Flora 206 (2011) 892–895

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.de/flora

Buddleja davidii and *Buddleja yunnanensis*: Exploring features associated with commonness and rarity in *Buddleja*

Gao Chen [a], Hang Sun [b], Weibang Sun [a,*], Eliane Norman [c]

[a] *Kunming Botanical Garden, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204, Yunnan, China*
[b] *Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204, Yunnan, China*
[c] *Department of Biology, Stetson University, Deland, FL 32723, USA*

ARTICLE INFO

Article history:
Received 21 December 2010
Accepted 17 March 2011

Keywords:
Buddleja davidii
Buddleja yunnanensis
Autogamy
Xenogamy
Floral volatiles
Dust seeds

ABSTRACT

Buddleja davidii is a widespread shrub in Asia while *B. yunnanensis* is a narrowly endemic species limited to Yunnan Province, China. To explore whether floral volatiles, morphological characters of flower and seed and breeding system are correlated with their distributions, we measured length and width of corolla, trichome density at corolla throat, level of stigma/anthers relationship, seed size and weight. The results indicated that these characteristics were significantly different between the two species ($P < 0.01$). Bagging experiments revealed that *B. davidii* is a self-incompatible plant while *B. yunnanensis* is self-compatible. Thick trichome density at the corolla throat may reduce out-crossing in *B. yunnanensis*. Autogamy plays an important role in fruit production of this species while *B. davidii* requires pollinators for fruiting. Scents were collected using dynamic headspace adsorption method and identified with coupled gas chromatography and mass spectrometry. In total, 27 floral scent compounds were identified. The volatile composition in the two species was very different. We attempted to determine if these features, associated with commonness and with rarity found in these two taxa, could also help to explain the distribution pattern of other species of the genus *Buddleja*.

Introduction

Buddleja davidii is a widespread plant in large areas of China. It is planted as an ornamental in many temperate countries and became a spreading weed in many of them (e.g. Clay and Drinkall, 2001; Ebeling et al., 2008; Tallent-Halsell and Watt, 2009). By contrast, the congeneric taxon *B. yunnanensis* is confined to a small area of Yunnan, China, where it grows at forest edges and thickets of the mountains (Li and Leeuwenberg, 1996). The two taxa differ in their chromosome numbers (Chen et al., 2007). In their native growing places both species are vital, but *B. yunnanensis* obviously is unable to spread vigorously. We suspected that this might result in part from reproductive peculiarities. The specific objectives of a pertinent study were: (1) to document morphological characteristics of the flowers and seeds of the two taxa; (2) to investigate their mating system and test levels of self-compatibility and probability of selfing; (3) to analyze the differences in their flowers' volatile compounds and (4) to determine how the data obtained in this study might help in understanding their present distributions and the distributions of some other species of the genus *Buddleja*.

Materials and methods

Distribution of B. davidii and B. yunnanensis

We obtained the detailed distribution of the two species using data from the Chinese Virtual Herbarium (http://www.cvh.org.cn/) as well as information from previous studies (Leeuwenberg, 1979; Li and Leeuwenberg, 1996; Tallent-Halsell and Watt, 2009). Experimentally used plants are growing in the Kunming Botanical Garden of Kunming Institute of Botany, The Chinese Academy of Sciences (25°8′48.9″N and 102°44′41.2″E, 1788 m).

Characteristics of flower, seed and breeding system

Fifty randomly selected flowers from 10 plants of each species were used in taking the following measurements: length and width of corolla, trichome density on inner surface of the corolla throat obtained from 10 flowers and seed length from 50 randomly selected mature and full seeds from different individuals (10 seeds per plant). The latter two measurements were made using an Olympus light microscope equipped with ocular micrometer. In order to obtain an average seed weight, 1000 seeds of each species were weighed on an analytical balance. The pollen–ovule ratio (P/O) was estimated following Cruden (1976), using 30 randomly selected flowers from *B. davidii* and *B. yunnanensis*. To test

* Corresponding author. Tel.: +86 871 5223622; fax: +86 871 5223622.
E-mail address: wbsun@mail.kib.ac.cn (W. Sun).

0367-2530/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.flora.2011.05.006

for self-compatibility in each taxon, we used populations growing in the Kunming Botanical Garden of Kunming Institute of Botany, The Chinese Academy of Sciences (25°8′48.9″N and 102°44′41.2″E, 1788 m). Fifty flowers (from 10 individuals) from each species were selected before they opened and surrounded with waterproof paper bags. Fifty other flowers were tagged for control measures of open pollination. Insect visitors and visitation rates (visits per inflorescence) were recorded from 12:00 to 15:00 for five days on three plants for each species in past two years (2009–2010). Three individuals of each floral visitor were captured with a net, and later identified.

Floral scent samples

Floral scents were investigated using the dynamic headspace adsorption method during the sunniest time of the day between 12:00 and 15:00, which coincided with the time of butterfly feeding activity. Five inflorescences of *B. davidii* and 40 of *B. yunnanensis* (approximately the same total number of flowers in each sample) were treated to compare their emission levels ($n = 3$). Newly opened inflorescences were enclosed in Tedlar bags (Dupont, USA) and volatiles were drawn from the enclosures into cartridges containing the adsorbent Porapak Q (150 mg, mesh 60/80, Waters Associates, Inc.) for 3 h, using a pump with an inlet flow rate of 300 ml min^{-1}. Prior to use, the adsorbent cartridges were cleaned with 2 ml of diethyl ether, dried with nitrogen gas. Trapped volatiles were eluted with 400 μl dichloromethane and concentrated to one-fifth the original volume by a gentle stream of nitrogen. 720 ng of *n*-nonane was added to each sample for quantification, and the samples were stored at −20 °C for subsequent analysis. Control samples were collected from the vegetative parts of the plant (Tedlar bags containing plants with no inflorescences).

Extracts from the inflorescences were analyzed 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 HP 5973 mass spectrometer. Helium was used as a carrier gas at a flow of 1 ml min^{-1}, and injector temperature was set to 250 °C. Column temperature was 40 °C and after injection, was increased to 250 °C at a rate of 3 °C min^{-1}. Compounds were identified by comparing mass spectra and retention times with those of reference compounds as well as with mass spectra in computer libraries (NIST, Wiley 7n.1.).

Results

Distribution of B. davidii and B. yunnanensis

Records from the Chinese Virtual Herbarium (http://www.cvh.org.cn/), our field exploration as well as the literature (Leeuwenberg, 1979; Li and Leeuwenberg, 1996; Tallent-Halsell and Watt, 2009) indicated that the natural distribution area of *B. davidii* includes India, Pakistan, Japan and at least 13 provinces of China (Fig. 1a), growing on mountain slopes and bottomlands at 360–3800 m while *B. yunnanensis* is limited to a small portion of Yunnan (Jingdong and Simao), China, at 1500–2300 m, growing at the edges of forests or thickets in the mountains (Fig. 1a).

Characteristics of flower, seed and breeding system

The inflorescences of *B. davidii* are terminal, narrowly paniculate thyrses, 10–30 cm long with 10–25 pairs of cymes (Fig. 1b), each cyme with 3–30 flowers. The inflorescences of *B. yunnanensis* are terminal and densely spicate, 2–6 cm long with 24–82 flowers (Fig. 1b and c). The floral traits, seed characters, and breeding systems are compared in Table 1 and illustrated in Fig. 1b–i.

Floral visitors of *B. davidii* included a diverse range of insects: butterflies (*Papilio*, *Vanessa*, *Argynnis*, *Argyronome*), moths (*Macroglossum*), bees (*Apis cerana*, *Xylocopa* spp.) and hover flies (Syrphidae). Although visitors varied in composition and number at different times, butterflies had the highest overall visitation rates (1.84 per inflorescence per hour, $n = 225$ inflorescences). Hawkmoth (0.54) and bees (0.49) also were conspicuous insect visitors. Floral visitors of *B. yunnanensis* were recorded only three times in the two years of observation (hover fly, 0.014 per inflorescence per hour, $n = 207$ inflorescences).

Scent composition in B. davidii and B. yunnanensis

Table 2 shows the detailed list of volatiles from *B. yunnanensis* and *B. davidii.* Altogether, 19 compounds were identified from the flowers of *B. davidii* and 11 from *B. yunnanensis.* Three of them (Methyl salicylate, Bergapten and α-Farnesene) occurred in both species. The emission rate of *B. yunnanensis* and *B. davidii* was 140 ng and 42,380 ng per inflorescence per hour, respectively ($n = 3$). It is notable that the emission levels are about 300 times stronger in *B. davidii* than in *B. yunnanensis*. When listed in terms of main volatile compounds in the two species, the order was as follows: α-Farnesene (30.9%), Ketoisophorone (30.1%), Epoxyoxophorone (11.1%), Benzaldehyde (10.1%) and β-Cyclocitral (2.4%) in *B. davidii* and Bergapten (55.8%), β-Caryophyllene (23.7%), Dimethyl phthalate (4.8%), α-Farnesene (3.6%) and tridecane (2.0%) in *B. yunnanensis*.

Discussion

Differences in distribution and flower biology of Buddleja davidii and B. yunnanensis

Buddleja davidii shows a wide distribution in Asia and is an invasive species in Europe, Africa, America, and Oceania (Clay and Drinkall, 2001; Ebeling et al., 2008; Tallent-Halsell and Watt, 2009; Thomas et al., 2008). By contrast, *B. yunnanensis* is an endangered species with limited distribution in Yunnan province (Fig. 1a). Flower biology may explain in part the differences in the distribution range of the two species.

Breeding system

There is a high percentage of fruit set in open pollinated samples of both taxa. However when the flowers were covered, so that selfing was obligatory, no fruit set occurred in *B. davidii.* By contrast, in *B. yunnanensis*, 76% of flowers set fruits. These results suggest that autogamy plays an important role in fruit production in *B. yunnanensis*, whereas *B. davidii* is self-sterile. This has already been pointed out by several authors (Moore, 1949; Tallent-Halsell and Watt, 2009). The proximity of the stigma to the stamens (Fig. 1e) in *B. yunnanensis* can promote self-pollination and it may be that the high density of trichomes at the corolla's throat (Fig. 1g) decreases the ability of potential pollinators to penetrate inside the corolla tube. The flowers of *B. davidii* are well adapted for butterfly visitations because of their long and narrow corolla tubes with nectar guides at the throat and large production of nectar (Tallent-Halsell and Watt, 2009).

Size and weight of seed

The mean seed size of *B. davidii* is 2.19 × 0.32 mm with long wings at both ends, and 0.46 × 0.36 mm for *B. yunnanensis*, without wings (Fig. 1h and i). The seeds of the latter are also approximately four times as heavy than those of the former. There is no doubt that the light seeds with long wings are a great advantage in the dispersal of *B. davidii* (3).

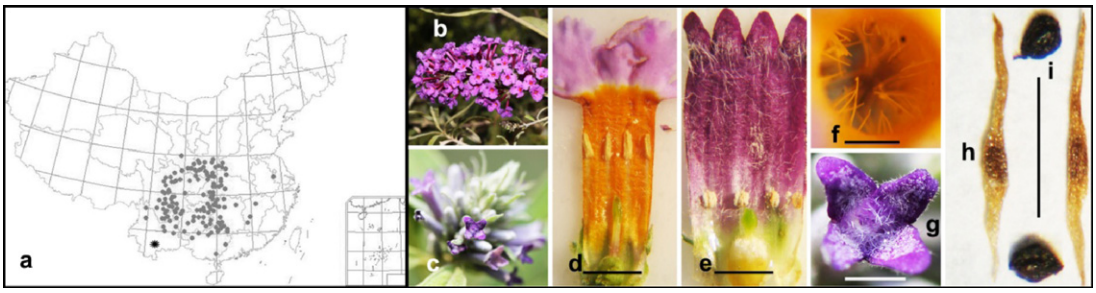

Fig. 1. Comparison of characteristics of distribution region (a) and flower and seed morphology between *B. davidii* and *B. yunnanensis*. *B. davidii*: a, b, d, f, h; *B. yunnanensis*: a, c, e, g, i. Asterisk and dots in the map of China indicate the distribution of *B. yunnanensis* and *B. davidii*, respectively. Bar: d, e, g, h, i = 2 mm; f = 0.5 mm.

Table 1
Comparison of morphological characteristics of flower and seed, breeding system of *B. davidii* and *B. yunnanensis*.

Character	*B. davidii*	*B. yunnanensis*	Significance
Ploidy level	Tetraploid	Diploid	
Length of corolla tube (mm)**	9.44	7.99	$P<0.01$
Width of corolla tube (mm)**	0.864	1.782	$P<0.01$
Trichome density of corolla (mm^2)**	58.3	256.7	$P<0.01$
Level of stigma/anthers**	1.92	1.04	$P<0.01$
Weight of 1000 seed s (mg)**	34.2	133.4	$P<0.01$
Seed size (mm)**	2.19×0.32	0.46×0.36	$P<0.01$
Compatibility	Self-incompatible	Self-compatible	
Pollen–ovule ratio (P/O)	106.59	74.82	
Fruit set under open-pollination	84.36%	90.75%	
Fruit set under self-pollination	0	76.26%	
Color of corolla	Violet: 84A, 84B	Purple: 79B	Color codes

** Mean significant at the 0.01 probability levels.

Flower volatiles

Flower volatiles were studied by Andersson et al. (2002) in 22 butterfly-pollinated species from 13 families in which they found that benzenoids (Phenylcetaldehyde and 2-Phenylethanol), monoterpenes (Linalool and Linalool oxide I and II) and an irregular terpene (Oxoisophorone) served as signals in attracting butterflies. Andersson and Dobson (2003) also found that Oxoisophoroneoxide, Benzaldehyde and trans-β-Ocimene elicited strong antennal responses in attracting potential butterfly pollinators. But sesquiterpenes, β-Caryophyllene, failed to elicit antennal responses in some butterflies. Andersson (2003) indicated that α-Farnesene, Benzaldehyde, Phenylacetaldehyde and irregular terpenes (Oxoisophorone, Oxoisophoroneoxide, Dihydroxoisophorone) from *B. davidii*

Table 2
Volatiles from flowers of *B. davidii* (*Bd*) and *B. yunnanensis* (*By*) ($n = 3$).

No.	Rt	Compound	CAS	*Bd* (%)	*By* (%)
1	6.55	2,6-Heptanedione	13505-34-5	0.27%	
2	7.36	3-Hexen-1-ol	928-96-1		1.88%
3	10.81	2,6-Dimethyl-2-heptanol	13254-34-7	1.09%	
4	11.38	Benzaldehyde	100-52-7	10.06%	
5	12.19	1-Octen-3-ol	3391-86-4	0.09%	
6	12.45	3-Octanone	106-68-3	0.18%	
7	12.46	Methyl heptenone	110-93-0		0.89%
8	12.85	Butyl butyrate	109-21-7	1.01%	
9	13.66	Ethyl acetate	141-78-6	0.27%	
10	14.99	Phenylacetaldehyde	122-78-1	1.63%	
11	15.16	β-Ocimene	3779-61-1	0.73%	
12	17.18	Undecane	1120-21-4		0.72%
13	17.26	Linalool	78-70-6		1.74%
14	18.60	Epoxyoxophorone	38284-11-6	11.11%	
15	19.16	Ketoisophorone	1125-21-9	30.11%	
16	19.62	Phenyl acetate	122-79-2	0.43%	
17	20.62	Methyl salicylate	119-36-8	0.14%	1.26%
18	20.70	Dodecane	112-40-3		1.83%
19	21.47	β-Cyclocitral	432-25-7	2.42%	
20	23.06	Cinnamaldehyde	104-55-2	0.18%	
21	23.78	Tridecane	629-50-5		2.00%
22	25.26	3-Hydroxy-4-phenyl-2-butanone	5355-63-5	0.98%	
23	27.26	β-Caryophyllene	87-44-5		23.68%
24	28.17	Dimethyl phthalate	131-11-3		4.76%
25	28.93	β-Ionone	14901-07-6	0.11%	
26	29.09	Bergapten	484-20-8	1.32%	55.77%
27	29.56	α-Farnesene	502-64-1	30.89%	3.59%
Total				92.03%	98.12%

Rt: retention time.

elicited strong antennal responses of butterflies when present both in natural and synthetic floral scents. In this study, we found volatile emission rates of *B. yunnanensis* and *B. davidii* to be 140 ng and 42,380 ng, respectively, per inflorescence per hour. It is worth noting that the difference in levels of volatiles between the two species is approximately 300 times. Certainly, the much stronger odor of *B. davidii* attracts butterflies much more easily than *B. yunnanensis*. Main compounds from *B. davidii* were found to be α-Farnesene (30.9%), irregular terpenes (30.1% for Ketoisophorone and 11.1% for Epoxyoxophorone) and Benzaldehyde (10.1%); these compounds elicit strong antennal responses to different butterflies according to Andersson's study. The main volatile compounds in *B. yunnanensis* are Bergapten (55.8%) and Dimethyl phthalate (4.8%); both have not been recorded to attract butterflies. For β-Caryophyllene, contributing 23.7% to the *B. yunnanensis* volatiles, the study Andersson and Dobson (2003) gave evidence that this compound fails eliciting antennal responses in butterflies.

Buddleja davidii ***vs.*** *B. yunnanensis****: outcrossing, easily dispersed vs. selfing without long-distance dispersal***

We conclude that the large distribution of *B. davidii* can be attributed at least in part to its flowers which have a strong ability by both morphology and scent, to attract butterflies, which in turn permits cross-pollination. Its numerous flowers yield many fruits which release a multitude of very light long-winged seeds, which can easily be distributed by wind or water. Apart from these characteristics, it is well known that this species thrives in disturbed areas and is very opportunistic. It is fast growing and can be considered a generalist as far as habitat is concerned, growing at a large range of altitudes and being rather cold and drought tolerant (Tallent-Halsell and Watt, 2009). In contrast, the relatively low number of flowers of *B. yunnanensis* with little scent fails to attract butterflies or other pollinators. It is primarily self-pollinated and its wingless seeds are relatively heavy. Its distribution may be limited by low genetic variability which is often found in autogamous species, poor seed dispersal and possibly a specialized habitat (Bevill and Louda, 1999).

Distribution and flower biology in other *Buddleja* ***species***

How do these features apply to other species of *Buddleja*? The most widespread species in Asia is the diploid *B. asiatica* which is found in Pakistan, Bangladesh, India, China, S.E. Asia up to the Philippines, at 200–2000 m (Leeuwenberg, 1979). It has not been studied in detail but is known to be self-incompatible (Moore, 1949), having rather small flowers but with a strong and pleasant smell, and winged seeds, although considerably shorter than those of *B. davidii*.

The most widely distributed species in the New World is *B. americana*, a tetraploid taxon which ranges from central Mexico through Central America to the western part of south America to northern Bolivia as well as Jamaica, Cuba and the Galapagos Islands, from sea level to 2500 m. The species is trioecious (Norman, 2000) with some plants having perfect flowers, while others only have male or female flowers. Although the flowers are very small, they have a pleasant fragrance which attracts small bees and flies. Thus in these taxa, pleasant odor, cross-pollination, self-sterility and winged seeds are associated with a large distribution.

There are probably three to four rare species of *Buddleja* in Asia and Africa, and approximately 15 in the Americas, but little is known about them. At least in America, these rare taxa are often associated with a specialized habitat. Ploidy levels do not seem to be correlated with a broad or narrow distribution pattern in this genus, although in both the Old and New World, the polyploids generally occur in mountains and at higher elevations than the diploid species (Chen et al., 2007; Norman, 2000).

Acknowledgements

This study was supported by grants-in-aid from the National Natural Science Foundation of China (NSFC30970192, 30625004, 40930209) and Natural Science Foundation of Yunnan Province (2009CD110).

References

Andersson, S., 2003. Antennal responses to floral scents in the butterflies *Inachis io*, *Aglais urticae* (Nymphalidae), and *Gonepteryx rhamni* (Pieridae). Chemoecology 13, 13–20.
Andersson, S., Dobson, H.E.D., 2003. Antennal responses to floral scents in the butterfly *Heliconius melpomene*. J. Chem. Ecol. 29, 2319–2330.
Andersson, S., Nilsson, L.A., Groth, I., 2002. Floral scents in butterfly-pollinated plants: possible convergence in chemical composition. Bot. J. Linnean Soc. 140, 129–153.
Bevill, R.L., Louda, S.M., 1999. Comparisons of related rare and common species in the study of plant rarity. Conserv. Biol. 13, 493–498.
Chen, G., Sun, H., Sun, W.B., 2007. Ploidy variation in *Buddleja* L. (Buddlejaceae) in the Sino-Himalayan region and its biogeographical implications. Bot. J. Linnean Soc. 154, 305–312.
Clay, D.V., Drinkall, M.J., 2001. The occurrence, ecology and control of *Buddleja davidii* in the UK. In: Conference Proceedings BCPC Conference Weeds, 1/2 , pp. 155–160.
Cruden, R.W., 1976. Intraspecific variation in pollen–ovule ratios and nectar secretion preliminary evidence of ecotypic variation. Ann. Mo. Bot. Gard. 63, 277–289.
Ebeling, S.K., Hensen, I., Auge, H., 2008. The invasive shrub *Buddleja davidii* performs better in its introduced range. Divers. Distrib. 14, 225–233.
Leeuwenberg, A.J.M., 1979. The Loganiaceae of Africa. XVIII. *Buddleja* II. Revision of the African and Asiatic species. Meded. Lanbouwhogeshool. 79, 1–163.
Li, P.T., Leeuwenberg, A.J.M., 1996. Loganiaceae. In: Wu, C.Y., Raven, P.H. (Eds.), Flora of China, vol. 15. Science Press, Beijing, pp. 320–332.
Moore, R.J., 1949. Cytotaxonomic studies in the Loganiaceae. III. Artificial hybrids in the genus *Buddleja* L. Am. J. Bot. 47, 511–517.
Norman, E.M., 2000. Buddlejaceae. Flora Neotrop. 81, 1–225.
Tallent-Halsell, N.G., Watt, M.S., 2009. The invasive *Buddleja davidii* (Butterfly Bush). Bot. Rev. 75, 292–325.
Thomas, M.M., Watt, M.S., Turnbull, M.H., Peltzer, D., Whitehead, D., 2008. Compensation in seasonal leaf area dynamics and leaf longevity in *Buddleja davidii*. Weed Res. 48, 340–348.