanoate and methyl (3Z)-hex-3-enoate were present in all *S. latifolia* taxa, but only in some *S. pannonica* (Table 3). On the other hand, some compounds shared by all parental taxa were bequeathed to none of the *S. latifolia* taxa, such as (E)-arbusculone and anisaldehyde (Table 3).

The close relationship between *S. adeana* and *S. cordigastensis* is reflected in several shared substance, namely methyl nicotinate (only in *S. torminalis* and two individuals of *S. aria* aff. *aria*), lilac alcohols, lilac aldehydes and an unidentified sesquiterpene (unk-St1750). All these substances despite lilac alochol D were not found in *S. franconica*.

Interestingly, sexual *S. aria* s.str. is differentiated against the other members of *S. aria* agg. predominantly by a lack of sesquiterpenes, such as α-longipinene, unk-St1693, α-copaene, unk-St1711 and the lack of unk-mt1402. The component unk-St1732 was found specifically in intermediates *S. aria* aff. *aria*.

Table 2: Sample size, average number of scent compounds and intra specific scent dispersion (mean distance to centroid, Jaccard similarity) in the eight *Sorbus* taxa analysed. Different upper script letters indicate significant differences.

***Sorbus* taxa**	**n**	**average number of scent substances and standard error of mean**	**average dispersion and standard error of mean**
S. aria s.str.	2	50 (1.5)^{b,c,d}	6.6 (0)^{a,d,e}
S. pannonica	6	57 (1.04)^{d}	9.6 (1.0)^{a,d}
Intermediates with affinity to *S. aria* s.str. (aff. *aria*)	4	53 (1.93)^{c,d}	10.2 (1.3)^{a,c,d,e}
S. torminalis	4	29 (1.88)^{a}	18.2 (4.3)^{a,b,c,d,e}
S. adeana	4	48 (1.7)^{c}	10.3 (0.8)^{a,c}
S. cordigastensis	8	39 (1.3)^{b}	14.7 (1.4)^{c}
S. franconica	5	31 (1.83)^{a}	16.4 (0.81)^{b}

The number of scent substances differed significantly among the taxa (ANOVA: $F_{df=6,26}$=44.18, p<0.001). With ca. 30 compounds, *S. torminalis* and *S. franconica* emitted only a little bit more than half of the substance number compared to the *S. aria* agg. (between 50 in *S. aria* s.str. and 57 in *S. pannonica*, Table 2). The *S. latifolia* taxa possess a number of scent compounds intermediate between those of the parental taxa *S. torminalis* and *S. aria* agg. (between 31 and 48, Table 2).

Scent dispersion was significantly different between the *Sorbus* taxa (Permdisp, Primer, 100000 permutations $F_{df=6,26}$=4.357; P=0.0239). It is highest in *S. torminalis*, followed by *S. franconica*, *S. cordigastensis* and *S. adeana* (Table 2). It is lowest in the subgroups of *S. aria* agg., intermediates aff. *aria, S. pannonica* and in *S. aria* s. str. In *S. aria* agg. however, the number of investigated individuals was very low (n=2).

Scent clustering

The scent cluster (Fig. 1) showed that all taxa of *S. latifolia* form groups of their own. *S. adeana, S. cordigastensis* and *S. franconica* cluster between their parental taxa *S. torminalis* and *S. aria* agg. In the UPGMA tree (Fig. 1), *S. adeana* and *S. cordigastensis* group closer to *S. aria* agg. than *S. franconica* and closer to each other than to *S. franconica.*

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 mostly between *S. aria* s.str. and *S. pannonica* except one individual that clusters within the *S. aria* s.str. group (Fig. 1).

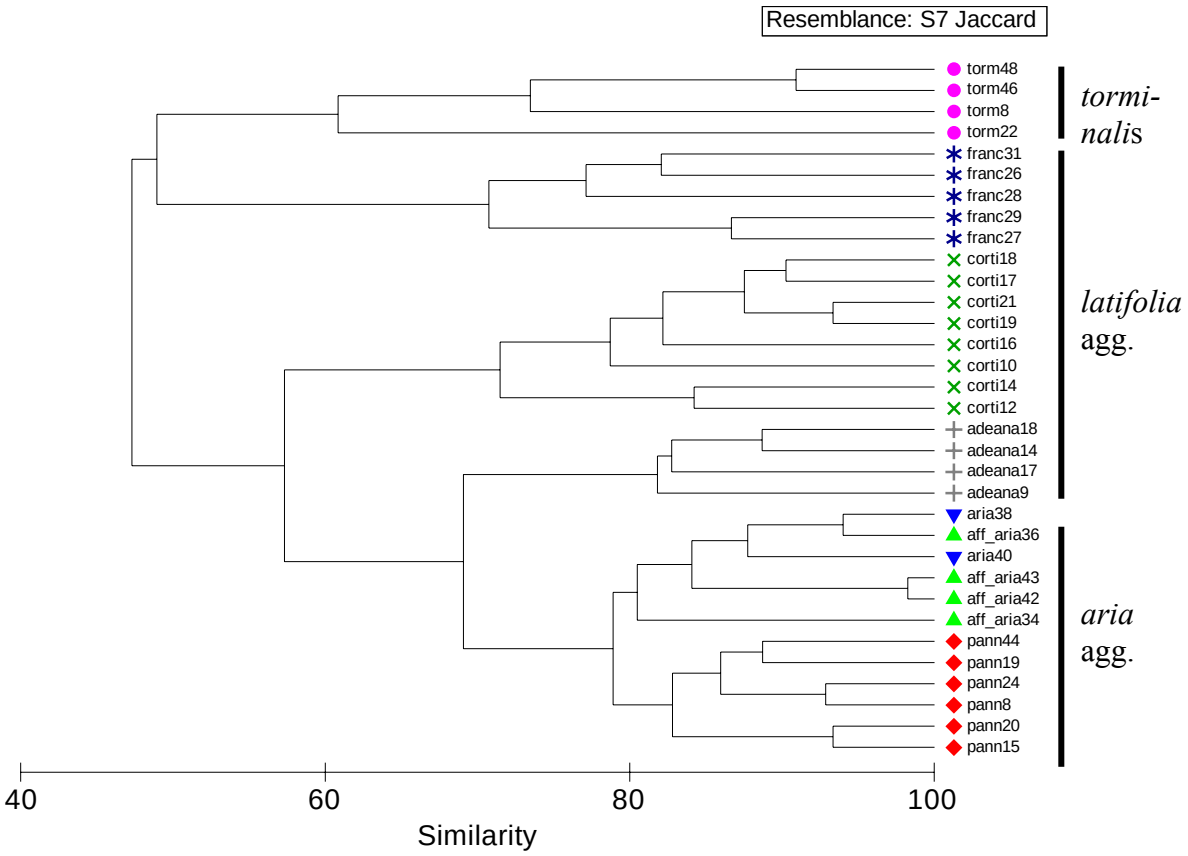

Fig. 1: Cluster analysis (UPGMA) of scent data based on Jaccard index of *S. latifolia* taxa *S. adeana* +, *S. franconica* * *and S. cordigastensis* x and their parental species groups *S. aria* agg. including *S. aria* s.str. ▼, *S. pannonica* ♦, intermediates aff. *aria* ▲ and *S. torminalis* ●.

Correlation between AFLP data and scent data

There was a significant correlation on the population (RELATE: R=0.683, p = 0.006, six populations included) and on the individual level (RELATE: R=0.823, p < 0.001, 11 individuals included) between AFLP and scent data. This indicates that genetically similar populations/taxa also emitted similar scents, whereas genetically dissimilar populations/taxa also emitted different scents.

Discussion

The present study documents a high correlation between scent and AFLP data in a *Sorbus* hybrid complex. For the *S. latifolia* taxa and their parental taxa the comparison between the grouping based on scent data (Fig. 1) and the AFLP phylogeny (comp. Feulner et al. 2013; submitted) revealed almost total congruency.

Parental *S. aria* agg. and *S. torminalis* are clearly differentiated by scent data as well as by molecular data (Feulner et al. 2013; submitted). The number of scent components of *S. tominalis* is significantly reduced compared to *S. aria* agg. (see Table 2). This is mainly due to a lack of lilac alcohols, lilac aldehydes and a reduced number of nitrogen-containing compounds in *S. torminalis* (Table 3). Such consistent differences between parental species are the predisposition for hybrid identification based on scent data (Feulner et al. 2009; 2011).

In both trees, derived from AFLP and from scent data, the *S. latifolia* taxa cluster between *S. aria* agg. and *S. torminalis*, showing that the *S. latifolia* hybrid taxa inherited scent compounds from both parental species (Table 3). Overall, the scent of the *S. latifolia* taxa was a somewhat unbalanced mixture of scent components of parental taxa as found in studies on hybrids before (comp. Gancel et al. 2002; Feulner et al. 2009; 2011; Vereecken et al. 2010). Two specific nitrogen-containing compounds were bequeathed from parental *S. torminalis* to *S. cordigastensis* and *S. adeana*, but not to *S. franconica*, in which none of these substances was found (Table 3).

Contrasting to other studies which could show that hybrids emit novel compounds besides the parental ones (Gancel et al. 2002; Feulner et al. 2009; 2011; Vereecken et al. 2010), flowers of *S. latifolia* taxa emitted no such compounds. However, substances such as methyl hexanoate and methyl (3Z)-hex-3-enoate 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.

In the AFLP study, a close relationship of *S. adeana* and *S. cordigastensis* was reported (Feulner et al. 2013; submitted). This is confirmed by scent chemistry since the two taxa share several compounds such as lilac alcohols, lilac aldehydes, methyl nicotinate and unk-St1750 (also shared with *S. aria* agg). All these substances besides one lilac alcohol D are missing in *S. franconica.* The lower number of inherited parental scent components in *S. franconica* compared to the other *S. latifolia* taxa might be explained by the assumption that this taxon is of an older hybrid origin than the other investigated *S. latifolia* members (Düll 1961; Feulner et al. 2013; submitted). Its parents may not have possessed and

bequeathed the same compounds which are emitted by recent populations of *S. torminalis* and *S. aria* agg. Alternatively, some scent genes may have become lost over the decades due to mutations.

For presumably apomictic taxa, the taxon-specific dispersion of scent was unexpectedly high for the investigated *S. latifolia* taxa, especially for *S. cordigastensis* and *S. franconica* (comp. Table 2). However, this was also in accordance with the AFLP study (Feulner et al. 2013; submitted) since *S. cordigastensis* and *S. franconica* showed higher genetic variability than *S. adeana*. Both studies - scent and AFLP - suggest that S. *cordigastensis* may not be an obligate apomict, but undergoes sporadic sexual events.

The differentiation within *S. aria* agg. into subgroups (Feulner et al. 2013; submitted) was confirmed by scent data although genetic distances between these groups are small (see Feulner et al. 2013; submitted). Even the intermediate state of the plants called "intermediates aff. *aria* s.str." was confirmed, since most individuals of these group cluster between *S. aria* and *S. pannonica,* but closer to *S. aria* s.str. (Fig. 1). Surprisingly, *S. pannonica* has the highest compound numbers of all investigated taxa. This fact is possibly correlated with its higher ploidy level since it is triploid, whereas *S. aria* s.str. is diploid (Feulner et al. 2013; submitted). Polyploid plants often show characters which are boosted in number or size compared to the diploid relatives. Since *S. pannonica* is thought to be an intermediate between *S. aria* s.str. and *S. graeca* (Spach) Lodd. ex S.Schauer (comp. Düll 1961), large compound numbers could be interpreted as effect of introgression from *S. graeca*. Here, it was not possible to investigate scent of *S. graeca* which is a very rare species of which the occurrence in the study area is doubtful (cf. Düll 1961; Meyer et al. 2005).

The *Sorbus aria* agg. and the *S. latifolia* agg. are examples of polyploid plant complexes. Such complexes show important differences compared to "truly sexual groups" that might be responsible for the strong phylogenetic signal of floral scent. Most members within such complexes are interconnected by ancient gene flow due to reticulate evolution. The number of hybrids that possess a mixture of scent of progeny taxa is high (see Gancel et al. 2002; Feulner et al. 2009; 2011). In sexual taxa, scent is much more variable and often interpopulation differentiation occurs as well (Dötterl et al. 2005). Such interpopulation variability can undermine the taxonomical signal of scent data. Instead, apomicts such as taxa of *Sorbus latifolia* agg. have a clonal population structure and the populations are genetically identical, which increases the taxonomic value of scent data. Together with a

reduced influence of pollinator selection this fact is leading to a strong correlation between scent data and genetic data, as has been shown above.

Most studies comparing scent and molecular data are based on Orchidaceae. However, this is a strongly contrasting system including many deceptive plants where pollinator selection plays a key role (Salzmann et al 2007; Vereecken et al. 2010). Deceptive plants usually have an increased variability of scent (Salzman et al. 2007), likely to avoid that pollinators can easily learn to discriminate between the reward and the mimic (Ackerman et al. 2011). Therefore it is not astonishing that in *Ophrys* no correlation of scent with genetic data was found (Stökl et al. 2008).

Table 3: 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	tormi-nalis	ade-ana	cordigast-ensis	franco-nica
aromatics							
benzaldehyde	4/4	2/2	6/6	4/4	4/4	8/8	5/5
benzeneacetaldehyde	3/4	1/2	3/6	3/3	4/4	4/8	-
methyl benzoate	4/4	2/2	6/6	-	4/4	8/8	5/5
2-phenylethyl alcohol	4/4	2/2	6/6	4/4	4/4	8/8	5/5
methyl phenylacetate	4/4	2/2	6/6	-	4/4	8/8	3/5
methyl salicylate	4/4	2/2	6/6	4/4	4/4	8/8	5/5
anisaldehyde	2/4	1/2	2/6	1/4	-	-	-
aliphatics							
methyl isovalerate	4/4	2/2	5/6	-	4/4	-	2/5
2,3-butandiol	4/4	2/2	6/6	-	-	-	-
(Z)-3-hexen-1-ol	4/4	2/2	6/6	3/4	4/4	8/8	5/5
methyl hexanoate	-	-	4/6	-	4/4	8/8	3/5
methyl (3Z)-hex-3-enoate	-	-	2/6	-	4/4	8/8	2/5
methyl 2-hydroxy-3-methylpentanoate	4/4	2/2	6/6	-	4/4	-	-
(Z)-3-hexen-1-ol acetate	4/4	2/2	6/6	4/4	4/4	8/8	5/5
acetic acid hexyl ester	-	-	4/6	3/4	1/4	8/8	-
(E)-2-hexen-1-ol acetate	-	-	4/6	-	-	-	5/5
octanal	4/4	2/2	6/6	4/4	4/4	-	5/5
Homoterpenes							
(E)-4,8-Dimethyl-1,3,7-nonatriene	4/4	2/2	6/6	4/4	4/4	8/8	5/5
Irregular monoterpenes							
4-oxoisophorone epoxide	4/4	2/2	6/6	4/4	4/4	8/8	4/5
4-oxoisophorone	4/4	2/2	6/6	4/4	4/4	8/8	5/5
dihydrooxoisophorone	4/4	2/2	6/6	4/4	4/4	8/8	5/5
monoterpenes							
α-pinene	4/4	2/2	6/6	4/4	4/4	8/8	5/5
camphene	3/4	2/2	6/6	2/4	4/4	8/8	-
β-pinene	4/4	2/2	6/6	3/4	4/4	8/8	5/5
(Z)-ocimene	4/4	2/2	6/6	4/4	4/4	8/8	5/5
limonene	4/4	2/2	6/6	4/4	4/4	8/8	5/5

eucalyptol	4/4	2/2	6/6	4/4	4/4	8/8	5/5
dihydro-5-methyl-5-vinyl-2(3H)-furanone	4/4	2/2	5/6	1/4	4/4	7/8	-
(E)-ß-ocimene	1/4	1/2	5/6	3/4	-	5/8	5/5
(Z)-arbusculone	4/4	2/2	6/6	1/4	1/4	-	-
(E)-arbusculone	4/4	2/2	6/6	1/4	-	-	-
(Z)-linalol-oxid furanoid	3/4	1/2	4/6	3/4	3/4	-	5/5
(E)-linalol-oxid furanoid	4/4	2/2	6/6	3/4	4/4	-	1/5
lilac aldehyde A	4/4	2/2	5/6	-	-	-	-
Lilak aldehyd B+C	4/4	2/2	6/6	-	3/4	8/8	-
lilac aldehyde D	4/4	2/2	6/6	-	3/4	3/8	-
unkn MT1402	3/4	-	6/6	-	-	1/8	4/5
lilac alcohol A	4/4	2/2	2/6	-	4/4	2/8	-
lilac alcohol BC	4/4	2/2	6/6	-	4/4	4/8	-
lilac alcohol D	4/4	2/2	4/6	-	4/4	2/8	2/5
isomenthone	4/4	1/2	4/6	4/4	3/4	4/8	1/5
lilac derivative	4/4	2/2	6/6	-	4/4	-	-
linalool	4/4	2/2	6/6	4/4	4/4	-	3/5
N-containing substances							
3-pyridinecarboxaldehyde	4/4	2/2	5/6	4/4	4/4	8/8	-
amyl/isoamyl-pyrrole	-	-	-	3/4	4/4	2/8	-
phenylacetonitrile	4/4	2/2	6/6	4/4	4/4	8/8	5/5
methyl nicotinate	2/4	-	-	2/4	4/4	8/8	-
unk-N1364 m/z 125, 81, 39	4/4	2/2	6/6	3/4	4/4	8/8	1/5
unk-N1377 m/z 151, 94	-	-	-	2/4	2/4	-	-
Unk-N1498 m/z 117,91,65,50,39	4/4	2/2	6/6	-	3/4	8/8	2/5
unk-N1530 m/z 117,91,59,50	4/4	2/2	6/6	-	4/4	7/8	2/5
Indole	4/4	2/2	6/6	-	3/4	-	-
1-nitro-2-phenylethane	4/4	2/2	6/6	-	4/4	8/8	3/5
Sesquiterpenes							
cf. α-longipinene	2/4	-	6/6	-	-	-	-
unk-ST1684 m/z 204,161, 91, 69, 55	1/4	-	2/6	-	-	-	-
unk-ST1690 m/z 161,119, 85, 73, 58	-	-	6/6	1/4	1/4	4/8	5/5
α-Copaene	3/4	-	4/6	-	-	3/8	-
β-bourbonene	3/4	2/2	6/6	1/4	4/4	5/8	5/5
unk-ST1711 m/z 161,123, 81, 67, 55	2/4	-	6/6	-	-	4/8	-
unk-ST1732 m/z 161,139, 93, 79	2/4	-	-	-	-	-	-
Longifolene	4/4	2/2	6/6	4/4	3/4	8/8	1/5
Isocomene	2/4	2/2	6/6	-	1/4	8/8	-
(E)-β-caryophyllene	4/4	2/2	6/6	4/4	3/4	6/8	5/5
α-gurjunene	4/4	2/2	6/6	-	3/4	5/8	3/5
unk-ST1808 m/z 204,161,143,133,105	-	-	2/6	1/4	1/4	4/8	1/5
unk-ST1831 m/z 204,189,161,133,119, 105 cf. Germacrene D	3/4	1/2	6/6	1/4	2/4	6/8	4/5
unk-ST1838 m/z 161,93,41	2/4	1/2	6/6	-	1/4	5/8	5/5
unidentified							
unk m/z 112,140,181	4/4	2/2	5/6	-	4/4	-	-

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Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die Arbeit selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

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Bayreuth, den