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Host Alternation by Gall Midges of the Genus *Asphondylia* (Diptera: Cecidomyiidae)[1]

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Abstract

Host alternation by *Asphondylia gennadii* (Marchal) and *Asphondylia baca* Monzen was studied. The DNA analysis revealed that at least *Ceratonia siliqua* Linnaeus (Fabaceae), *Urginea maritime* (Linnaeus) Baker (Liliaceae), and *Capparis spinosa* Linnaeus (Capparidaceae) were included in the host range of *A. gennadii* and that this gall midge can complete its annual life cycle by utilizing these host plants alternatively. The morphological study of pupal frontal area revealed that a gall midge that is responsible for leaf bud galls on *Weigela* species (Caprifoliaceae) in Japan had been misidentified as a North American species, *Asphondylia diervillae* Felt. DNA analysis, together with morphological, ecological, and distributional information, indicated that the weigela leaf bud gall midge is identical with *Asphondylia baca* Monzen that produces fruit galls on *Ampelopsis brevipedunculata* (Maximowicz) Trautvetter var. *heterophylla* (Thunberg) Hara (Vitaceae) and *Cayratia japonica* (Thunberg) Gagnepain (Vitaceae) in Japan. The identity of the two species indicated that *A. baca* exhibits host alternation, using *Ampelopsis* and *Cayratia* fruit for galling in summer-autumn and *Weigela* leaf buds in winter-spring. This is the third finding of host alternation by *Asphondylia* species, suggesting further detections of host alternation in the genus.

Introduction

Host alternation is common to a number of Aphididae and Pemphigidae (Homoptera) but is unusual for other insect groups. In the order Diptera, host alternation has never been reported (A. Freidberg, D. Henshaw, A. Pont, M. Suwa, and M. v. Tschirnhaus, 2002, pers. comm. at the 5th International Congress of Dipterology, Brisbane, Australia) except for 2 gall midge species of the genus *Asphondylia* (Cecidomyiidae) (Harris, 1975; Orphanides, 1975; Yukawa *et al*., 2003).

Asphondylia contains 271 nominal species in the world (Gagné, in press). They are most often responsible for bud, flower, and fruit galls on various plant species. Most of them are monophagous or oligophagous within a single plant genus or family (e.g., Skuhravá, 1986; Gagné & Orphanides, 1992; Gagné, 1994). The annual life cycle of monophagous or oligophagous species can be easily clarified when they are univoltine.

Multivoltine species do not appear to be dominant in *Asphondylia*, but overwintering hosts or spring-summer-autumn hosts still remain unknown for many species. Usually their spring-summer-autumn hosts die back in late autumn and the emergent summer-autumn generation has nowhere to lay their eggs on those hosts. The emergent winter generation has also nowhere to lay its eggs because its winter hosts do not proffer the proper organ for oviposition at the time of adult emergence in spring. Therefore, the multivoltine species require some alternative hosts on which to lay their eggs.

There are 2 known instances of host alternation by *Asphondylia* gall midges. In Cyprus, *Asphondylia gennadii* (Marchal) utilizes carob *Ceratonia siliqua* Linnaeus (Fabaceae) as a winter

1. Nucleotide sequence data used in this paper are available in the DDBJ/ EMBL/ GenBank databases under the following accession numbers: AB115562-AB115589, AB086426-AB086428, AB085773-AB085775, AB085777, AB085865, AB085868, AB085874, and AB085877.

host, and many other plants, including pepper *Capsicum annuum* Linnaeus (Solanaceae), caper *Capparis spinosa* Linnaeus (Capparidaceae), and sea squill *Urginea maritime* (Linnaeus) Baker (Liliaceae), as summer hosts (Harris, 1975; Orphanides, 1975; Gagné & Orphanides, 1992). However, host alternation by *A. gennadii* has never been confirmed at the DNA level. In Japan, Yukawa *et al*. (2003) confirmed by DNA analysis that the soybean pod gall midge, *Asphondylia yushimai* Yukawa & Uechi, produces fruit galls on *Prunus zippeliana* Miquel (Rosaceae) in winter and pod galls on soybean *Glycine max* (Linnaeus) Merrill (Fabaceae) or wild fabaceous plants in summer and autumn. On the basis of these examples, Yukawa *et al*. (2003) pointed out that the host alternation might occur elsewhere in the genus *Asphondylia* and that morphologically similar nominal species that utilize different groups of host plant may be synonymized in the future by DNA analysis.

Besides *A. yushimai*, at least 5 nominal species and 14 unidentified segregates of *Asphondylia* have been known to occur in Japan (Yukawa & Masuda, 1996; Yukawa *et al*., 2003). Three species and 1 segregate of them are univoltine and monophagous or oligophagous, but the remainder are multivoltine and part of their life history has been unknown (Yukawa & Masuda, 1996; N. Uechi & J. Yukawa, unpubl. data). Even though these multivoltine species or segregates possibly utilize different groups of host plants, they are morphologically quite similar to each other (Yukawa, 1971; Yukawa & Masuda, 1996).

Sunose (1992) tried to confirm the identity of the ampelopsis fruit gall midge, *Asphondylia baca* Monzen, with the weigela leaf bud gall midge, *Asphondylia* sp., which had been misidentified by Shinji (1938) as a North American species, *Asphondylia diervillae* Felt. Sunose (1992) observed that females of the weigela leaf bud gall midge laid their eggs into fruit of *Ampelopsis brevipedunculata* (Maximowicz) Trautvetter var. *heterophylla* (Thunberg) Hara (Vitaceae) when they were introduced into a small cage covering the fruit. He then suspected that the 2 species might be identical although gall formation was not confirmed on the fruit.

This paper proposes: (1) to reconfirm the host alternation by *A. gennadi* at the DNA level, (2) to revise, by morphological studies, the specific position of the weigela leaf bud gall midge that had been identified as *A. diervillae,* (3) to confirm the identity of the weigela leaf bud gall midge with *A. baca* based on morphological, molecular, ecological, and biogeographical information, and (4) to make remarks about host alternation by *Asphondylia* gall midges.

In order to show clearly the results of the aforementioned purposes, this paper is divided into the following 3 parts: I. *Asphondylia gennadii* from Cyprus; II. *Asphondylia baca* and the weigela leaf bud gall midge in Japan; and III. General remarks.

I. *Asphondylia gennadii* from Cyprus

Material and Methods

Asphondylia gennadii specimens stored in 75% ethanol were sent from Cyprus by Dr. N. Seraphides and Mr. A. Georghiou in March 2003. They consist of 41 pupae from *Ceratonia*, 20 pupae, and 1 larva from *Urginea*, and about 50 pupae from *Capparis*. Unfortunately we could not obtain *A. gennadii* individuals from the following known summer host plants: pepper *C. annuum* (Solanaceae), potato *Solanum tuberosum* Linnaeus (Solanaceae), garden rocket *Eruca vesicaria* Linnaeus (Brassicaceae), mustard *Sinapis* spp. (Brassicaceae), asphodel *Asphodelus fistulosus* Linnaeus (Liliaceae), and St. Johnswort *Hypericum crispum* Linnaeus (Hypericaceae).

Three individuals on *Ceratonia* and 5 on *Urginea* and *Capparis*, respectively, were submitted to DNA analysis. For every individual, total DNA was extracted from the whole body with the Dneasy tissue kit (Qiagen, Japan), following the manufacturer's instructions. A region of the cytochrome oxidase subunit I (COI) gene of mtDNA was amplified, purified, sequenced, and electrophoresized following the methods described by Yukawa *et al*. (2003). This region, together with other regions, has been effectively used for the analysis of intra- or interspecific variations in vari-

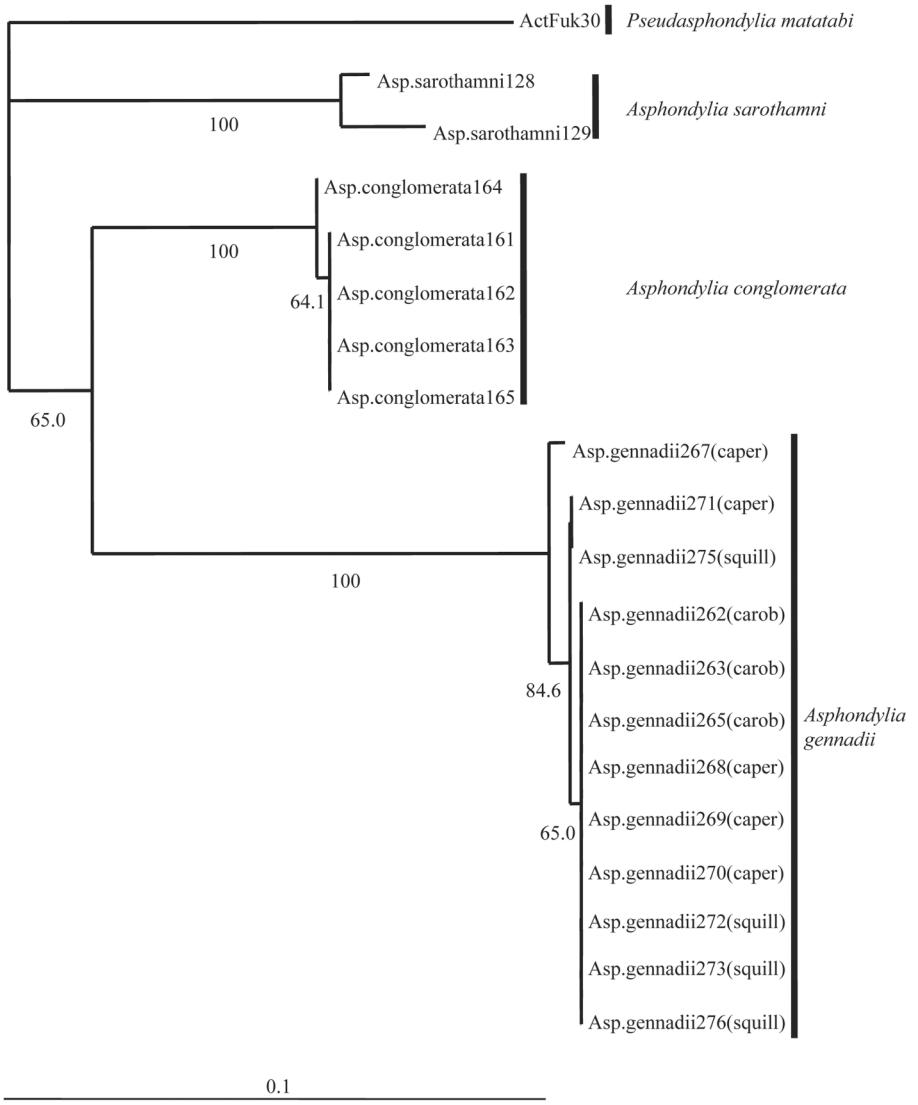

Figure 1. Neighbor-joining tree based on 439 bp of the mtDNA COI gene for *Asphondylia gennadii* on various hosts. Bootstrap values are indicated for nodes gaining more than 60% support (1000 replications). *Pseudasphondylia matatabi*, *A. sarothamni*, and *A. conglomerata* were used as outgroup species. Sample names correspond to the respective isolation names registered in DNA database.

ous insect orders: e.g., Hymenoptera (Scheffer & Grissell, 2003), Heteroptera (Damgaard & Sperling, 2001), Diptera: Cecidomyiidae (Shirota *et al*., 1999; Yukawa *et al*., 2003), Tephritidae (Jamnongluk *et al*., 2003) and Lepidoptera (Andolfatto *et al*., 2003).

The primers used for the amplification were as follows: forward; 5´-GGATCACCTGATATAGCATTCCC-3´ and reverse; 5´-CCCAAAATTAAAATATAAACTTC-3´. Both strands of the PCR products were sequenced.

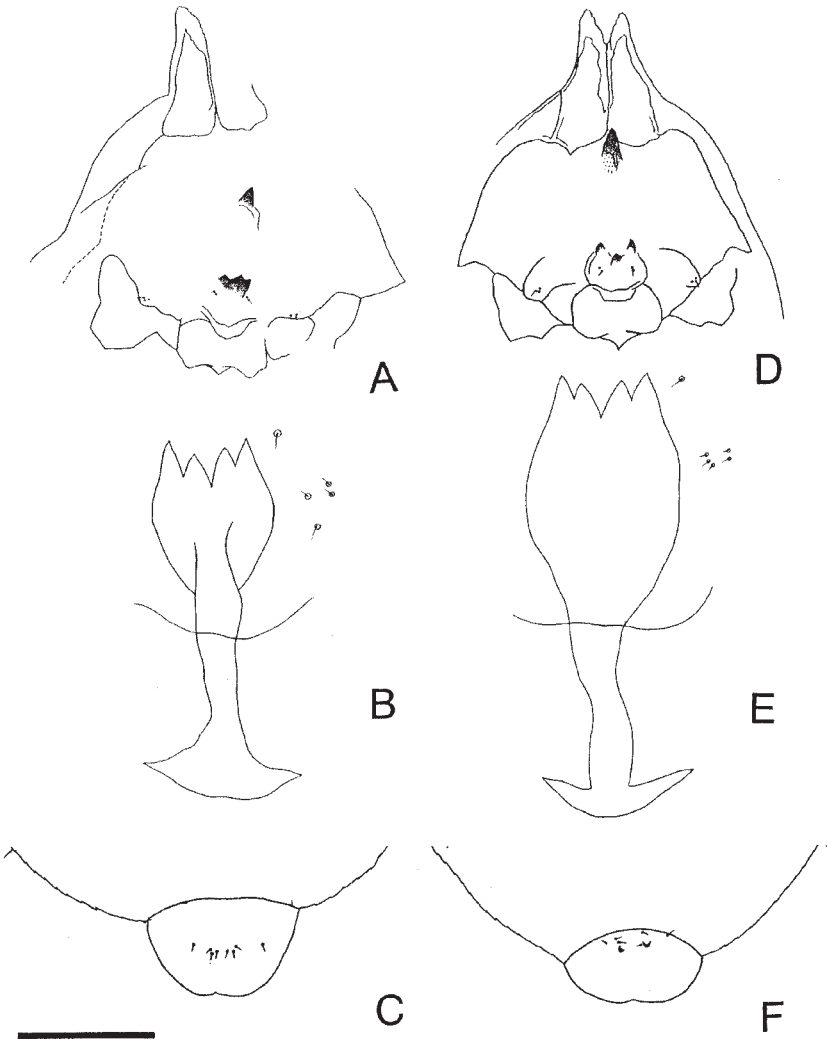

Figure 2. A–C, *Asphondylia diervillae*, **D–F**, the weigela leaf bud gall midge. **A, D**, ventral view of pupal head. **B, E**, larval sternal spatula and adjacent papillae. **C, F**, larval terminal segment and papillae, dorsal view. Scale: 0.8 mm for A, D, 0.2 mm for B, E, and 0.26 mm for C, F.

The sequence data were analyzed by the neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) methods, and unweighted pair-group method using arithmetic means (UPGMA) with the software package PHYLIP Version 3.573c (Felsenstein, 1993). Evolutionary distances were computed by Kimura's two-parameter distances (Kimura, 1980). The resulting trees were evaluated by the bootstrap test (Efron, 1982; Felsenstein, 1985) based on 1,000 replications for the NJ, MP, and UPGMA trees and 100 replications for the ML tree. The MP, ML, and UPGMA trees are not shown in this paper, since these are quite similar to the NJ tree.

As an outgroup in the above analysis, the following 2 European species of *Asphondylia* and a

Table 1. Gall midges used for DNA analysis

Gall midge	Host plant	English name	Collection site (Collector)	n	Accession No.
Asphondylia gennadii	*Ceratonia siliqua*	carob	Zygi, Cyprus (N. Seraphides & A. Georghiou)	3	AB115569-AB115571
	Capparis spinosa	caper	Ay. Theodovas, Cyprus (N. Seraphides & A. Georghiou)	5	AB115572-AB115576
	Urginea maritime	squill	Mazotos, Cyprus (N. Seraphides & A. Georghiou)	4	AB115577-AB115580
A. conglomerata	*Atriplex halimus* (bud)	tree purslane	Sede Boqer, Israel (N. Dorchin)	5	AB115562-AB115566
A. sarothamni	*Cytisus scoparius*	common broom	Guildford, UK (K. M. Harris)	2	AB115567, AB115568
A. baca	*Ampelopsis brevipedunculata*		Sapporo City, Hokkaido, Japan (N. Uechi)	1	AB115589
			Fukuoka City, Fukuoka Pref., Japan (N. Uechi)	3	AB115581-AB115583
			Hisayama Town, Fukuoka Pref., Japan (N. Uechi)	1	AB115585
			Wakamiya Town, Fukuoka Pref., Japan (N. Uechi)	1	AB115584
			Ume Town, Oita Pref., Japan (J. Yukawa)	1	AB115587
			Kitagawa Town, Miyazaki Pref., Japan (J. Yukawa)	1	AB115588
	Cayratia japonica		Sendai City, Miyagi Pref., Japan (J. Yukawa)	1	AB115586
The weigela leaf bud gall midge	*Weigela hortensis*		See Yukawa *et al.* (2003)	3	AB086426-AB086428
	Weigela coraeensis		See Yukawa *et al.* (2003)	3	AB085773-AB085775
Asphondylia yushimai	*Glycine max*	soybean	See Yukawa *et al.* (2003)	3	AB085777, AB085865, AB085868
The hedera flower bud gall midge	*Hedera rhombea*		See Yukawa *et al.* (2003)	2	AB085874, AB085877
Pseudasphondylia matatabi	*Actinidia polygama*	silver vine	See Yukawa *et al.* (2003)	1	AB085873

Japanese species of *Pseudasphondylia* were used (Table 1): *Asphondylia conglomerata* Stefani galling on *Atriplex halimus* Linnaeus (Chenopodiaceae), *Asphondylia sarothamni* Loew galling on *Cytisus scoparius* (Linnaeus) Link (Fabaceae), and *Pseudasphondylia matatabi* (Yuasa & Kumazawa) (Diptera: Cecidomyiidae), which is responsible for fruit galls on *Actinidia polygama* (Siebold & Zuccarini) Planchon & Maximowicz (Actinidiaceae).

Results

The amplified mitochondrial COI gene fragment was 439 bp long. This region corresponded to the bases 1752–2190 of the genome of *Drosophila yakuba* Burla (Diptera: Drosophilidae) (Clary & Wolstenholme, 1985).

The monophyly of the clade *A. gennadii* on *Ceratonia*, *Capparis*, and *Urginea* was supported by a 100% bootstrap value (Fig. 1). The sequences of *A. gennadii* were distinctly different from those of the 2 European congeners in the outgroup species, *A. conglomerata* and *A. sarothamni* (Fig. 1). There were 51 (11.62% of 439 bp) to 69 bp (15.72%) differences between *A. gennadii* and the 2 species (Table 1) and 6 (4.11%) to 7 (4.80%) differences in the 146 deduced amino acid residues.

Three haplotypes of *A. gennadii* were recognized in this study. However, sequential variations between those on different host plants were very small, 0 (0%) to 4 bp (0.91%) differences, and there were no differences in the 146 deduced amino acid residues.

Discussion

In contrast to the big differences between *A. gennadii* and the outgroup species, intraspecific variations among *A. gennadii* individuals from different host plants are small enough to consider that they are identical species. Thus, the current DNA analysis indicates that at least *Ceratonia, Urginea,* and *Capparis* are included in the host range of *A. gennadii*. As shown in Orphanides (1975), *A. gennadii* can complete its annual life cycle by utilizing these host plants alternatively. This provides the confirmation at the DNA level of host alternation by *A. gennadii*.

Orphanides (1975) mentioned a possible existence of host races or sibling species on summer hosts. They have been temporarily called by common names based on their main summer host plants as the caper midge, the pepper midge, and the squill midge. However, our sequencing data did not indicate the existence of host races or sibling species because there were more than one haplotype on a single summer host and they were not always included in one clade associated with one host species (Fig. 1).

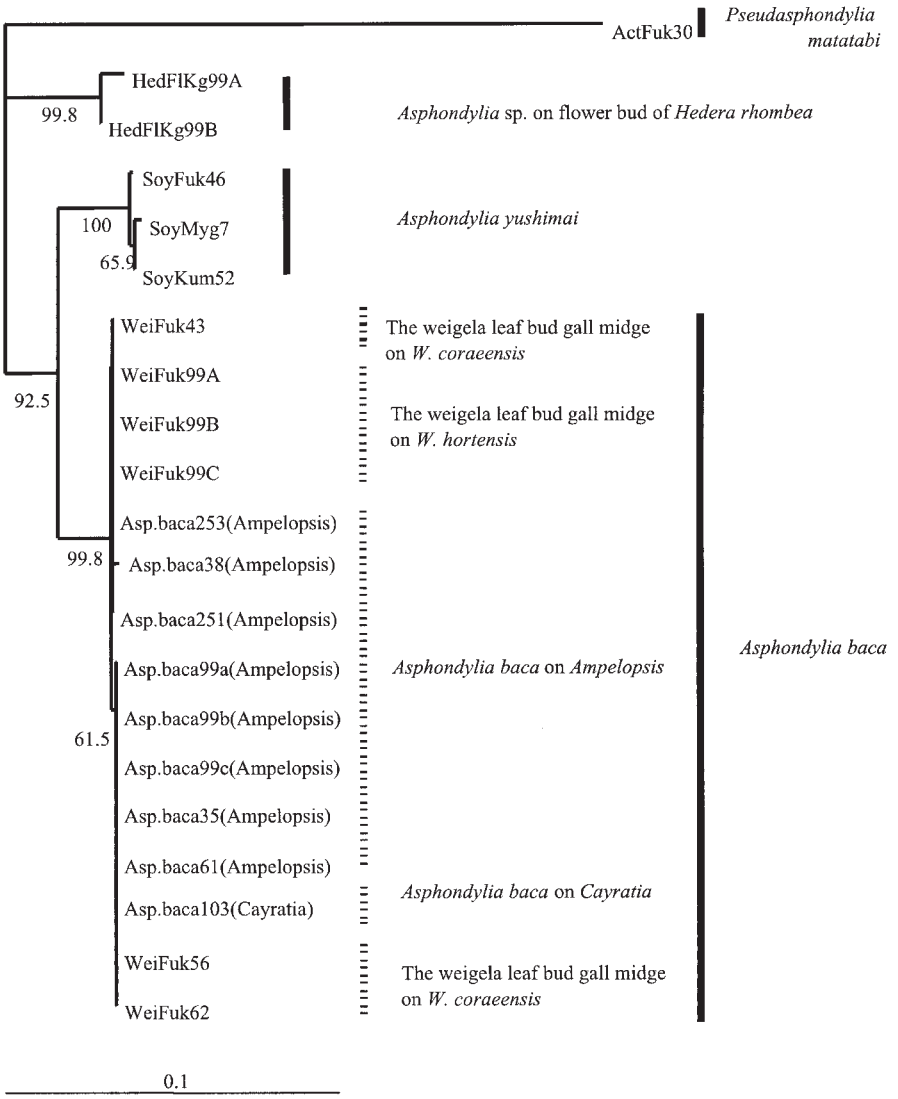

Figure 3. Neighbor-joining tree based on 439 bp of the mtDNA COI gene for *Asphondylia baca* on various hosts, *A. yushimai*, and *Asphondylia* sp. on flower buds of *Hedera rhombea*. Bootstrap values are indicated for nodes gaining more than 60% support (1000 replications). *Pseudasphondylia matatabi* was used as outgroup species. Sample names correspond to the respective isolation names registered in DNA database.

However, with regard to the pepper midge, we require specimens from the Mediterranean for DNA analysis to determine whether or not it is identical with *A. gennadii*, because there were distinct differences in the sequencing data (N. Uechi & J. Yukawa, unpubl. data) between *A. gennadii* and the Indonesian chili pod gall midge that had been identified as *Asphondylia capsici* Barnes (Aunu Rauf, 2001, pers. comm.; see also Skuhravá, 1986 for the occurrence of *A. capsici* in the Oriental Region).

Table 2. ***Asphondylia diervillae*****: fronto-clypeal, mesopleural, and mesepimeral setal counts, and measurements of wing, third flagellomere, ovipositor, and female seventh sternite.**

		Male				Female			
		n	mean	s.d.	range	n	mean	s.d.	range
Setal counts	Fronto-clypeal setae	2	37.0	1.4	36-38	2	30.0	7.1	25-35
	Mesopleural setae	1	18.0	-	18	2	38.0	14.1	28-48
	Mesepimeral setae	1	35.0	-	35	2	42.5	5.0	39-46
Wing	Length (μm)	2	3122	332	2887-3357	2	3194	134	3100-3289
	Width (μm)	2	1444	140	1344-1543	2	1511	132	1418-1604
	Length/width	2	2.2	0.02	2.1-2.2	2	2.1	0.1	2.1-2.2
Third flagellomere	Basal enlargement (μm)	2	191.0	26.9	172-210	2	197.0	7.1	192-202
	Width (μm)	2	49.5	13.5	40-59	2	54.5	9.2	48-61
Ovipositor	Length (μm)	-	-	-	-	2	1247.5	36.1	1222-1273
7th sternite	Length (μm)	-	-	-	-	2	318.0	7.1	313-323
	Ovipositor/7th sternite	-	-	-	-	2	3.9	0.2	3.8-4.1

II. *Asphondylia baca* and the weigela leaf bud gall midge in Japan

Material and Methods

Collection and preservation of Japanese Asphondylia *species*

Fruit galls produced by *Asphondylia baca* on *Ampelopsis brevipedunculata* and *Cayratia japonica* (Thunberg) Gagnepain (Vitaceae) and leaf bud galls by *Asphondylia* sp. on *Weigela hortensis* K. Koch and *Weigela coraeensis* Thunberg (Caprifoliaceae) were collected from various localities in Japan (Table 1). In addition to our collecting efforts, many people cooperated in collecting *Asphondylia* galls and gall midges at various localities in Japan. Distribution information was accumulated by literature surveys (Yukawa & Masuda, 1996; Uechi *et al.*, 2002) besides the collecting data of this study.

Some of the collected galls were dissected under a binocular microscope to obtain larval and pupal specimens. Remaining galls were maintained in plastic containers (10 cm in diameter, 6 cm in depth) to rear adult midges. Mature larvae, pupae, or emerged adults were kept in 70 to 75% ethanol for morphological observation or 99.5% acetone for DNA analysis.

The following slide-mounted specimens were examined: 8 males and 8 females, galls collected from Mt. Hikosan, Fukuoka Pref., Japan, 23 May 1965, J. Yukawa leg., emerged on 23 June–3 July 1965, reared by A. Taketani (host plant: *Weigela japonica* Thunberg) Cecid. No. A1601–A1616, A1621; 7 males and 1 female, galls collected from Iino, Miyazaki Pref., Japan, 25 May 1963, J. Yukawa leg., emerged on 9–20 June 1963, reared by J. Yukawa (host plant: *ibid.*), Cecid. No. A1631–A1637, A1640; 5 females, galls collected from Mt. Nyûtô, Akita Pref., Japan, 30 June 1965, J. Yukawa leg., emerged on 3–12 July 1965, reared by J. Yukawa (host plant: *W. hortensis*), Cecid. No. A1656-A1659; 6 males and 5 females, galls collected from Mt. Inunaki, Fukuoka Pref., Japan, 19 June 2000, N. Uechi leg., emerged on 21–23 June 2000, reared by N. Uechi (host plant: *W. coraeensis*), Cecid. No. A1671–A1681. These specimens are kept in the collection of the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Japan.

Morphological Comparison between Asphondylia diervillae *and the weigela leaf bud gall midge in Japan*

In June 2001, one of us, JY, and Dr. R. J. Gagné (Systematic Entomology Laboratory, USDA) visited Albany, NY and the Appalachian Mountains, WV and MD, USA in search of leaf bud galls produced by *Asphondylia diervillae* on *Diervilla lonicera* Miller (Caprifoliaceae) to obtain fresh larvae or pupae for DNA analysis. However, no galls were found on *D. lonicera* in these areas, so DNA could not be analyzed for *A. diervillae*. For morphological comparison between *A. diervillae* and the weigela leaf bud gall midge in Japan, we borrowed 2 males, 2 females, 2 pupae, and 1 larva of *A.*

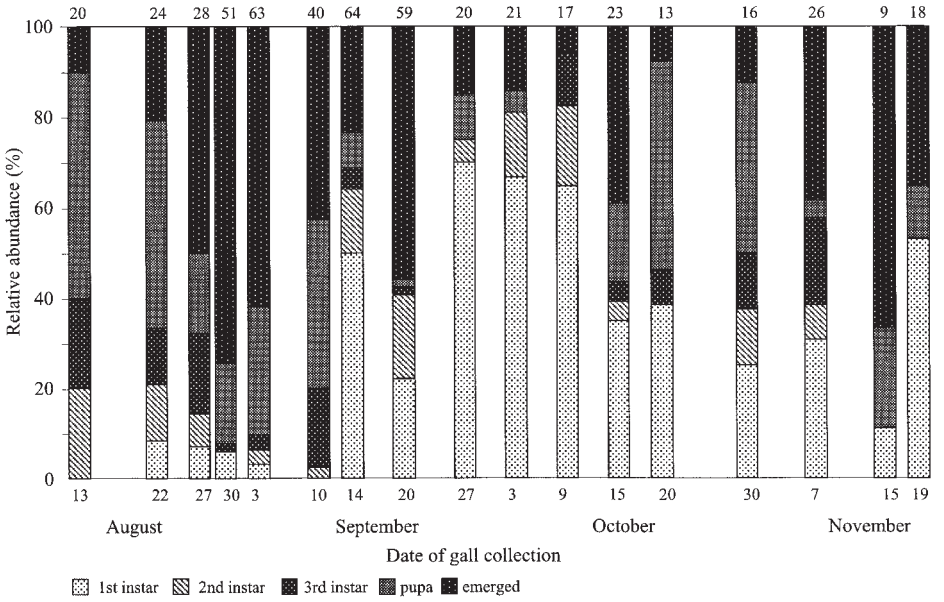

Figure 4. Changes in the age structure of *Asphondylia baca* on *Ampelopsis brevipedunculata* during the period from 13 August to 19 November 1998 at Inunaki, Wakamiya Town, Fukuoka Prefecture, Japan. Numerals above each bar indicate the number of individuals examined.

diervillae from the Felt collection that is presently in the Department of Entomology, Smithsonian Institution, Washington, D.C. Their collection data are as follows (Reference No. a1469 for all the specimens): 1 male, Albany, 29 May 1907; 1 male and 1 pupa, Albany, 17 Aug. 1907; 1 female, Albany, 22 May 1907; 1 female, Albany, 12 Aug. 1907; 1 pupa, Albany, 24 May 1907; 1 larva, Albany, 12 May 1907.

Comparison in the DNA sequencing data and morphological features between Asphondylia baca *and the weigela leaf bud gall midge in Japan*

In order to confirm the identity between *Asphondylia baca* and the weigela leaf bud gall midge, a partial COI region of mtDNA was analyzed by the aforementioned methods applied to *A. gennadii* from Cyprus. Eight individuals of *A. baca* on *Ampelopsis* and 1 on *Cayratia* were submitted to the analysis (Table 1). DNA sequence data for 3 individuals of the weigela leaf bud gall midge on *W. hortensis* and 3 on *W. coraeensis* were available from Yukawa *et al*. (2003) (Table 1). For comparison with *A. baca* and the weigela leaf bud gall midge, we used DNA sequence data for the following Japanese species and segregate that were available from Yukawa *et al*. (2003): *A. yushimai*, *Asphondylia* sp. on flower bud of *Hedera rhombea* (Miquel) Bean (Araliaceae), and *P. matatabi* (Table 1).

Morphological similarity between *A. baca* and the weigela leaf bud gall midge was confirmed by examining the slide-mounted specimens of *A. baca* used in Yukawa (1971). In addition, we examined at least 20 of ethanol-stored larval specimens of *A. baca* to confirm whether or not the shape of apical lobe of sternal spatula is consistent in the species. The larvae were collected by one of us, NU, in July to August 1998 from fruit galls produced on *A. brevipedunculata* at Mt. Inunaki and Mt. Hikosan, Fukuoka Prefecture, Japan.

Table 3. The weigela leaf bud gall midge: fronto-clypeal, mesopleural, and mesepimeral setal counts, and measurements of wing, third flagellomere, ovipositor, and female seventh sternite

		Male				Female			
		n	mean	s.d.	range	n	mean	s.d.	range
Setal counts	Fronto-clypeal setae	4	40.3	12.2	22-48	7	35.7	10.3	25-52
	Mesopleural setae	12	34.3	14.5	13-52	9	45.1	5.1	36-52
	Mesepimeral setae	14	42.1	7.4	28-51	12	45.8	11.0	22-58
Wing	Length (μm)	8	3532	219	3188-3813	8	3867	408	2938-4250
	Width (μm)	8	1367	108	1250-1563	8	1625	88	1438-1688
	Length/width	8	2.6	0.2	2.2-2.7	8	2.4	0.3	1.7-2.8
Third flagellomere	Basal enlargement (μm)	14	253.2	33.0	200-305	14	216.1	25.4	170-250
	Width (μm)	14	59.3	10.5	45-80	14	51.1	12.0	35-75
Ovipositor	Length (μm)	-	-	-	-	6	1378.8	29.8	1333-1424
7th sternite	Length (μm)	-	-	-	-	6	382.2	28.1	343-414
	Ovipositor/7th sternite	-	-	-	-	6	3.6	0.3	3.2-4.1

Changes in the age structure of Asphondylia baca

Ten trees of *Ampelopsis brevipedunculata* were established as census trees at Inunaki, Wakamiya Town, Fukuoka Prefecture, Japan. From August to November 1998, 30–150 fruit galls produced by *A. baca* were collected by two of us, NU and DY, from these trees, 3–5 times a month. They were measured (diameter, height) and dissected to record developmental stages of the gall midge.

Adult emergence season of the weigela leaf bud gall midge

To monitor adult emergences of the weigela leaf bud gall midge, a total of 48 twigs of *W. hortensis* were numbered on 5 May 2000 with plastic labels at Nagatani Dam Park, Fukuoka City, Fukuoka Prefecture, Japan. There were at least 140 bud galls on these twigs. The number of adult emergences was recorded in the field every other day. After recording the number, pupal cases left on the galls were removed and emergence holes were marked with a felt pen to avoid double counting.

Results

Morphological comparison between Asphondylia diervillae *in North America and the weigela leaf bud gall midge in Japan*

Adults were morphologically quite similar to each other. There were no distinct differences between *Asphondylia diervillae* and the weigela leaf bud gall midge in the setal counts and the measurements of wing and third flagellomere (Tables 2, 3). The measurements of ovipositor and seventh sternite were a little shorter in *A. diervillae* than in the weigela leaf bud gall midge, but there were no differences between them in the relative length of ovipositor to the seventh sternite.

In contrast to the adult morphology, the arrangement of 3 lobes of lower frontal horn of pupa clearly indicated that they are different species. The lobes are arranged in a row in *A. diervillae* (Fig. 2A), while the central lobe is located posterior to the 2 outer lobes in the weigela leaf bud gall midge (Fig. 2D). Such a nonlinear arrangement of lobes of lower frontal horn is common in Japanese *Asphondylia* gall midges (Yukawa, 1971; Yukawa & Miyamoto, 1979). In addition, the total number of inner and outer lateral papillae of all larval thoracic segments is 4 in *A. diervillae* (Fig. 2B), while 5 in the weigela leaf bud gall midge (Fig. 2E). All 6 terminal papillae have a short seta, respectively, in the larva of *A. diervillae* (Fig. 2C). In contrast, 2 of 6 terminal papillae are cone-shaped and each of remaining 4 papillae has a short seta in the larva of the weigela leaf bud gall midge (Fig. 2F).

Based on these differences, we considered that the weigela leaf bud gall midge in Japan is not identical with *A. diervillae* in North America.

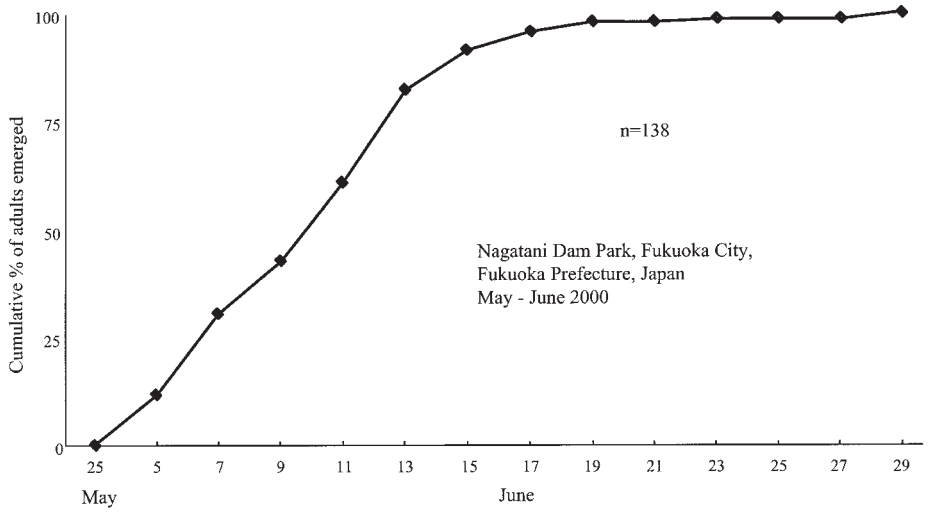

Figure 5. Cumulative percentage emergence curve for the weigela leaf bud gall midge.

Comparison in the DNA sequencing data and morphological features between Asphondylia baca *and the weigela leaf bud gall midge in Japan*

Three haplotypes were recognized in *Asphondylia baca* on *Ampelopsis* (Fig. 3), and there were only 1 to 2 bp differences among them. The sequence data of an individual on *Cayratia* coincided with 1 of the 3 haplotypes of *A. baca* on *Ampelopsis* (Fig. 3). In the weigela leaf bud gall midge, there were 2 haplotypes (Fig. 3), which were only 1 bp different from one another. One of the haplotypes of the weigela leaf bud gall midge was identical with 1 of the 3 haplotypes of *A. baca* on *Ampelopsis* (Fig. 3). All 146 deduced amino acid residues were identical between *A. baca* and the weigela leaf bud gall midge. Monophyly of the clade including the weigela leaf bud gall midge and *A. baca* on *Ampelopsis* and *Cayratia* was supported by a high bootstrap value (Fig. 3).

We examined the redescriptions of *A. baca* and the weigela leaf bud gall midge in Yukawa (1971), and could not find any features to distinguish them except the shape of apical lobe of sternal spatula. However, we confirmed by the observation of further larval specimens that there were individual variations in the apical lobe of sternal spatula from pointed to slightly rounded shape.

Changes in the age structure of Asphondylia baca

On 13 August 1998, all of the developmental stages except 1st instar were found in the galls on *Ampelopsis brevipedunculata* (Fig. 4). Thereafter the relative abundance of adults emerged to other stages increased and reached 75% on 30 August and numbers of adults continued to emerge until late September. In early October, the relative abundance of 1st instars increased, and then a small peak of adult emergence appeared in mid October. From late October to mid November, the rate of adults emerged increased again (Fig. 4). These data indicated that *A. baca* had at least 3 generations on *Ampelopsis* during the period from August to November. In late November, a considerable number of galls contained first instars, but these galls decayed or dropped to the ground in December before the larvae became full-grown.

Adult emergence of the weigela leaf bud gall midge

A total of 138 adults were recognized to have emerged from 116 galls out of about 140 that had been monitored in the field. The remaining 24 galls decayed or withered before emergence. The emer-

gence started on 25 May 2000 and continued until 29 June 2000 (Fig. 5). The percentage of accumulated emergences reached 50% on 11 June, 3 weeks after starting of emergence.

Distributional information

Galls of both *Asphondylia baca* and the weigela leaf bud gall midge have been recorded from various localities in Japan (Yukawa & Masuda, 1996; Uechi *et al*., 2002). In the present study, we newly found these galls in the following places: Mt. Tarumaesan, Hokkaido on *Weigela middendorffiana* (Carriére) K. Koch (Japanese name: Ukon-utsugi) (new host record); Futo, Ito City, Shizuoka Prefecture on *W. coraeensis*; Mt. Azumasan, Hiroshima Prefecture on *W. hortensis*. These collection records indicate that the distribution range of both *Asphondylia baca* and the weigela leaf bud gall midge widely overlap in Japan.

Discussion

Species identification of the weigela leaf bud gall midge

Shinji (1938) identified the host plant of the weigela leaf bud gall midge in Japan as *Diervilla japonica* Candolle (Caprifoliaceae), instead of *W. hortensis*. Based on the similarity of host plant, he believed that the gall midge is identical with a North American species, *A. diervillae*, which produces bud galls on *D. lonicera*. Yukawa (1971) and Yukawa & Masuda (1996) tentatively followed Shinji's identification, because the larval and pupal stages of the Japanese specimens were not precisely compared with those of *A. diervillae* at that time.

However, we found clear morphological differences between *A. diervillae* and the weigela leaf bud gall midge in Japan by comparing the Felt's specimens from North America with Japanese specimens (Fig. 2). This result is quite natural, because there is little possibility that North American and Eurasian (including Japanese) gall midge species are identical, although many genera are common on the 2 continents (Gagné, 1989). This shows that species identification based only on gall resemblance and host plant information can lead to misidentification.

Now that the weigela leaf bud gall midge and *A. diervillae* are shown to be distinct species, the next step is a comparison between the weigela leaf bud gall midge and *A. baca* to confirm their identity suspected by Sunose (1992).

Comparison in the DNA sequencing data and morphological features between Asphondylia baca *and the weigela leaf bud gall midge in Japan*

An *Asphondylia* segregate inhabiting fruit galls on *Cayratia japonica* was identical with *A. baca* in sequencing data (Fig. 3) and morphological features, so we recognized *A. brevipedunculata* and *C. japonica*, both Vitaceae, as summer-autumn host plants of *A. baca*.

We confirmed by our DNA analysis that at least 2 species of the genus *Weigela*, *W. hortensis* and *W. coraeensis*, are winter hosts of the weigela leaf bud gall midge (Fig. 3). These results suggest that other *Weigela* species, *W. middendorffiana*, *W. japonica*, and *Weigela decora* (Nakai) Nakai, are possibly included in the winter host plant range of the gall midge, although the DNA of *Asphondylia* gall midges reared from leaf bud galls on these plants could not be analyzed.

Since at least 1 haplotype of the weigela leaf bud gall midge coincided with 1 of the haplotypes of *A. baca* (Fig. 3) and there were no differences in all 146 deduced amino acid residues between them, we confirmed that the weigela leaf bud gall midge is identical with the ampelopsis fruit gall midge, *A. baca*.

In addition, there were no morphological differences between *A. baca* and the weigela leaf bud gall midge. The only morphological difference, the shape of apical lobe of larval sternal spatula (Yukawa, 1971), was shown to be insignificant in distinguishing the segregates. Thus, the field observation by Sunose (1992) was supported both by the DNA analysis and by the morphological study.

The DNA sequences of the weigela leaf bud gall midge (now identified as *A. baca*) are quite different from those of *A. yushimai* (Fig. 3) and many other *Asphondylia* segregates in Japan

(Yukawa *et al*., 2003; N. Uechi & J. Yukawa, unpubl. data). This means that *A. baca* is a distinct species and does not use known host plants of other *Asphondylia* species or segregates in Japan.

Life history of Asphondylia baca

On the basis of the aforementioned results, we consider that *Asphondylia baca* exhibits host alternation, utilizing *A. brevipedunculata* and *Cayratia japonica* as summer-autumn hosts and *Weigela* species as winter-spring hosts. Although *Ampelopsis* or *Cayratia* fruit do not exist from winter to spring, *Weigela* overwintering leaf buds are available for the gall midge. *Asphondylia baca* can overwinter inside *Weigela* overwintering leaf buds as first instars. Adults of *A. baca* emerge in June from *Weigela* leaf bud galls. Most of them leave *Weigela* and lay their eggs into young fruit of *Ampelopsis* or *Cayratia*. The infested young fruit grow into galls, in which the larvae mature and pupate. After spending 2 or more generations on summer-autumn hosts, adults of *A. baca* emerge in middle to late autumn, but there are no young fruit on *Ampelopsis* and *Cayratia* available for them to oviposit in this season. Instead, they lay their eggs in the overwintering buds of *Weigela* species.

III. General remarks

The example of host alternation by *Asphondylia baca* is the third finding in an *Asphondylia* species, following *A. gennadi* (Orphanides, 1975) and *A. yushimai* (Yukawa *et al*., 2003). The current findings, together with the preceding instances, indicate that host alternation may occur elsewhere in multivoltine species of the genus *Asphondylia* and that morphologically similar nominal species that utilize different groups of host plants may be found to be synonymous through DNA analysis.

It is too premature to remark on the evolution of host alternation by gall midges because information available for discussion is limited. However, we can naturally suspect that the host alternation has ecological significance such as an increase of voltinism that enhances reproductive potential, escape from parasitoid attacks by changing habitats, seeking for more fresh and nutritive host as has been noted for aphids.

Host alternation could evolve in groups of gall midges with strong flight ability. For example, the flight ability of the soybean pod gall midge, *Asphondylia yushimai*, is known to be strong enough to search for the winter hosts away from the summer hosts and *vice versa* (Yukawa *et al*., 2003).

The galls of *Asphondylia* species are always accompanied by a fungal symbiont (Gagné, 1989). There are various papers (e.g., Bronner, 1992; Richter-Vollert, 1964; Rohfritsch, 1992) suggesting, *in vitro* or indirectly, that larvae of ambrosia gall midges take nutritive substances not only from their host plants but also from associated fungi. Since we do not know enough about the role of the fungus, we need further studies to examine whether or not the fungal association has allowed *Asphondylia* species to utilize plant species across different plant families.

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