

On elytral color dimorphism of sweet potato weevil, *Cylas formicarius* (Fabricius), in the Southwest islands, Japan

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Synopsis

Sweet potato weevil, *Cylas formicarius*, is a serious pest of sweet potato, distributed widely in the tropic and subtropic areas of the world, containing Southwest islands in Japan. We recognized a dimorphism of elytral color, bluish and greenish elytra (BE and GE), in weevils collected in Southwest islands of Japan. This study examined the geographic distribution in Southwest islands of Japan, an inheritance and a cold tolerance of these forms of *C. formicarius* and obtained following results. 1) GE occupied more than 96% of wild male weevils collected in Miyakojima and Ishigakijima, while it was inversely minor in Amami-Oshima and Okinawa-Hontou. 2) The color forms of *C. formicarius* would be genetically determined and GE would be a genetically dominant to BE. 3) BE would be more suitable to acquire a tolerance to a lower temperature. This property corresponds well with a remarkably high frequency of BE of wild weevils found in Amami-Oshima and Okinawa-Hontou.

Introduction

Sweet potato weevil, *Cylas formicarius* (Fabricius), is a serious pest of sweet potato which is distributed widely in the tropic and subtropic areas of the world¹⁾, containing Southwest islands in Japan²⁻³⁾. Since 1950 the Japanese plant quarantine regulation has prohibited to transport its host plants from the distributed areas to others in Japan⁴⁾.

The taxonomy of *C. formicarius* has been confused because of a polymorphism of its elytral color and treated as three distinct taxa, *C. f. formicarius* (brownish or piceous elytra), *C. f. elegantulus* (bluish elytra; abbreviated as BE), and *C. turcipennis* (greenish elytra; abbreviated as GE)⁵⁻⁶⁾. However, *C. turcipennis* was considered

conspecific with *C. formicarius*⁷⁾, and also the above classification of the subspecies has been questioned⁸⁾. Recently, Wolfe⁹⁾ recognized these taxa as a synonymy and assigned them to *C. formicarius* group.

Although specimens of *C. formicarius* collected in Japan were described as BE form¹⁰⁾, we found both BE and GE forms in weevils which had been reared for a long time in Kinki University after collecting them in Southwest islands.

In the present study, we first investigated the geographic distribution of the color forms of *C. formicarius* in Southwest islands of Japan, and also examined an inheritance and a cold tolerance of these forms.

Materials and Methods

1. Geographic variation

Wild weevils Males of sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae), were collected using pheromone trap (Lure-II, Sankei Chem. Co., Japan) in Amami-Ohshima island in Kagoshima Prefecture, Okinawa-Hontou, Miyakojima and Ishigakijima islands in Okinawa Prefecture, in 2002 and 2003 (Fig. 1). After these weevils were preserved in 99.5% ethanol soon after collecting, they were dried on the Kimwipe® (Crecia Co., Japan), and classified into the two color forms by eye (Fig. 2). No change of the original color of their elytra was observed in these treatments.

Reared weevils Weevils collected in Amami-Ohshima and Okinawa-Hontou islands in 1996, and in Miyakojima and Ishigakijima islands in 1997, had been reared successively over five years on sweet potato tubers in a bio-safety incubator controlled at 27°C, 70% RH and 14L-10D in Kinki University, Nara Prefecture, Japan, with special permission of the Japanese government. A plastic

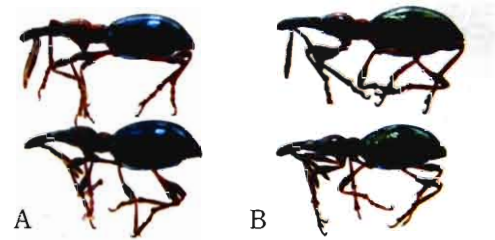


Fig. 2. Adults of *C. formicarius*.
A; BE form, B; GE form.

container, 300 mm (length) × 110 mm (width) × 100 mm (depth), was used for rearing weevils.

2. Inheritance of color forms

BE and GE weevils were isolated from reared weevils originated from Amami-Ohshima and Miyakojima islands. In order to obtain non-mated weevils, pupae which were picked out from sweet potato tuber, were settled one by one in each hole of 96-well tissue culture plate (Falcon, Becton Dickinson and Co., USA). The plates were kept in a bio-safety incubator controlled at 27°C, 70% RH and 14L-10D until adults emerged. Adult pairs of the same color were reared together for reproduction on sweet potato tuber (about 50g) in

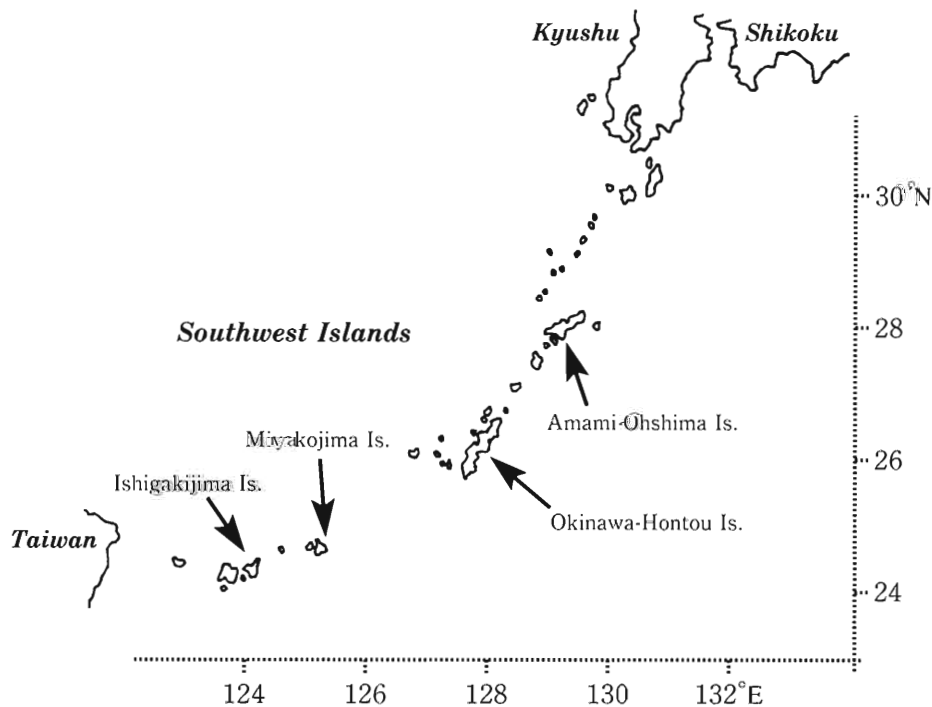


Fig. 1. Map showing the localities which *C. formicarius* was collected in this study.

a plastic cup, 95 mm (diameter) × 60 mm (depth), the bottom of which was filled with wet sawdust for keeping wet. In the next generation non-mated adult progenies with the same color as their parents were also reared in the same condition described above. This treatment was repeated successively over 10 generations. In the crossing experiment we used the weevil strain which was not found any progenies of the other color for five to eight generations.

Various pairs of non-mated adults, 10 and 15 day old after an adult emergence, were introduced into the plastic cup with a piece of sweet potato tuber. These tubers were renewed every 14 days, and were kept under the same conditions until adult progenies emerged. Progenies were classified into BE and GE forms. This experiment was replicated at three times in each pairs.

3. Cold tolerance

Five matured females or males, 10 and 15 day old, were introduced into a small plastic cup, 70

mm (diameter) × 40 mm (depth), with a piece of sweet potato tuber and wet sawdust. Those weevils were stored during 5, 7 or 10 days at 0°C, and checked whether alive or not. This experiment was replicated at 10 times in each storage period.

Results

1. Geographical variation

Four and two percent of wild males collected in Miyakojima and Ishigakijima was BE respectively, while 90 and 69% of those collected in Amami-Ohshima and Okinawa-Hontou was BE, respectively (Table 1). Thus, the frequencies of two color forms were significantly different among islands except between Miyakojima and Ishigakijima (χ^2 -test with Bonferroni correction, $p < 0.05$). With regard to reared weevils, frequencies of BE and GE were not significantly different between both sexes regardless of their origin islands (χ^2 -test with Bonferroni correction,

Table 1. Observed frequencies of BE and GE forms in wild and reared weevils of *C. formicarius*.

	Origin	Sex	No. (%) of weevils tested		
			BE ^a	GE ^a	
Wild	Amami-Ohshima	♂	188 (90.4)	20 (9.6)	a
	Okinawa-Hontou	♂	122 (69.3)	54 (30.7)	b
	Miyakojima	♂	9 (3.8)	227 (96.2)	c
	Ishigakijima	♂	5 (2.0)	245 (98.0)	c
Reared	Amami-Ohshima	♀	95 (88.0)	13 (12.0)	a
		♂	90 (88.2)	12 (11.8)	a
	Okinawa-Hontou	♀	74 (66.7)	37 (33.3)	b
		♂	73 (62.9)	43 (37.1)	b
	Ishigakijima	♀	11 (9.2)	109 (90.8)	c
		♂	7 (7.1)	91 (92.9)	c

^a BE and GE: Weevils with bluish and greenish.

Numbers followed by the same letter are not significantly different at $p = 0.05$ (χ^2 -test with Bonferroni correction).

$p > 0.05$), but significantly different among islands regardless of sexes (χ^2 -test with Bonferroni correction, $p < 0.05$). In addition, with regard to each of Amami-Ohshima, Okinawa-Hontou and Ishigakijima, these frequencies were not significantly different between wild and reared male weevils (χ^2 -test with Bonferroni correction, $p > 0.05$). Reared weevils from Miyakojima could not be examined because of a heavy infection of entomogenous fungus.

2. Inheritance of color forms

Results of a crossing experiment are shown in Table 2. One of three pairs examined in $GE \text{♀} \times GE \text{♂}$ in Amami-Ohshima and $BE \text{♀} \times BE \text{♂}$ in Miyakojima were excluded because these females were too short-lived due to unknown reasons. All of F_1 progenies from $BE \text{♀} \times BE \text{♂}$ and $GE \text{♀} \times GE \text{♂}$ were BE and GE forms, respectively, in both islands. In both reciprocal crosses, GE occupied more than 66%. The segregation ratio (BE:GE) of two color forms was most fitted to 1:3 in Amami-Ohshima, and to 1:2 in Miyakojima (χ^2 -

test for goodness of fit, $p > 0.01$, $df = 1$).

3. Cold tolerance

The survival rate of BE females originated from Amami-Ohshima was highest of tested weevils from two islands in 5 days storage (χ^2 -test with Bonferroni correction, $p < 0.05$) (Table 3). Also, the survival rate of BE males originated from Amami-Ohshima was highest of tested weevils from two islands in 5 and 7 days storage (χ^2 -test with Bonferroni correction, $p < 0.05$) (Table 3).

Discussion

The color polymorphism in *Atrachya menetriesi*¹¹⁾, *Chrysolina aurichalcea*¹²⁾ and *Conocephalus maculatus*¹³⁻¹⁴⁾ would be controlled by genetic factors. In the present study, we were able to isolate BE and GE from reared weevils originated from Amami-Ohshima and Miyakojima. Also, no significant difference of the frequencies of two color forms was observed between sexes in reared weevils originated from Amami-Ohshima, Okinawa-Hontou and Ishigakijima islands (Table

Table 2. Numbers (%) of BE and GE progenies obtained in various parent pairs of BE and GE forms of *C. formicarius*.

Origin	Crosses ^a	No. of pairs tested	No. (%) of progenies obtained		χ^2 -value ^b	
			BE ^a	GE ^a	1:2	1:3
Amami-Ohshima	$BE \text{♀} \times GE \text{♂}$	3	110 (27.2)	294 (72.8)	$\chi^2 = 6.777^*$	$\chi^2 = 1.069$ N.S.
	$GE \text{♀} \times BE \text{♂}$	3	69 (22.7)	235 (77.3)	$\chi^2 = 15.475^*$	$\chi^2 = 0.860$ N.S.
	$BE \text{♀} \times BE \text{♂}$	3	348 (100)	0 (0)	-	-
	$GE \text{♀} \times GE \text{♂}$	2	0 (0)	144 (100)	-	-
Miyakojima	$BE \text{♀} \times GE \text{♂}$	3	129 (32.9)	263 (67.1)	$\chi^2 = 0.032$ N.S.	$\chi^2 = 13.075^*$
	$GE \text{♀} \times BE \text{♂}$	3	154 (33.6)	304 (66.4)	$\chi^2 = 0.017$ N.S.	$\chi^2 = 18.169^*$
	$BE \text{♀} \times BE \text{♂}$	2	161 (100)	0 (0)	-	-
	$GE \text{♀} \times GE \text{♂}$	3	0 (0)	386 (100)	-	-

^a BE and GE: Weevils with bluish and greenish.

^b χ^2 -test for goodness of fit of the observed frequencies of BE and GE to 1:2 and 1:3. * significant at $p < 0.01$, N.S.: not significant.

Table 3. Survival of BE and GE forms of *C. formicarius* at 0 °C.

Sex	Origin	Color form ^a	No. of weevils tested	No. (%) of progenies obtained		
				5th day	7th day	10th day
♀	Amami-Ohshima	BE	50	40 (80.0) a	25 (50.0) a	1 (2.0) a
		GE	50	28 (56.0) ab	22 (44.0) a	0 (0) a
	Miyakojima	BE	50	26 (52.0) b	17 (34.0) a	1 (2.0) a
		GE	50	24 (48.0) b	19 (38.0) a	1 (2.0) a
♂	Amami-Ohshima	BE	50	40 (80.0) a	35 (70.0) a	4 (8.0) a
		GE	50	37 (74.0) ab	29 (58.0) ab	2 (4.0) a
	Miyakojima	BE	50	25 (50.0) b	19 (38.0) b	2 (4.0) a
		GE	50	27 (54.0) b	21 (42.0) b	1 (2.0) a

^a BE and GE: Weevils with bluish and greenish.

Numbers followed by the same letter are not significantly different at $p = 0.05$ (χ^2 -test with Bonferroni correction).

1). Thus, the color forms of *C. formicarius* would be genetically determined regardless of sexes like above described insects.

In the cross experiment, all of F₁ progenies from parents of the same color form had the same elytral color as their parents. In both reciprocal crosses 66-77% of F₁ progenies obtained was GE (Table 2). The segregation ratio of BE:GE in the reciprocal crosses was estimated as 1:3 in Amami-Ohshima and 1:2 in Miyakojima (Table 2). This suggests a genetic dominance of GE to BE though an inheritance pattern of color forms was not determined in the present study.

With regard to a geographic distribution of color forms of *C. formicarius* in Southwest islands, GE occupied more than 96% of wild male weevils collected in Miyakojima and Ishigakijima, while it was inversely minor in Amami-Ohshima and Okinawa-Hontou (Table 1). In spite of genetic dominance of GE, BE occupied more than 69% of wild males collected in Amami-Ohshima and Okinawa-Hontou (Table 1). This suggests that there will be any factors to inhibit the propagation of GE in these northern islands and that BE would

be more adaptive to these northern areas.

Regarding the geographic distribution of bronze and purple-green forms of *Chrysolina quadrigemina*, Peschken ¹⁵⁾ reported that the proportion of bronze form was correlated with a minimum temperature in winter at their collection sites. In the present experiment, when stored weevils originated from Amami-Ohshima during 5 days at 0°C, the survival rate of BE was slightly higher than GE regardless of sexes (Table 3). Also, at 5 days storage the survival rate of BE of Amami-Ohshima was higher than both forms of Miyakojima regardless of sexes (Table 3). These facts indicate that BE of Amami-Ohshima will be superior in a cold tolerance to both forms of Miyakojima. Hence, these results suggest that BE would be more suitable to acquire a tolerance to a lower temperature. This property of BE would not be activated in more warm areas like Miyakojima but would be liable to be activated in cooler areas like Amami-Ohshima and Okinawa-Hontou. This inference corresponds well with a remarkably high frequency of BE of wild weevils found in Amami-Ohshima and Okinawa-Hontou.

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南西諸島におけるアリモドキゾウムシの色彩2型について

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要 約

アリモドキゾウムシはサツマイモの大害虫であり、熱帯・亜熱帯地域に広く分布しており、わが国では南西諸島に分布している。本種には鞘翅の色彩多型(黒青色；bluish elytra, 以下BE, 黒緑色；greenish elytra, 以下GE)が認められている。本研究では、これら色彩2型について南西諸島における地理的分布、遺伝性および耐寒性について調査し、以下の結果を得た。1) GE型が宮古島と石垣島で96%以上を占めたのに対し、沖縄本島と奄美大島では逆にBE型の割合が圧倒的に高かった。2) 本種の色彩2型が遺伝的に決定されており、GE型がBE型に対して優性であった。3) BE型がGE型に比べて耐寒性を獲得しやすい特性をもつ可能性を示し、南西諸島の北部の島でBE型の割合が高かったことと符号した。