Biochemical Systematics and Ecology 55 (2014) 322–328

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

# Comparison of floral scent between and within *Buddleja fallowiana* and *Buddleja officinalis* (Scrophulariaceae)

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ARTICLE INFO

*Article history:*
Received 4 December 2013
Accepted 29 March 2014
Available online 4 May 2014

*Keywords:*
*Buddleja*
Intra-specific
Floral scent
GC–MS
Hybridization

ABSTRACT

Floral scents are important olfactory signals for communication between plants and pollinators. Several studies have focused on inter-specific variation of floral scents, but little is known about the intra-specific variation, especially in some polychromic species. In this study, we investigated the floral scent compositions of *Buddleja fallowiana* and *Buddleja officinalis in situ* by dynamic headspace collection and coupled GC–MS. Variations of scent compositions within and between populations as well as among species were compared. In spite of substantial intra- and inter-population variability, *B. fallowiana* and *B. officinalis* were clearly differentiated in their scent profiles. In *B. fallowiana*, obvious differentiation was found between studied populations, while all investigated populations in *B. officinalis* are part of a metapopulation. These high intra-specific variations are discussed in relation to the introgression through hybridization and founder effects from different populations.


## 1. Introduction

Floral scent is an important olfactory cue for communication between flowering plants and their pollinators (Raguso, 2008), and several studies have been conducted to identify species-specific scent compositions that are attractive to visitors (Schlumpberger and Raguso, 2008; Shuttleworth and Johnson, 2009). Studies on intra-specific variations of floral scent compositions have been conducted on a limited number of plant species (Knudsen, 2002; Suinyuy et al., 2012). In a few cases, no intra-specific differences in floral scent were found (Knudsen, 2002). However, in most cases, high intra-specific variations in floral scent have been reported (Moya and Ackerman, 1993; Azuma et al., 2001; Suinyuy et al., 2012). Generally, intra-specific variation has been notably explained by hypotheses such as relaxed selective pressure, genetic drift, introgression of scent traits through hybridization, gene pleiotropic effects, or phenotypic plasticity (Raguso, 2008; Majetic et al., 2010). The exploration of intra-specific variability in floral scent may supply important information to the evolution and diversification of plant taxa.

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http://dx.doi.org/10.1016/j.bse.2014.03.029
0305-1978/

*Buddleja* L. (Scrophulariaceae) comprises about 100 species, most of them are native to the tropical and temperate zones, including Asia, Africa, and America (Leeuwenberg, 1979; Norman, 2000). In Asia, most species of *Buddleja* exhibit polychromic inflorescences with a strong floral scent (Li and Leeuwenberg, 1996). Floral color and odor are the dominant pollinator attractants to flowers, and the emission of floral volatiles is closely related to the variations of floral color because of the shared biochemical pathway (Delle-Vedove et al., 2011). Furthermore, floral volatile compositions have been reported to vary according to changes in floral color for numerous plant taxa (Delle-Vedove et al., 2011). However, variability in floral odor in polychromic *Buddleja* species has not been studied.

In this study, scent variations within and between populations and among species of *Buddleja fallowiana* Balf.f. & W.W.Sm. and B. officinalis Maxim. were analyzed. Both *B. fallowiana* and B. officinalis could emit strong floral odor, with notable intraspecific color variation according to our field investigations. Hand-pollination revealed that both *B. fallowiana* and *Buddleja officinalis* are self-incompatible (Wei-Chang Gong et al., unpublished data), suggesting that they are actually pollinator-dependent. In addition, *B. fallowiana* and *B. officinalis* flower in different seasons with distinct corolla colors, lavender pink and purple, respectively. We here focused on floral scent composition and variation between and within *B. fallowiana* and *B. officinalis* to characterize scent profiles and to determine variation extent and pattern within the two species.

## 2. Materials and methods

### 2.1. Species and study area

Both *B. fallowiana* and *B. officinalis* are polychromic, and they are mainly distributed in the Sino-Himalayan region in Asia, flowering in late summer and early spring, respectively. Floral scent of *B. officinalis* was collected from 4 February to 29 March 2011 at five different sites in Yunnan province. Floral scent of *B. fallowiana* was collected from 17 July to 15 August 2011 at three different sites mainly at Lijiang, Yunnan province, southwest of China. Detailed information is listed in Table 1. At each site, 2–5 plant individuals with distinct floral color were sampled. Nine individuals of *B. fallowiana* were sampled in total and 14 individuals for *B. officinalis*. Three to eight different inflorescences from the same plant individual were collected as a sample. Floral scents were collected using dynamic headspace adsorption method during daytime. All studies described herein were conducted in natural populations that generally occurred near the road. Vouchers specimens of each species were deposited at the Herbarium of the Kunming Institute of Botany (KUN), the Chinese Academy of Sciences, China.

### 2.2. Collection of floral scent

Inflorescence of *B. fallowiana* and *B. officinalis* during peak flowering were enclosed in a modified vacuum dryer. The inflorescence peduncles were covered with absorbent cotton soaked in a 10% sucrose solution. The scent-containing air was sucked through a glass cartridge containing adsorbent Porapak Q (150 mg, mesh 60/80, waters Associates, Inc.) for 3–5 h (depending on the relative strength of the scent to the human nose) using a pump with an outlet flow rate of 350 ml/min$^{-1}$. The ambient air pumped into the vacuum dryer was purified with activated carbon. To identify background contamination, ambient air (purified air) was collected as a control.

The adsorbed scent components were eluted with ~0.8 ml dichloromethane and collected in a 1.5 ml Agilent vial. In each sample, 4320–7200 ng *n*-nonane was added as an internal standard (IS) for quantification. The vials were kept in a freezer (−20 °C) until analysis. After odor collecting, the total numbers of open flowers in each sample were counted.

### 2.3. Chemical and data analysis

The extracts were analyzed by coupled gas chromatography and mass spectrometry (GC–MS). Samples were analyzed by using an Agilent Technologies HP 6890 gas chromatograph, equipped with a HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness), and linked to a HP5973 mass spectrometer. Helium (He) was used as a carrier gas at a flow of 1 ml/min$^{-1}$, and the injector temperature was set to 250 °C. The column temperature was first set at 40 °C, and then programmed to 250 °C at a rate of 3 °C/min$^{-1}$.

**Table 1**
Study sites of *B. fallowiana* and *B. officinalis* from the Sino-Himalayan region.

| Population | Location | Geographic coordinate | Elevation (m) | Population size (no. of plants) | Voucher numbers |
|---|---|---|---|---|---|
| *B. officinalis* | | | | | |
| OF1 | Lijang | N26°52′44.21″; E100°13′51.52″ | 2421 | >50 | GWC-BU-OFLJ-001 |
| OF2 | Lijang | N27°08′23.45″; E100°03′17.7″ | 1822 | >50 | GWC-BU-OFLJ-005 |
| OF3 | Yimen | N24°44′41″; E102°11′15″ | 1786 | >50 | GWC-BU-OFYM-001 |
| OF4 | Kunming | N24°56′42.04″; E102°38′31.79″ | 1896 | >100 | GWC-BU-OFKM-001 |
| OF5 | Kunming | N25°03′41″; E102°42′11″ | 1902 | >100 | GWC-BU-OFKM-012 |
| *B. fallowiana* | | | | | |
| FA1 | Lijiang | N27°0′47.10″; E100°15′18.87″ | 2667 | >10,000 | GWC-BU-FALJ-001 |
| FA2 | Lijiang | N27°10′13.27″; E100°15′42.16″ | 3170 | >50 | GWC-BU-FALJ-023 |
| FA3 | Daju | N27°15′03.32″; E100°14′38.89″ | 2680 | >50 | GWC-BU-FADJ-001 |

Compounds were identified by comparing their retention times (RT) and mass spectra with those of authentic compounds, or by comparisons with MS spectra from the Wiley 7 n.1 mass spectral library and the associated retention indexes reported both in the NIST Chemistry Web Book (http://webbook.nist.gov) and in the RI database (Adams, 2001). Proportional abundance of compounds (relative amounts with respect to aggregate peak areas, excluding contaminants) of the floral scents was calculated based on the absolute amounts of the internal standard compound.

To characterize floral scent dissimilarity among samples, a non-metric Multi-Dimensional Scaling (nMDS) ordination was performed based on a matrix of Euclidean dissimilarity calculated on the relative amount of odor compounds (in % of the total blend). The stress value generated by the nMDS analysis reflects how well the ordination summarizes the observed distances among the samples. The dissimilarity of floral scents was also quantified through one-way SIMPER analysis in PAST (Version 2.08). In addition, data were rigorously compared using a one-way analysis similarity (ANOSIM; maximum permutations =1000) to determine if differences in similarities occurred among all samples, and the Bray–Curtis similarity matrix was created.

Finally, the contribution of each compound among individuals was determined by using Principal Component Analysis (PCA) based on the relative amount of each scent compound in PAST (Version 2.08). The variance-covariance matrix of the floral scents was used. The Jolliffe cut-off value gives an informal indication of how many principal components should be considered significant (Jolliffe, 1986).

## 3. Results and discussion

Chemical compositions of inflorescence odor emitted by *B. fallowiana* and *B. officinalis* are given in Table 2. In total, 55 different volatile compositions were identified from these two *Buddleja* species, including fatty acid derivatives (28), benzenoids (4), and terpenoids (23). In *B. fallowiana*, 41 different scent compounds were identified, while only 33 compounds were found in *B. officinalis*. Eighteen compounds were shared by both species (Table 2). Most of these volatile compounds found in the extracts have been recorded from other angiosperms (Knudsen et al., 2006). Terpenoids were the most dominant class of compounds in *B. fallowiana* and *B. officinalis* and contributed 75.1 ± 3.6% and 52.0 ± 6.3% of the total blend, respectively. Only seven compounds had large relative amounts over 10% (four compounds for *B. fallowiana* and three for *B. officinalis*), while the majority of the compounds were present at levels lower than 10% (Table 2).

### 3.1. Inter-specific variation in floral scent

To characterize floral scent profile of *B. fallowiana* and *B. officinalis*, multivariate analyses was performed on the relative amount of compositions for each individual sample in the two species. The euclidean nMDS analysis of the inflorescence odor between the two species showed a separation of floral scents, because of high linear and non-metric fits ($R^2 = 0.79$ and 0.34, respectively) and a low stress value (0.09; Fig. 1). This divergence between *B. fallowiana* and *B. officinalis* was confirmed by the results of one-way ANOSIM analysis ($R = 0.87$, $P$(0.001). Inter-specific dissimilarity of floral scent compositions is 83.5% (Fig. 1), and seven different compounds are mainly responsible for this inter-specific dissimilarity of floral scent, including benzaldehyde, linalool, *trans*-β-ocimene, lilac aldehyde, 4-oxoisophorone, *cis*-β-ocimene, and α-farnesene (see Table S1). Furthermore, *B. fallowiana* emitted large amounts of 4-oxoisophorone (21.7 ± 2.1%), *trans*-β-ocimene (19.8 ± 4.3%), *cis*-β-ocimene (13.9 ± 3.2%) and α-farnesene (11.5 ± 4.2%; Table 2). In contrast, benzaldehyde (28.8 ± 5.1%), linalool (25.4 ± 7.7%), and lilac aldehyde (15.6 ± 1.9%) characterized the floral scent profile of *B. officinalis* (Table 2).

In *B. officinalis*, the large emissions of 4-oxoisophorone, α-farnesene, *trans*-β-ocimene and *cis*-β-ocimene have been repeatedly found in plant species pollinated by butterflies and bees (Svensson and Bergström, 1977; Andersson, 2003; Mant et al., 2005; Dötterl and Schäffler, 2007). Likewise, benzaldehyde, linalool and lilac aldehyde in *B. officinalis* have been reported in butterfly or moth pollinated species (Schulz et al., 1993; Andersson, 2003; Dötterl et al., 2006). Floral scent is considered an important target of pollinator-mediated selection, and that the large emissions of specific compositions are related to their predominant pollinators (Parachnowitsch et al., 2012). These potential insect visitors in *B. fallowiana* and *B. officinalis* are consistent with two years of field observations (Wei-Chang Gong et al., unpublished data), implying that inter-specific differences in floral scent were needed to attract distinct visitor fauna. This further supported our hypothesis that floral scents, especially the compositions with large relative amounts in the *Buddleja* species, can successfully predict their potential pollinators (Gong et al., 2014).

### 3.2. Intra-specific variation in floral scent

The multivariate analysis (nMDS) performed on floral scents emitted by *B. fallowiana* showed that all individuals within a population clustered together (Fig. 1), suggesting scent differentiation among the studied populations. The results of ANOSIM further confirmed the significant population differentiation in *B. fallowiana* (ANOSIM similarity, $R = 0.65$, $P = 0.005$). Moreover, one-way SIMPER analysis quantified 49.5% of the inter-population dissimilarity in *B. fallowiana* (Fig. 1). Four major compounds primarily accounted for the population variation: *trans*-β-ocimene, α-farnesene, *cis*-β-ocimene and 4-hydroxy-4-methylpentan-2-on (Table S1). In general, population differentiation may arise through selective pressures by pollinators on distinct scent compounds and may represent an adaptation to local pollinators (Tollsten and Bergström, 1993; Dötterl et al., 2005). However, this does not seem to be the case in *B. fallowiana*, since the high intra-population variability (e.g., 28.1% in *B. fallowiana*; Fig. 1) contrasted the idea of adaptation to a particular local pollinator fauna (Azuma et al., 2001).

**Table 2**
Mean relative amount (%) of floral scent compounds from *B. fallowiana* and *B. officinalis*.

| Compounds | CAS | RI | *B. fallowiana* | *B. officinalis* |
|---|---|---|---|---|
| Fatty acid derivatives | | | 18.8 ± 4.2 | 14.9 ± 6.7 |
| 3-Hydroxy-2-butanone | 513-86-0 | 684 | 0.3 ± 0.2 | 0.1 ± 0.1 |
| Heptane | 142-82-5 | 699 | 0.2 ± 0.1 | – |
| 2,3-Heptanedione | 96-04-8 | 841 | 0.3 ± 0.1 | – |
| n-Butyl ether | 142-96-1 | 860 | – | 1.4 ± 1.1 |
| Hexanal | 66-25-1 | 893 | 0.2 ± 0.1 | 0.8 ± 0.2 |
| Isoamyl acetate | 123-92-2 | 903 | – | 1.5 ± 0.8 |
| 2,2,4,6,6-Pentamethylheptane | 13475-82-6 | 930 | 1.1 ± 0.8 | – |
| Butyl acrylate | 141-32-2 | 932 | – | 1.5 ± 1.2 |
| Butyl acetate | 123-86-4 | 935 | – | 0.2 ± 0.2 |
| 2,6-Dimethyl-4-heptanol | 108-82-7 | 944 | 1.7 ± 0.7 | – |
| 3-Methyl-1-butanol | 123-51-3 | 953 | 0.1 ± 0.1 | 2.7 ± 0.8 |
| Butyl propionate | 590-01-2 | 956 | – | 0.8 ± 0.6 |
| 2-Methyl-1-butanol | 137-32-6 | 965 | – | 0.1 ± 0.1 |
| 1-Octen-3-ol | 3391-86-4 | 998 | 0.5 ± 0.3 | – |
| 3-Octanone | 106-68-3 | 1006 | 0.2 ± 0.2 | – |
| 6-Methyl-5-hepten-2-one | 110-93-0 | 1006 | 0.3 ± 0.1 | 0.1 ± 0.0 |
| 4-Hydroxy-4-methylpentan-2-on | 123-42-2 | 1018 | 6.7 ± 2.1 | 1.6 ± 0.5 |
| Octanal | 124-13-0 | 1027 | – | 0.2 ± 0.2 |
| 3-Hexen-1-ol, acetate | 1708-82-3 | 1033 | 0.2 ± 0.1 | 0.1 ± 0.1 |
| Hexyl acetate | 142-92-7 | 1043 | 0.1 ± 0.1 | – |
| Isoundecane | 34464-43-2 | 1051 | 0.6 ± 0.6 | – |
| 2-Ethyl-1-hexanol | 104-76-7 | 1064 | 0.7 ± 0.7 | – |
| 1-Hexanol | 111-27-3 | 1107 | 0.3 ± 0.2 | – |
| Dodecane | 112-40-3 | 1199 | 2.6 ± 1.1 | – |
| Tridecane | 629-50-5 | 1298 | 0.2 ± 0.2 | – |
| 2-Decenoic acid | 3913-85-7 | 1341 | – | 1.5 ± 1.5 |
| Tetradecane | 629-59-4 | 1398 | 2.6 ± 0.6 | – |
| 5-Methyl-Tetradecane | 25117-32-2 | 1459 | 0.1 ± 0.1 | – |
| **Benzenoids** | | | 6.1 ± 1.5 | 33.1 ± 6.7 |
| Phenethylene | 100-42-5 | 884 | 0.5 ± 0.5 | – |
| Benzaldehyde | 100-52-7 | 1055 | 4.4 ± 1.4 | 28.8 ± 5.1 |
| Phenylacetaldehyde | 122-78-1 | 1083 | 0.7 ± 0.3 | 4.1 ± 1.7 |
| Phenethyl alcohol | 60-12-8 | 1168 | 0.4 ± 0.3 | 0.2 ± 0.2 |
| **Terpenoids** | | | 75.1 ± 3.6 | 52.0 ± 6.3 |
| α-Pinene | 80-56-8 | 930 | 0.8 ± 0.4 | 0.2 ± 0.1 |
| Camphene | 79-92-5 | 944 | – | 0.1 ± 0.1 |
| β-Myrcene | 123-35-3 | 989 | 0.5 ± 0.2 | 0.03 ± 0.02 |
| Limonene | 138-86-3 | 1028 | 0.2 ± 0.2 | – |
| *cis*-β-Ocimene | 3338-55-4 | 1040 | 13.9 ± 3.2 | 0.4 ± 0.1 |
| Eucalyptol | 470-82-6 | 1047 | 0.1 ± 0.1 | – |
| *trans*-β-Ocimene | 3779-61-1 | 1051 | 19.8 ± 4.3 | 0.8 ± 0.5 |
| *cis*-Linalol oxide (furan isomer) | 5989-33-3 | 1073 | – | 0.1 ± 0.1 |
| *trans*-Linalool oxide (furanoid) | 34995-77-2 | 1088 | – | 0.5 ± 0.2 |
| Linalool | 78-70-6 | 1103 | 0.1 ± 0.1 | 25.4 ± 7.7 |
| 4,8-Dimethylnona-1,3,7-triene | 19945-61-0 | 1117 | 1.4 ± 0.6 | – |
| neo-Allooctmene | 7216-56-0 | 1125 | 0.3 ± 0.2 | – |
| 1,3,3-Trimethyl-7-oxabicyclo[4,1,0]heptane-2,5-dione | 38284-11-6 | 1132 | 1.5 ± 0.4 | 1.4 ± 0.3 |
| Lilac aldehyde | 67920-63-2 | 1137 | 0.5 ± 0.4 | 15.6 ± 1.9 |
| 4-Oxoisophorone | 1125-21-9 | 1142 | 21.7 ± 2.1 | 8.6 ± 1.7 |
| 2,2,6-Trimethyl-1,4-cyclohexanedione | 20547-99-3 | 1165 | 1.8 ± 0.5 | – |
| Lilac alcohol A | 33081-34-4 | 1193 | – | 0.9 ± 0.2 |
| β-Cyclocitral | 432-25-7 | 1200 | 0.8 ± 0.3 | 0.1 ± 0.04 |
| *trans*-2-Decenal | 3913-81-3 | 1230 | – | 0.1 ± 0.1 |
| β-Caryophyllene | 87-44-5 | 1419 | 0.1 ± 0.1 | – |
| *cis*-Thujopsene | 470-40-6 | 1433 | – | 0.1 ± 0.1 |
| 6,10-Dimethyl-5,9-undecadien-2-one | 689-67-8 | 1451 | 0.1 ± 0.1 | – |
| α-Farnesene | 502-61-4 | 1509 | 11.5 ± 4.2 | 0.1 ± 0.1 |

"–", Not detected. Retention index (RI) reference according to HP-5MS column data in NIST Chemistry WebBook (http://webbook.nist.gov) and published RI values in R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Illinois, USA, 2001.

*Buddleja officinalis* showed a different pattern of intra-specific variation in the floral scent. The results of one-way SIMPER showed 50.0% inter-population dissimilarity, larger than that in *B. fallowiana* (Fig. 1). Linalool and benzaldehyde mainly accounted for this population dissimilarity (Table S1), while also making an important contribution to individual differentiation (Fig. 2). However, results of ANOSIM indicated that there was not a significant population differentiation in *B. officinalis* (ANOSIM similarity, $R = 0.14$, $P = 0.20$), and the results of nMDS also showed that all individual samples among different

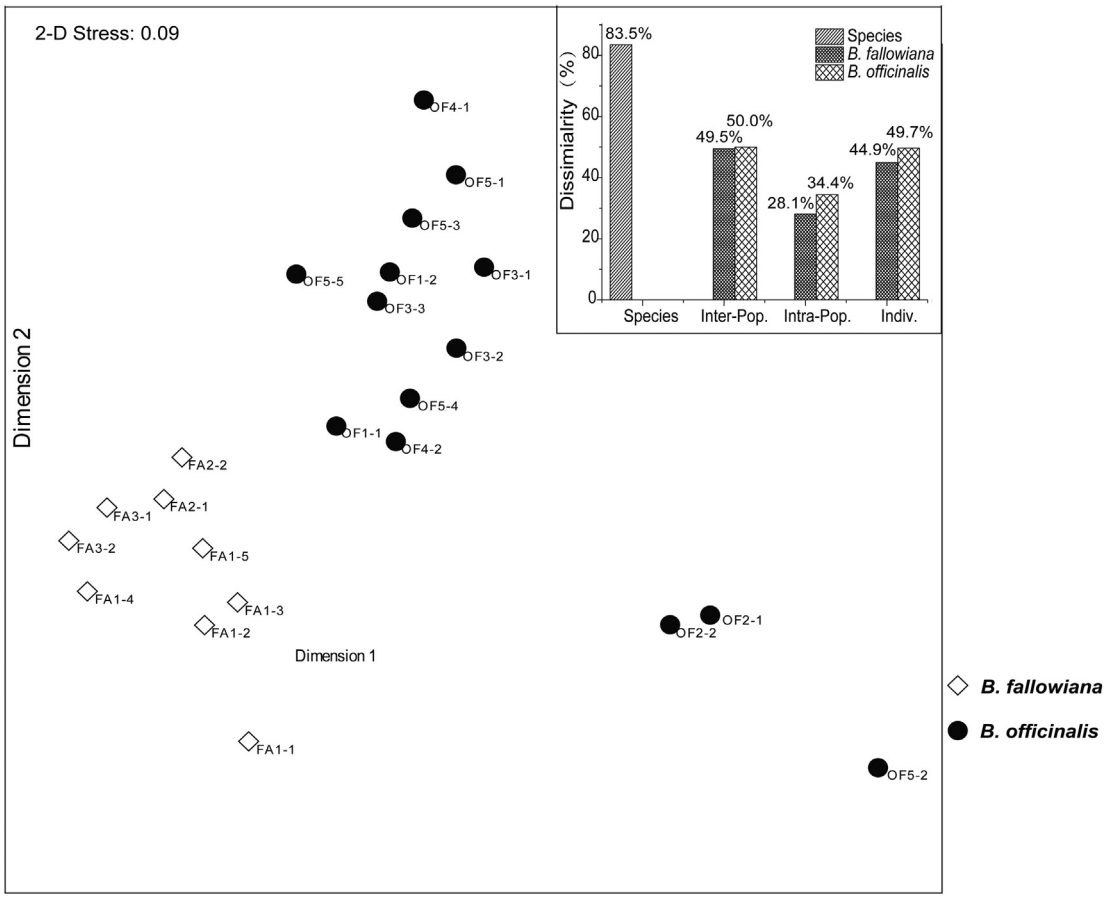


**Fig. 1.** nMDS (non-metric Multi-Dimensional Scaling) based on Euclidean similarities of scent composition from *B. fallowiana* (FA) and *B. officinalis* (OF). Inserted graph shows the dissimilarity level among samples calculated through one-way SIMPER.

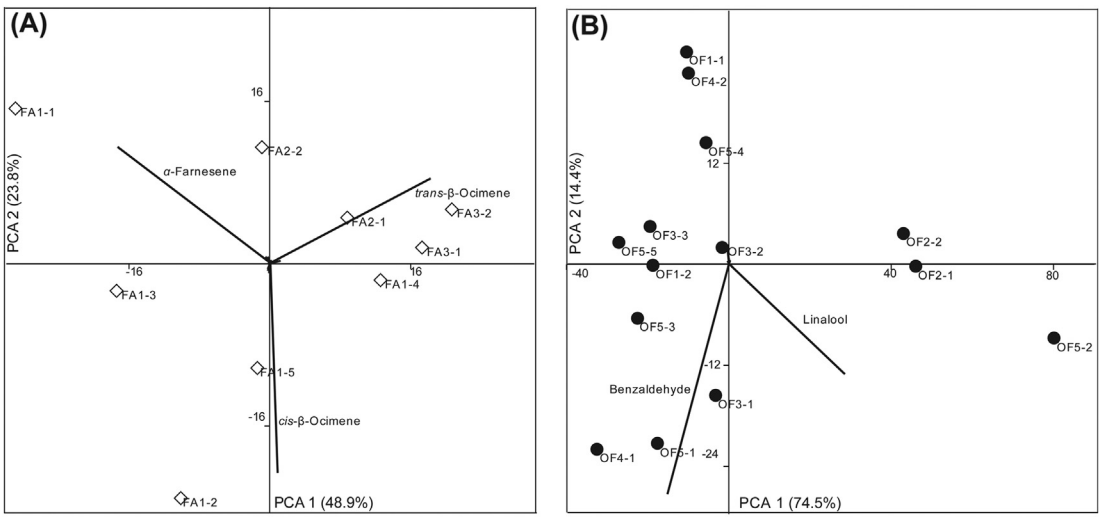


**Fig. 2.** PCA (Principal Component Analysis) biplot based on floral scent data of two *Buddleja* species investigated in the Sino-Himalayan region. (A) *B. fallowiana*, (B) *B. officinalis*.

populations were discrete (Fig.1). This suggested that all the investigated populations are part of a large metapopulation. In spite of high population dissimilarity in floral volatiles, we found that individuals of *B. fallowiana* and *B. officinalis* clustered in species groups with high individual variations (Fig. 1). Lack of population differentiation in *B. officinalis* may be attributed to gene flow among populations, which is occurring or has occurred recently.

To study intra-specific variation in detail, floral scent variations at individual level were also examined in the two species. Our results showed that individual dissimilarity within *B. fallowiana* and *B. officinalis* could reach to 44.9% and 49.7%, respectively (Fig. 1). According to the results of PCA, individuals within a species could be classified into distinct scent-types (Fig. 2). For example, individuals were characterized separately by *trans*-β-ocimene, α-farnesene, and *cis*-β-ocimene in *B. fallowiana*; linalool and benzaldehyde in *B. officinalis* (Fig. 2). In addition, Chen and Sun (2011) reported that butyl ester acetic acid (81.6%) was a major floral scent in *B. officinalis* individuals. The present study of intra-specific variation in floral scent compositions, especially the high inter-individual variations is among the largest ever performed. Thus, floral scent in *B. fallowiana* and *B. officinalis* might not be good to use as a taxonomic trait. In fact, this high scent variation among sampled individuals is often regarded as a by-product of hybridization and subsequent introgression, and a predominant mechanism proposed to explain scent variability (Tollsten and Bergström, 1993).

### 3.3. Geographic variation in floral scents of polychromic Buddleja species

Floral scent may vary depending on the number, composition, relative amounts of different compounds, and their temporal and spatial emission patterns in a given plant species (Knudsen et al., 2006; Suinyuy et al., 2012), however, such striking intra-specific variations in floral scents have seldom been reported (Azuma et al., 2001; Suinyuy et al., 2012). And the reasons for these large geographic variations of floral scents have not been explored in *Buddleja* species. So far, genetic structures of *B. fallowiana* and *B. officinalis* have not been reported, and genetic differentiation between populations in the two species is likewise unclear. Therefore, we tentatively hypothesized that phenotypic plasticity, shift of pollinator assemblages, introgression of inter-specific hybridization, and founder effect from discrete populations may influence floral scent compositions of the *Buddleja* species. The evidence for each of these hypotheses needs to be investigated in forthcoming work.

In our system, morphological observations suggested that both *B. fallowiana* and *B. officinalis* exhibited a broad floral color range, even within a population. The production of floral scent is involved in floral color due to their shared biochemical pathway (Delle-Vedove et al., 2011). Thus, the varied scent must be associated with this abundant color phenotypic plasticity in both *Buddleja* species. Nonetheless, the relationship between floral odor and color was unclear, and further studies with regard to the relationship are required. Generally, pollinator shift may also lead to floral scent change, while, floral scent variation does not seem to attribute to this in our study because no apparent pollinator differentiation was found among populations (Wei-Chang Gong, personal observation). In genus *Buddleja*, hybridization can easily occur, and play an important role in the occurrence of *Buddleja* species in the Sino-Himalayan region (Dirr, 1998; Norman, 2000; Elliott et al., 2004; Chen et al., 2007). Thus, combined with the high scent variation among individuals within a species, we inferred that the hybridization and subsequent introgression of scent traits contributed greatly to the substantial intra-specific scent variations within *B. fallowiana* and *B. officinalis*. Lastly, *Buddleja* species often distribute along riversides and roads (Li and Leeuwenberg, 1996), and the light weight, winged seeds in both species can also help in long-distance dispersal. Therefore, a given population of *Buddleja* species can have several founders, which may also impact the floral scent compositions in the different populations.

In conclusion, *B. fallowiana* and *B. officinalis* are clearly distinct in their floral scent. High population and individual variations in floral scents were found, with different patterns of intra-specific variation. High intra-specific scent variability may be associated with polychromic floral color, while further work would be required to investigate that relationship. Introgression through hybridization and founder effects from different populations are the primary factors accounting for the high intra-specific scent variation in the two *Buddleja* species.

## Acknowledgments

This study was supported by grants-in-aid from the National Natural Science Foundation of China (NSFC 30970192, 31100177 and NSFC-YUNN U1302262), and West Doctor Foundation of Chinese Academy of Sciences (Y02F7311W1).

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2014.03.029.

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