Host-Associated Differences in *Aspidiotus cryptomeriae*

KUWANA (Homoptera: Coccoidea: Diaspididae)

I. Adult Morphology and Host Preference

Akihiro MIYANOSHITA,^{1,2} Shozo KAWAI³ and Koichi FUJII⁴

¹ Department of Environmental Sciences, University of Tsukuba,
Tsukuba, Ibaraki 305, Japan

³ Laboratory of Tropical Plant Protection, Faculty of Agriculture,
Tokyo University of Agriculture, Setagaya-ku, Tokyo 156, Japan

⁴ Institute of Biological Sciences, University of Tsukuba,
Tsukuba, Ibaraki 305, Japan

(Received 28 May, 1992; Accepted 21 September, 1992)

Morphological differences and host preference in two forms of *Aspidiotus cryptomeriae* KUWANA associated with *Cryptomeria japonica* D. DON and *Torreya nucifera* SIEB. et ZUCC. were examined in the Kanto district, Japan. Adult males and females showed statistically significant differences in some characters. However, it was impossible to divide specimens perfectly by using any one of these characters. Linear discriminant analysis using combinations of several characteristics successfully (>99%) discriminated them. Two sympatric populations of adult females collected at the same stand were correctly classified by linear discriminant functions. Tests of forced host preference to five conifers indicated that each population was monophagous. The present study suggests strongly that these two forms are distinct host races. If the same holds true in other parts of Japan, these races might be considered to have differentiated into distinct species.

Key words: host race, *Aspidiotus cryptomeriae*, morphology, host preference, Diaspididae

INTRODUCTION

Aspidiotus cryptomeriae KUWANA occurs in China, Taiwan, Korea, South Sakhalin, (Russia), Japan and North America (STOETZEL and DAVIDSON, 1974; KAWAI, 1980; TANG, 1984). It is an important pest of many conifers in forestry, especially of *Cryptomeria japonica* in Japan. TAKAGI (1957) reported that there were two morphologically different types in adult females of this species. KAWAI (1980) divided it into three types on the basis of the adult female, locality, and host plant. MIYANOSHITA et al. (1991) showed that there were differences in esterase isozyme bands between populations on *Cryptomeria japonica* and *Torreya nucifera*. Nevertheless, all these forms have been considered conspecific, and morphological differences among them have not yet been analyzed quantitatively. Furthermore, neither host preference nor adult males have been studied. This paper deals with the morphological differences and host preference

² Present address: Laboratory of Applied Entomology, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Table 1. Collection data of the two forms of *Aspidiotus cryptomeriae* (adult male and female)

Host species	Location	Date	Number	Sex
<i>Cryptomeria japonica</i>	Yorii, Saitama	Apr. 13, 1986	67	♀
		Jun. 26, 1987		
	Atsugi, Kanagawa Tsukuba, Ibaraki	Apr. 14, 1986	54	♀
		Mar. 2, 1989 ^a	42	♀
		Apr. 4, Sep. 21, 1989	64	♀
		Apr. 8, 21, 1989		
		Apr. 6, 1989		
<i>Torreya nucifera</i>	Yorii, Saitama	Apr. 14, 1986	104	♀
		May 9, 1987		
	Kawasaki, Kanagawa	May 15, 24, 1989 ^a	26	♀
		Apr. 8, 21, 1989		
	Tsukuba, Ibaraki	Apr. 8, 21, 1989	59	♀
		Feb. 16, 21, 1989 ^a		
	Hino, Tokyo	Feb. 16, 21, 1989 ^a	64	♀
		Jul. 29, 1989		
	Hino, Tokyo	Feb. 19, 1989 ^a	13	♂
	Kawasaki, Kanagawa	Mar. 25, 1989 ^a	12	♂

^a Emerged at 25°C in the laboratory.

between the two forms associated with *Cryptomeria japonica* D. DON and *Torreya nucifera* SIEB. et ZUCC.

MATERIALS AND METHODS

Morphological differences. Adult males and females of the two forms were collected from *Cryptomeria japonica* and *Torreya nucifera* at several localities in the Kanto district, Japan (Table 1). Adult males preserved in 70% ethanol were soaked in lactic acid, mounted on a microscope slide, and measured under a microscope for five characters: forewing length (A), forewing width (B), body length (from head to the apex of penial sheath) (C), antenna length (from the segment III to X) (D), and penial sheath length (E) (Fig. 1).

Adult females preserved in 70% ethanol were boiled in 10% KOH solution, mounted on a microscope slide (KAWAI, 1980), and measured under a microscope for six characters: pygidium length (A), anal location (the distance from the base of median lobe to posterior margin of anal opening) (B), length of the dorsal macroduct which opens between the median lobes (C), number of dorsal macroducts (except the one which opens between the median lobes) (D), number of perivulvar pores (E), and body length (from head to the apex of median lobes) (F) (Fig. 2). Morphological measurements of the two forms were compared by *t*-test. For complete discrimination, we used linear discriminant analysis. The analysis is a multivariate statistical technique that can be used to identify an object as belonging to one of two or more previously defined groups (BLACKMAN, 1987; DIETRICH et al., 1991). In addition, the length of the plates between the median lobes (hereafter termed "median plates") relative to the length of the median lobes was compared between the two forms. We classified the specimens into three types: type I (plates longer than the lobes), II (plates as long as the lobes) and III (plates shorter than the lobes) (G in Fig. 2).

Fig. 1. Morphological characters of *Aspidiotus cryptomeriae* (adult male) used in the study. A: forewing length, B: forewing width, C: body length, D: antenna length (from the segment III to X), E: penial sheath length.

Fig. 2. Morphological characters of *Aspidiotus cryptomeriae* (adult female) used in the study. A: pygidium length, B: anal location, C: dorsal macroduct length (opening between median lobes), D: number of dorsal macroducts (except one which opens between median lobes), E: number of perivulvar pores, F: body length, G: type of median plates.

Table 2. Comparison of morphological characters between the two forms of *Aspidiotus cryptomeriae* (adult male)

Characters ^a	<i>C. japonica</i> form ^b	<i>T. nucifera</i> form ^c	Difference ^d
A: Forewing length	780.0±35.1	906.3±33.5	126.3**
B: Forewing width	381.4±20.1	441.9±17.4	60.5**
C: Body length	938.3±47.4	1141.9±60.5	203.6**
D: Antenna length	243.8±14.4	288.2±11.7	44.4**
E: Penial sheath length	504.7±33.8	611.1±21.8	106.4**

^a See Fig. 1, Mean±S.D. (μm), ^b N=30–31, ^c N=25, ^d **: $p < 0.01$ (*t*-test).

Table 3. Discriminant function and percentage of correct discrimination by linear discriminant analysis between the two forms of *Aspidiotus cryptomeriae* (adult male)

Combined character ^a (Length, μm)	Discriminant function (Z)	Correct discrimination (%)
Body (C) × antenna (D)	$Z = 0.00504 C + 0.00768 D - 9.54$	100
Forewing width (B) × body (C)	$Z = 0.00986 B + 0.0059 C - 10.20$	100
Forewing (A) × body (C)	$Z = 0.00489 A + 0.00668 C - 10.73$	100
Forewing (A) × penial sheath (E)	$Z = 0.00558 A + 0.01594 E - 10.06$	100

^a See Fig. 1, $Z > 0$: *T. nucifera* form, $Z < 0$: *C. japonica* form,
T. nucifera form: N=25, *C. japonica* form: N=24.

Morphological differences between two populations occurring sympatrically. Two sympatric populations were collected from a small stand containing *Cryptomeria japonica* and *Torreya nucifera* on April 8 and 21, 1989 at Tsukuba, Ibaraki. They were compared with respect to six morphological characters (A–F in Fig. 2) of adult females.

Host preference. Two test populations were collected in Saitama. For tests of host preference, young plants of five conifer species, *Cryptomeria japonica* D. DON, *Torreya nucifera* SIEB. et ZUCC., *Abies firma* SIEB. et ZUCC., *Taxus cuspidata* SIEB. et ZUCC. and *Pinus thunbergii* PARL, were used. These plants were inoculated with adult females, which had eggs under the scale, in order to settle crawlers on the test plant. Host preference was determined by examining the occurrence of oviposition in the following generation. The test plants were kept at $27 \pm 2^\circ\text{C}$, $69 \pm 8\%$ R.H., under natural photoperiod in a glass room.

RESULTS

Morphological differences

Adult males showed significant differences between the two forms in the mean of each of the five characters (Fig. 1; A–E and Table 2). The *T. nucifera* form was significantly larger than the *C. japonica* form. However, it was impossible to divide them perfectly by any one of these characters. To discriminate them, we performed linear discriminant analysis using combinations of several characters (Table 3).

Linear discriminant analysis showed that the function $Z = 0.00489A + 0.00668C - 10.73$ (A: forewing length (μm), C: body length (μm)) is the most useful to discriminate the two (correct discrimination 100%) ($Z > 0$ for the *T. nucifera* form and $Z < 0$ for the *C. japonica* form (Fig. 3)). Other combinations of the characters could also classify

Fig. 3. Relationship between body length and forewing length and linear discriminant function between the two characters in *Aspidiotus cryptomeriae* adult males. *C. japonica* form: $N=31$ ○, *T. nucifera* form: $N=25$ ●.

Table 4. Comparison of morphological characters between the two forms of *Aspidiotus cryptomeriae* (adult female)

Characters ^a	<i>C. japonica</i> form ^b	<i>T. nucifera</i> form ^c	Difference ^e
A: Pygidium length ^d	208.4 ± 14.1	256.1 ± 17.4	47.7**
B: Anal location ^d	63.8 ± 5.6	89.5 ± 9.2	25.7**
C: Dorsal macroduct length ^d	44.2 ± 3.8	61.7 ± 5.1	17.5**
D: Number of dorsal macroducts	42.1 ± 6.1	44.0 ± 7.8	1.9**
E: Number of perivulvar pores	35.0 ± 7.6	40.4 ± 10.6	5.0**
F: Body length ^d	660.5 ± 101.6	746.8 ± 114.1	86.3**

^a See Fig. 2, Mean $\pm$ S.D., ^b $N=213-227$, ^c $N=226-253$, ^d μm , ^e **: $p < 0.01$ (t -test).

the two perfectly, but the characters used are less easily measurable.

Adult females also showed significant differences in the six characters (Fig. 2; A-F) between the two forms (Table 4). The *T. nucifera* form had a longer pygidium (A), dorsal macroduct (C) and body (F); a more distant anal opening (B); and more numerous dorsal macroducts (E) and perivulvar pores (D). However, since it was impossible to divide them perfectly by any one of these characters, linear discriminant analysis was performed on a combination of several characters (Table 5).

Linear discriminant analysis showed that the function $Z = 0.03596B + 0.05212C - 5.59$ (B : anal location (μm), C : dorsal macroduct length (μm)) is the most useful to

Table 5. Discriminant function and percentage of correct discrimination by linear discriminant analysis between the two forms of *Aspidiotus cryptomeriae* (adult female)

Combined characters ^a	Discriminant function (Z) ^b	Correct discrimination (%)
Anal location (B) $\times$ Dorsal macroduct length (C)	$Z=0.03596 B+0.05212 C-5.59$	99.12
Pygidium length (A) $\times$ Anal location (B) $\times$ Dorsal macroduct length (C)	$Z=0.00197 A+0.003253 B$ $+0.05183 C-5.77$	98.90
Pygidium length (A) $\times$ Dorsal macroduct length (C)	$Z=0.00847 A+0.07996 C-6.27$	97.59
Pygidium length (A) $\times$ Anal location (B)	$Z=0.00257 A+0.06353 B-5.54$	96.93

^a See Fig. 2, μm , ^b $Z>0$: *T. nucifera* form, $Z<0$: *C. japonica* form,
T. nucifera form: $N=244$, *C. japonica* form: $N=212$

Fig. 4. Relationship between anal location and dorsal macroduct length and linear discriminant function between the two characters in *Aspidiotus cryptomeriae* adult females. *C. japonica* form: $N=212$ ○, *T. nucifera* form: $N=244$ ●, Large dots: More than 5 specimens.

discriminate the two (correct discrimination 99.12%) ($Z>0$ for the *T. nucifera* form and $Z<0$ for the *C. japonica* form (Fig. 4)).

In the *T. nucifera* form, most individuals (99.6%) had longer median plates (type I). On the other hand, in the *C. japonica* form, 73.3% of the individuals belonged to type II, 20.4% to type I, and 6.3% to type III (Table 6).

Table 6. Comparison of median plates between the two forms of *Aspidiotus cryptomeriae* (adult female)

Type of plates ^a	<i>C. japonica</i> form ^b	<i>T. nucifera</i> form ^c
I	45	249
II	162	1
III	14	0

^a See G in Fig. 2, ^b *N*=221, ^c *N*=250.Table 7. Comparison of morphological characters between the two populations of *Aspidiotus cryptomeriae* occurring sympatrically (adult female)

Characters ^a	<i>C. japonica</i> population ^b	<i>T. nucifera</i> population ^c	Difference ^e
A: Pygidium length ^d	212.6±10.9	259.7±17.4	47.1**
B: Anal location ^d	67.0±5.2	91.3±8.1	24.3**
C: Dorsal macroduct length ^d	43.3±3.6	60.6±5.0	17.3**
D: Number of dorsal macroducts	44.7±4.5	39.6±8.6	5.1**
E: Number of perivulvar pores	34.8±4.1	32.4±14.3	2.4 N.S.
F: Body length ^d	757.9±92.4	760.5±89.9	2.6 N.S.

^a See Fig. 2, Mean±S.D., ^b *N*=48–52, ^c *N*=43–45, ^d μm , ^e **: $p < 0.01$ (*t*-test), N.S.: Not significant difference. Fig. 5. Relationship between anal location and dorsal macroduct length in *Aspidiotus cryptomeriae* adult females occurring sympatrically, and linear discriminant function from Fig. 4. *C. japonica* population: *N*=52 ○, *T. nucifera* population: *N*=48 ●.

Table 8. Comparison of host preference between the two populations of *Aspidiotus cryptomeriae* on five conifer species (adult female)

Conifers tested	<i>C. japonica</i> population		<i>T. nucifera</i> population	
	Inoculated ^a	Parasitized ^b	Inoculated ^a	Parasitized ^b
<i>Cryptomeria japonica</i>	300	591	38	0
<i>Torreya nucifera</i>	370	0	40	254
<i>Taxus cuspidata</i>	350	0	20	0
<i>Abies firma</i>	250	0	18	0
<i>Pinus thunbergii</i>	350	0	16	0

^a Number of gravid adult females.

^b Number of adult females of next generation which oviposited.

Morphological differences between two populations occurring sympatrically

Adult females showed significant differences in the four characters (A, B, C, D in Fig. 2) between the two populations collected in the same stand (Table 7). The *T. nucifera* population had a longer pygidium (A) and dorsal macroduct (C), more distant anal opening (B), and more numerous dorsal macroducts (D). There were no significant differences in the body length (F) and the number of perivulvar pores (E). When the linear discriminant function $Z=0.03596B+0.05212C-5.59$ (B : anal location (μm), C : dorsal macroduct length (μm)) was applied to specimens of the two populations, it classified them correctly with only one exception (Fig. 5).

Difference in host preference

The *C. japonica* population could live on *Cryptomeria japonica* only, while the *T. nucifera* population could live only on *Torreya nucifera*; each population was monophagous (Table 8). Neither of the two populations could live on the other three conifers tested, *Abies firma*, *Taxus cuspidata*, and *Pinus thunbergii*. Although some crawlers of both populations could settle on other plants, most of them could not grow into second instars and none of them became adults. Thus, the two populations were also distinguishable by their host preference.

DISCUSSION

Some morphological types have been known in adult females of *Aspidiotus cryptomeriae*. TAKAGI (1957) reported that the type collected from *Taxus cuspidata* at Sapporo, Hokkaido, had much shorter pygidial lobes than that from *Torreya nucifera* at Mt. Amagi, Shizuoka. Also the pygidium of the former was less protruding than of the latter. WATANABE and TAKAGI (1967) stated that the species could be divided into two types in Japan; one type occurring in Hokkaido and in the mountains of Honshu, and the other in the lowlands of Honshu. KAWAI (1980) reported that this species could be divided into three types by host plant and by morphological characters, i.e., pygidium shape, length and number of dorsal macroducts, and number of perivulvar pores. One of them has a small, round pygidium and was collected on *Taxus cuspidata*, *Picea jezoensis* and *Tsuga sieboldii* from lowlands and mountains in Hokkaido, northern Honshu, and high mountains west of the Kanto district (cold-mountain region type). Another type has a long pygidium, short dorsal macroducts, and relatively few perivulvar pores.

It was collected from *Cryptomeria japonica* in the western Kanto lowlands (*Cryptomeria* type). The third type has a long pygidium, long dorsal macroducts, and many perivulvar pores, and was collected from *Torreya nucifera* and *Cephalotaxus harringtonia* in the western Kanto lowlands (plain type).

These studies suggest that *A. cryptomeriae* in Japan consists of some morphologically different forms. The two forms examined in this study correspond to the *Cryptomeria* type (*C. japonica* form) and the plain type (*T. nucifera* form) of KAWAI's (1980) classification.

In the present study, there were significant differences between the two forms with respect to morphological characters of adult males and females (Tables 2, 4 and 7). However, it was impossible to classify specimens into two distinct groups perfectly by using any one of these characters. We found that the two forms are almost perfectly distinguishable in both males and females by linear discriminant functions using two characters (Tables 3 and 5, Figs. 3 and 4).

The two populations of adult females occurring sympatrically can be correctly distinguished in almost every case by this method (Table 7, Fig. 5). This conclusion suggests the presence of reproductive isolation between the two populations at the same localities, because the collection sites of the two populations were only about 2 m apart. This distance would not prevent them from cross mating. Also, the two populations were monophagous (Table 8). Even if crawlers can settle on other plants, they will not develop to the adult stage. It seems that the populations are effectively isolated by their host plants. Electrophoretic assay (MIYANOSHITA et al., 1991) showed differences in esterase isozyme bands between a population on *C. japonica* and that on *T. nucifera*. Each population was characterized by the frequency and activity of a specific band, suggesting the presence of host specificity within this species. *A. cryptomeriae* generally is considered polyphagous; 13 species in 5 families have been recorded as its host plants (KAWAI, 1980). The present study has revealed that there are at least two monophagous forms, the *Cryptomeria japonica* form and the *Torreya nucifera* form.

Based on these morphological differences and host preference, we think that there are distinct host races. If the same holds true in other parts of Japan, these races might be considered to have differentiated into distinct species.

ACKNOWLEDGEMENTS

The authors wish to thank Professor T. KUSANO, Institute of Agriculture and Forestry, University of Tsukuba, for valuable advice. We are very grateful to Dr. S. TATSUKI, The University of Tokyo, for his helpful advice and reviewing of the manuscript.

REFERENCES

- BLACKMAN, R. L. (1987) Morphological discrimination of a tobacco-feeding form from *Myzus persicae* (SULZER) (Homoptera: Aphididae), and key to new world *Myzus* (Nectarosiphon) species. *Bull. Entomol. Res.* **77**: 713-730.
- DIETRICH, C. H., T. H. EMIGH and L. L. DEITZ (1991) Morphometric discrimination among females of sibling species of Aconophorini (Homoptera: Membracidae). *Sys. Entomol.* **16**: 311-318.
- KAWAI, S. (1980) *Scale insects of Japan in colors*. Nat. Soc. Agric. Educ.: Dai Nippon Print, Tokyo, 445 pp. (in Japanese).
- MIYANOSHITA, A., S. TATSUKI, T. KUSANO and K. FUJII (1991) Variation of esterase isozymes in *Aspidiotus cryptomeriae* KUWANA (Homoptera: Diaspididae). *Jpn. J. Appl. Entomol. Zool.* **35**: 317-321 (in Japanese with English summary).

- STOETZEL, M. B. and J. A. DAVIDSON (1974) Biology, morphology and taxonomy of immature stages of 9 species in the Aspidiotini (Homoptera; Diaspididae). *Ann. Entomol. Soc. Am.* **67**: 475–509.
- TAKAGI, S. (1957) A revision of the Japanese species of the genus *Aspidiotus*, with descriptions of a new genus and a new species. *Insecta Matsumurana* **21**: 31–40.
- TANG, F.-T. (1984) *The scale insect of horticulture and forest of China*. Volume II. Shanghai Agriculture University Press, Shanghai, 133 pp. (in Chinese).
- WATANABE, T. and S. TAKAGI (1967) Scale insects of forest. *Shinrin-Boeki-News* **16**: 2–6 (in Japanese).