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Stereoisomeric pattern of lilac aldehyde in *Silene latifolia*, a plant involved in a nursery pollination system

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Abstract

The monoterpene lilac aldehyde is found in floral scent of several plants species, among them *Silene latifolia*. This plant is involved in a nursery pollination system, because a noctuid moth, *Hadena bicruris*, is not only pollinator but also seed predator. Lilac aldehyde is the key floral scent compound of *S. latifolia* for attracting *Hadena*. This monoterpene has three stereogenic centers, and eight different isomers are possible. Here, we analysed the ratio of lilac aldehyde isomers from plants originating from 18 different populations of *S. latifolia* using enantioselective multidimensional GC–MS (enantio-MDGC–MS), and compared resulting variability with variability found in total scent emitted by specimen under study. Though variability in total emitted scent was high, ratio of lilac aldehyde isomers was a more conservative trait. There was no correlation between the ratio of lilac aldehyde isomers and the total emitted floral scent pattern. Both, ratio of stereoisomers and total emitted scent were independent from the geographic origin of the plants. In conclusion, the ratio of lilac aldehyde stereoisomers in *S. latifolia* is a reliable trait, and may used by the nursery pollinator *H. bicruris* for host-plant detection.
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Keywords: *Silene latifolia*; Caryophyllaceae; White campion; Headspace; Enantio-MDGC–MS; Flower scent; Lilac aldehyde stereoisomers; *Hadena bicruris*; Nursery pollination

1. Introduction

Plant volatiles are important signals for host-location of phytophagous insects, and often specific blends of ubiquitous compounds and not host-plant specific compounds are used by insects for host-plant detection (Bruce et al., 2005). Many herbivorous insects deposit their eggs on plant foliage, and volatiles emitted from these green parts are often essential for host-plant finding (Visser, 1988; Bernays and Chapman, 2001). On the other hand, nursery pollinators, such as *Hadena bicruris* Hufnagel, lay their eggs in flowers, which additionally are used as nectar source (Brantjes, 1976a), and floral scent therefore may serve as cue for finding both nectar and larval host-plants. The noctuid moth *Hadena bicruris* accepts few Caryophyllaceae species as larval host-plants, with dioecious *Silene latifolia* Poiret ssp. *alba* (Miller) Greuter & Burdet (hence *S. latifolia*) being its most important (Bopp and Gottsberger, 2004). Female moths pollinate the flowers, lay their eggs exclusively in female flowers, and the larvae subsequently feed on the developing seeds (Brantjes, 1976a,b; Bopp, 2003). Flowers of *S. latifolia* emit a strong scent at night, which is known to attract *H. bicruris* (Brantjes, 1976b). However, in a previous study, Dötterl et al. (2005) found that flower scent profiles between and within populations differ in quality and relative amount of emitted compounds. Only 2 out of 58 detected compounds were found in all flowers studied, i.e. the oxygenated monoterpene lilac aldehyde and the benzenoid benzaldehyde, and the ratio of these two compounds was quite variable. Therefore, host-plant detection on the basis of a specific blend of com-

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pounds seems to be most likely impossible in this nursery pollination system. Indeed, wind tunnel biotests demonstrated that lilac aldehyde alone was as attractive as the scent of whole flowers of *S. latifolia* (Dötterl et al., 2006b). Interestingly, lilac aldehyde can be found in the floral scent of several plant families, which makes its use as key compound for host-plant detection by *H. bicruris* doubtful. However, this oxygenated monoterpene is among the most complicated floral scent compounds due to three stereogenic centres. Therefore, eight different stereoisomers are conceivable, and a study on the isomeric composition of lilac aldehyde stereoisomers in 15 different plant species revealed species-specific patterns (Dötterl et al., 2006a). Different plant species emitted different isomers or different ratios of isomers. Nevertheless, only few specimen (1–2) per species were analysed in this study, and little is known about intraspecific variability of the stereoisomeric pattern.

It is imaginable that an undefined ratio of lilac aldehyde stereoisomers is sufficient to attract *Hadena* from long distances, but a specific ratio may be necessary to enable final host-plant recognition. Host-plant finding may therefore not only be achieved by recognition of species-specific odours or specific compound-ratios, but also by different ratios of stereoisomers of an ubiquitous compound. The first important prerequisite for the use of a specific ratio of lilac aldehyde stereoisomers by *Hadena* would be that the pattern of stereoisomers within *S. latifolia* specimens is constant and intraspecific variability is smaller than interspecific variability. The second prerequisite would be that *H. bicruris* can discriminate between different ratios of stereoisomers. The aim of this study was to get an insight into the role and function of floral scents and chiral compounds in attracting *Hadena* moths by determining the variability in isomeric pattern of lilac aldehyde in specimens of different European populations (Fig. 1) of *S. latifolia*. Further, this intraspecific variability is compared with variability in total emitted scent (relative amount of compounds) of studied plants, and is discussed with results obtained by Dötterl et al. (2006a) describing interspecific variability of isomeric patterns of lilac aldehydes.

This is the first study analysing in detail the intraspecific variability of a chiral floral scent compound, which is important for plant-pollinator interactions, from specimens covering a large distribution area of the species.

2. Results

In all samples only the (5′*S*)-configured stereoisomers were detected, and no (5′*R*)-configured stereoisomer was found. The most abundant isomer in all samples was either the (2*S*, 2′*S*, 5′*S*)- or the (2*R*, 2′*S*, 5′*S*)-configured stereoisomer (Fig. 2). Both isomers reached a relative amount of ca. 40%, followed by isomer (2′*S*, 2′*R*, 5′*S*) with ca. 20% relative amount. The fourth isomer, (2*R*, 2′*R*, 5′*S*), occurred only in small amounts (0.7–3.0%).

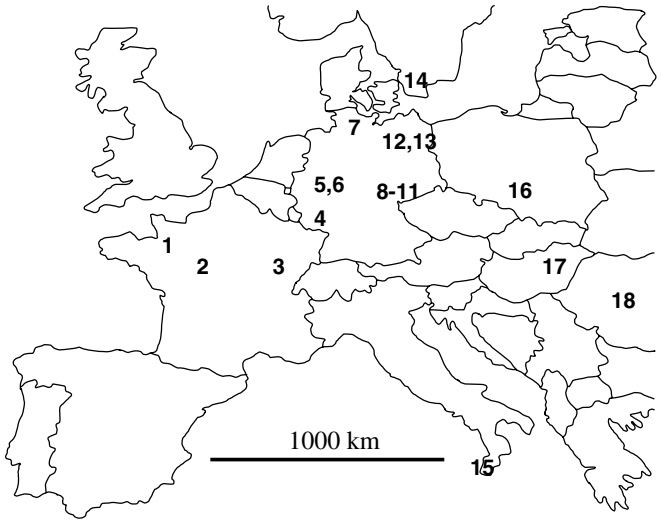

Fig. 1. Geographic origin of the 18 populations analysed. One specimen per population was studied in populations 3, 4, 6, 9, 14, 18, two specimens were studied in populations 1, 7, 8, 11, 15, 17, three specimens were studied in populations 5, 10, 12, 13, 16, and four specimens were sampled in population 2.

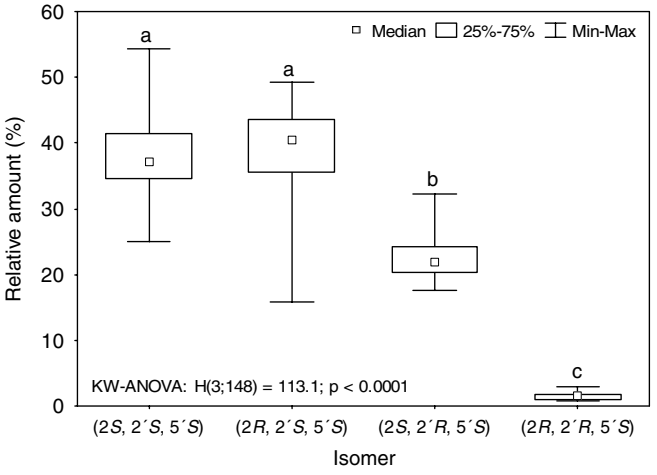

Fig. 2. Relative amount of the four isomers of lilac aldehyde found in the 37 specimens under study.

The pattern of lilac aldehyde stereoisomers was population specific (ANOSIM: $R = 0.52$, $p < 0.0001$), and variability within populations was significant lower than variability between populations (Fig. 3). Similarly, total emitted floral scent (relative amount of compounds) was population specific (ANOSIM: $R = 0.35$, $p = 0.001$), however, a significant difference in variability within and between populations was only found when omitting the variability of isomers from the analyses, and comparing only variability in total scent within and between populations (*U*-Test: $Z_{27,639} = -3.1$, $p < 0.002$), but not when including all four groups in the analysis (Fig. 3).

When comparing the dissimilarity matrix (CNESSm1) resulting from the isomeric pattern with the distance matrix

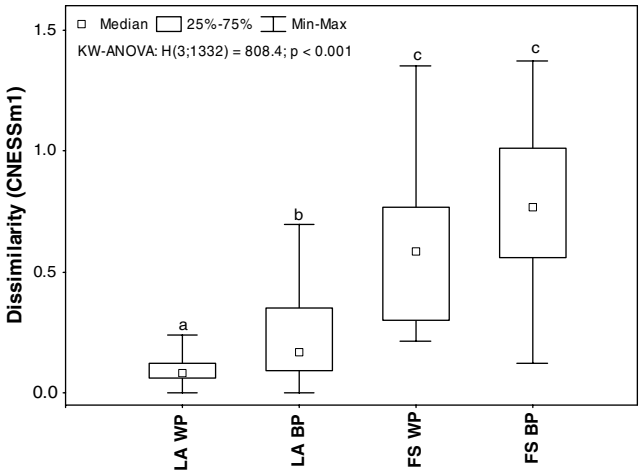

Fig. 3. Mean dissimilarity in pattern of lilac aldehyde isomers (LA) and whole flower scent (FS) within (WP) and between (BP) populations.

(km) of the populations no correlation was found (RELATE: $\rho = 0.18$, $p = 0.13$). Similarly, there is no correlation between the totally emitted scent and the origin of the plants (RELATE: $\rho = 0.15$, $p = 0.16$). These results indicate that populations located in close proximity are not more similar to each other than distant populations. The variability in isomeric pattern of lilac aldehydes within the western (1–3) and within the eastern (16–18) populations is relatively high, whereas patterns found in the different western populations could also be found in eastern populations (Fig. 4A).

There is also no correlation between the dissimilarity matrix (CNESSm1) resulting from the pattern of lilac aldehyde isomers of the 37 samples, and the dissimilarity matrix (CNESSm1) resulting from the relative amount of all floral scent compounds emitted (RELATE: $\rho = -0.06$, $p = 0.75$). Though all samples were characterised by similar patterns of stereoisomers, the total scent was partly quite different, and variability in pattern of lilac aldehyde isomers was significant lower than variability in total floral scent (Fig. 3). In Fig. 4, the isomeric patterns of the 18 populations under study are compared with the most abundant floral scent compounds (including lilac aldehyde) found in these populations. Although being quite differently scented (total emitted compounds) in general, populations 5 and 6, e.g. were very similar in their pattern of lilac aldehyde stereoisomers. While plants of population 5 emitted high amounts of veratrole, the single plant studied in population 6 emitted high amounts of phenylacetaldehyde. Further, populations 4 and 15 were similar in their pattern of lilac aldehyde isomers, but population 4 was dominated by phenylacetaldehyde, and population 15 emitted almost exclusively lilac aldehyde, and no phenylacetaldehyde at all. On the other hand, populations 2 and 3 were similar in their scent with high amounts of phenylacetaldehyde, benzyl acetate, and lilac aldehyde, though they were somewhat different in their patterns of lilac aldehyde stereoisomers.

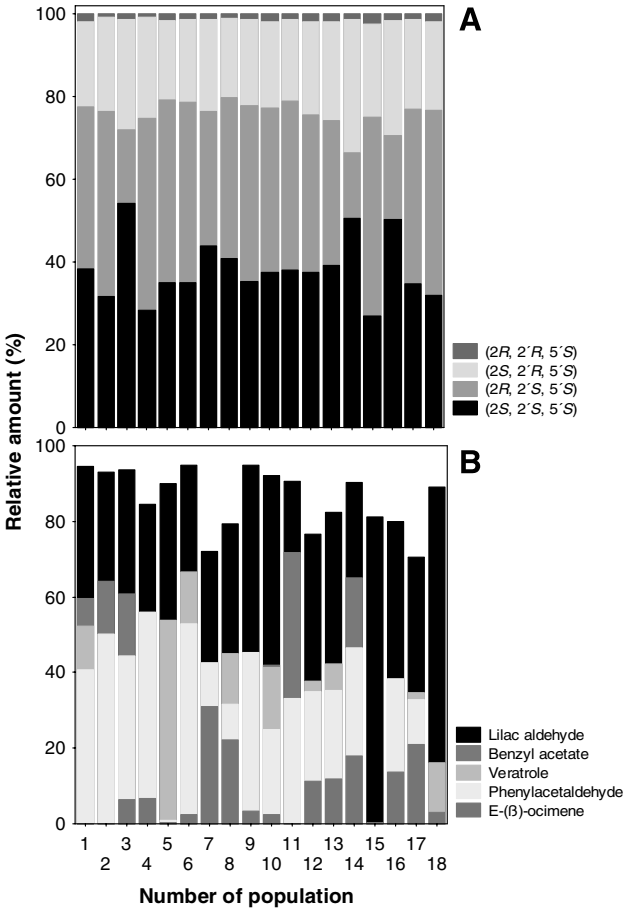

Fig. 4. Mean relative amounts of the (A) individual stereoisomers of lilac aldehyde and (B) the most dominant compounds (reaching a relative amount of 20% at least in one sample) in the 18 studied populations. The population numbers correspond to the populations presented in Fig. 1.

3. Discussion

Many flower scent compounds have chiral centres, and are optically active (Ohloff, 1994). Compounds with stereogenic centres are often found within isoprenoids, and especially monoterpenoids are known to be present in essential oils/flower scents in specific enantiomeric proportions, which may serve as "fingerprint" (König and Hochmuth, 2004). Such specific enantiomeric proportions may by used by insects, such as nursery pollinators, for host-plant finding or recognition.

However, in most studies on flower scent the stereoisomeric configuration is not determined (but see for exceptions, e.g. Kaiser and Nussbaumer, 1990; Borg-Karlson et al., 1994, 1996; Bartschat et al., 1995), though it is known to play a role at least in specialised pollination systems (Ayasse et al., 2003). From electrophysiological studies it is known that insects differently respond to different enantiomers of a specific compound (e.g. Stranden et al., 2002, 2003), and further, studies on female sex pheromones of insects show that chirality is an important factor for chemical communication within and between species (Tumlinson et al., 1977; Leal, 1996). As for insects, chiral-

ity also plays a role for human beings, and it is known that the sense of smell in humans is enantio-selective. A prominent example is the smell of the carvone stereoisomers. (+)-Carvone smells caraway-like and (−)-carvone has a sweet spearmint odour (Abate et al., 2004). The lilac aldehyde stereoisomers also seem to have different smells to human beings (Kreck and Mosandl, 2003).

Here, we compared the variability of isomeric pattern of lilac aldehyde with variability of total emitted scent in different populations of *S. latifolia*, and found comparatively low variability in isomeric pattern and high variability in total emitted scent. Further, the ratio of stereoisomers was independent from the geographic origin of the plants, and there was no correlation between the total flower scent and the pattern of lilac aldehyde isomers. These results indicate that the ratios of the four different lilac aldehyde isomers occurring in *S. latifolia* are a more conservative trait compared to the total scent, making the proportions of isomers a more reliable cue for host-plant detection by *H. bicruris*.

In all samples four of the eight conceivable stereoisomers were found, and all of them were (5′*S*)-configured. Recently, it was shown that the four (5′*R*)-configured stereoisomers occur in flower scents of different plants. However, the four (5′*S*)-configured isomers are more often found compared to the four (5′*R*)-configured isomers (Dötterl et al., 2006a). In *Syringa vulgaris*, an enantiomeric high purity of linalool with *C*-3 (*S*)-configuration leads solely to the four (5′*S*)-configured isomers, while the metabolism of linalool leading to lilac aldehyde shows a low enantioselectivity. In this plant (*S*)-linalool is enzymatically hydroxylated to 8-hydroxylinalool, followed by an oxidation step leading to 8-oxolinalool, which is finally cyclised to the four (5′*S*)-configured lilac aldehyde stereoisomers (Kreck et al., 2003). This biosynthetic route is also proposed for the other lilac aldehyde producing plants. The cyclisation enzyme responsible for the last step of biosynthesis seems to be responsible for the different patterns of lilac aldehyde stereoisomers observed within *S. latifolia* in the present study (it seems that this enzyme is exerting limited control on the cyclisation process, and allows some variability in isomeric pattern), and between the different plant species studied by Dötterl et al. (2006a). However, nothing is known about the structure of this enzyme, and it would be worthwhile to isolate it, and to identify the gene responsible for expression to be able to understand the genetic basis responsible for variability of isomeric patterns within and between plant species.

The isomeric pattern of lilac aldehyde as well as the total scent profile was independent from geographic origin. Western populations were not separable from the eastern populations. This result is in contrast to studies analysing the geographic variation of morphological and/or genetical characters of *S. latifolia* in Europe (Prentice, 1979; Mastenbroek et al., 1983, 1984; Prentice et al., 1984; Vellekoop et al., 1996). Western and eastern races (clusters) of *S. latifolia* were found in all these analyses.

The small variability in isomeric pattern of lilac aldehyde within *S. latifolia* even becomes more clear when comparing

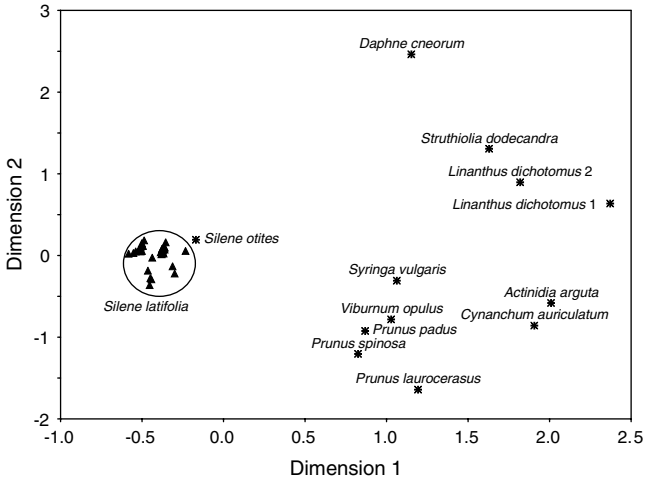

Fig. 5. Nonmetric multidimensional scaling (stress = 0.03) of lilac aldehyde pattern of 37 studied specimen of *S. latifolia* and of 11 further species containing solely (5′*S*)-configured isomers (from Dötterl et al., 2006a) based on the CNESSm8 index. Two specimen of *Linanthus dichotomus* were included.

isomeric pattern of the 37 specimens of *S. latifolia* studied here with the isomeric pattern found in specimens of 11 other plant species studied (Dötterl et al., 2006a), all containing the four (5′*S*)-isomers. The ratio found in the other plants was quite different to the ratio found in *S. latifolia*, and therefore the samples of *S. latifolia* are quite separated from the samples of the other species in Fig. 5. Only in the closely related *S. otites* was the pattern of stereoisomers comparable to the pattern found in *S. latifolia,* with high amounts of stereoisomers (2*S*, 2′*S*, 5′*S*) and (2*R*, 2′*S*, 5′*S*). However, there was still a difference in the samples of both species: in all samples of *S. latifolia* isomer (2*S*, 2′*R*, 5′*S*) was found in higher amounts than in the *S. otites* sample.

Of special interest is the comparison of isomeric pattern in *S. latifolia* with isomeric pattern in *Silene vulgaris*. Both plant species are very similarly scented (Jürgens et al., 2002), grow sympatrically, and often in the same habitat. However, *H. bicruris* is strongly preferring *S. latifolia* over *S. vulgaris* as larval host-plant (e.g. Steiner and Ebert, 1998; Dötterl, unpublished data). The isomeric pattern of both plants are quite different (Dötterl et al., 2006a): in contrast to *S. latifolia*, all eight stereoisomers occur in *S. vulgaris*. *Hadena bicruris* can electrophysiologically detect all eight stereoisomers (Dötterl et al., 2006a), and may use the different isomeric patterns for discrimination between *S. latifolia* and *S. vulgaris*. Empirical data are now needed to test our hypothesis that *H. bicruris* uses the specific ratio of lilac aldehyde stereoisomers for host-plant recognition.

4. Experimental

4.1. Plant material and volatile collection

Floral scent was collected from 37 specimens of 18 different populations of *S. latifolia* Poiret ssp. *alba* (Miller)

Greuter & Burdet (=*S. alba* L.) (Fig. 1). Seeds of the different populations were provided by several botanical gardens. The plants were grown in the greenhouse for ca. 8 weeks until they built up a rosette, and were then placed in a flower bed. Vouchers of all studied populations are housed in the University of Bayreuth (UBT). For each sample floral scent was collected from 10 to 40 flowers of single plants for ca. three hours using dynamic headspace methods. The living flowers were enclosed in glass cylinders and the emitted volatiles were trapped in an adsorbent tube (pasteur pipette, filled with 100 mg of a 1:1 mixture of Tenax-TA 60–80 and Carbotrap 20–40). Air was sucked from the glass cylinder over the trap by means of a membrane pump (G12/01 EB, Rietschle Thomas, Puchheim, Germany). Samples were collected at night, when *S. latifolia* is emitting most of its floral volatiles (Jürgens et al., 2002; Dötterl et al., 2005). Volatiles were eluted with 200–300 µl of acetone (SupraSolv, Merck, Germany).

4.2. Chemical analyses

4.2.1. GC–MS

The composition of the floral scent solvent extracts (1 µl per sample) was analysed on a Varian Saturn 2000 mass spectrometer and a Varian 3800 gas chromatograph fitted with a 1079 injector (Varian Inc., Palo Alto, USA). The injector split vent was opened (1:10) and the injector heated initially at 150 °C. The injection temperature increased during 30 s to 250 °C and was held for 2 min. A ZB-5 column was used for the analyses (length 60 m, 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 2 min at 40 °C, then increased with 5 °C min^{-1} to 240 °C and held for 3 min. The MS interface was heated to 260 °C and the ion trap worked at 175 °C. The mass spectra were taken at 70 eV with a scanning speed of 1 scan s^{-1} from *m/z* 40–350.

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 data base, and confirmed by comparison of retention times with published data (Adams, 1995), or by comparison of both mass spectrum and GC retention data with those of authentic standards. For statistical analyses (see below) relative amounts of compounds (%) were used because there was high variability in the total emitted amount of floral scent (see also Dötterl et al., 2005).

4.2.2. Enantio-MDGC–MS

The lilac aldehyde isomers were analysed with a Siemens SiChromat 2–8, with two independent column oven programs and a live T-switching device, coupled to the transfer line of a Finnigan MAT GCQ, using an open split interface.

GC conditions were as follows. Pre column: self prepared fused silica capillary (30 m × 0.23 mm i.d.), coated with a 0.23 µm film of SE 52; carrier gas helium, 1.85 bar; injector temperature 250 °C; detector FID 250 °C. Main column: self-prepared fused silica capillary (30 m × 0.23 mm i.d.), coated with a 0.23 µm film of 4‰ heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (DIME-β-CD) (30%) in SE 52 (70%), carrier gas helium, 1.45 bar, MS-detector; transfer line temperature 250 °C, open split interface 250 °C; helium sweeping flow 1 ml min^{-1}; ion trap manifold 200 °C; EI 70 eV; oven temperature program: precolumn: 60 °C (5 min isothermal), raised at 3 °C min^{-1} to 250 °C (30 min isothermal); main column temperature program: 60 °C (25 min isothermal), raised at 1.5 °C min^{-1} to 200 °C. Cut times: lilac aldehyde 26.5–28.0 min. Data acquisition were done with the Xcalibur instrument software (Finnigan).

4.3. Statistical analyses

Kruskal–Wallis-ANOVA (hence KW-ANOVA) was used to compare the mean relative amounts of the four stereoisomers occurring in floral odour of *S. latifolia*, and to compare variability (dissimilarity) in isomeric pattern of lilac aldehyde and in whole floral scent of *S. latifolia*. The Tukey–Kramer test for nonparametric data, provided also in the STATISTICA package (StatSoft Inc., 2004), was used as a post hoc test.

The quantitative dissimilarity index CNESS (see also Trueblood et al., 1994; Dötterl et al., 2005) was calculated using COMPAH96 privided by Gallagher at UMASS/Boston (http://alpha.es.umb.edu/faculty/edg/files/edgwebp.htm) to determine differences in patterns of lilac aldehyde stereoisomers between the different samples or between the different populations under study. For the latter analysis, mean relative amounts of stereoisomers were determined and used for calculation of CNESS, if more than one sample per population was available.

CNESS was also used to determine differences in total floral scent between the different samples or populations of *S. latifolia*, and to determine differences in ratios of lilac aldehyde stereoisomers between *S. latifolia* samples and samples of 11 other plants emitting the same four isomers as *S. latifolia* (see Dötterl et al., 2006a). Then nonmetric multidimensional scaling (NMDS) in the STATISTICA package (StatSoft Inc., 2004) was used to detect meaningful underlying dimensions and to visualise similarities between samples (see Borg and Lingoes, 1987).

The significance of differences in isomeric pattern of lilac aldehyde and total floral scent (where lilac aldehyde stereoisomers were pooled) between different populations was assessed by one-way ANOSIM (Clarke and Gorley, 2001; Clarke and Warwick, 2001) with 100,000 random permutations. For these analyses, the rank similarities within populations are compared to the rank similarities between populations.

RELATE analyses (Clarke and Gorley, 2001; Clarke and Warwick, 2001) were performed to test for correlation of stereoisomeric patterns of lilac aldehyde (CNESSm1 matrix) and patterns of total emitted scent compounds

(CNESSm1 matrix) as well as for the scent matrices and the distance matrix (km) of populations.

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