Geographic variation of elytral color in the sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae), in Japan

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Abstract

To use elytral color morphs of *Cylas formicarius* as a genetic marker in its eradication using the sterile insect technique, the geographic distribution of the morphs was examined in the three main infested areas of Japan (Central Ryukyus, Southern Ryukyus, and Ogasawara Islands). In addition to the two known color morphs in Japan (bluish elytra (BE) and greenish elytra (GE)), the piceous elytra (PE) was found mainly in the Southern Ryukyus at low frequency. The color morph frequencies varied between the Central and Southern Ryukyus and between locations within the two areas, while only BE was found in the Ogasawara Islands. PE would be useful for genetically marking sterile weevils to be released. Similarly, GE would be useful in some islands where GE is rarely or not found. BE frequencies on two islands in the Central Ryukyus (Amami and Okinawa) and two islands in the Southern Ryukyus (Miyako and Ishigaki) in 2006–2007 were higher than in 2002–2003.

Key words: Eradication program; genetic marking; quarantine pest; Ryukyus; sterile insect technique

INTRODUCTION

The sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae), is a serious pest of sweet potato, *Ipomoea batatas* (L.) Lam. (Convolvulaceae), in tropical and subtropical areas (Sugimoto, 1990; International Institute of Entomology 1993; Setokuchi et al., 1996; Alonso-Zarazaga, 2004). It is thought to have originated from India and to have been dispersed on a regional scale mainly by human transportation of infested sweet potato (Wolfe, 1991) because of its limited dispersal ability (Moriya, 1995; Moriya and Hiroyoshi, 1998). In Japan, this weevil was first recorded on Okinawa Island in 1903 (Nawa, 1903) and is currently distributed throughout the Southwest Islands and Ogasawara Islands. Owing to the serious damage caused to sweet potato by this weevil, Japanese plant quarantine regulations

have prohibited the transport of host plants from infested areas to other areas in Japan since 1950. To solve this problem, the Kagoshima and Okinawa prefectural governments initiated the *C. formicarius* eradication project using a sterile insect technique (SIT) with the support of the Japanese government (Ohmura, 2000). Sterilized weevils are currently marked with a fluorescent powder dye to distinguish them from wild weevils when they are recaptured by monitoring traps in the field (Kuba et al., 2003); however, it is necessary to develop a better marking technique because the marker can be removed (Kuba et al., 2003).

In insect marking methods, visible genetic variations, such as body and eye color, have often been used as genetic markers to identify insects (reviewed by Hagler and Jackson, 2001), for example, the ebony body coloration of the boll weevil, *Anthonomus grandis*, was used to study its dispersal

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and mating behavior (Bartlett, 1967; Bartlett et al., 1968). *C. formicarius* shows three elytral color polymorphisms: bluish elytra (BE), greenish elytra (GE), and piceous or piceous-brown elytra (PE) (Pierce, 1918, 1940; Kissinger, 1968; Wolfe, 1991). PE, BE, and GE *C. formicarius* were recorded in India, USA and the Caribbean; and Indonesia and the Philippines, respectively (Pierce, 1918, 1940; Kissinger, 1968; Wolfe 1991); however, their detailed geographic distribution and color morph frequencies have not yet been examined. The BE morph of *C. formicarius* in Japan was described by Sutherland (1986) and later by Kawamura et al. (2005), who reported the co-occurrence of BE and GE weevils on four islands in the Southwest Islands (Amami Island, Okinawa Island, Miyako Island, and Ishigaki Island) with geographic variation in their frequency. They observed the genetic dominance of GE over BE regardless of sex, but the inheritance pattern of the color morphs was not determined.

To use elytral color morphs of *C. formicarius* as genetic markers, it is necessary to further understand BE and GE geographic distribution, including the exploration of additional color morphs. In the present study, we examined the color morphs of *C. formicarius* collected in 29 locations that almost covered its distribution range within Japan.

MATERIALS AND METHODS

C. formicarius were collected using pheromone lures (Lure-II, Sankei Chem. Co., Japan) in the Central and Southern Ryukyus, which are in the Southwest Islands, and in the Ogasawara Islands:

13 locations from the Central Ryukyus in 2006–2007, 12 from the Southern Ryukyus in 2006–2007, and four from the Ogasawara Islands in 2004 and 2007 (Fig. 1; Appendix). Since the color morph frequencies of this weevil did not vary between sexes in the previous study (Kawamura et al., 2005), only males were used in the present study. The collected weevils were preserved in 99.5% ethanol, air-dried on Kimwipes (Crecia Co., Japan), and classified into color morphs by eye (Fig. 2). When weevils were collected in two or more locations on a single island, we referred to them as different populations based on their distance because of their poor dispersal ability; their maximum flying ability is about 2 km (Moriya, 1995; Miyatake et al., 1997; Moriya and Hiroyoshi, 1998). The statistical analyses were the Yates chi-square test for color morph frequencies in different periods and locations within single islands and among populations within the main island group, and the Tukey-Kramer test for color morph frequencies among the main island group. To examine

Fig. 1. Locations where *C. formicarius* males were collected in this study.

Fig. 2. Elytral color polymorphism in *C. formicarius*. A. Bluish elytra (BE). B. Greenish elytra (GE). C. Piceous elytra (PE). Scale bar: 5.0 mm.

Table 1. Within-island comparisons of different periods and locations with regard to color morph frequencies^a

Tested group	df	χ^2	<i>p</i>
Comparison among periods			
Yomitan Village (Okinawa Island)	6	4.590	0.597
Uruma City (Okinawa Island)	6	4.962	0.548
Iheya Island	2	5.062	0.790
Miyako Island	6	10.769	0.095
Ishigaki Island	6	8.365	0.212
Funauki (Iriomote Island)	2	0.240	0.886
Taketomi Island	2	5.429	0.066
Kohama Island	4	5.720	0.221
Kuro Island	2	2.966	0.226
Hateruma Island	2	0.176	0.915
Yonaguni Island	4	2.213	0.696
Comparison among locations			
Amagi Town vs. Isen Town (Tokunoshima Island)	2	0.728	0.694
Nago City vs. Yomitan Village vs. Uruma City (Okinawa Island)	4	0.567	0.966
Toyohara vs. Funauki (Iriomote Island)	2	48.825	<0.001

^aDetailed sampling data are shown in Appendix.

temporal changes in color morph frequencies, the frequencies on Amami Island, Okinawa Island, Miyako Island, and Ishigaki Island, were also compared to those in 2002–2003 (Kawamura et al., 2005) using the Yates chi-square test.

RESULTS

C. formicarius of non-BE/GE morphs were found on the following nine islands: Kouri Island (Central Ryukyus) and Tarama Island, Ishigaki Island, Iriomote Island, Taketomi Island, Kohama Island, Kuro Island, Hateruma Island, and Yonaguni Island (Southern Ryukyus) (Fig. 2c). These beetles were characterized by a piceous or reddish purple elytra. We referred to this color morph as piceous elytra (PE) according to the description by Pierce (1918, 1940) and Kissinger (1968).

Color morph frequencies did not change during 2006–2007 in the eleven populations examined (Yates chi-square test, $p > 0.05$) (Table 1; Appendix); hence, the data for these periods were pooled. There was no significant difference in color morph frequencies within islands between two populations on Tokunoshima Island (Amagi Town and Isen Town) and among three populations on Okinawa Island (Nago City, Yomitan Village, and Uruma City) (Yates chi-square test, $p > 0.05$), while there was a significant difference between two populations on Iriomote Island (Toyohara and Funauki)

(same test, $p < 0.001$) (Table 1; Appendix). Figure 3 shows the geographic distribution of the three color morphs of *C. formicarius* in the Southwest Islands and the Ogasawara Islands of Japan. In the Southwest Islands, BE accounted for 80.2–98.9% in the Central Ryukyus, but for 15.0–83.8% in the Southern Ryukyus. PE was mainly found in locations where BE frequencies were 50–60% and accounted for only 0.1–1.1%. In contrast, BE accounted for 100% in four of the Ogasawara Islands. There were significant differences in BE frequencies among the Central Ryukyus, the Southern Ryukyus, and Ogasawara Islands (Tukey-Kramer test, $p < 0.05$) (Table 2). The difference in color morph frequencies was also significant within the Central and Southern Ryukyus (Yates chi-square test, $p < 0.001$) (Table 2). BE frequencies of Amami Island, Okinawa Island, Miyako Island, and Ishigaki Island in 2006–2007 were significantly higher than in 2002–2003 (Kawamura et al., 2005) (Yates chi-square test, $p < 0.05$) (Table 3).

DISCUSSION

In the present study, *C. formicarius* showing PE were found at a low frequency (1% or less). PE is probably genetically determined because both BE and GE have a genetic basis (Kawamura et al., 2005). In fact, we have recently established a PE-monomorphic population from weevils collected in

Fig. 3. Geographic distribution of elytral color morphs of *C. formicarius* in Japan. White represents BE frequency; grey: GE frequency; and black: PE frequency. Numbers near pies represent PE frequencies (in %). Isls.: Islands; Is.: Island.

Table 2. Comparisons of BE frequencies among and within island groups^a

Locality	Among island groups		Among localities within an island group ^c		
	No. of collection sites	BE frequency (Mean $\pm$ SD) ^b	df	χ^2	<i>p</i>
Ogasawara Islands	4	100 $\pm$ 0.0 a	—	—	—
Central Ryukyus	16	90.0 $\pm$ 6.4 b	30	276.518	<0.001
Southern Ryukyus	13	47.3 $\pm$ 21.7 c	24	1101.921	<0.001

^a Detailed sampling data are shown in Appendix.

^b Numbers followed by different letters are significantly different at the 5% level (Tukey-Kramer test on arcsine-transformed data).

^c Yates' chi-square test.

Table 3. Comparisons of color morph frequencies from four islands between 2002–2003 and 2006–2007

Island	Collection year	No. of weevils examined	No. (%) of weevils ^b			df	χ^2	p
			BE	GE	PE			
Amami Island	2002–2003 ^a	208	188 (90.4)	20 (9.6)	0 (0)	2	6.326	0.042
	2007	502	480 (95.6)	22 (4.4)	0 (0)			
Okinawa Island	2002–2003 ^a	176	122 (69.3)	54 (30.7)	0 (0)	2	48.086	<0.001
	2006–2007	1,054	939 (89.1)	115 (10.9)	0 (0)			
Miyako Island	2002–2003 ^a	236	9 (3.8)	227 (96.2)	0 (0)	2	26.551	<0.001
	2006–2007	614	109 (17.8)	505 (82.2)	0 (0)			
Ishigaki Island	2002–2003 ^a	250	5 (2.0)	245 (98.0)	0 (0)	2	45.499	<0.001
	2006–2007	726	146 (20.1)	578 (79.6)	2 (0.3)			

^a Refers to the results of Kawamura et al. (2005).

^b BE, GE, and PE: Weevils showing bluish elytra, greenish elytra, and piceous elytra, respectively.

Yonaguni Island (Kawamura et al., unpublished), strongly supporting the genetic basis of PE. Therefore, PE would be useful for genetically marking sterile weevils to be released in the *C. formicarius* eradication project using SIT in Kagoshima and Okinawa Prefecture (Southwest Islands) or in other areas (Ogasawara Islands) if the eradication project is extended in the future. Similarly, GE would be useful in some locations where GE is rare (e.g., Kikai Island and Minamidaitou Island) or does not exist (e.g., Ogasawara Islands).

Because the quality of mass-reared insects affects the efficacy of a SIT program (Huetzel, 1976), the ecological and physiological characteristics (e.g., sexual competitiveness, dispersal ability, and longevity) have been examined using mass-reared insects to be released as sterilized insects (e.g., Nakamori and Soemori, 1981; Koyama et al., 2004; Matsuyama and Kuba, 2004; Kumano et al., 2007). To successfully introduce *C. formicarius* showing PE as a genetic marker in SIT, it is important to compare these characteristics between PE weevils and other colored weevils, and to clarify the inheritance pattern of the three color morphs.

In contrast to the Central and Southern Ryukyus (Southwest Islands), only BE existed in the Ogasawara Islands. In addition, color morph frequencies varied both between the Central and Southern Ryukyus and between locations within the same areas. A possible factor influencing monomorphism in the Ogasawara Islands is the difference in its invasion source. Kawamura et al. (2007) suggested that the sources of populations of the Ogasawara Islands differed from those of the South-

west Islands based upon differences in the nucleotide sequences of the internal transcribed spacer-1 region. *C. formicarius* in the Ogasawara Islands may have originated from a monomorphic population of elytral color or from a small number of BE weevils in a polymorphic population.

The present study also revealed that BE frequencies increased from 2002–2003 to 2006–2007 in all four islands examined, although it is uncertain why they increased. We need to continue research to clarify temporal changes in color morph frequencies of the respective islands to introduce elytral color morphs of *C. formicarius* as genetic markers in SIT.

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Appendix. List of color morph frequencies of *C. formicarius* males collected from the Southwest Islands and Ogasawara Islands

Collection site	Collector ^a	Collection date	No. of weevils examined	No. (%) of weevils ^b		
				BE	GE	PE
Southwest Isls.						
Central Ryukyus						
Amami Is.						
Tatsugo Town	S. Kawashima	27–29 August 2007	502	480 (95.6)	22 (4.4)	0 (0)
Kikai Is.	S. Kawashima	5–7 September 2007	375	371 (98.9)	4 (1.1)	0 (0)
Tokunoshima Is.						
Amagi Town	T. Shibata	8–15 June 2007	279	269 (96.4)	10 (3.6)	0 (0)
Isen Town	S. Ito	8–15 June 2007	75	70 (93.3)	5 (6.7)	0 (0)
		Total	354	339 (95.8)	15 (4.2)	0 (0)
Yoron Is.	S. Taniyama	22–29 June 2007	67	65 (97.0)	2 (3.0)	0 (0)
Okinawa Is.						
Nago City	K. Kawamura, S. Ohno, T. Ganaha, D. Haraguchi, N. Kumano, M. Hokama	10 January 2007	76	70 (92.1)	6 (7.9)	0 (0)
Yomitan Village	OPPPC	18 August–4 September 2006	118	112 (94.9)	6 (5.1)	0 (0)
	OPPPC	21 November–5 December 2006	174	155 (89.1)	19 (10.9)	0 (0)
	OPPPC	20 February–6 March 2007	196	170 (86.7)	26 (13.3)	0 (0)
	OPPPC	22 May–6 June 2007	165	145 (87.9)	20 (12.1)	0 (0)
		Total	653	582 (89.1)	71 (10.9)	0 (0)
Uruma City	OPPPC	22 August–7 September 2006	143	119 (83.2)	24 (16.8)	0 (0)
	OPPPC	30 November–20 December 2006	22	21 (95.5)	1 (4.5)	0 (0)
	OPPPC	8–22 March 2007	62	57 (91.9)	5 (8.1)	0 (0)
	OPPPC	30 May–14 June 2007	98	90 (91.8)	8 (8.2)	0 (0)
		Total	325	287 (88.3)	38 (11.7)	0 (0)
Kouri Is.	K. Kawamura, S. Ohno, T. Ganaha, D. Haraguchi, N. Kumano, M. Hokama	10 January 2007	616	574 (93.2)	41 (6.7)	1 (0.1)
Yabuchi Is.	OPPPC	22 August–7 September 2006	168	150 (89.3)	18 (10.7)	0 (0)
Miyagi Is.-Henza Is.	OPPPC	22 August–7 September 2006	175	141 (80.6)	34 (19.4)	0 (0)
Iheya Is.	M. Yamagishi	21 October 2006	264	225 (85.2)	39 (14.8)	0 (0)
	M. Yamagishi	19–20 February 2007	116	87 (75.0)	29 (25.0)	0 (0)
		Total	380	312 (82.1)	68 (17.9)	0 (0)
Tonaki Is.	Y. Ohshiro	9–11 August 2006	87	73 (83.9)	14 (16.1)	0 (0)
Aguni Is.	D. Haraguchi	19–20 July 2006	647	519 (80.2)	128 (19.8)	0 (0)
Kitadaitou Is.	N. Kirihara	17–24 July 2007	69	57 (82.6)	12 (17.4)	0 (0)
Minamidaitou Is.	D. Haraguchi, S. Ohno, N. Kumano	20–22 June 2006	36	35 (97.2)	1 (2.8)	0 (0)

Appendix. (Continued)

Collection site	Collector ^a	Collection date	No. of weevils examined	No. (%) of weevils ^b		
				BE	GE	PE
Southern Ryukyus Miyako Is.	OPPPC	24°43'N, 125°17'E	165	25 (15.2)	140 (84.8)	0 (0)
		28 August–13 September 2006	211	27 (12.8)	184 (87.2)	0 (0)
		22 November–11 December 2007	76	15 (19.7)	61 (80.3)	0 (0)
		7–23 March 2007	162	42 (25.9)	120 (74.1)	0 (0)
		28 May–11 June 2007	614	109 (17.8)	505 (82.2)	0 (0)
		Total	128	19 (15.0)	109 (85.0)	0 (0)
		28 August–13 September 2006	70	31 (44.3)	39 (55.7)	0 (0)
		19 May 2007	405	90 (22.2)	315 (77.7)	0 (0)
		8–26 June 2007	633	346 (54.7)	282 (44.5)	5 (0.8)
		24 August 2006	255	51 (20.0)	203 (79.6)	1 (0.4)
Kurima Is. Oogami Is. Irabu Is.-Shimoji Is. Tarama Is. Ishigaki Is.	OPPPC T. Ohishi T. Uesato N. Kumano OPPPC OPPPC OPPPC OPPPC	24°42'N, 125°14'E	201	30 (14.9)	171 (85.1)	0 (0)
		24°54'N, 125°18'E	79	14 (17.7)	64 (81.0)	1 (1.3)
		24°48'N, 125°9'E	191	51 (26.7)	140 (73.3)	0 (0)
		24°38'N, 124°41'E	726	146 (20.1)	578 (79.6)	2 (0.3)
		24°21'N, 124°9'E	306	192 (62.7)	112 (36.6)	2 (0.7)
		12–13 July 2006	385	320 (83.1)	65 (16.9)	0 (0)
		18–20 August 2006	220	187 (85.0)	33 (15.0)	0 (0)
		21 December 2007	605	507 (83.8)	98 (16.2)	0 (0)
		Total	501	261 (52.1)	239 (47.7)	1 (0.2)
		12 October 2006	323	196 (60.7)	127 (39.3)	0 (0)
Iriomote Is. Toyohara Funauki	S. Ohno S. Ohno, Y. Tamura S. Ohno K. Kawamura K. Kawamura S. Ohno K. Kawamura K. Kawamura	24°15'N, 123°51'E	824	457 (55.5)	366 (44.4)	1 (0.1)
		24°18'N, 123°42'E	352	195 (55.4)	156 (44.3)	1 (0.3)
		24°18'N, 124°5'E	294	181 (61.6)	113 (38.4)	0 (0)
		24°19'N, 123°58'E	129	86 (66.7)	43 (33.3)	0 (0)
		24°13'N, 124°E	775	462 (59.6)	312 (40.3)	1 (0.1)
		12–13 October 2006	330	171 (51.8)	157 (47.6)	2 (0.6)
		17–19 January 2007	337	197 (58.5)	140 (41.5)	0 (0)
		Total	667	368 (55.2)	297 (44.5)	2 (0.3)
		24°13'N, 124°E	330	171 (51.8)	157 (47.6)	2 (0.6)
		17–19 January 2007	337	197 (58.5)	140 (41.5)	0 (0)
Taketomi Is. Kohama Is. Kuro Is.	K. Kawamura K. Kawamura S. Ohno K. Kawamura K. Kawamura K. Kawamura, S. Ohno K. Kawamura, T. Ganaha, S. Ohno	24°18'N, 124°5'E	501	261 (52.1)	239 (47.7)	1 (0.2)
		24°19'N, 123°58'E	323	196 (60.7)	127 (39.3)	0 (0)
		24°15'N, 123°51'E	824	457 (55.5)	366 (44.4)	1 (0.1)
		24°18'N, 123°42'E	352	195 (55.4)	156 (44.3)	1 (0.3)
		24°18'N, 124°5'E	294	181 (61.6)	113 (38.4)	0 (0)
		24°19'N, 123°58'E	129	86 (66.7)	43 (33.3)	0 (0)
		24°13'N, 124°E	775	462 (59.6)	312 (40.3)	1 (0.1)
		12–13 October 2006	330	171 (51.8)	157 (47.6)	2 (0.6)
		17–19 January 2007	337	197 (58.5)	140 (41.5)	0 (0)
		Total	667	368 (55.2)	297 (44.5)	2 (0.3)

Appendix. (Continued)

Collection site	Collector ^a	Collection date	No. of weevils examined	No. (%) of weevils ^b		
				BE	GE	PE
Hateruma Is.	S. Ohno, N. Kumano K. Kawamura	11 July 2006 24 June 2007 Total	127 258 385	79 (62.2) 166 (64.3) 245 (63.6)	47 (37.0) 92 (35.4) 139 (36.1)	1 (0.8) 0 (0) 1 (0.3)
Yonaguni Is.	K. Kawamura, T. Kohama K. Kawamura, S. Ohno, D. Haraguchi T. Kohama	22 August 2006 14–15 February 2007	331 284	201 (60.7) 165 (58.1)	127 (38.4) 114 (40.1)	3 (0.9) 5 (1.8)
Ogasawara Isls.						
Mukojima Is.	S. Sato	19–21 June 2007 Total	210 825	135 (64.3) 501 (60.7)	74 (35.2) 315 (38.2)	1 (0.5) 9 (1.1)
Otoutojima Is.	S. Sato	26–29 October 2004	122	122 (100)	0 (0)	0 (0)
Chichijima Is.	S. Sato T. Hiramoto	25 November 2004 9 May 2007	46 159	46 (100) 159 (100)	0 (0) 0 (0)	0 (0) 0 (0)
Hahajima Is.	S. Sukenari	27 November 2007	85	85 (100)	0 (0)	0 (0)

^a OPPPC: Okinawa Prefectural Plant Protection Center.^b BE, GE, and PE: Weevils with bluish elytra, greenish elytra, and piceous elytra, respectively.