The role of floral scent of the cherry tree, *Prunus yedoensis*, in the foraging behavior of *Luehdorfia japonica* (Lepidoptera: Papilionidae)

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Abstract

The olfactory response of adults of *Luehdorfia japonica* (Papilionidae) to floral volatiles from *Prunus yedoensis* (Rosaceae) was investigated by means of a behavioral test (reflex extension of proboscis: REP) and electroantennographic (EAG) responses. Chemical analysis of the flower extract revealed that the floral scent consisted mainly of aromatic compounds, among which benzaldehyde (BAL, ca. 33%) was the dominant component. Of 10 floral volatiles tested, phenylacetaldehyde (PAL) elicited the highest REP response from both sexes. The REP-eliciting activities of benzyl alcohol, 2-phenylethanol and BAL, though a little lower than that of PAL, were also relatively high (ca. 50%) compared with those of other floral components. These four compounds, therefore, were considered to be the olfactorily active principles that stimulate foraging behavior in the butterfly. In EAG experiments with ten test chemicals, differences in responsive intensities among these compounds were not remarkable at a dose of 1 µg. In contrast, males displayed stronger EAG responses to ethyl phenylacetate, linalool, BAL and PAL at a dose of 100 µg. The present findings indicate that the antennal sensitivity of the butterfly is not necessarily correlated with the REP behavior. Although we found no explanation for conspicuous male EAG responses to particular components, this phenomenon seems suggestive of additional and as yet unknown function(s) of these compounds in the life of the male butterfly.

Key words: *Luehdorfia japonica*, *Prunus yedoensis*, floral scent, foraging behavior, reflex extension of proboscis

INTRODUCTION

Even when several species of flowers are simultaneously in bloom, most flower-visiting insects utilize not all flowers but specific ones for energy resources. Although such flower selection of insects is determined by various information from flowers (e.g. color, shape, scent, nectar and phenology), visual and olfactory cues are thought, in general, to be the main stimuli that attract insects to flowers (Dobson, 1994).

It is well known that several nocturnal moths utilize floral scents in their location of flowers (Cantelo and Jacobson, 1979; Haynes et al., 1991; Heath et al., 1992; Gabel et al., 1992, 1994; Zhu et al., 1993; Raguso et al., 1996; Shaver et al., 1997). Although earlier studies (Kay, 1976; Miyakawa, 1976; Kandori and Ohsaki, 1996, 1998) demonstrated that flower colors as visual

stimuli are important in foraging by butterflies, relatively fewer studies have been addressed to floral scents as olfactory stimuli (Honda, 1973, 1976; Pellmyr, 1986). In our continuous study of chemical interactions between butterflies and flowers (Honda et al., 1998; Ômura et al., 1999), we showed that floral scents served as significant cues in the foraging behavior of the cabbage butterfly, *Pieris rapae* (Pieridae). However, no information is available at present about the effect of floral scent on the foraging behavior of papilionid butterflies.

Luehdorfia japonica Leech (Papilionidae) is a typical flower-visiting butterfly. Since this univoltine butterfly, emerging in early spring, often forages on various flowers including *Erythronium japonicum* (Liliaceae), *Viola violacea* (Violaceae), *Prunus yedoensis* (Rosaceae), we examined the olfactory responsiveness of the butterfly to floral

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volatiles of the cherry tree, *P. yedoensis*, and discussed the role of the floral scent in its foraging behavior.

MATERIALS AND METHODS

Insects. Adults of *L. japonica* used for the experiments were 2- to 10-days old, and were from our laboratory stock cultures. Larvae were reared on fresh leaves of *Heterotropa hexaloba* (Aristolochiaceae) at 17°C with a 16-h light : 8-h dark regime. Adults were fed 10% aq. sucrose solution once daily and were not allowed to contact real flowers throughout the experiments.

Both REP and EAG experiments were conducted at 25°C in an air-conditioned room with good ventilation.

Collection of floral volatile compounds. Flowers of *P. yedoensis* (81.9 g), collected in Higashihiroshima City in April, 1998, were extracted with 1 l of isopentane for 1 h at 20°C. The extract was concentrated *in vacuo* at 15°C to yield an orange-colored residue (412 mg).

Chemical analysis of floral volatiles. Chemical composition of the floral volatiles was examined by GC and GC-MS. GC analyses were conducted with a Shimadzu GC-14A gas chromatograph on an FFAP fused-silica capillary column (0.25 mm I.D. $\times$ 50 m). The injection temperature was 250°C, and the oven temperature was programmed from 50°C to 220°C at a rate of 5°C/min. The flow rate of the carrier gas (N₂) was 1 ml/min. EI-MS spectra were recorded at an ionization potential of 70 eV on a Shimadzu QP2000 mass spectrometer connected to the same GC model, using the same capillary column and under similar operational conditions to those shown above. Identification of the components was based on a comparison of GC retention data and mass spectra with those of authentic samples.

Reflex extension of proboscis (REP). The REP responses of *L. japonica* to the floral scent of *P. yedoensis* were measured by an analogous method to that described previously (Honda et al., 1998). Before the bioassay, butterflies that had been starved for about 20 h were permitted free-flight for 90 min in a plastic chamber (30 cm $\times$ 25 cm $\times$ 20 cm) under fluorescent lamps (1,100 lx) and were subsequently fed with water only. One individual was introduced into a

plastic mesh cage (15 cm $\times$ 13 cm $\times$ 8 cm) under incandescent lamps (3,500 lx). A test chemical (5 μ l) was applied on a strip of filter paper (5 mm $\times$ 30 mm) which was attached to the end of a glass tube (5 mm I.D. $\times$ 30 mm). Crystalline compounds were dissolved in dichloromethane and tested at a dose of 5 mg, while the flower extract dissolved in the same solvent was tested at a dose of 50 μ g. The odor plume of a test chemical was delivered onto the antenna for 30 s together with a deodorized and humidified air stream (1,400 ml/min) blowing through the glass tube. The REP response to a test chemical was scored as follows: 100(%) for full extension of the proboscis, 50(%) for half-unrolling the proboscis and 0 for no response. Each individual was assayed twice daily, and the experiments were performed for two consecutive days. REP-eliciting activity of each chemical was expressed as the mean percentage of responses recorded from 15 individuals of each sex. Multiple comparison of REP activities of the respective compounds was made by an LSD-test at the 1% level, and the sexual difference in REP responsiveness was analyzed by a *t*-test.

Electroantennogram (EAG) recording. The antennal sensitivity to floral volatiles was examined electroantennographically according to the method of Honda et al. (1998). EAGs were recorded using a DPZ-115 input probe coupled with a DPA-100E amplifier (Dia Medical System), which was interfaced to a personal computer. A test compound was applied on a strip of filter paper (5 mm $\times$ 30 mm). An odor puff of 1 ml was mixed with a deodorized and humidified air stream blowing continuously over the antenna at a rate of 350 ml/min through a 6-mm-I.D. glass tube, which terminated 1 cm from the antennal preparation. Each compound was tested at three doses (1, 10, 100 μ g) on five antennae of both sexes, each of which originated from a different butterfly. EAG response to a compound of a given dose was measured three times for each preparation. The responses were averaged and expressed as percentages of the response to a standard sample (1 μ g of hexan-1-ol). Sexual differences in EAG intensity were analyzed by a *t*-test.

Test chemicals. Authentic chemicals used as olfactory stimuli were commercially purchased

(Tokyo Chemical Industry, Nacalai Tesque and Aldrich). Their purities as assessed by GC were all above 97%.

RESULTS

Chemical composition of floral volatiles

Ten aromatic and one terpenoid compounds were identified as characteristic components from the extract of cherry blossoms (Table 1). The extract contained a large amount of benzaldehyde (20.8 μg per flower), which comprised more than 30% of the total floral volatiles detected. Benzyl alcohol and 2-phenylethanol were also identified as additional major components. The floral scent was abundant in aromatic compounds, and linalool was the one terpenic component detected.

REP responses to floral components

The REP response (Fig. 1) evoked by the flower extract exceeded 50% (51% for males and 67% for females) at a dose of 50 μg , therefore, the floral scent was regarded as containing active compound(s) for proboscis extension. Of 10 chemicals tested, phenylacetaldehyde elicited the highest REP response (ca. 75%) from both sexes. Benzyl alcohol, 2-phenylethanol and benz-

Table 1. Chemical composition of floral volatiles from *Prunus yedoensis*

Compound	[μg] ^a	Amount (%) ^b
Terpenoids		
Linalool	0.01	0.02
Aromatics		
Benzyl alcohol	0.54	0.83
2-Phenylethanol	0.71	1.10
4-Methoxybenzyl alcohol	0.03	0.04
Benzaldehyde	20.84	32.67
Phenylacetaldehyde	0.09	0.13
4-Methoxybenzaldehyde	0.12	0.18
Ethyl phenylacetate	0.06	0.09
Methyl 4-methoxybenzoate	0.01	0.02
Methyl 3,5-dimethoxybenzoate	0.42	0.67
Benzoic acid	0.04	0.07
Aliphatic acids (C ₆ -C ₁₆)	1.59	2.41
Aliphatic hydrocarbons (C ₂₃ -C ₃₀)	25.07	39.17
		(total) 77.40

^a Amount per flower.

^b GC area %.

Fig. 1. REP responses (mean $\pm$ SE) of male and female *L. japonica* adults to ten floral components (5 μl or 5 mg) and the flower extract of *P. yedoensis* (50 μg). The compounds followed by the same letter (roman type for males and italic type for females) are not significantly different (LSD test, $p < 0.01$).

aldehyde elicited ca. 50% responses from both sexes. Since their activities, except for that of benzaldehyde to females, were appraised as rank-A by an LSD test for each sex, these components were regarded as the major active compounds in the floral scent. In contrast, methyl 4-methoxybenzoate, ethyl phenylacetate and benzoic acid were weakly active to both sexes. Sexual differences in REP activity of each chemical were not significant.

EAG responses to floral components

Male butterflies tended to display significantly higher EAG responses to certain compounds than females (Fig. 2). EAG activities of test chemicals were not greatly different from one another at a dose of 1 μg , except for phenylacetaldehyde to which males responded significantly stronger than females ($p < 0.05$). At a dose of 10 μg , male responses to phenylacetaldehyde and ethyl phenylacetate were very strong and significantly higher than females' ($p < 0.01$ for phenylacetaldehyde and $p < 0.05$ for ethyl phenylacetate). Linalool and benzaldehyde elicited intermediate responses from both sexes. Benzyl alcohol exerted a weak response, though the female response was significantly larger than the male's ($p < 0.05$). At a dose of 100 μg , linalool, benzaldehyde, phenylacetaldehyde and ethyl phenylacetate evoked significantly higher responses from males than from females ($p < 0.01$).

Fig. 2. Relative EAG responses (mean $\pm$ SE vs 1 μ g of hexan-1-ol) of *L. japonica* adults to ten floral components. Significance of sexual difference (for the same compound at the same dose) is represented by an asterisk (*t*-test: * for $p < 0.05$ and ** for $p < 0.01$).

for benzaldehyde, and $p < 0.05$ for linalool, phenylacetaldehyde and ethyl phenylacetate).

DISCUSSION

L. japonica gave relatively high (over 50%) REP responses to benzyl alcohol, 2-phenylethanol, benzaldehyde and phenylacetaldehyde. The authors (Honda et al., 1998; Ômura et al., 1999) have demonstrated that the cabbage butterfly utilizes floral components of high REP activity for both recognition and location of flowers in its foraging behavior. It is, therefore, very feasible that foraging adults of *L. japonica* should also make use of these floral components to locate cherry blossoms.

The above four compounds are ubiquitous floral volatiles present in many other plant species (Knudsen et al., 1993). 2-Phenylethanol, benzaldehyde and phenylacetaldehyde are components characteristic of *Ligustrum japonicum* (Oleaceae) (Honda et al., 1998) and *Brassica rapa* (Cruciferae) (Ômura et al., 1999) flowers for which *P. rapae* showed high preference. *Sasakia charonda charonda* (Nymphalidae) exhibited high REP responses to 2-phenylethanol and phenylacetaldehyde (Honda, 1973), while another nymphalid, *Nymphalis xanthomelas japonica*, also displayed a high preference for 2-phenylethanol (Honda, 1976). Several species of moths were lured by 2-phenylethanol and/or phenylacetaldehyde emanating from flowers of *Abelia grandiflora* (Haynes et al., 1991), *Araujia*

sericofera (Cantelo and Jacobson, 1979) and *Cestrum nocturnum* (Heath et al., 1992). Since such particular aromatic compounds are highly preferred by many flower-visiting lepidopterans and/or attract them, these compounds are likely to act as common olfactory cues by which they recognize the presence of food sources.

L. japonica showed considerably high REP responses to phenylacetaldehyde and 2-phenylethanol (ca. 75% and 55%, respectively), while *P. rapae* responded less sensitively to these compounds (ca. 30% and 25%, respectively) (Honda et al., 1998; Ômura et al., 1999). This appears to suggest that *L. japonica* is more dependent on olfaction than *P. rapae* in foraging.

Although linalool and ethyl phenylacetate evoked high EAG responses especially from males at 100 μ g dose, they induced moderate REP responses. In contrast, benzaldehyde and phenylacetaldehyde eliciting high EAG responses displayed high REP activities. These results indicate that in *L. japonica*, there is little correlation between the degree of preference (REP response) for and olfactory sensitivity (EAG response) to an odor. This finding is consistent with many other investigations (Evans and Allen-Williams, 1992; White and Hobson, 1993; Raguso et al., 1996; Honda et al., 1998). Interestingly enough, males were more sensitive to these four compounds than females. Similar results have been reported for EAG responses of *P. rapae* to 2-phenylethyl acetate and ethyl

phenylacetate (Honda et al., 1998). Such sexual differences might suggest additional and as yet unknown function(s) of these compounds that are specific to males. Morphological study of antennal olfactory sensilla is necessary to elucidate the reason for such sexual differences.

With respect to preference for flower colors, *L. japonica* has been shown to primarily prefer purple-colored flowers in the field and to exhibit strong preference for blue coloration in laboratory experiments (Fujii, 1996). Although pale pink flowers of *P. yedoensis* appear less attractive to the butterfly than blue or purple flowers (*E. japonicum* and *V. violacea*, for example), the butterfly often forages on cherry blossom. Spectroscopic and UV-photographic examinations of *P. yedoensis* flowers revealed that the petal, though slightly reflecting UV-rays, has no apparent UV-nectar guide (unpublished). Therefore, the scent of cherry blossoms may serve as a potent olfactory stimulus that guides the butterfly to the flower and may compensate for the lack of visual attractiveness of the flower. Further study is needed to clarify the combined effect of olfactory and visual stimuli on their foraging behavior.

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