Environmental Factors Affecting Black/White Coloration of the Silken Girdle in the Swallowtail Butterfly, Atrophaneura alcinous (Lepidoptera: Papilionidae)

Authors: Saito, Masami, Yamanaka, Akira, Masaki, Hisashi, Nishijima, Ayako, Harada, Yumiko, et al.

Source: Zoological Science, 22(11) : 1259-1263

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.22.1259

ZOOLOGICAL SCIENCE **22**: 1259–1263 (2005)

Environmental Factors Affecting Black/White Coloration of the Silken Girdle in the Swallowtail Butterfly, *Atrophaneura alcinous* (Lepidoptera: Papilionidae)

Masami Saito[1], Akira Yamanaka[1*], Hisashi Masaki[1], Ayako Nishijima[1], Yumiko Harada[1], Chisato Kitazawa[2], Hirokazu Abe[2], Masao Watanabe[1] and Katsuhiko Endo[1]

[1] *Department of Physics, Biology and Informatics, Faculty of Science, Yamaguchi University, Yamaguchi 753-8512, Japan*
[2] *Biological Institute, Faculty of Education, Yamaguchi University, Yamaguchi 753-8513, Japan*

ABSTRACT—The silken girdles of pupae of the swallowtail butterfly *Atrophaneura alcinous* show black and white color diphenism. Field observations revealed that all pupae observed on non-food plants and the leaves and stems of the larval food plant *Aristolochia debilis* were classified as a silken girdle of a black type, while a large portion of pupae pupating on the twigs and trunks of cherry trees in close proximity to *A. debilis* were classified as a silken girdle of a black type. Additionally, all pupae observed on the surfaces of artificial objects in areas where there are no surrounding plants or trees were classified as a silken girdle of a white type. We demonstrated the effect of day length and the texture, light, plant odor and humidity of pupation sites on the coloration of the silken girdle in *A. alcinous*. Regardless of long-day or short-day day length conditions, light conditions of constant light or dark, or the presence of a plant odor of *A. debilis* as environmental cues, all larvae placed at over 80% relative humidity (R.H.) developed into pupae with a silken girdle of a black type. However, all larvae developed into pupae with a silken girdle of a white type when R.H. was below 75%. Furthermore, when pupae with a silken girdle of a white type were transferred to conditions of 90% R.H. within 24 hr of pupation, the white color of the silken girdle changed into a black type within 24 hr of the transfer. The present data suggest that the induction of a black coloration of the silken girdle in *A. alcinous* requires a R.H. of approximately 80% or more as an environmental factor.

Key words: *Atrophaneura alcinous*, silken girdle, coloration, relative humidity

INTRODUCTION

In butterflies, most larvae enter a wandering stage after a gut purge in order to find a suitable place to spin a thread of silk from silk glands to attach themselves to a plant or other object and form a silken girdle and/or silken pad before pupation. They then split open and lose their exoskeleton and pupate. In general, the role of the silken girdle and/or silken pad of butterflies is to hold their bodies to a plant or other object during the pupal stages, and these features are used as one index for the classification or characterization of butterflies (Mosher, 1916; Borror *et al*., 1976).

Depending on the species, larvae of many Lycaenidae, all Pieridae, and most Papilionidae spin a thin girdle of silk that supports them in a more or less upward position about the middle of the body, in addition to forming a silken pad, with the pupae finally being attached by the joint between the cremaster and silken pad, and the silken girdle. The pupae of some butterflies (Danaidae, Nymphalidae, Satyridae, and Libytheidae) are attached to a twig or leaf of a plant by the joint between the cremaster of the posterior end of the body and the silken pad that was spat on a plant, and hang upside down from this pad. Most Hesperiidae pupate inside a thin covering of silk, often among a few leaves that have been tied loosely together (Scott, 1986; Scoble, 1992).

The swallowtail butterfly *Atrophaneura alcinous* has pupae of the former type, and the silken girdle of *A. alcinous* shows a black and white color diphenism (Fig. 1). It has been reported that the color of the silken girdle in *A. alcinous* only appeared black, brown and white as a result of differences in the quality of foods, such as the fresh leaves of the larval host plant *Aristolochia onoei arimaensis*, or in

* Corresponding author. Phone: +81-83-933-5720;
Fax : +81-83-933-5720;
E-mail: yamanaka@yamaguchi-u.ac.jp

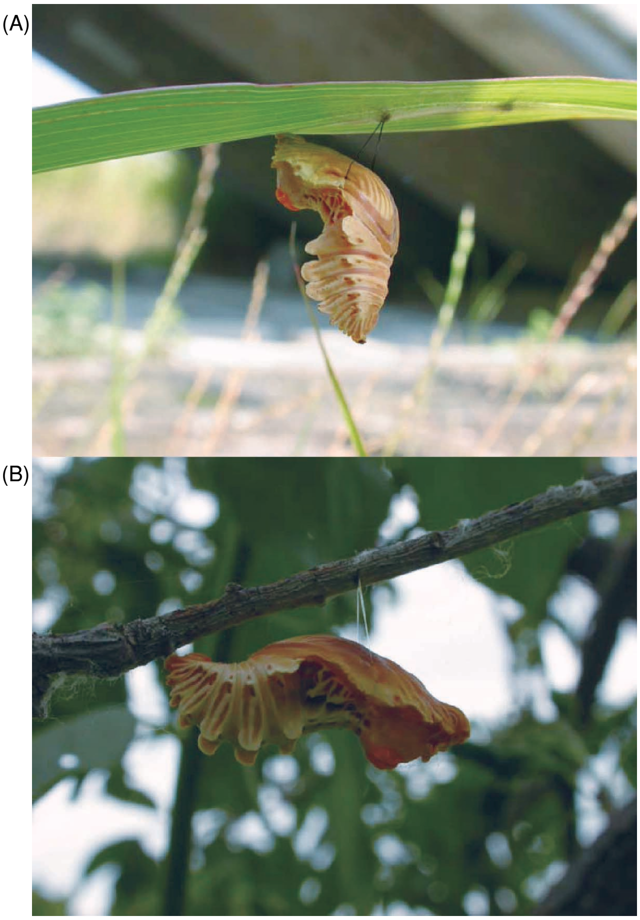

Fig. 1. Photographs of the coloration of the silken girdle in the swallowtail butterfly *Atrophaneura alcinous*. (A) A pupa with a silken girdle of the black type. (B) A pupa with a silken girdle of the white type.

response to an artificial diet containing powdered dry leaves of *A. onoei arimaensis* given as larval food during rearing periods (Kohzuki *et al*., 1994). However, the regulatory mechanism controlling the coloration of the silken girdle in *A. alcinous* has not been determined. In contrast, many moth larvae make cocoons using silk instead of the silken girdle and/or silken pad of many butterflies, and transform into pupae inside such structures. In the wild silkworm *Antheraea yamamai*, the green color of cocoons is produced in response to light of high intensity, which acts an environmental stimulus (Kato *et al*., 1989; Kato, 1991).

It is now known that the color change and color polymorphism of pupal bodies (or adult wings) in many butterfly species are controlled by environmental factors such as photoperiods, temperature, humidity, odor and background color (Müller, 1955; Ishizaki and Kato, 1956; Ohnishi and Hidaka, 1956; Shapiro, 1976; Hidaka, 1961; Honda, 1979, 1981; Usui *et al*., 2004). However, there is no information about environmental factors affecting the coloration of the silken girdle and/or silken pad, which are important formations during the pharate and pupal stages of many butterflies.

In this study, we aimed to determine the environmental factors affecting the coloration of the silken girdle in *A. alcinous*.

MATERIALS AND METHODS

Field observation

Field observations were carried out in the Hirai area (39°9'N, 131°27'E), Yamaguchi City, Western Honshu, Japan. This area includes the Fushino River, with the riverbank being covered mostly with weeds and low grasses dominated by the food plant of *Atrophaneura alcinous* larvae, *Aristolochia debilis*. Cherry trees are also found all along the riverbanks of this area.

Insect

Adult females of the swallowtail butterfly *A. alcinous* were initially collected from suburbs of Yamaguchi, Japan. Female butterflies were fed with a 10% sugar solution daily to ensure reproductive success. Two to four female butterflies were released in a cage (16×27×18 cm) and allowed to lay eggs on the leaves of the larval food plant, *A. debilis*. Newly hatched larvae kept in a transparent plastic container (13×20×6 cm) were fed on fresh leaves of *A. debilis*, which were exchanged during the light period on a daily basis, under either long-day (LD) conditions (alternating 16-hr light and 8-hr dark period, 16L: 8D) at 23°C, or short-day (SD) conditions (8L: 16D) at 20°C.

Classification of the color of the silken girdle

Larvae of *A. alcinous* entering the wandering stage after a gut purge were selected from each stock culture in the morning, transferred in transparent plastic containers, and allowed to pupate at 20°C under either constant light or constant dark conditions. After pupation, the color of the silken girdle of each *A. alcinous* pupa was classified into one of two types, black or white, because larvae of *A. alcinous* are easily disturbed and discontinue spinning a thread after an artificial vibration resulting from an attempt to check the color of the silken girdle during the spinning stages, and begin to wander again to seek another pupation site.

Humidity

A 90% R.H. was used for an examination of the influence of humidity on silken girdle coloration, and was attained by lining the container with moistened paper. When fresh plant was placed in the container alone, the humidity level inside the container was 80–90% R.H. To maintain a R.H. of 55–75% inside a container containing fresh plant of *A. debilis*, the top surface of the container was covered with a net of glass fibers (16×18 meshes).

Plants

Leaves of the larval food plant *A. debilis* were collected from suburbs of Yamaguchi City and Hofu City, Japan, and kept at 4°C until used.

Transfer experiment

Pupae with a silken girdle of a white type (or black type) and maintained under constant light at 20°C and 55% R.H. (or at 20°C and 90% R.H.) were transferred within 24 hr of pupation to conditions of constant light at 20°C and 90% R.H. (or 20°C and 55% R.H.). After one day, the color of the silken girdle of each *A. alcinous* pupa was classified into one of two types, black or white.

RESULTS

Field observations

Table 1 shows the color types of the silken girdles of *A.*

Table 1. Color types of silken girdle in *A. alcinous* pupae observed in the suburbs of Yamaguchi, Japan

Pupation sites	Number of pupae observed	Number of silken girdles of each color type	
		Black	White
Fresh leaf and string			
Larval food plant*[1]	10	10	0
Larval non-food plants	11	11	0
Twig or trunk			
Cherry tree	18	16	2
Concrete wall or wooden wall*[2]	2	0	2

Field observations of *A. alcinous* pupae were carried out along a riverbank of the Fushino River, Hirai, Yamaguchi City, between June and October 2004. *[1] Food plants for *A. alcinous* larvae only consisted of *Aristrochia debilis* in the observation area. *[2]The surroundings of these artificial objects did not include green plants.

Table 2. Color types of silken girdle in *A. alcinous* pupae observed under different pupation conditions

Pupation sites	Number of LD (SD) insects	Number of silken girdles of each color type	
		Black	White
Constant light conditions at 20°C and 55% R. H.			
Transparent plastic container	38 (34)	0 (0)	38 (34)
Constant dark conditions at 20°C and 55% R.H.			
Transparent plastic container	35 (23)	0 (0)	35 (23)
Cardboard box	23 (20)	0 (0)	23 (20)

LD and SD larvae after a gut purge were kept under each condition and allowed to pupate, as described in MATERIALS AND METHODS.

alcinous pupae observed along the riverbank of the Fushino River, Hirai, Yamaguchi City, between June and October 2004.

All silken girdles of *A. alcinous* pupae observed on the leaves and stems of the larval food plant *A. debilis*, and on the leaves and stems of larval non-food plants in close proximity to larval food plants, were classified as black types. On the other hand, 16 individuals of 18 *A. alcinous* pupae observed on the twigs and trunks of cherry trees situated near larval food plants were classified as a silken girdle of a black type, while the remaining two *A. alcinous* pupae were classified as a silken girdle of a white type. Additionally, all *A. alcinous* pupae observed on either concrete or the wooden walls of houses, such that the pupation site was not in close proximity to green plants or larval food plants, were classified as a silken girdle of a white type.

These results indicate that the color of the silken girdle in *A. alcinous* in the field shows color diphenism, as represented by black and white types, and that the induction of a black silken girdle in *A. alcinous* may be associated with light or other factors derived from green plants that function as environmental cues.

Effect of light and texture of the pupation site on the coloration of the silken girdle

Table 2 shows the effect of light and texture of the pupation site on the coloration of the silken girdle in *A. alcinous*. All LD and SD larvae developed into pupae with a silken girdle of a white type in containers of either smooth-surfaced transparent plastic or cardboard under conditions of constant light or constant dark at 20°C and 55% R.H.

These results show that all larvae kept within artificial objects in laboratory experiments developed into pupae with silken girdles of a white type, regardless of the light conditions to which the larval and pharate pupal stages were exposed, or the texture of the pupation sites (smooth or rough).

Effect of humidity and plants on the coloration of the silken girdle

We next investigated the effect of humidity and plants on the coloration of the silken girdle in *A. alcinous*. All LD larvae kept in transparent plastic containers without plants under constant light at 20°C and 90% R.H. or with fresh host plant *A. debilis* under constant light at 20°C and 80–90% R.H. developed into pupae with silken girdles of a black

Table 3. Effect of relative humidity on coloration of the silken girdle in *A. alcinous* pupae

R. H. conditions of pupation sites	Number of LD pupae	Number of silken girdles of each color typ Black	White
Without plant			
90% R. H.	32	32	0
With a fresh *A. debilis*			
80–90% R. H.	21	21	0
55–75% R. H.	43	0	43

LD larvae were kept in transparent plastic containers under constant light conditions and allowed to pupate, as described in MATERIALS AND METHODS.

Table 4. Change of the coloration of silken girdle in *A. alcinous* pupae by changing of humidity

Transfer conditions	Number of LD pupae	Number of silken girdles of each color type: Before transfer		After transfer	
		Black	White	Black	White
From 55% to 90% R. H.	20	0	20	20	0
From 90% to 55% R. H.	25	25	0	25	0

LD larvae were kept in transparent plastic containers under constant light conditions and allowed to pupate, as described in MATERIALS AND METHODS.

type, while all LD larvae kept in transparent plastic containers with fresh host plant under constant light at 20°C and 55–75% R.H. developed into pupae with silken girdles of a white type (Table 3).

These results indicate that the black coloration of the silken girdle in *A. alcinous* is induced by a R.H. of approximately 80% or more as an environmental cue, regardless of the presence of an odor of a fresh host plant.

Dose the white silken girdle change to black?

To investigate whether a white silken girdle can change to a black type, pupae with a silken girdle of a white type were transferred to conditions of 90% R.H.

When pupae with a silken girdle of a white type and maintained at 55% R.H. were transferred to conditions of 90% R.H. within 24 hr of pupation, all white silken girdles changed into a black type within 24 hr of the transfer. On the other hand, when pupae with a silken girdle of a black type and maintained at 90% R.H. were transferred within 24 hr of pupation to conditions of 55% R.H., all black silken girdles remained black after the transfer until adult emergence (Table 4).

These results indicate that larvae of *A. alcinous* spin a thread of silk of a white type after a wandering stage, and the black coloration of the silken girdle is induced by a reaction between the silken girdle and moisture under conditions of high humidity at least within 24 hr of pupation.

DISCUSSION

In the present study, we firstly examined the color changes of the silken girdle in *A. alcinous* resulting from differences in pupation site because environmental characteristics of pupation sites such as the photoperiod, texture, curvature, humidity, and odor have been shown to affect pupal coloration in some swallowtail butterfly species (Ishizaki and Kato, 1956; Ohnishi and Hidaka, 1956; Hidaka, 1961; Smith, 1978; Honda, 1979, 1981; Yamanaka *et al*., 1999, 2004). As shown in Table 2, light conditions during larval and pharate pupal stages and the texture of the pupation site were not important factors in inducing a silken girdle of a black type in *A. alcinous*, even though we found that *A. alcinous* pupae with silken girdles of a black type were more common in the field than the white type (Table 1), and that light intensity is a key factor in producing a green cocoon in the wild silkworm, *A. yamanai* (Kato *et al*., 1989; Kato, 1991). However, as shown in Table 3, we found that high R.H. is essential as an environmental cue for the induction of a silken girdle of a black type in *A. alcinous*, regardless of plant odor. Therefore, our results suggest that moisture (or the water molecule) might qualify as an effective inducer, for high humidity acts as one of the environmental factors determining coloration of the silken girdle in *A. alcinous*.

Additionally, when pupae with a silken girdle of a white type and maintained under conditions of 55% R.H. were transferred to conditions of 90% R.H. within 24 hr of pupation, the coloration of the silken girdle changed to black (Table 4). Hence, these results suggest that larvae might spin silk of a white type to produce a silken girdle/silken pad after wandering, and if they pupate under environmental conditions of high humidity such as under the leaf of fresh plants, grass wet with dew, or during the rainy season, the silken girdle has the potential to change to a black type until the early pupal stages, although the mode of action of moisture (or the water molecule) on a silken girdle of *A. alcinous* remains unclear. Furthermore, we do not know why *A. alcinous* larvae might change the coloration of the silken girdle from a white to black type from a biological and ecological aspect. Interestingly, however, preliminary observations revealed that the color of silken pads produced by young larvae for larval molting is of a white type under conditions of high humidity (data not shown).

On the basis of the data presented here, we are now investigating the physicochemical properties concerning the relationship between the silken girdle and moisture (or the water molecule), with emphasis on the reaction mechanism inducing black pigmentation and the strength of a silk girdle.

ACKNOWLEDGMENTS

This research is supported by a Grant-in-Aid for Young Scientists (B) (No. 12740462) from the Japan Society for the Promotion of Science (JSPA) to Akira Yamanaka.

REFERECES

Borror DJ, Delong DM, Triplehorn CA (1976) An Introduction to the Study of Insects, 4th ed. Holt, Rinehart and Winston, New York, 852 pp

Hidaka T (1961) Recherches sur le mechanism endocrine de l'adaptation chromatique morphologique chez les nymphes de *Papilio xuthus* L. J Fac Sci Univ Tokyo Sec IV Zool 9: 223–261

Honda K (1979) Environmental factors affecting the pupal coloration in *Papilio protenor demetrius* Cr. (Lepidoptera: Papilionidae) I. Effect of chemical stimuli (odor). Kontyû 47: 191–195

Honda K (1981) Environmental factors affecting the pupal coloration in *Papilio protenor demetrius* Cr. (Lepidoptera: Papilionidae) II. Effect of physical stimuli. Appl Entomol Zool 16: 467–471

Ishizaki H, Kato M (1956) Environmental factors affecting the formation of orange pupae in *Papilio xuthus*. Mem Coll Sci Univ Kyoto Ser B 23: 11–18

Kato Y (1991) Light-stimulated green colouration of silk glands in the saturniid moth, *Antheraea yamamai*: influence of ligation and parabiosis. Zool Sci 8: 665–672

Kato Y, Onuma Y, Sakurai K, Yamada H (1989) Role of light in the green pigmentation of cocoons of *Antheraea yamamai* (Lepidoptera: Saturniidae). Appl Ent Zool 24: 398–406

Kohzuki K, Omata K, Takeda M (1994) A simple artificial diet for *Atrophaneura alcinous* and its performance. J Appl Entomol Zool Chugoku-Branch 36: 17–23 (in Japansese with English summary)

Mosher E (1916) A classification of the Lepidoptera based on characters of the pupa. Bull Ill State Lab Nat His 12: 15–159

Müller HJ (1955) Die Saisonformenbildung von *Araschnia levana*, ein photoperiodish gesteuerter Diapause-effekt. Naturwissenschaften 42: 134–135

Ohnishi E, Hidaka T (1956) Effect of environmental factors on the determination of pupal types in some swallowtail, *Papilio xuthus* L. and *P. protenor demetrius* Cr. Zool Mag 65: 185–187 (in Japanese with English summary)

Scoble MJ (1992) The Lepidoptera; form, function, and diversity. Oxford Univ Press, Oxford

Scott JA (1986) The butterflies of North America. A natural history and field guide. Stanford Univ Press, Stanford

Shapiro AM (1987) Seasonal polyphenism. Evol Biol 9: 259–333

Smith AG (1978) Environmental factors influencing pupal colour determination in Lepidoptera I. Experiments with *Papilio polytes*, *Papilio demoleus* and *Papilio polyxenes*. Proc R Soc Lond B 200: 295–329

Usui Y, Yamanaka A, Islam ATMF, Shahjahan R, Endo K (2004) Photoperiod- and temperature-dependent regulation of pupal beige/black polymorphism in the small copper butterfly, *Lycaena phlaeas daimio* Seitz. Zool Sci 21: 835–839

Yamanaka A, Endo K, Nishida H, Kawamura N, Hatase Y, Kong W, Kataoka H, Suzuki A (1999) Extraction of partial characterizations of pupal-cuticle-melanizing hormone (PCMH) in the swallowtail butterfly, *Papilio xuthus* L. (Lepidoptera: Papilionidae). Zool Sci 16: 261–268

Yamanaka A, Imai H, Adachi M, Komatsu M, Islam ATMF, Kodama I, Kitazawa C, Endo K (2004) Hormonal control of the orange coloration of diapause pupae in the swallowtail butterfly, *Papilio xuthus* L. (Lepidoptera: Papilionidae). Zool Sci 21: 1049–1055

(Received February 16, 2005 / Accepted September 14, 2005)