Volatile compounds from a Parnassiinae butterfly, *Luehdorfia japonica* (Lepidoptera, Papilionidae)

Kento Yoshimori\(^1\), Taro Gochu\(^2\), Shinji Ohta\(^2\) and Hisashi Ōmura\(^2\)

\(^1\) Faculty of Applied Biological Science, Hiroshima University, Higashihiroshima, 739-8528 Japan
\(^2\) Graduate School of Biosphere Science, Hiroshima University, Higashihiroshima, 739-8528 Japan

**Abstract** Male adults of several species in the family Papilionidae emit characteristic odors. However, it remains unclear whether the presence of male-specific volatiles is a common trait in this family, because the chemistry of adult odors has been investigated in only a limited number of species belonging to the Papilioninae subfamily. We conducted gas chromatography–mass spectrometry analyses of the highly volatile substances of the Parnassiinae butterfly, *Luehdorfia japonica* (Lepidoptera, Papilionidae), using solvent extracts of laboratory-reared adults. Although this species lacks a strong odor detectable by the human nose, a total of 24 highly volatile compounds were identified from both sexes, and 17 of these compounds were discovered in adult papilionid butterflies. The most abundant component was limonene, which was present at a level of 1 μg per individual on average. Both sexes showed similar compositions of these volatile compounds, with no significant differences in the quantity of each compound between sexes. These results suggest that *L. japonica* adults possess a common odor in both sexes and lack male-specific volatiles.

**Key words** GC–MS analysis, *Luehdorfia japonica*, scent substance, solvent extraction.

**Introduction**

Adult lepidopteran insects produce sex-specific odors for mating; female moths emit blends of volatile compounds that serve as sex pheromones, and male butterflies have characteristic scents (Boppré, 1984; Vane-Wright and Boppré, 1993). Several butterfly species whose males possess scents that are perceptible to the human nose have been subjected to chemical analyses, and various compounds have been identified as male-specific volatiles (Honda, 1986 a,b; Ōmura, 2015). In several butterfly species, male-specific volatiles are produced by specialized alar organs (androconia) and play important roles in mating (Kristensen and Simonsen, 2003). However, information on scent chemistry is available for only a limited number of butterfly species, unlike moths.

In the family Papilionidae, chemical profiles of adult odors have been determined only in species belonging to the subfamily Papilioninae, in which particular terpenoids have been identified as male-specific volatiles. *Byasa alcinous alcinous* (Klug) males are characterized by a strong musk-like odor, which consists of γ-himachalene acetate (the predominant component) and several sesquiterpene hydrocarbons, while males of the subspecies *Byasa alcinous loochooana* (Rothschild) have an odor with a slightly different composition (Itoh and Ōmura, 2015; Ōmura et al., 2016). Male *Papilio machaon hippocrates* C. & R. Felder emit a fragrant smell containing linalool and geranyl acetone as the major substances (Ōmura et al., 2001). The scents of male *Papilio polytes* L. and *Papilio protenor demetrius* Stoll contain small amounts of linalool (Ōmura and Honda, 2005; Ōmura et al., 2012). However, whether the presence of male-specific volatiles is common in other subfamilies such as Baroiniinae and Parnassiinae remains to be investigated. In this study, we examined the adult scent chemistry of a Parnassiinae butterfly, *Luehdorfia japonica* Leech using gas chromatography-mass spectrometry (GC–MS). *L. japonica* is a univoltine, spring-flying species, endemic to Honshu, Japan. Its larvae feed on leaves of wild ginger, particular species of the genus *Asarum* (Aristolochiaceae). Because the essential oils of their host plants are abundant in odoriferous compounds, we also examined the relationship between the odor of the butterfly and the volatiles of its host plant.

**Materials and methods**

**Insects**

*Luehdorfia japonica* adults were raised from eggs in the laboratory. Two gravid females were collected in the field at Higashihiroshima (34.23′N, 132.42′E, Hiroshima Prefecture) and placed in plastic containers (35 × 50 × 25 cm) to allow them to lay eggs on fresh leaves of *Asarum hexalobum* (synonym *Heterotropa hexaloba*) F. Maek. under incandescent lamps. The larvae were reared on fresh leaves at 20°C under a 16 L: 8 D photoregime. The adults, provided with 15 % aqueous sucrose once daily, were maintained individually in plastic cases (9 cm in height, 12 cm in inner diameter).
Extraction

At 3 days after eclosion, adult butterflies were killed by freezing at –20°C and subjected to 3 min extraction with purified dichloromethane. Without dissection, individual adults (10 males and 8 females) were extracted intact with 5 mL solvent at room temperature. The crude extracts were subsequently filtered, concentrated to 100 µL under a stream of nitrogen gas, and stored at –20°C until chemical analyses were conducted.

Chemical analyses

The concentrated extracts were subjected to GC–MS using a Shimadzu QP5000 mass spectrometer equipped with a Shimadzu GC-17A gas chromatograph equipped with an Agilent J&W HP-5 MS capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness) at an electron ionization (EI) voltage of 70 eV. All samples were injected in the splitless mode at 280°C, using helium as the carrier gas. The oven temperature was programmed from 40°C (2 min isothermal) to 300°C (10 min isothermal) at a rate of 10°C/min. For three compounds, high-resolution gas chromatography-time-of-flight mass spectrometry (HR–GC–TOF–MS) was performed, using a Jeol JMS-T100GCV 4G mass spectrometer equipped with an Agilent J&W HP-5 capillary column (30 m × 0.22 mm ID, 0.25 µm film thickness) at an EI voltage of 70 eV or a chemical ionization (CI) voltage of 200 eV (with isobutane as the CI gas). Compounds were identified by comparing retention times and mass spectra with those of authentic samples. As authentic samples for two compounds were unavailable, tentative identification was performed using the NIST/EPA/NIH mass spectral library version 2.0. Quantitative estimates of individual compounds were obtained by comparing peak intensities with those of authentic linalool.

Authentic samples

The authentic samples of four alkanes (decane, undecane, dodecane, and tridecane), three aliphatic aldehydes (heptanal, nonanal, and decanal), one aliphatic ketone (2,5-hexadione), two aliphatic esters (isobutyl isobutyrate and ethyl 2-acetyl-3-methylbutyrate), one aliphatic lactone (γ-dodecalactone), three aromatic hydrocarbons (α-, m-, and p-xylene), three oxygenated aromatic compounds (benzaldehyde, benzo furan, and acetophenone), and four monoterpenoids (p-cymene, limonene, rose oxide, and pulegone) were commercial products from Tokyo Chemical Industry (Tokyo, Japan) or Sigma-Aldrich (St. Louis, MO). Authentic longifolene, a component of an essential oil from the Atlas cedar (Cedrus atlantica), was supplied by Pranarôm International (Ghislenghien, Belgium).

Results

The total ion chromatograms of the male and female extracts showed few large peaks within the retention time range of 4–20 min, indicating that adult L. japonica of both sexes have small amounts of highly volatile compounds (Fig. 1A). This result is consistent with the observation that adults of this species lack strong odors detectable by the human nose. However, comparing the retention times and mass spectra with those of authentic samples, we first identified 21 volatile compounds: four alkanes (peaks 8, 13, 18, and 21), three aliphatic aldehydes (4, 14, and 19), one aliphatic ketone (6), two aliphatic esters (5 and 15), one aliphatic lactone (23), three aromatic hydrocarbons (1–3), three oxygenated aromatics (7, 8, and 12), and four monoterpenoids (9, 10, 16, and 20) (Fig. 1B, Table 1). Then three other compounds (11, 17, and 22) were analyzed by high-resolution mass spectrometry.

Peak 11 had a base ion at m/z 55 (100%), a possible molecular ion at m/z 156 (15%), and fragment ions at m/z 141 (11%), 127 (7%), 111 (26%), 95 (28%), 83 (59%), 67 (50%), and 41 (98%) in GC–MS. The molecular formula was established as C₉H₁₅O₂ from the molecular ion [M]+ at m/z 156.11517 in HR–GC–TOF–MS in EI mode (calcd for C₉H₁₅O₂: 156.11503). This compound was tentatively identified as ethyl 3, 4-dimethyl-2-pentenoate.

Peak 17 showed a base ion at m/z 57 (100%, relative ion...
intensity) and fragment ions at \( m/z \) 43 (14%), 73 (18%), 103 (5%), 129 (7%), and 159 (2%), without a clear molecular ion. This fragmentation pattern suggested a possible aliphatic acetal structure. However, we could not obtain \([M]^+ \) and \([M+H]^+ \) ions of this compound in exact mass measurements. Following a mass spectral library search, 1,1-dibutoxybutane was tentatively assigned to it as its structure.

The EI–MS fragmentation pattern of peak 22 was typical of a cyclic sesquiterpene, although a molecular ion peak was not observed at \( m/z \) 204. As HR–GC–TOF–MS in the CI mode showed a \([M+H]^+ \) ion at \( m/z \) 205.19617 (calced for \( \text{C}_{21}\text{H}_{34}: 205.19563 \)), its molecular formula was established as \( \text{C}_{21}\text{H}_{34} \). This compound was identified as longifolene by comparing the EI–MS spectrum and retention index of the component in Atlas cedar oil.

Of the 24 compounds identified, limonene was the most abundant component, with an average of > 1 \( \mu \text{g} \) per individual in both sexes, followed by 1,1-dibutoxybutane and 2,5-hexadione. The chemical composition of highly volatile compounds was very similar between sexes, with no significant differences in the quantity of each compound between the sexes (\( p > 0.05, \) Mann–Whitney \( U \) test).

**Discussion**

*Luehdorfia japonica* adults, even males, had little odor detectable by the human nose. However, crude extracts of it contained various highly volatile substances, such as aliphatic, terpenoid, and aromatic compounds, although in small amounts. Of the 24 compounds identified, heptanal, benzaldehyde, \( \alpha \)-cymene, limonene, nonanal, dodecane, and decanal have previously been reported as adult scent substances in the subfamily Papilioninae, while the remaining 17 compounds were discovered in the
family Papilionidae. It is intriguing that the adult odor of a Parnassiinae butterfly, *L. japonica*, has a unique chemical composition.

The essential oils of the genus *Asarum*, including host plants of *L. japonica*, have been well investigated and are characterized by sharing particular monoterpens (e.g., β-pinene and borneol), sesquiterpenoids (e.g., carophyllene and β-farnesene), and phenol ethers (e.g., safrole and elemicin) (Hayashi et al., 1984, 1988, 1990). It is noteworthy that the major volatile components of *A. hexalobum* (1-allyl-2,3,4,5-tetramethoxybenzene, elemicin, and asatone) were not detected in the present study (Hayashi et al., 1980), suggesting that the scent substances of *L. japonica* adults are not sequestered through larval feeding on host plants but rather are biosynthesized after adult emergence.

There were no significant sex-related differences in the quantity of each compound identified in this study, indicating that the scent profile of *L. japonica* adults is qualitatively and quantitatively similar between males and females, in contrast to the male-specific compounds found in the Papilioninae species examined previously. However, this species shows conspicuous geographical variation in several traits, such as larval host usage and adult wing pattern and coloration (Watanabe, 1996). Because the scent profiles of *L. japonica* adults also may show large fluctuations, further comparative studies using adults from different populations are required.

Acknowledgments

We thank Dr Tomoko Amimoto, the Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University, for the measurement of HR–GC–TOF–MS.

References


Ōmura, H. 2015. Insect Nat. 50(9): 4-8. (In Japanese)


摘 要

ウスバアケハ亜科ギフチョウ Luehdorfia japonica の成虫揮発性成分（吉森健人・野口太郎・太田伸二・大村 尚）

ある種のアケハチョウは雌成虫が雌特有の香気性の匂いをもつことで知られる。しかし、匂いの化学成素除はアケハチョウ亜科に属すずあか数種で調べられたに過ぎず、雌特有の匂いがアケハチョウ科に広くみられる共通形質であるのかどうかはよくわからていない。我々は、研究室で飼育したギフチョウ（ウスバアケハ亜科）の成虫を溶媒抽出し、その高揮発性成分の化学組成をGC-MS で調べた。ヒトの嗅覚では本種成虫から強い匂いを感じ取ることはできなが、雌雄成虫の溶媒抽出物から24の高揮発性化合物を同定した。その17成分はアケハチョウ亜科成虫の匂い成分としては初めての発見であった。最も含有量の多い成分は limonene で、雌雄1個体につき約1μg 含まれていた。一方、各成分の雌雄の含有量に有意差はなく、同定した24成分について化学組成は雌雄でほぼ類似であった。これらの結果より、ギフチョウ成虫は雌雄共通の匂いを有し、雌に特異的な匂い成分をもっていないと考えられる。

（Received November 11, 2016. Accepted April 4, 2017）