Doctoral Thesis

Two types of mimicry rings at the immature stages of Lepidoptera with black spots on the yellow body and the red/black/white marking

Makoto Tsubuki

Department of Biology
Tokyo Metropolitan University
Tokyo
Japan

February in 2023

Contents

8-2. Title and summary in Japanese

Abstract

Insects have usually cryptic colors to avoid detection by the predators that use their vision to search for food. However, if the insects acquire toxic or repellent substances against predators, some of them develop conspicuous coloration to exhibit their unpalatability (called aposematism). Such warning colors allow insects to survive in nature exposed to predators. However, the immature stages of insects have little studied from this viewpoint of aposematic coloration despite the larvae and pupae exhibit colorful bodies in Lepidoptera.

First, I compared the pupal color patterns of the nine-spotted diurnal moths (Arctiidae: Syntominae) that differ in pupation sites, above ground or on the ground surface. Pupae of the two species (*Amata germana* and *A. flava*) were conspicuous in their color pattern with seven black dotted lines longitudinally on their pale-yellow bodies. These pupae were exposed to the aerial predators in a coarse silk mesh hanging from leaves and/or branches. The other two species (*A. fortunei* and *Syntomoides imaon*) pupated in spaces under stones, fallen twigs and leaves on the ground surface, and the pupae in a coarse silk cocoon were cryptic dark brown. Their pupation site selections were reproduced in the rearing glass vessels. Palatability assessment using the lizards as a potential predator suggested that pupae of *A. germana*, *A. flava* and *A. fortunei* are unpalatable and the lizard's feeding response decreases with experience (i.e., warning color with learning). However, pupae of *S. imaon* were all eaten (palatable). The evolutionary scenario of pupal coloration may be first palatable and cryptic on the ground (*S. imaon*), next unpalatable but still cryptic on the ground (*A. fortunei*), and last unpalatable and with warning color above the ground where the pupae are exposed to visually searching predators (*A. germana* and *A. flava*).

Next, the pupal color pattern, pupation site, palatability of pupae, and predator's learning to avoid unpalatable pupae were examined and compared among the three species of *Cystidia* moths (Geometridae: Ennominae). Pupae of *C. couaggaria* and *C. truncangulata* had a yellow surface dotted with black spots just like the pupae of the above-mentioned *Amata germana* and *A. flava*. *Cystidia couaggaria* and *C. truncangulata* make the naked pupae on the surface of branches and leaves above the ground, where they are more conspicuous against the predators. Pupae of *C. stratonice*

also have a yellow surface with black spots, but the head, thorax, wing pads, and abdominal end are uniformly blackish. This species also differs from the other two species in that it pupates in an above-ground pouch made of leaves by spinning with course silk. In the predation experiment using lizards as one of the potential predators, pupae of all three species were repelled, and repeated feeding decreased the lizard's trials to attack the pupae. These results suggested that the conspicuous coloration of unpalatable pupae of *Cystidia* may function as a warning color. However, *C. stratonice* pupating in the leaf pouch may halve the function of the warning color because the dark color expands widely on the pupal surface to be concealed in the rolled leaf.

Finally, the causes and maintenance mechanisms of aposematic mimicry seen in phylogenetically different three species of lepidopteran larvae, *Asota plana* (family Erebidae), *Brithys crini* (Noctuidae) and *Choaspe benjaminii* (Hesperiidae), were examined. These larvae have similar size and body color pattern of the red tip and repeated black/white bands. Palatability tests using lizards suggested that the former two species are unpalatable but the latter is palatable. The lizards that learned unpalatability of *A. plana* or *B. crini* missed in rapid discrimination of palatable *C. benjaminii* and silkworms painted with red and black as similar to *C. benjaminii*, but discriminated perfectly the silkworms painted entirely with brown (a mix of red and black) and without painting. Thus, the potential predators can learn and indiscriminate such a conspicuous color pattern of the three species of larvae. The field experiments using artificial clay models of caterpillars showed that red/black/white color-banded models were pecked less frequently by the wild birds than the control grey models in March at the southern Japan, and also in June at central Japan in which the resident and summer birds that learned unpalatable caterpillars were abundant but before naïve fledglings appear. However, the frequencies of pecking did not differ between the color-banded and control grey models in April at central Japan and in July at northern Japan in which unlearned resident and winter birds were abundant. In palatable *C. benjaminii* and unpalatable *A. plana* and *B. crini* are distributed sympatrically in southern Japan where the aposematic Batesian and Müllerian mimicries occur. In most part of central Japan, only palatable *C. benjaminii* is distributed. However, Batesian mimicry is probably maintained there by predation avoidance for *C. benjaminii* via the memory of the migratory birds from the south where they have a chance to learn unpalatable *A. plana* and/or *B. crini*.

Thus, the dotted blackish lines on the yellow surface of pupae form the mimicry rings including *Amata* species (Arctiidae: Syntominae) and *Cystidia* species (Geometridae: Ennominae). This mimicry ring also includes larval *Pryeria sinica* (Zygaenidae) and pupal *Ivela auripes* (Lymantridae) that are known to be unpalatable and have the dotted blackish lines on the yellow body surface. On the other hand, the common color pattern, reddish tip and black/white color banding, of the three species of *A. plana*, *B. crini* and *C. benjaminii* form another type of mimicry ring. Such mimicry rings among the aposematic larvae and pupae might be more common and include more species in the field, and which are maintained via the memory of insectivorous avian and non-avian reptiles and mammals. These mimicry rings are advantageous to understand the dynamic interactions between multiple species of prey and predators that occur in the nature.

1. General introduction

1-1. Aposematic mimicry rings

In the wild, some animals have conspicuous colors or color patterns. In general, conspicuous colors are more disadvantageous than cryptic colors, because the former are easily detected by the predators that search for prey visually. However, if these animals are poisonous or unpalatable, the predators can quickly learn such unprofitable prey, and thus survival of individuals with conspicuous colors are expected to be higher than those without conspicuous colors. This is the simple mechanism that the warning colors evolve, i.e., aposematism, although the study from the predator side is rare (Ruxton *et al*. 2018). The complex processes and mechanisms of prey choice, prey cognition, learning, and sensitivity to toxins (or palatability) are the problems to be understood from the predator side.

In the situation that the aposematic colored prey species is little eaten by the predators that learned it unprofitable, survival of the other palatable prey species with the similar color to the aposematic prey species may become higher than the conspecific other individuals with a different color. Thus, mimicry occurs between these unprofitable species (the model) and profitable species (the mimic), which is called Batesian mimicry (Bates 1862; Ruxton *et al*. 2018). Profitable (Palatable) prey species experience the selection that the potential predators tend to refrain from attacking them when they resemble some unprofitable (harmful and/or unpalatable) species. However, mimicry among unpalatable or otherwise unpalatable species is known as Müllerian mimicry based on Müller's idea reported in 1878 (Ruxton *et al*. 2018). Theoretically, if all other things are equal, the rarer unpalatable species face selection to resemble the more common unpalatable species (Ruxton *et al*. 2018). The Batesian and Müllerian mimicries are considered to be the extremes of a mimicry spectrum with aposematic color patterns (Huheey 1976). Weakly defended prey could act as Batesian mimics of better-defended models for hungry predators, and therefore, the relationships between co-mimics can sometimes switch from mutualistic (Müllerian) to parasitic (Batesian) according to predator's nutritional conditions (Ruxton *et al*. 2018).

If two or more mimetic species share the similar aposematic color patterns, they form the aposematic mimicry ring. The mimicry ring is defined as groups of unpalatable species, together with some palatable species, that have converged on the same warning color pattern (Mallet & Gilbert 1995), or as communities that contain at least well-defended prey species that has a warning signal and experiences reduced predation pressure owing to its aposematism and at least one more associated prey species that derive a fitness benefit from mimicking the well-established aposematic signals (Kunte *et al*. 2021). Mimicry rings are also known as mimicry complexes, in which mimics may be Batesian or Müllerian (Kunte *et al*. 2021). The mimicry rings sometimes consist of a great number of species and they interact to each other. Dynamics of their interactions, also including the effect of divergent predators, are now subject to be a real community that should be resolved (Kunte *et al*. 2021).

1-2. Mimicry of lepidopteran larvae and pupae

Insects use color and pattern as camouflage to blend into their environment and avoid detection by visually hunting predators. Many insect bodies and wings resemble fresh or dead leaves and tree trunks or branches, which is a phenomenon known as cryptic coloration (review by Caro & Koneru 2021). However, as mentioned in the above section, if the insect is equipped with defenses against predators such as toxins or inappetence (bad odor), selection may favor warning colorations (aposematism), i.e. conspicuous colors signaling that the prey is unprofitable (Caro & Koneru 2021). Cryptic coloration is found in all developmental stages, but the studies of warning coloration is generally restricted to adults (e.g., Ruxton *et al*. 2018).

In Lepidoptera, larvae of most species have a cryptic color, and a relatively few species exhibit a conspicuous color (Mappes *et al*. 2014; Robinson *et al*. 2023). However, little Batesian and Müllerian mimicries have been suggested even in the larvae with conspicuously colors (Willmott *et al*. 2011). Pupae are also mostly cryptic and hidden under the substrate or in inconspicuous cocoons. (Wiklund & Sillén-Tullberg 1985; Edmunds 1990; Janzen *et al*. 2010; Paul *et al*. 2018). The known example of pupal warning coloration is the unpalatable moth, *Ivela auripes* (Butler, 1877), which pupates at the exposed sites such as tree trunk and stone surface above the ground and displays the warning color pattern, seven blackish longitudinal lines on the dorsal yellow body surface (Yazaki *et al*. 2019), and the toxic two-spotted ladybird beetle, *Adalia bipunctata* (Linnaeus, 1758), which has warning colors at all stages of its life cycle (Paul *et al*. 2018), although not Lepidoptera. Thus, little attention has been paid to the mimicry of lepidopteran larvae and pupae.

In the field surveys and checking of the literature with color illustrations of the immature stages of Japanese Lepidoptera, I noticed that several larvae and pupae have markedly colorful marking patterns. Some of them apparently resemble each other and are grouped into two color patterns. One is the larvae and pupae with dotted blackish longitudinal lines on the yellow body surface (Fig. 1-1A) and the other is the larvae with a reddish tip and repeated black/white bands (Fig. 1-1B). These color patterns are likely to form the mimicry rings, respectively. In this study, therefore, those larvae and pupae with such two types of color patterns are examined whether they are the potential species of each aposematic mimicry ring or not. The first group consists of pupae of the two species of the nine-spotted diurnal moths (family Arctiidae), *Amata germana* Felder, 1862 and *A. flava* (Wileman, 1910); the three species of *Cystidia* (family Geometridae), *C. couaggaria* (Guenée, 1858), *C. truncangulata* Wehrli, 1934, and *C. stratonice* (Stoll, 1782); and the pupae of *Ivela auripes* (Butler, 1877) (family Lymantriidae) and the last-instar larvae of *Pryeria sinica* Moore, 1877 (family Zygaenidae) which already reported by us (Yazaki *et al*. 2019). The second group consists of the larvae of *Asota plana* (Walker, 1854) (family Erebidae), *Brithys crini* (Fabricius, 1775) (family Noctuidae), and *Choaspe benjaminii* (Guérin-Méneville, 1843) (family Hesperiidae).

It is usually difficult to get direct evidences to demonstrate the aposematic mimicries between two or more species, particularly to identify that Batesian or Müllerian mimicry occurs. Therefore, we must determine it based on a series of experimental evidences in the laboratory and field. First, we need information of palatability of the possible mimetic species. Insectivorous avian and non-avian reptiles and mammals are potential predators searching for and feeding on lepidopteran larvae and pupae. These animals are usually generalist predators, and the lizards are used as the potential predators in this study. The lizards have visual cue to perceive prey, and are often used as one of the potential predators of insects (Sexton 1960; Boyden 1976; Johki & Hidaka 1979; Mclain 1984; Hasegawa & Taniguchi 1994; Thery & Gomez

2010; Yazaki *et al*. 2019).

Second, morphological and color similarities are an essential factor to suggest the mimicry between two or more species. However, more directly evidences are needed to demonstrate that the predators can learn unpalatable types of prey (models) and once learned predators cannot discriminate the mimetic species (mimics) from the model. In this study, learning abilities are assessed using lizards by giving the same prey repeatedly (daily). The discrimination ability between model and mimic prey species is examined by giving the mimic to the lizards that learned the model. Moreover, the silkworms with different color decoration are used to confirm the color pattern itself functions in discriminating the mimic from the model.

Finally, to understand the spatiotemporal dynamics of the mimicry rings, predation pressures to model and mimic species must be assessed (Kunte *et al*. 2021). In this study, the predation pressures were assessed using the artificial clay models with different color decoration by setting them in the field where the avian communities differ seasonally and geographically.

This doctoral dissertation includes the following studies of aposematic coloration and mimicry in lepidopteran larvae and pupae by paying attention to the above mentioned issues; first, pupal warning color development in above-ground pupating species but cryptic color in ground-surface pupating species of the nine-spotted diurnal moths *Amata* and *Syntomoides* (Arctiidae); second, pupal warning coloration of three species of *Cystidia* (Geometridae) in relation to their pupation sites; and third, aposematic mimicry rings among larvae of *Asota plana*, *Brithys crini* and *Choaspe benjaminii* which may be maintained over their sympatric areas via memory of the migratory birds; and finally, the two types of mimicry rings are summarized and discussed.

Figure 1-1. Examples of two types of markedly colorful lepidopteran immature stages. A, a pupa of *Ivela auripes* (Butler, 1877) with longitudinally dotted blackish lines on yellow body surface. B, a larva of *Asota plana* (Walker, 1854) with a reddish marking and repeated black/white bandings.

2. Pupal warning color development in above-ground pupating species but cryptic color in ground-surface pupating species of the nine-spotted diurnal moths (Arctiidae: Syntominae)

2-1. Introduction

Insects use color and pattern as camouflage to blend into their environment and avoid detection by predators. Many insect bodies and wings resemble fresh or dead leaves and tree trunks or branches, which is a phenomenon known as cryptic coloration (review by Caro & Koneru 2021). However, if the insect is equipped with defenses against predators such as toxins or inappetence (bad odor), selection may favor warning colorations (aposematism), i.e. conspicuous colors signaling that the prey is unprofitable (Caro & Koneru 2021). Cryptic coloration is found in all developmental stages, but warning coloration is generally restricted to larvae and adults. Pupae are usually cryptic or hidden under the substrate or in inconspicuous cocoons. (Wiklund & Sillén-Tullberg 1985; Edmunds 1990; Janzen *et al*. 2010; Paul *et al*. 2018). The known example of pupal warning coloration is in the toxic two-spotted ladybird beetle, *Adalia bipunctata* (Linnaeus, 1758), which has warning colors at all stages of its life cycle (Paul *et al*. 2018) and in the unpalatable moth, *Ivela auripes* (Butler, 1877), which pupates at the exposed sites such as tree trunk and stone surface above the ground and displays the warning color of yellow bodies with seven blackish longitudinal lines on its dorsal surface (Yazaki *et al*. 2019).

In general, the pupation site selection is critical for the moths to survive at the pupal stage. In the nine-spotted diurnal moths (Arctiidae: Syntominae), I noticed that the above-ground pupating species make colorful pupae, but the ground-surface pupating species make dark brown pupae. The former two species pupate in a course silk net hanging from the leaves and twigs, whereas the latter two species pupate in a rough cocoon under stones and fallen twigs and leaves on the ground surface. Colorful pupae suggest they are unpalatable for predators, whereas dark brown pupae may not rink with unpalatability. Pupae in the aerial course net are exposed to flying birds and tree-climbing lizards that use their vision to search for food (Knowlton 1938; Waldbauer & Sternburg 1967). In this situation, unpalatable pupae would be advantageous to exhibit a warning color. If not unpalatable, the pupae may be advantageous to be cryptic on the ground surface where the ground-roaming predators such as small mammals (mice and shrews), birds and lizards who search for food visually (Liebhold *et al.* 1998, 2000).

On the ground, there may be other serious risks for pupal survival to avoid attacks by carnivorous insects, spiders and centipedes (Tanhuanpää *et al.* 1999) and by microorganisms such as bacteria and fungi under high humidity (Kamata *et al.* 1997). Probably to avoid these risks, several species of ground-dwelling lizards (Mount 1963; Hasegawa 1985), earwigs (Boos *et al.* 2014; Van Meyel *et al.* 2019), and centipedes (Machado 2000) protect their eggs on the ground surface by cleaning their egg surface until hatching. The pupae spending on the ground are also expected to defend to such attacks morphologically by making dense cocoons and/or thick pupal shells and chemically using anti-predatory/microbial secretions (Dossey 2011; Sugiura 2020).

In this study, I focus on the relationship between pupal colors and pupation sites among the four species of the nine-spotted diurnal moths belonging to the genera *Amata* and *Syntomoides*. First, their size, color, and shape including cocoon structure are described. Second, pupation site selection is examined in the laboratory cages and their palatability is examined using the lizard as a potential predator. Finally, the possible scenario of pupal warning coloration is discussed in relation to pupation site selection and palatability.

2-2. Materials and methods

Insects

Four species of the nine-spotted diurnal moths were used in this study: *Amata germana* Felder, 1862 and *A. flava* (Wileman, 1910) and the ground-surface pupating *A. fortunei* De L'Orza, 1869 and *Syntomoides imaon* (Cramer, 1779).

Amata germana is distributed in southeast Russia, China, Korea, Japan, Vietnam, Thailand, and Indonesia, and adults appear from July to September in Japan (Kishida 2011; Przybylowicz *et al.* 2016). Eight females collected at Hino, Tokyo, central Japan (35°39'54.0"N, 139°25'32.3"E) on 3 July 2019, 12 June 2021, and 23 August 2021 laid eggs and a total of ca. 300 hatched larvae were reared in the laboratory. Several larvae were placed together in the same plastic cup (130 mm in diameter, 55 mm in depth). Our preliminary observations revealed that larvae of these species feed on dead plant materials and animal carcasses on the ground surface. As also suggested by KonDo *et al.* (2011), a 1:1 mixture of two artificial foods was given to larvae: one is used for rearing silkworm larvae (Silk Mate; Nihon Nosan Kogyo Co. Ltd., Yokohama, Japan) and the other is water-soaked carp food (Swimmy; Nippon Pet Food Co. Ltd., Tokyo, Japan). They were reared at 25 ± 1°C with a 14 h light and 10 h dark cycle in all experimental treatments.

Amata flava is distributed in Taiwan and the Ryukyus and adults appear twice, May and October (Kishida 2011). Three females collected at Yonaguni-jima, the Ryukyus, southern Japan (24°27'08.8"N, 123°00'21.2"E) on 3 May 2021 laid eggs. A total of 96 hatched larvae were reared. The mass cultured ca. 200 larvae derived from Yonaguni-jima were provided at Tottori University in April 2022. The rearing methods were the same as in *A. germana*.

Amata fortunei is distributed in China, Korea, Russia (Kuril Island), and Japan and adults appear twice, June and August to September, in Japan (Kishida 2011; Przybylowicz *et al.* 2016). Eight females collected at Yawata, Chikuma, Nagano, central Japan (36°30'10.8"N, 138°05'52.3"E) on 12 September 2020 and at Hino, Tokyo, central Japan (35°40'31.0"N, 139°25'23.4"E) on 19 August 2021 laid eggs and a total of ca. 360 hatched larvae were reared by the same methods as in *A. germana*.

Syntomoides imaon is distributed widely in India, Southeast Asia, Southern China, and southern Japan and adults appear in June and July in southern Japan where this moth established recently (Kishida 2011; KonDo *et al.* 2012a). Four females collected on Okinawa-jima, southern Japan (26°11'48.4"N, 127°40'03.5"E) in June 2021 laid eggs and a total of ca. 160 hatched larvae were reared by the same methods as in *A. germana*.

Adults of these four species are well known to wasp-mimicking moths and the wing and body marking patterns also function in mate choice (KonDo *et al.* 2012b). However, the morphological and ecological information of their immature stages is scarce. Only the useful information to this study is that the pupal color is whitish yellow in *A. germana* whereas brown in *A. fortunei* (Okagaki 1956; Nakamura 2007), and pupation of *A. germana* occurs at tree branches (Okagaki 1956).

Morphological measurements

Body size were measured using the free software, ImageJ (version 1.53e; National Institutes of Health, Bethesda, Maryland, USA) based on the photographs of randomly chosen 14 final-instar larvae, 31 pupae, 12 male adults and 12 female adults of *A. germana*; 12 final-instar larvae, 35 pupae, 11 male adults and 10 female adults of *A. flava*; 12 final-instar larvae, 29 pupae, 11 male adults and 11 female adults of *A. fortunei*; and 14 final-instar larvae, 34 pupae, 11 male adults, and 10 female adults of *S. imaon*. I measured the body length (from the front of head to the body end) and width (at the widest part of body) of larvae and pupae from the dorsal side, and the forewing length (from base to the tip) of adults.

To know the pupal color patterns in ultraviolet light range, the pupal photographs of *A. germana* and *A. flava* were taken using an ultraviolet filter (U330; HOYA Co. Ltd., Tokyo, Japan) to reproduce the visual perception of birds and lizards.

The mass of a whole pupa, a total of silk, and a pupal exuvium was measured to compare their allocation rates between above-ground pupating *A. germana* ($N = 24$) and ground surface pupating *A. fortunei* ($N = 16$). Four to five days after pupation, silk threads around the pupa were removed carefully and the fresh weight of the silk and pupa was separately measured to the nearest 0.01 mg. On the day of emergence, the pupal exuvium was also weighed to the nearest 0.01 mg after excluding meconial fluid inside the exuvium by absorbing it with a paper string.

Pupation site selection

The final-instar larvae of *A. germana* ($N = 39$), *A. flava* ($N = 55$), *A. fortunei* ($N = 26$) and *S. imaon* ($N = 21$) were individually placed in a glass vessel (75 mm in diameter, 90 mm in height) with a glass lid. The bottom of the vessel was filled up to 5 mm with a 1:1 mixture of peat moss and red ball earth. A plastic rod (3 mm in diameter, 100 mm in length) was put into the vessel for larvae to climb up to the lid. The food was supplied fully in the bottom. After pupation, the height was measured from the bottom to the center of the pupa in the dorsal view. If not pupated or died within two weeks, those larvae were omitted from the data analysis.

Assessment of palatability

A total of 52 lizards, *Plestiodon finitimus* Okamoto & Hikida, 2012, were used as a potential predator. These lizards were caught at Tama, Hino, and Minamiosawa Cities in Tokyo, central Japan, on September 10, 13 and 17 in 2019; June 26 and August 28 in 2020; April 18, 27, July 25, and August 25, 30 in 2021; September 4, 7, and April 28 in 2022. The mean snout to vent length (SVL) of them was 59.9 mm (SD = 5.1, $N = 52$) and total tail length was 88.5 mm (SD = 18.0, $N = 52$). They were kept individually in plastic cages (200 mm x 350 mm, 200 mm in depth) at 17-35°C with natural daylength. Each cage was filled up to 10 mm with a mixture of peat moss and red ball soil in a 1:1 ratio and an unglazed shelter. Water was supplied constantly in a cup (90 mm in diameter, 50 mm in depth). Two mealworms (larvae of *Tenebrio molitor* Linnaeus, 1758) were fed during the 3-h basking period with artificial light every other day.

Feeding experiments were conducted after maintained in the cage for at least a week. After 30-min basking, one of larvae, pupae, male adults or female adults of the moth was given to one lizard per day. The same staged moth was given to the same lizard for five consecutive days. The feeding willingness of each lizard was checked by giving one mealworm just before the first day trial and by giving three mealworms just after the last (fifth) day trial. To prevent the adult moth from flying out of the cage bottom, adults were given after cutting the forewings partly at their edges. Pupae were presented to the lizards suspended by a black polyester thread (0.15 mm in diameter). All tests were terminated if there was no approach to the prey for 20 s. Lizard behavior was recorded with a video camera (12 MPx Camera, Apple Inc., Cupertino, CA, USA) of the Apple iPhone 8. The lizard's behavior was discriminated into seven categorized sequences: no response (0), heading toward prey (1), licking prey (2), pecking prey with their mouthparts (3), biting prey but immediately releasing it (4), swallowing prey but regurgitating it (5), and complete feeding (6).

Three lizards were tested for 5-day feeding responses to each stage of *A. germana*, *A. flava*, and *A. fortunei*. In *S. imaon*, however, the feeding responses to female adults were tested only the first day for seven lizards. Once the lizards were used for this 5-day feeding experiment, they were released to the capture sites.

Statistics

Values are shown as mean ± standard deviation (SD). To compare the relative silk weight to pupal weight between above-ground pupating *A. germana* and the ground-surface pupating *A. fortunei*, first Pearson's correlation analysis was used to examine the log-log relationship between pupal weight and silk weight for all combined data of *A. germana* and *A. fortunei*, and next the residuals were calculated from this common regression equation. Thus, the obtained residuals compared between these two species using Mann–Whitney *U*-tests. The relative exuvium weight to pupal weight was also compared between these two species in the same method. In the analysis of the pupation site selection among four species, the Kruskal-Wallis test and the following Scheffe’s multiple comparison test were used.

2-3. Results

Morphological comparisons

As shown in Fig. 2-1, the larva of *A. germana* is elongated, blackish in color, and covered with short hairs. The mean larval body length is 15.0 mm (SD = 1.2, Range 13.0-18.0, N = 14). Its pupa is oval and pale yellowish with seven black dotted longitudinal lines on the dorsal surface. The mean pupal length is 13.5 mm (SD = 1.1, Range 11.5-15.5, N = 31). The pupa makes no clear cocoon but fixed in a course silk net that hang from the leaves and/or tree branches. The adult has seven yellow bands on the abdomen. The mean forewing length is 14.6 mm (SD = 0.8, Range 13.0-15.2, N = 12) in males and 13.6 mm (SD = 0.5, Range 12.5-14.1, N = 12) in females.

The larva of *A. flava* is elongated, blackish in body, but brown in head. The dorsal surface is covered with short hairs. The mean larval body length was 15.7 mm (SD = 4.5, Range 10.2-23.1, N = 12). The pupa and adult are similar in shape and color to those of *A. germana*. The mean pupal length is 14.7 mm (SD = 1.4, Range 11.4 -18.8, N = 35). The mean forewing length is 16.9 mm (SD = 0.7, Range 16.0-18.0, N = 11) in males and 15.9 mm (SD = 1.1, Range 15.0-18.1, $N = 10$) in females.

The larva of *A. fortunei* is elongated, blackish, and covered with short hairs. The mean larval body length is 16.3 mm (SD = 2.3, Range 13.0-20.0, $N = 12$). The pupa is oval and entirely dark. The mean pupal length is 14.6 mm (SD = 1.5, Range 11.5-17.4, $N = 29$). The final-instar larva makes a simple cocoon threated with silk to which the surrounding fallen leaf debris and soil particles attach. The adult has two yellow abdominal bands. The mean forewing length is 12.8 mm (SD = 1.2, Range 11.0-14.2, $N = 11$) in males and 11.8 mm (SD = 0.5, Range 11.0-12.5, $N = 11$) in females.

The larva of *S. imaon* is elongated and blackish in color, but slightly slender and having longer hairs than the other three species. The mean body length is 21.4 mm (SD = 3.2, Range 16.0-25.0, $N = 14$). The pupa and cocoon are similar in shape and color to those of *A. fortunei*. The mean pupal length is 13.9 mm (SD = 0.9, Range 11.9-15.8, $N = 34$). The adult has two yellow bands on the abdomen and one on the head. The mean forewing length is 13.6 mm (SD = 0.5, Range 12.5-14.2, $N = 10$) in males and 14.1 mm (SD = 0.7, Range 12.8-15.5, $N = 11$) in females.

Thus, *A. germana* and *A. flava* differed greatly in pupal coloration from *A. fortunei* and *S. imaon* (Fig. 2-1). In the pupal photographs of *A. germana* and *A. flava* taken through an ultraviolet filter, there was no other marking pattern on the pupal surface (Fig. 2-1).

At pupation in the rearing glass vessel, silk thread weight relative to the pupal weight did not differ between the above-ground pupating *A. germana* and ground-surface pupating *A. fortunei* (Fig. 2-2 upper: Mann–Whitney *U*-test; $z = 0.387$, $N_1 = 24$, $N_2 = 16$, $P = 0.700$). At adult emergence, the exuvium weight relative to the pupal weight did not differ between these two species (Fig. 2-2 lower; $z = 0.463$, $N_1 = 23$, $N_2 = 15$, $P = 0.463$). Thus, the proportion of silk materials to surround the pupae and the pupal cuticles weight did not differ between above-ground pupating and ground-surface pupating species.

Pupation site selection

The mean pupation height in the rearing glass vessels were 71.1 mm (SD = 26.3, $N = 34$) in *A. germana*, 75.6 mm (SD = 13.4, $N = 33$) in *A. flava*, 1.3 mm (SD = 1.6, $N =$

21) in *A. fortunei*, and 1.4 mm (SD = 1.4, $N = 15$) in *S. imaon*. Thus, the former two species pupated at the upper sites, but the latter species did on the bottom (Fig. 2-3: Kruskal-Wallis test, $\chi^2 = 69.9$, df = 3, $P < 0.001$). Three pupae of *A. germana* and one pupa of *A. flava* pupated in the lower position in the experimental vessel, adults did not emerge. The pupae in contact with soil (one in *A. germana* and one in *A. fkava*) seemed to be infected by fungi.

Palatability

All stages of *A. germana*, *A. flava*, and *A. fortunei*, did not eaten by the lizards (Table 2-1). In the 1st- to 3rd-days, several lizards reach the feeding sequences of pecking with their mouthparts (3), biting (4), and swallowing but regurgitating (5), but after that such positive responses to the prey did not occur (Table 2-1). In *S. imaon*, however, one of three lizards ate three larvae and all lizards ate all given pupae (Table 2-1). Adult females were also eaten by three of seven lizards (Table 2-1). This result shows that *S. imaon* is more palatable, particularly in pupae and adult females, than the other three species.

2-4. Discussion

Warning coloration of pupae

The pupae of both *A. germana* and *A. flava* are similar in shape, size and color; with seven black dotted lines longitudinally on the dorsal of the pale-yellowish oval body (Fig. 2-1). These species do not make the typical cocoons but pupate in the course silk mesh hanging from the plant leaves and/or twigs (Fig. 2-1). The above-ground pupation of these two species was reproduced in the rearing glass vessels (Fig. 2-3). Above-ground pupation of *A. germana* is already reported based on the single field example of a pupa hanging from silk thread among the tree branches, from which the adult emerged and identified as this species (Okagaki 1956). The above-ground pupation by *A. flava* may be the first finding.

On the other hand, the pupae of *A. fortunei* and *S. imaon* were dark over their oval bodies (Fig. 2-1). They make a rough cocoon made of silk thread to which fallen leaf debris and soil particles are attached. Their pupation sites are at the bottom of the rearing glass vessels (Fig. 2-3). In the field, we could find three pupae of *A. fortunei* under stones on the ground.

In general, pupae of most lepidopterans are cryptic in color and make a cocoon hidden under the ground substrates such as stones and fallen leaves and twigs to avoid predation by the ground-roaming small mammals, birds and lizards searching prey by eyes (Waldbauer & Sternburg 1967). Other lepidopterans that pupate above the ground are also cryptic in color that serve as camouflage to deceive the eyes of predators (Wiklund & Sillén-Tullberg 1985; Mayekar & Kodandaramaiah 2017; Yumnam *et al*. 2021). Pupae with warning colors are not reported except for the toxic two-spotted ladybird beetle *A. bipunctata* which has warning colors at all developmental stages (Paul *et al*. 2018) and the unpalatable moth *I. auripes* (Yazaki *et al*. 2019). Warning colors evolved to warn predators that the prey is not profitable (Mappes *et al*. 2005, 2014). The benefit of conspicuous coloration strongly promotes predator learning in association with repellency (Mappes *et al*. 2005, 2014). The pupae of *A. germana* and *A. flava* are exposed to predators above the ground and their color is conspicuous just as in the ladybird beetle *A. bipunctata* and the moth *I. auripes*.

All larval, pupal and adult stages of *A. germana*, *A. flava* and *A. fortunei* seem to be unpalatable for the lizards (Table 2-1). European and Australian congeneric species, feeding on non-poisonous plants, are known to contain a histamine-like compound or pyrazines in their bodies which function as repellent to many avian predators (Rothschild *et al*. 1973, 1984). However, *S. imaon* are more palatable for the lizards particularly in pupae (Table 2-1). In our experiments, the lizards became to reject with their feeding experience of unpalatable pupae, suggesting that they learn the prey unpalatability. Thus, the unpalatable pupae potentially show warning colors.

On the ground, attacks to the pupae by microorganisms such as bacteria and fungi may be serious particularly in wet seasons, compared with the conditions above the ground. This may be another reason to pupate above the ground, predicting that the pupae on the ground surface would require more pupal cuticle and silk than those above the ground to prevent microorganism's attack. However, a comparison of above-ground pupating *A. germana* and ground-surface pupating *A. fortunei* showed no statistically significant difference in these allocation rates (Fig. 2-2).

Mimicry of pupae

When mimicry occurs via warning color, there are two types of mimicry, Müllerian and Batesian. In Müllerian mimicry, there is close similarity in color and shape (as well as in other defensive features) between cohabiting unpalatable prey species (Rettenmeyer 1970; Mallet & Mathieu 1999; Sherratt 2008). Due to the similar color and shape between the sympatric two species, the warning signal to predators is shared and learned more quickly at less cost to each species, which increases expected individual survival rates. Batesian mimicry refers to cases where a palatable, non-poisonous species resembles an unpalatable species with warning coloration, thereby increasing its survival rate (Rettenmeyer 1970). When cohabiting animal species share a warning color pattern, the animal community is referred to as a mimicry ring (Mallet & Mathieu 1999; Sherratt 2008). However, these phenomena are still descriptive, and there are very few quantitative studies on the toxicity, color similarity, and learning mechanisms of each species (Rammert 1992). Descriptions of mimicry rings, or called mimicry complexes, are also limited to similarities in colors and patterns of larval (Willmont *et al*. 2011) and adult insects (Edmunds 1990; Joron & Mallet 1998).

The warning color of the pupal *A. germana* is likely to be Müllerian mimicry with the pupae of the unpalatable moth *I. auripes* and the last-instar larvae of *Pryeria sinica* Moore, 1877, because these three species are distributed widely in Japan and they are seen in late spring to early summer (Yazaki *et al*. 2019). These pupae and larvae have seven black dotted lines longitudinally on the dorsal part of pale-yellowish body color. The shape is oval and the size is similar; 13.5 mm in pupal *A. germana*, 21.7 mm in pupal *I. auripes*, and 13.8 mm in larval *P. sinica* (Yazaki *et al*. 2019). They are all unpalatable, and therefore, these three species are thought to form a Müllerian mimicry ring among different families of *A. germana* (Arctiidae), *I. auripes* (Lymantriidae), and *P. sinica* (Zygaenidae).

Although *A. fortunei* is the closely related congener and is rejected by the lizards at all stages, their pupae are not conspicuous but cryptic in color and conceal under the substrate on the ground surface. The pupae of *S. imaon* are palatable for the lizards and exhibit cryptic dark color on the ground-surface. The effects of warning colors are established by the acquisition of unpalatability and following the conspicuous colors. By acquiring both of these traits, the predator learns the unpalatability of the prey (Mappes *et al*. 2005). As a result, insects have evolved and maintained the behavioral trait of shifting their living habitat to areas that are well visible to predators. Applying this evolutionary sequence of pupal warning color in the four studied moth species, the common ancestor species may have pupae without repellent materials and with cryptic coloration under the substrate on the ground surface as *S. imaon* does. Acquisition of repellent materials would then occur, and the pupae with repellent material and cryptic coloration, such as *A. fortunei*, would emerge. However, the lack of warning colors does not facilitate predator learning. Therefore, the behavior of hiding and pupating under the substrate on the ground surface would be chosen. If moths subsequently acquire distinctive conspicuous colors that facilitate predator learning, they may select for pupation above the ground where the pupae are exposed to the predators as in *A. germana* and *A. flava*. The microhabitat differences may promote such a shift of pupation sites from ground surface to above the ground in relation to pupal color. The suggested scenario of pupal warning coloration in this study should be examined in the future, based on the robust phylogenetic tree of the subfamily Syntominae in the world.

Table 2-1 Five-day feeding behaviors of individual lizards (#1-52) given larvae (L), pupae (P), adult males (Am), and adult females (Af) of four species of the nine-spotted diurnal moths, *Amata germana*, *A. flava*, *A. fortunei*, and *Syntomoides imaon* (but seven lizards were tested only in the first day for adult female *S. imaon*). Feeding behavior is shown by the six sequences to which lizards reach: no response (0), heading toward prey (1), licking prey (2), pecking prey with their mouthparts (3 in yellow), biting prey but immediately releasing it (4 in yellow), swallowing prey but regurgitating it (5 in yellow), and complete feeding (6 in blue). SVL shows the lizard snout to vent length in mm.

Lizaed ID	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12				
Lizaed SVL (mm)	61	57	60	62	65	70	61	63	65	61	65	63				
Stage of *A. germana*	L	L	L	P	P	P	Am	Am	Am	Af	Af	Af				
1st day	4	5	2	4	5	2	1	2	2	2	2	2				
2nd day	2	2	2	4	2	0	1	1	1	1	1	0				
3rd day	4	0	1	2	2	0	1	1	1	1	1	1				
4th day	2	1	0	2	2	0	1	0	0	1	2	1				
5th day	1	0	1	1	2	0	0	0	0	2	0	1				
Lizaed ID	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22	#23	#24				
Lizaed SVL (mm)	55	60	64	64	65	59	52	54	44	60	64	60				
Stage of *A. flava*	L	L	L	P	P	P	Am	Am	Am	Af	Af	Af				
1st day	5	2	2	3	3	2	2	2	2	3	3	2				
2nd day	2	2	2	2	4	2	0	1	1	1	2	2				
3rd day	0	2	0	2	1	1	0	0	0	2	2	1				
4th day	1	2	0	2	0	0	0	0	1	1	2	2				
5th day	1	1	2	1	0	0	1	0	0	0	2	1				
Lizaed ID	#25	#26	#27	#28	#29	#30	#31	#32	#33	#34	#35	#36				
Lizaed SVL (mm)	57	55	63	54	63	68	62	60	65	52	58	57				
Stage of *A. fortunei*	L	L	L	P	P	P	Am	Am	Am	Af	Af	Af				
1st day	2	2	4	5	5	4	2	2	2	4	2	2				
2nd day	2	2	2	2	2	5	2	1	3	0	3	1				
3rd day	2	1	1	5	2	2	4	1	2	1	2	2				
4th day	2	1	2	0	2	2	1	1	1	0	2	1				
5th day	1	1	0	0	0	2	0	2	1	1	2	1				
Lizaed ID	#37	#38	#39	#40	#41	#42	#43	#44	#45	#46	#47	#48	#49	#50	#51	#52
Lizaed SVL (mm)	56	54	55	56	52	66	67	61	64	60	58	62	68	55	52	64
Stage of *S. imaon*	L	L	L	P	P	P	Am	Am	Am	Af	Af	Af	Af	Af	Af	Af
1st day	6	4	4	6	6	6	2	3	2	6	2	6	4	3	6	4
2nd day	6	4	2	6	6	6	2	2	0	-	-	-	-	-	-	-
3rd day	6	2	2	6	6	6	3	2	1	-	-	-	-	-	-	-
4th day	1	4	1	6	6	6	0	1	0	-	-	-	-	-	-	-
5th day	1	0	4	6	6	6	0	1	0	-	-	-	-	-	-	-

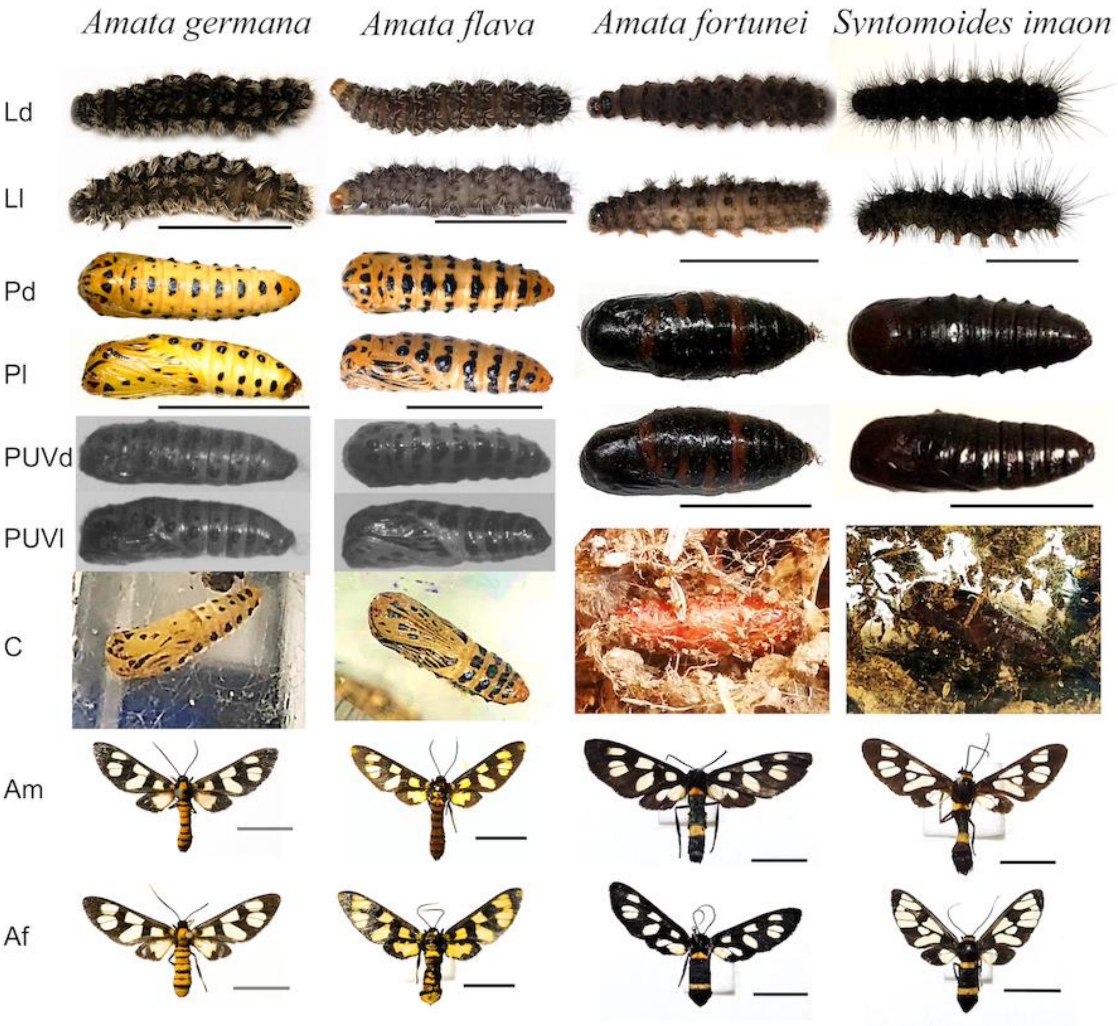

Figure 2-1. Larvae (L), pupae (P), cocoons (C), adult males (Am) and adult females (Af) of the nine-spotted diurnal moths, *Amata germana*, *A. flava*, *A. fortunei*, and *Syntomoides imaon*. Each photograph shows the dorsal (d) or lateral (l) view, and in *A. germana* and *A. flava*, the pupal color through the ultraviolet (UV) light is shown. Scale bars show 10 mm.

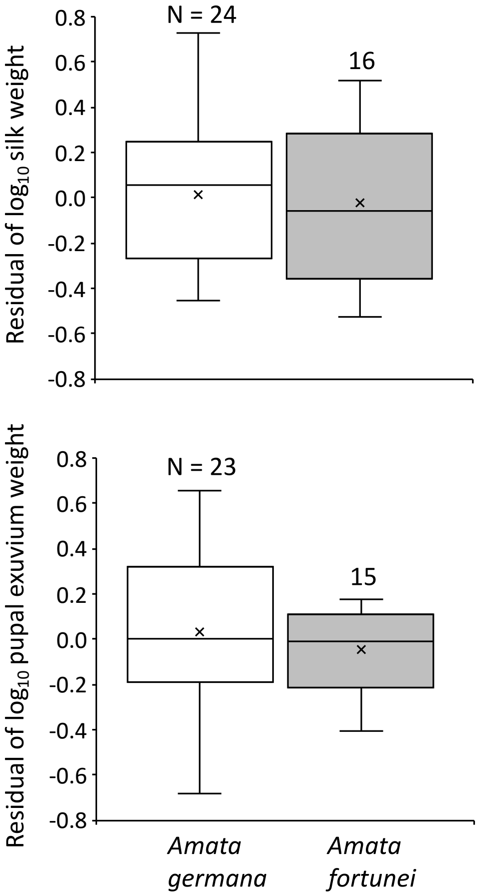

Figure 2-2. Silk weight (upper) and pupal exuvium weight (lower) of the above-ground pupating *Amata germana* and ground-surface pupating *A. fortunei*. These weights are shown as the residual of the log-log regression lines calculated for the combined data. Closes indicate the mean value.

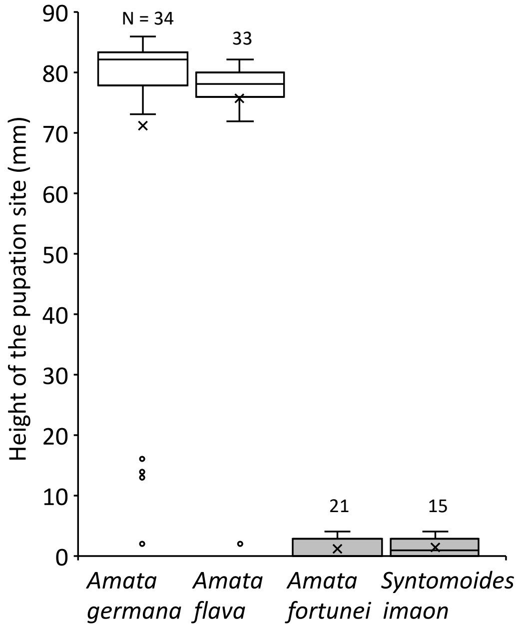

Figure 2-3. Height of the pupation sites in the rearing glass vessels of the nine-spotted diurnal moths, *Amata germana*, *A. flava*, *A. fortunei*, and *Syntomoides imaon*. Open bars and shaded bars show the above-ground pupating and the ground-surface pupating species, respectively. Closes indicate the mean value. Bars followed by the same alphabet are not significantly different (*P* > 0.05) in Scheffe’s multiple comparison test.

3. Pupal warning coloration of three species of *Cystidia* (Geometridae: Ennominae) in relation to their pupation sites

3-1. Introduction

Predation avoidance of the pupal stage in insects has received little attention in comparison to larval and adult stages (Lindstedt *et al*. 2019). In insect life cycles, the pupae are an immobile stage and the pupation site selection is critical to survive this stage. Pupae of most insect species are cryptic in color and hide under the substrates or in inconspicuous cocoons (Wiklund & Sillén-Tullberg 1985; Edmunds 1990; Janzen *et al*. 2010; Paul *et al*. 2018; Lindstedt *et al*. 2019). However, if insects have toxins or bad odor, they can develop warning coloration (aposematism). Such a prominent color signals to visually hunting predators that the prey is unprofitable through the process of predator's experience and learning (Caro & Koneru 2021). However, the warning coloration of the pupal stage is little known. The rare example of pupal warning coloration is reported in the toxic two-spotted ladybird beetle, *Adalia bipunctata* (Linnaeus, 1758), which has warning colors at all stages of its life cycle (Paul *et al*. 2018) and in the unpalatable moth, *Ivela auripes* (Butler, 1877), which pupates at the exposed sites such as tree trunk and rock surface above the ground and displays the conspicuous yellow pupal body with seven longitudinal stripes of blackish dots (Yazaki *et al*. 2019).

The moths of *Cystidia* (Lepidoptera: Geometridae: Ennominae) make colorful pupae exceptionally among Ennominae with cryptic brown pupae (Nakamura 2004). I noticed that the colorful pupae of *Cystidia* are exposed on the surface of leaves and twigs above the ground and are likely to be warning coloration. If so, they should be unpalatable for the predators, such as birds, mammals and lizards, that use their vision to search for food. In Japan, three species of *Cystidia* are distributed widely (Nakamura 2004): *C. couaggaria* (Guenée, 1858), *C. truncangulata* Wehrli, 1934, and *C. stratonice* (Stoll, 1782). This genus belongs to the tribe Cystidiini in Ennominae (Anikin *et al*. 2016; Zheng *et al*. 2022). These species are distributed from Far East Russia to Japan (Anikin *et al*. 2016) and adults appear in early summer (Sato & Nakajima 1975). The external morphology of larvae and pupae of these species are described in detail and each species can be identified based on their morphology and color patterns (Isshiki 1969; Sato & Nakamura 1975; Nakamura 2004). Adults fly and mate in the daytime using female sex pheromone (hydrocarbons) (Yamakawa *et al*. 2012; Naka 2018). Six species of hymenopteran parasitoids are recorded from the pupae of *C. couggaria* (Konno *et al*. 2002). These are the information on *Cystidia* known so far.

In this study, to examine the possibility of warning coloration of these *Cystidia* pupae, first, the pupal size, color, and marking pattern are compared based on their DNA Barcoding results. Second, pupation sites in the field are compared among the three species. Third, palatability of these pupae is assessed using the lizard as a potential predator. Finally, the observed trend of variation in pupal coloration is discussed in relation to pupation site selection and palatability.

3-2. Materials and methods

Insects

Larvae, pupae and adults of *C. couaggaria* were collected in the wood at Hino, western Tokyo, in May and June 2020. They were placed individually in a plastic cup (130 mm in diameter, 55 mm in depth) and kept at natural temperatures and daylength. Leaves of the tree *Armeniaca mume* (Rosaceae) were given to the larvae. Larvae of *C. truncangulata* were collected in the wood at Moriya, Ibaraki Prefecture, on 24 April 2021, and reared at natural temperatures and daylength, giving leaves of the tree *Celastrus orbiculatus* (Celastraceae). Field-caught pupae from the same locality on 24 May 2020 and adults from Ueda, Nagano Prefecture, on 24 June 2020, were also used for experiments. Larvae, pupae and adults of *C. stratonice* were collected in the wood at Ueda, Nagano Prefecture, in June 2020. The larvae were reared at natural temperatures and daylength, giving leaves of *C. orbiculatus*.

Morphological measurements

Body size were measured using the free software, ImageJ (version 1.53e; National Institutes of Health, Bethesda, Maryland, USA) based on the photographs of randomly chosen 6 final-instar larvae, 11 pupae, 5 male adults, and 5 female adults of *C. couaggaria*; 6 final-instar larvae, 25 pupae, 8 male adults, and 10 female adults of *C. truncangulata*; 8 final-instar larvae, 10 pupae, 6 male adults, and 4 female adults of *C. stratonice*. I measured the body length (from the front of head to the body end) and width (at the widest part of body) of larvae and pupae from the dorsal side, and the forewing length (from base to the tip) of adults (Fig. 3-1).

Visual pigments of the potential predators such as birds and lizards can absorb ultraviolet light (Chen *et al*. 1984; Fleishman *et al*. 1993). Therefore, to know the pupal color patterns under ultraviolet light, the pupal photographs of the three species of *Cystidia* were taken using an ultraviolet filter (U330; HOYA Co. Ltd., Tokyo, Japan).

DNA barcoding

Legs of the dried adult specimens of *C. couaggaria* collected at Hino, western Tokyo, and *C. truncangulata* and *C. stratonice* both collected at Ueda, Nagano Prefecture, were used for DNA barcoding and molecular phylogenetic analysis. Total genomic DNA was extracted from these 3 individuals, using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The mitochondrial cytochrome c oxidase subunit I (COI) gene fragment was amplified using Ex Taq® (TaKaRa, Tokyo, Japan) by the primer set forward 5´-TTATTTTGGAATTTGAGC-3´and reverse 5´-CCTGTTAATCCTCCTACTGT-3´ (Yamamoto & Sota 2009). The PCR reaction mix (total volume 10 μL) contained 1.0 μL 10× Ex Taq Buffer, 0.8 μL 25 mM dNTP mix, 0.5 μL of each the forward and reverse primer (10 pM), 0.05 μL Taq polymerase, 6.15 μL distilled deionized water, and 1.0 μL template DNA. The PCR protocols implemented using a T100™ thermal cycler (Bio-Rad, Hercules, CA, USA) were as follows: an initial 3-min denaturing step at 94°C; 35 cycles of 20 sec at 94°C, 20 sec at 50°C, and 30 sec at 72°C; with a final 5-min extension at 72°C. The PCR products were purified with illustra™ ExoProStar™ 1-Step (GE Healthcare, Buckinghamshire, UK) and sequenced using BigDye® Terminator ver. 3.1 (Applied Biosystems, Foster City, CA, USA) on an ABI 3130xl Genetic Analyzer (Applied Biosystems).

Direct sequencing data were aligned with the outgroup data using MEGA X (Kumar *et al.* 2018). The sequences were aligned to 585 bp without gaps (GenBank accession numbers LC742382 in *C. couaggaria*, LC742380 in *C. truncangulata*, and LC742381 in *C. stratonice*) and the outgroup *Abraxas miranda* Butler, 1878 (JN087399, Lee *et al*. 2012) belonging to the tribe Abraxini of the subfamily Ennominae and *Geometra papilionaria* (Linnaeus, 1758) (GU655815, Hausmann *et al*. 2011) belonging to the subfamily Geometrinae. Phylogenetic trees were constructed by the neighbor-joining (NJ) method based on p-distance (bootstrap replication = 1000) and maximum likelihood (ML) estimation (bootstrap replication = 1000) based on the General Time Reversible model following the gamma distribution with invariant sites using MEGA X (Kumar *et al*. 2018). The best-fit nucleotide substitution models were estimated based on the corrected Akaike's information criterion (AICc) (Sugiura 1978) using MEGA X (Kumar *et al*. 2018).

Pupation site selection

We searched prepupae, pupae and pupal exuviae of *C. truncangulata* and *C. stratonice* in the wood at Ueda, Nagano Prefecture, on 31 May and 2 June 2020 and 13 June 2021. Those of *C. couaggaria* were searched in the wood at Ichikawa, Chiba Prefecture, on 13 May 2021. If they were found, the height from the ground was measured (0 m if pupated on the ground surface) and the pupation site was recorded by distinguishing the situations into five categories; on twig surface, on artificial substrate surface (wire net and concrete pillar), upside a leaf, underside a leaf, and within leaves stitched together with silk (Fig. 3-2).

Assessment of palatability

A total of 36 lizards, *Plestiodon finitimus* Okamoto et Hikida, 2012, were used as a potential predator. These lizards were caught at Inagi, Tama, Hino, and Hachioji Cities, western Tokyo, in May 3, 7, 13, 27, 29, and June 15, 24, 26, 2020 and April 12, 16, 18, 2021. The mean snout to vent length (SVL) of them was 60.4 mm (SD = 4.1, $N = 36$) and total tail length was 76.3 mm (SD = 22.0, $N = 36$). They were kept individually in plastic cages (200 mm × 350 mm, 200 mm in depth) at 17–33°C with natural daylength. Each cage was filled up to 10 mm with a mixture of peat moss and red ball soil in a 1:1

ratio and an unglazed shelter. Water was supplied constantly in a cup (90 mm in diameter, 50 mm in depth). Two mealworms (larvae of *Tenebrio molitor* Linnaeus, 1758) were fed during the 3-h basking period with artificial light every other day.

Feeding experiments were conducted after maintained in the cage for at least one week. After 30-min basking, one of larvae, pupae, male adults, or female adults of the moth was given to one lizard per day. The same staged moth was given to the same lizard for five consecutive days. The feeding willingness of each lizard was checked by giving one mealworm just before the first-day trial and by giving three mealworms just after the fifth-day trial. To prevent the adult moth from flying out of the cage bottom, adults were given after cutting the forewings partly at their edges. Pupae were presented to the lizards suspended by a black polyester thread (0.15 mm in diameter). All tests were terminated if there was no approach to the prey for 20 s. Lizard behavior was recorded with a video camera (12 MPx Camera, Apple Inc., Cupertino, CA, USA) of the Apple iPhone 8. The lizard's behavior was discriminated into seven categorized sequences: no response (0), heading toward prey (1), licking prey (2), pecking prey with their mouthparts (3), biting prey but immediately releasing it (4), swallowing prey but regurgitating it (5), and complete feeding (6).

This 5-day feeding experiment was conducted to three lizards for each stage of the three species. Once the lizards were used for the feeding experiment, they were released to the capture sites in the field.

Statistics

Values are shown as mean ± standard deviation (SD) with sample size (*N*). To compare the height of pupation sites from the ground surface among the three species, the Kruskal-Wallis test and the following Steel-Dwass multiple comparison test were used.

3-3. Results

Morphological comparison

Larvae are elongated in the three species, but the color patterns differed among the species (L in Fig. 3-1). Body length of the last-instar larvae is 32.1 ± 4.5 mm ($N = 6$) in *C. couaggaria*, 30.6 ± 5.7 mm ($N = 6$) in *C. truncangulata*, and 31.8 ± 3.3 mm ($N = 8$) in *C. stratonice*. Their body width is 3.3 ± 0.7 mm ($N = 6$), 3.2 ± 0.4 mm ($N = 6$), and 3.5 ± 0.4 mm ($N = 8$), respectively.

Pupae of the three species similarly have blackish dotted lines longitudinally on the yellow body (P in Fig. 3-1). However, the blackish markings differ among the species; larger marking in *C. couaggaria*, smaller dotted marking in *C. truncangulata*, and totally blackish in the head, thorax including wing pads, and caudal segments of the abdomen in *C. stratonice*. Such blackish markings are kept during the pupal stage, except for the stage near adult emergence at which the adult wing marking is formed in wing pads (P1–P3 in Fig. 3-1). The blackish marking patterns do not differ between the photographs taken under visible and ultraviolet light conditions (PUV in Fig. 3-1). The pupal length is 21.0 ± 1.3 mm ($N = 11$) in *C. couaggaria*, 17.7 ± 3.0 mm ($N = 25$) in *C. truncangulata*, and 18.2 ± 1.8 mm ($N = 10$) in *C. stratonice*. Their width is 5.3 ± 0.4 mm ($N = 11$), 4.8 ± 1.1 mm ($N = 25$), and 4.6 ± 0.4 mm ($N = 10$), respectively.

The adult commonly has white and black stripes on the wings and yellow and black bands on the abdomen. Male forewing length is 24.0 ± 1.0 mm ($N = 5$) in *C. couaggaria*, 25.4 ± 0.9 mm ($N = 8$) in *C. truncangulata*, and 26.7 ± 0.8 mm ($N = 6$) in *C. stratonice*. Female forewing length is 22.2 ± 1.5 mm ($N = 5$), 26.8 ± 1.8 mm ($N = 10$), and 25.9 ± 0.7 mm ($N = 4$), respectively.

Phylogenetic relationships of DNA barcoding results suggest that *C. truncangulata* and *C. stratonice* are more closely related among the three species of *Cystidia* (the tribe Cystidiini), although not supported strongly (Fig. 3-1). These relationships are also reflected in morphological differences. The dotted black marking pattern on the yellow pupa and the blackish striped pattern on wings are closely similar between *C. truncangulata* and *C. stratonice*, but such patterns differ in *C. couaggaria*. In the former two species, however, the areas of pupal blackish marking are much wider in *C. stratonice*. The pupae of *Abraxas miranda* belonging to the different tribe Abraxini is entirely brown (Fig. 3-1). This pupa was obtained by rearing a field-caught last-instar larva in Hachioji, western Tokyo, on 9 October 2021, and pupated on the bottom of the rearing cage.

Pupation site

All pupae were found above the ground in the field (Fig. 3-2). The median of pupation height was 1.4 m in *C. couaggaria*, 1.7 m in *C. truncangulata*, and 0.5 m in *C. stratonice* (Fig. 3-3). The former two species pupated higher sites than the latter species (Kruskal-Wallis test, $\chi^2 = 14.7$, df = 2, $p < 0.001$). Pupae of *C. couaggaria* and *C. truncangulata* were exposed on the surface of twigs, leaves, and artificial wire mesh fences and stone blocks, whereas those of *C. stratonice* were hidden within the leaves roughly stitched together with silk (Table 3-1, Fig. 3-2).

Palatability

All mealworms given at the 1st- and 5th-days were eaten by the lizards, whereas all stages of *C. couaggaria*, *C. truncangulata*, and *C. stratonice* were not eaten, suggesting these moths unpalatable during their development for the lizards (Table 3-2). In the 1st- to 3rd-day of feeding trials, most lizards showed the feeding sequences of pecking with their mouthparts (3) and biting (4), but after that such positive responses to the prey did little occur (Table 3-2). This result suggests that the lizards gradually learned and discriminated these unpalatable larvae, pupae, and adults.

3-4. Discussion

Bodies and wings of most insect species resemble fresh or dead leaves and tree trunks and branches, which is a phenomenon known as cryptic coloration to avoid detection by the predators that use their vision to search for food (Caro & Koneru 2021). Almost all pupae of 119 genera of Japanese Ennominae are known to be uniformly brown (pale, reddish, or dark brown) or green in *Auaxa sulphurea* (Nakamura 2004). The brown and green pupae may be cryptic to the background color. Among these 119 ennomine genera, pupae of the three species of *Cystidia* are reported to be exceptionally yellow with some black spots (Nakamura 2004). We confirmed this fact that pupae of *C. couaggaria*, *C. truncangulata*, and *C. stratonice*, are similar to each other in shape, size, color, and marking pattern (Fig. 3-1). The marking pattern did not differ between visible and ultraviolet lights (Fig. 3-1), suggesting that there was a similar image for birds and lizards that can use ultraviolet vision. However, there are some species-specific differences in pupal marking patterns (Fig. 3-1). The black markings of *C. couaggaria* are usually larger and form at least three bands in the abdomen. The pupae of *C. truncangulata* have a smaller black spot and are widely yellowish, whereas the pupae of *C. stratonice* are more blackish throughout the pupal stage.

The conspicuous color common to *Cystidia* species is likely to be warning coloration. If the insect is equipped with defenses against predators such as toxins or inappetence, selection may favor warning colorations (Caro & Koneru 2021). Warning coloration is a widespread strategy to alert predators about such prey unprofitability. The success of this strategy partly depends on predators being able to learn and recognize certain signals as indicators of toxicity by encountering frequently (Arenas & Stevens 2017). Thus, to discuss the possibility of pupal warning coloration, we need information of encountering rates to the predators, unpalatability of the pupae, and the ability of learning by the predators.

All three species of *Cystidia* pupate above the ground (Fig. 3-2). Pupae of *C. couaggaria* and *C. truncangulata* are exposed on the surface of twigs, leaves, and artificial materials such as wire mesh fences and stone blocks, but those of *C. stratonice* are all hidden inside the leaves roughly spun with silk (Table 3-1). The height of pupation sites from the ground surface is greater in the former two species than the latter (Fig. 3-3). Thus, the pupae of *C. couaggaria* and *C. truncangulata* seem to be more exposed to the predators and detected by the predators more frequently than those of *C. stratonice*. Most lepidopterans that pupate above the ground are cryptic in color that serve as camouflage to deceive the eyes of predators (Wiklund & Sillén-Tullberg 1985; Mayekar & Kodandaramaiah 2017; Yumnam *et al*. 2021). Pupae with warning colors are rarely reported even in the case of above ground pupation. The exceptional examples are the toxic two-spotted ladybird beetle *Adalia bipunctata*, which has warning colors at all developmental stages (Paul *et al*. 2018), and the unpalatable moth *Ivela auripes* (Yazaki *et al*. 2019). Warning colors evolved to warn predators that the prey is not profitable (Mappes *et al*. 2005, 2014). The benefit of conspicuous coloration strongly promotes predator learning in association with repellency (Mappes *et al*. 2005, 2014). The pupae of *C. couaggaria* and *C. truncangulata* are exposed to predators above the ground and their color is conspicuous just as the pupae of the ladybird beetle *A. bipunctata* and the moth *I. auripes*. In contrast, the pupae of *C. stratonice* are inside of the roughly-spun leaves at the lower site above the ground. The pupal color of *C. stratonice* is likely to have a dual meaning as cryptic and warning colors. The more blackish pupa may be inconspicuous when hidden within the leaf space, but once detected by the predators, the yellow color of the pupa may function as a warning color. To understand such interspecific differences in pupal colors, further attention should be paid to the effects of the pupation site environment on the predation intensity.

All developmental stages of larvae, pupae and adults of the three species of *Cystidia* are unpalatable for the lizards (Table 3-2). Under the experimental conditions, the lizards became to ignore the pupae with their feeding experience. Such a tendency suggests that they learn unpalatability of the pupae by their conspicuous coloration. Thus, the unpalatable pupae potentially show warning colors. The chemical analysis has never been conducted for *Cystidia*, but one of the ennomine moth of the tribe Boarmiini, *Arichanna gaschkevitchii* (= *A. jaguararia*), is reported strongly toxic (Nishida *et al.* 1990; Nishida 1994). Adults of this species include grayanoid diterpenes that are acquired by larval host plant *Pieris japonica* (Ericaceae) and are effective in escape from predation by birds and geckos. The adult of this moth exhibits an aposematic color pattern with orange-yellow hindwings and flies in daytime like a butterfly, suggesting the function of a warning color (Nishida 1994). However, *A. gaschkevitchii* pupate in the soil of the ground (Issiki 1969) and the pupae are uniformly brown (Nakamura 2004). The adults of *Cystidia* fly in the daytime, and the pupae are also exposed to the predators on the branches and leaves above the ground, excluding *C. stratonice* with a partially conspicuous color. Thus, the development of warning coloration of lepidopteran pupae seems to be affected by pupation site selection which is exposed to the predators or not after acquisition of unpalatability. This is a testable hypothesis of the warning color evolution in the pupal stages.

In conclusion, pupae of the geometrid moth *Cystidia* were unpalatable to lizards, one of the potential predators. When the pupae were repeatedly fed to the lizards, they gradually began to ignore the pupae. This suggests that the lizards learn unpalatability of the pupae through experience. Pupae were yellow with black spots, making them conspicuous. Furthermore, pupae are located on the surface of tree branches and leaves above the ground, where they are exposed to predators that search for prey with their eyes. These results indicate that the conspicuous color of *Cystidia* pupae functions as a warning color. However, the pupae inside the leaves spun with silk show more blackish marking pattern on the yellow body than the exposed pupae. This tendency to darken is thought to have two effects: one was to conceal the pupae to make it inconspicuous within the leaves, and the other was to serve as a warning color when predators detect them. To understand the interspecific differences in pupal colors, further attention should be paid to the relationship between the environment of the pupation site and pupal coloration.

Table 3-1. Pupation sites of the three species of *Cystidia* moths; *C. couaggaria*, *C. truncangulata*, and *C. stratonice* in the field. The number of pupae found at five categories of pupation sites is shown (see Fig. 3-2).

Pupation site	On twig surface	On artificial substrate surface	Upside a leaf	Underside a leaf	In leaves stitched together with silk	Total
C. couaggaria	8	1	47	0	0	56
C. truncangulata	4	5	0	3	0	12
C. stratonice	0	0	0	0	17	17

Table 3-2. The lizard feeding behaviors in the five-day feeding trials of individual lizards (#1–36), when given larvae (L), pupae (P), adult males (Am), and adult females (Af) of the three species of *Cystidia* moths; *C. couaggaria*, *C. truncangulata*, and *C. stratonice*. Feeding behavior is shown by the six sequences to which lizards reach: no response (0), heading toward prey (1), licking prey (2), pecking prey with their mouthparts (3), biting prey but immediately releasing it (4), swallowing prey but regurgitating it (5), and complete feeding (6). Categories (5) and (6) are not observed, and more active responses (3) and (4) are shaded. SVL shows the lizard snout to vent length in mm.

Lizaed ID	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Lizaed SVL (mm)	59	55	64	55	64	69	60	66	60	55	61	59
Stage of *C. couaggaria*	L	L	L	P	P	P	Am	Am	Am	Af	Af	Af
1st day	4	4	2	3	4	4	4	4	4	4	4	4
2nd day	4	0	0	4	4	2	4	4	2	3	2	2
3rd day	4	1	1	2	0	2	4	4	4	1	2	0
4th day	3	2	1	1	0	0	0	0	0	0	1	0
5th day	2	2	0	0	0	0	0	0	1	0	2	2
Lizaed ID	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22	#23	#24
Lizaed SVL (mm)	64	59	57	58	58	54	57	55	58	64	61	60
Stage of *C. truncangulata*	L	L	L	P	P	P	Am	Am	Am	Af	Af	Af
1st day	4	4	4	4	4	4	4	4	4	4	4	4
2nd day	2	2	2	1	2	4	4	4	4	4	4	4
3rd day	2	2	1	0	0	1	1	4	4	0	4	3
4th day	0	0	1	1	0	1	1	2	4	0	0	0
5th day	0	1	2	2	0	2	1	1	2	0	0	0
Lizaed ID	#25	#26	#27	#28	#29	#30	#31	#32	#33	#34	#35	#36
Lizaed SVL (mm)	66	65	61	61	58	60	70	58	55	64	68	57
Stage of *C. stratonice*	L	L	L	P	P	P	Am	Am	Am	Af	Af	Af
1st day	4	4	4	4	4	4	4	3	2	3	1	4
2nd day	2	4	2	2	2	2	4	4	2	1	1	4
3rd day	2	4	2	0	1	2	4	2	0	0	0	2
4th day	2	1	0	0	1	1	2	2	1	0	0	1
5th day	2	4	1	1	1	1	1	3	0	1	1	1

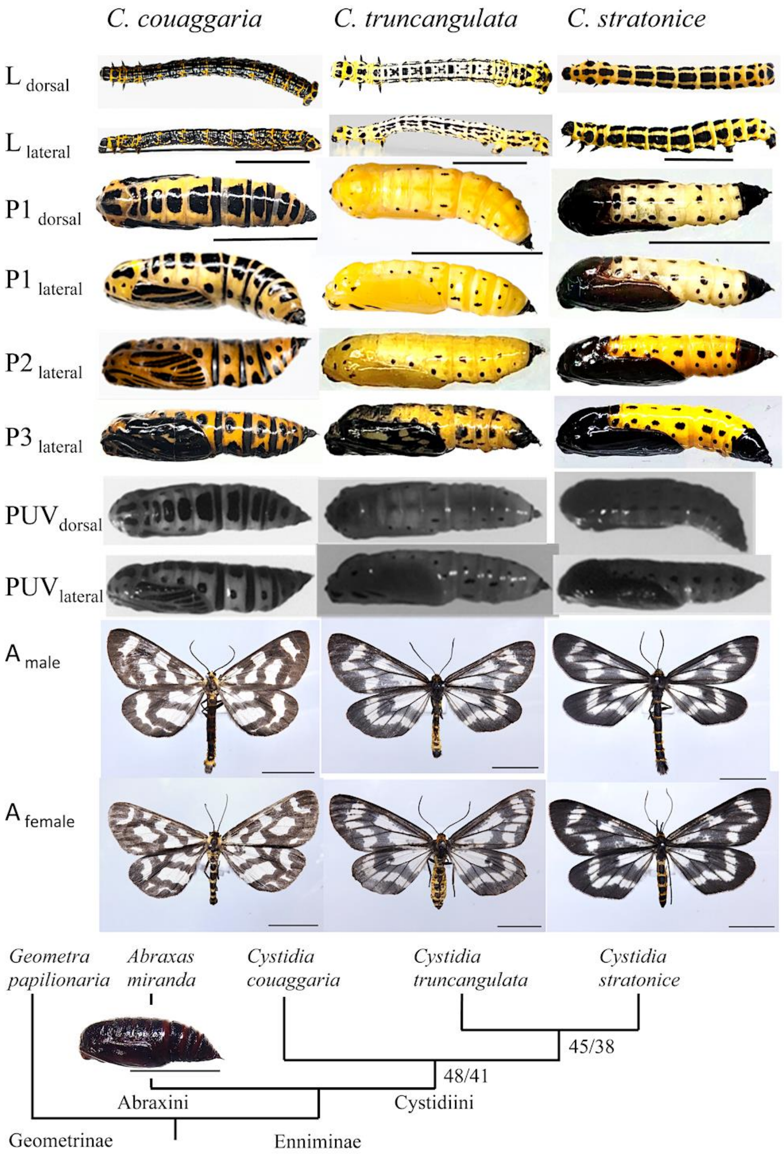

Figure 3-1. Larvae (L), pupae (P), and adults (A) of the three species of *Cystidia* moths; *C. couaggaria*, *C. truncangulata*, and *C. stratonice*. Each photograph shows the dorsal or lateral view under visible and ultraviolet (UV) light. In pupal stage, P1–P3 shows the pupal color patterns in the day of pupation, 6th or 7th day since pupation, and 1 day before adult emergence. The DNA barcoding result is drawn for three species of *Cystidia* and the outgroup *Abraxas miranda* (JN087399) belonging to the tribe Abraxini of the subfamily Ennominae and *Geometra papilionaria* (GU655815) belonging to the subfamily Geometrinae. The numerals near the branches present % probabilities in the neighbor-joining/maximum likelihood methods. Scale bars show 10 mm.

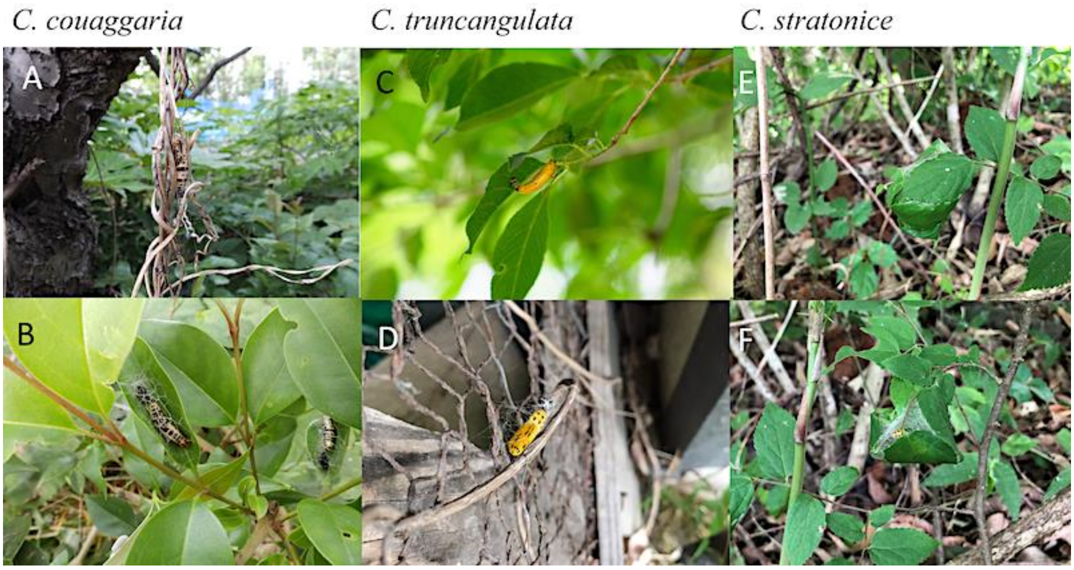

Figure 3-2. Pupation sites in the field of the three species of *Cystidia* moths; *C. couaggaria* (A, B), *C. truncangulata* (C, D), and *C. stratonice* (E, F). They pupate on the surface of twigs (A, C), upside a leaf (B), underside a leaf (C), and within the leaves stitched with silk (E, F).

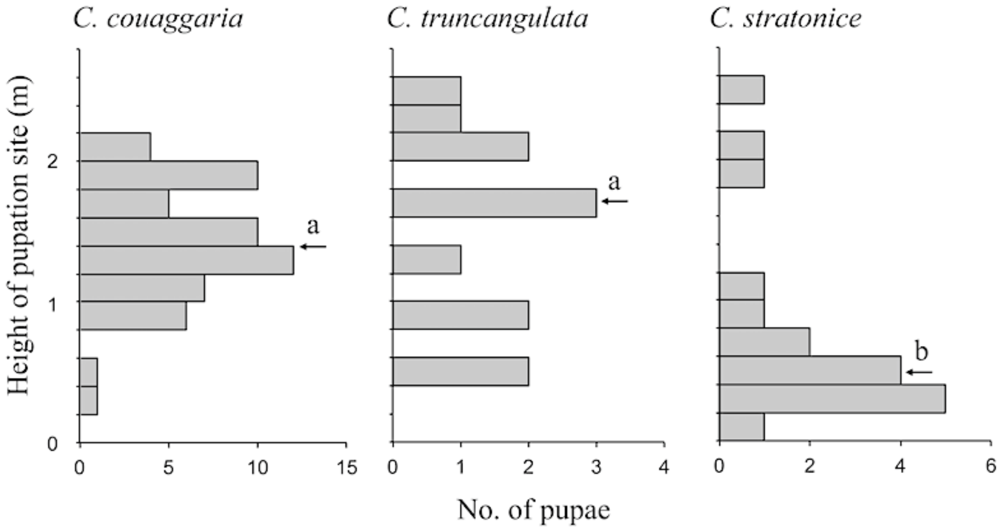

Figure 3-3. Height of the pupation sites from the ground surface of the three species of *Cystidia* moths; *C. couaggaria* ($N = 56$), *C. truncangulata* ($N = 12$), and *C. stratonice* ($N = 17$). Arrows indicate medians and the same alphabet means not significantly different ($p > 0.05$) in the Steel-Dwass multiple comparison test after the Kruskal-Wallis test ($p < 0.001$).

4. Aposematic mimicry rings among lepidopteran larvae maintained over their sympatric areas via memory of the migratory birds

4-1. Introduction

Mimicry of prey species with aposematic coloration is separated classically into two extremes, Batesian and Müllerian mimicries. Batesian mimicry is the case that a palatable, non-poisonous species resembles cohabiting unpalatable species. If predators learn unpalatability of model species, the survival rate of mimic species increases (Ruxton *et al*. 2018). Müllerian mimicry occurs among cohabiting unpalatable species with similar colorations. Inter-specifically similar warning colors warn predators more frequently that the prey is not profitable and promote predator learning, and so increasing individual survival rate of the mimicry type (Mappes *et al*. 2005; Ruxton *et al*. 2018). In both mimicry types, sympatry between the mimetic species is the basic assumption (Wallace 1867; Poulton 1890; Wickler 1968; Pfennig & Mullen 2010). However, the mimetic species appear often in the different time of day, in different seasons, and in different geographic ranges. Selection pressures to cause aposematic prey mimicry are predation by the predators searching prey visually, such as avian and non-avian reptiles and mammals. Once these predators experience unpalatable prey, they can learn and remember it for longtime. Social learning on avoidance of aposematic prey occurs in birds (Landová *et al*. 2017). Long-term memory in the scale of years is known in birds (Waldbauer 1988; Blum *et al*. 2020; Kashetsky *et al*. 2021), in non-avian reptiles (Cooper *et al*. 2020; Szabo *et al*. 2021), and mammals (Saliveros *et al*. 2020; Kashetsky *et al*. 2021; Dixon *et al*. 2023).

In the environment where most predators are still naïve to unpalatable prey, the mimic species can avoid predation when they appear later of the day than the model species, because the predators learn unpalatable model before the mimic is exposed to them (Pinheiro 2007). This type of asynchronies is reported in Brazilian butterfly mimicry rings in which the Batesian mimic species tend to fly in later time than the Müllerian mimic species (Pinheiro 2007). This expands to the seasonal asynchronies (Huheey 1980). Batesian mimicry species are beneficial to reduce predation by retarding their appearance until after the models appear (Rothschild 1963; Huheey1980;

Brodie 1981; Prusa & Hill 2021). In seasonal environments, the naïve predators, particularly the bird fledging, increase in summer and the abundance of wasp-mimic insects decreases in summer as unlikely to avoid predation (Waldbauer 1988) and also the warning colored lepidopteran caterpillars is rare in summer (Mappes *et al*. 2014). In summer, the survival of artificial caterpillar models with an aposematic color is lower than the survival of non-warning-colored models because of their conspicuousness (Mappes *et al*. 2014). Thus, mimic species avoid appearance in the season when naïve predators are abundant.

Geographical asynchronies are the phenomena that the mimic and model species are distributed allopatrically or the mimic's range expands the area of the model (Huheey 1980; Waldbauer 1988). The predation avoidance by mimicry is expected to breaks down where the model is absent (Pfennig & Mullen 2010). If the rejection of capturing the mimic species is innate (requiring no learning), allopatric distributions of mimic and model species is not contradicted (Waldbauer 1988). If the rejection occurs by the memory after learning unpalatability of the model species, the range expansion of such learned predators would be needed. Periodically migrating insectivorous birds are the most important candidates (Poulton 1908; Wickler, 1968; Waldbauer 1988; Pfennig & Mullen 2010). Another hypothesis to explain geographical asynchrony is gene flow widely within the mimic species (Pfennig & Mullen 2010). In general, the aposematic color pattern is genetically determined and each species range of the mimic and model species is not fixed but changed by different dispersal ability and habitat preference.

These temporal (daily and seasonal) and distributional synchronies between mimetic species are still unresolved for most cases. To understand such temporal and spatial dynamics of mimic and model species, we need the data of palatability and distribution ranges of mimic and model species, the predators' ability to discriminate the species and to learn unpalatability, and the predation pressures that changes temporally and spatially in the field.

In general, larvae of most species of Lepidoptera have a cryptic color, and a relatively few species exhibit a conspicuous color (Mappes *et al*. 2014; Robinson *et al*. 2023) and only few Batesian and Müllerian mimicries have been suggested among the conspicuously colored species (Willmott *et al*. 2011). In Japanese lepidopteran larvae, I noticed that larvae of the moths *Asota plana* (Walker, 1854) (Erebidae) and *Brithys crini* (Fabricius, 1775) (Noctuidae) and the butterfly *Choaspe benjaminii* (Guérin-Méneville, 1843) (Hesperiidae) are aposematic in having the red/black/white banding in their body (Fig. 4-1). These three species are distributed sympatrically in southern Japan, but allopatrically in northern Japan. In this study, first, the morphological features were described; second, the palatability was assessed using the non-avian reptile (lizard); and third, the ability of learning and discrimination of unpalatable prey was examined using silkworms with different color patterns; and finally, the predation pressures were assessed using the artificial models set in the field where the combination of these three larvae and the avian communities differ.

4-2. Materials and methods

Insects

The moth *A. plana* is distributed in southern Japan, Taiwan, southern China, Southeast Asia and India (Bayarsaikhan *et al*. 2016). The adults appear almost all year round (Kishida 2011). A total of 62 larvae and ca. 200 eggs were collected on Okinawajima Island, southern Japan (26°21'07.2"N, 127°48'35.2"E) in May 2020 and in June 2021. In the laboratory, ca. 190 hatched larvae were reared giving leaves of the tree *Ficus microcarpa* (Moraceae). Several larvae were put together in a plastic cup (130 mm in diameter, 55 mm in depth) and placed in the incubator set at 25 ± 1°C with a 14 h light and 10 h dark cycle.

The moth *B. crini* is distributed in southwestern Japan, Taiwan, southern China, Southeast Asia, India, Australia, southern Europa, and Africa and adults appear from April to October in Japan (Kishida 2011). A total of 123 larvae were collected at Yokosuka, Kanagawa Prefecture, Japan (35°13'13.6"N, 139°37'16.6"E) on 26 August 2021 and on 17 August 2022, and reared at natural temperatures and daylengths, giving leaves of the perennial plant *Crinum asiaticum* (Amaryllidaceae).

The butterfly *C. benjaminii* is distributed in Japan, Taiwan, southern China, Southeast Asia, and India (Chiba 2009). Adults appear from May to August in central Japan but all seasons except winter in the Ryukyus, southern Japan (Shirôzu 2006). A total of 33 larvae were collected at Hino, Tokyo (35°38'37.5"N, 139°23'56.3"E) on 17, 21 and 27 May 2020 and Machida, Tokyo (35°35'57.9"N, 139°25'13.9"E) on 26 May 2021 and 26 May 2022. These larvae were reared at natural temperatures and daylengths, giving leaves of the tree *Meliosma myriantha* (Sabiaceae).

Morphological measurement

Body size was measured using the free software, ImageJ (version 1.53e; National Institutes of Health, Bethesda, Maryland, USA) based on the photographs of randomly chosen 24 final-instar larvae of *A. plana*, 37 final-instar larvae of *B. crini*, 11 final-instar larvae of *C. benjaminii*. I measured the body length (from the front of head to the body end) and width (at the widest part of body) of larvae (Fig. 4-1). Visual pigments of the potential predators such as avian and non-avian reptiles can absorb ultraviolet light (Chen *et al*. 1984; Fleishman *et al*. 1993). Therefore, to know the larval color patterns under ultraviolet light, the larval photographs of the three species were taken using an ultraviolet filter (U330; HOYA Co. Ltd., Tokyo, Japan).

Assessment of palatability

A total of 9 (#1–9) lizards, *Plestiodon finitimus* Okamoto et Hikida, 2012 were used as a potential predator. These lizards were captured at Hachioji, Tokyo, on April 20, 27 and June 10 in 2021. The mean snout to vent length (SVL) of them was 61.9 mm (SD = 4.6, *n* = 9) and the tail length (TL) was 76.8 mm (SD = 17.9, *n* = 9). They were kept individually in a plastic cage (200 mm × 350 mm, 200 mm in depth) at 21.5–37.0°C with natural daylength. Each cage was filled up to 10 mm with a mixture of peat moss and red ball soil in a 1:1 ratio and an unglazed shelter. Water was always supplied in a cup (90 mm in diameter, 50 mm in depth). Two mealworms (larvae of *Tenebrio molitor* Linnaeus, 1758) were fed during the 3-h basking period with artificial light every other day. The mean body length of the mealworms was 17.1 mm (SD = 1.0, *n* = 20) and their width was 2.4 mm (SD = 0.2, *n* = 20).

Feeding experiments were conducted for lizards maintained in the cage for at least one week. To test palatability of larval *A. plana*, after 30-min basking, one larva was given to the lizards #1–3 daily for five consecutive days (1st- to 5th-day trials). This 5-day feeding experiment was conducted also in *B. crini* using the lizard #4–6 and in *C. benjaminii* using those #7–9. The feeding willingness of each lizard was checked by giving one mealworm just before the 1st-day trial and by giving three mealworms just after the 5th-day trial. All tests were terminated if there was no approach to the prey for 20 s. Lizard behavior was recorded with a video camera (12 MPx Camera, Apple Inc., Cupertino, CA, USA) of the Apple iPhone 8. The lizard's behavior was discriminated into seven categorized sequences: no response (0), heading toward prey (1), licking prey (2), pecking prey with their mouthparts (3), biting prey but immediately releasing it (4), swallowing prey but regurgitating it (5), and complete feeding (6). Once the lizards were used for the 5-day feeding experiment, they were released to the capture sites in the field.

Prey learning and discrimination

A total of 66 lizards (#10–75) were captured at Hachioji, Tokyo, on May 17 in 2020; July 10, August 25, 30, and September 4, 7 in 2021; and September 25 and October 23 in 2022. SVL was 60.0 mm (SD = 4.5, n = 66) and TL was 79.3 mm (SD = 18.2, n = 66). All lizards were reared in the same methods as used in palatability assessment and the following experiments were conducted after they were maintained in the cage for at least 1 week.

In Experiment 1, I examined whether the lizards can discriminate the aposematic larvae of *A. plana* from the others. The four types of larvae were presented to individual lizards on the 4th day after the 3-day learning period of larval *A. plana* (Fig. 4-2B). The four types are the larvae of *C. benjaminni* and the three types of silkworm larvae, *Bombyx mori* Linnaeus, 1758, painted with red and black colors as similar to *C. benjaminni* larvae, painted with the mixed colors of red and black (brown), and unpainted (Fig. 4-2B). I used the paints for red food coloring (HOME MADE cake Food Coloring Red; Kyoritsu Foods Service Co. Ltd., Tokyo, Japan) and black food coloring (Icing Color, 1 oz. – Food Coloring; Wilton Brands LLC, USA). The lizards #10–16 (n = 7) were tested to discriminate *C. benjaminii* from the experienced *A. plana*, those #17–23 (n = 7) to discriminate color-banded silkworms, those #24–26 (n = 3) to discriminate brown silkworms, and those #27–29 (*n* = 3) to discriminate intact silkworms. The feeding willingness of each lizard was checked by giving one mealworm just before the 1st-day trial and by giving three mealworms just after the 4th-day trial. All tests were terminated if there was no approach to the prey for 120 s. Lizard feeding behavior was categorized from (1) to (6) by the same way as in palatability assessment, and when the lizards swallow the prey item (i.e., feeding categories 5 and 6), the duration from heading toward the prey to bite it was measured. After the 4-day feeding experiment, they were released to the capture sites in the field.

In Experiment 2, the same learning experiment as Ex. 1 was conducted by conditioning to the larvae of *B. crini* (Fig. 4-2C). The lizards #30–36 (*n* = 7) were tested to discriminate *C. benjaminii* from the experienced *B. crini*, those #37–43 (*n* = 7) to discriminate color-banded silkworms, those #44–46 (*n* = 3) to discriminate brown silkworms, and those #47–49 (*n* = 3) to discriminate intact silkworms.

As Control of Exs. 1 and 2, the larvae of *C. benjaminii*, color-banded silkworms, brown silkworms, and intact silkworms were given at the 1st day to the lizards #50–54 (*n* = 5), #55–61 (*n* =7), #62–68 (*n* =7) and # 69–75 (*n* =7), respectively (Fig. 4-2A).

Predation pressures in the field

The artificial clay models have been used recently to assess predation pressures in the field (Mappes *et al*. 2014; Roslin *et al*. 2017; Aslam *et al*. 2020; Hernández-Agüero *et al*. 2020; Zvereva & Kozlov 2021). I also used the two types of larval models made by plastic clay, to determine whether or not insectivorous wild birds attack the prey with red/black/white color bands. One was the color-banded model consisting of one red tip and 6 black and white bands (Fig. 4-3A) which was made by the odorless plastic clays (Polymer clay for handicrafts red, black and white, Daiso Sangyo Co. Ltd., Hiroshima, Japan). The other is the entirely gray model (Fig. 4-3E) made by mixing the same amount of red, black and white clays used for the first model. The photographs of these two models did not show any different markings if taken under the ultraviolet light (Fig. 4-3A, E). The length of these two models was 30 mm and the width was 5 mm. A fine stainless wire (0.28 mm in diameter, 60 mm long) was buried longitudinally in the model body to fix it on the surface of tree blanches of 4–15 mm in diameter (Fig. 4-3B, F). In the field study site, 100 color-banded and 100 grey models were set at 1.0–1.5 m in height above the ground alternately at more than 2 m intervals along both sides of the narrow road in the forest. The photographs of all models set were taken so as not to overlook them. The marks on the model taken by birds' beaks were checked at every noon from the next day of setting for 3 consecutive days. If marked, the model was replaced with a new one (so available always 100 color-banded and 100 grey models).

This field experiment was conducted at three sites of Japan; Yonaguni Island of the Ryukyus, southern Japan (24°27'06.1"N, 123°01'19.4"E), Ueda in central Honshu, central Japan (36°19'53.2"N, 138°13'30.4"E), and Tomakomai in Hokkaido, nouthern Japan (42°40'37.4"N, 141°35'45.2"E).

In Yonaguni, the moths of *A. plana*, *B. crini*, and *C. benjaminii* are all distributed, and the experiment was conducted in spring (25 to 28 March 2022) when the resident and some winter birds (staying from autumn to the next spring for overwintering) are present. The study site was 300 m along the road in evergreen broadleaf forest dominated by *Ficus* (*F. macrocarpa*, *F. septics* and *F. superba*) and *Arenga engleri*. Air temperatures during the experiment ranged from the maximum 23.7°C (SD = 0.04, n = 4) to the minimum 19.6°C (SD = 0.34, n = 4). The weather was fine although fogged in early morning. Morning fogging may help to keep softness of clay models.

In Ueda, only *C. benjaminii* is distributed and the experiment was done twice, in spring (7–10 April 2022) when the resident and some winter birds are present, and in early summer (9–12 June 2022) when the resident and some summer birds (migrated from the southern region for breeding) are present, but the newly born chicks still stay in the nests, as predicted from ca. 1.5-month fledging period from nest building until the young birds begin foraging (Kawaji *et al*. 1996; Suzuki *et al*. 2010; Takagawa *et al*. 2011; Tamada 2013; Kawaji and Kawaji 2015; Igarashi *et al*. 2015; Yagi 2020) and the known dates of the first call of individual species of the summer birds seen there (Hotta *et al*. 2012). The study site was 330 m along the road in deciduous broadleaf forest dominated by *Quercus* (*Q. acutissima* and *Q. serrata*), *Cerasus jamasakura*, and *Zelkova serrata*. In the spring experiment, the mean maximum temperature was 16.7°C (SD = 0.25, n = 4) and the mean minimum temperature was 2.8°C (SD = 0.24, n = 4). The weather was clear although fogged in early morning. In early summer, the maximum was 25.7°C (SD = 0.08, n = 4) and the minimum was 14.6°C (SD = 0.22, n =

4). The weather was clear although fogged in early morning.

In Tomakomai, none of the three species of moths is distributed and the experiment was conducted in summer (26–29 July 2022) when the resident and only a few summer birds are present but the chicks may little fledge. The site was 330 m along deciduous broadleaf forest dominated by *Quercus crispula*, *Prunus ssiori* and *Tilia japonica*. During the experiment, air temperatures ranged from the maximum 22.4°C (SD = 0.19, *n* = 4) and to the minimum 17.6°C (SD = 0.14, *n* = 4). The weather was fine with fog in early morning.

On each experimental occasion, the birds and bird songs observed along the study site were identified to know the bird fauna. These birds were categorized into residents, winter birds, and summer birds (Mizutani & Kanouchi 2020) to estimate the possible number of species that learned and unlearned the color patterns of unpalatable *A. plana* and *B. crini* larvae.

Statistics

Values are shown as mean ± standard deviation (SD) with sample size (*n*). The frequency of eaten prey and the duration from heading toward the prey to biting of it by the lizard were compared between the control (unlearned) and learned lizards to both unpalatable *A. plana* and *B. crini* larvae using Fisher's exact tests and Mann-Whitney *U*-tests, respectively. The frequency of bird pecking on the artificial prey models set in the field was compared between the grey and color-banded models using Fisher's exact test.

4-3. Results

Morphology

Larvae of the three species have apparently similar color marking patterns, red in the tip and repeated black/white bandings in the body, although the reddish part of larval *A. plana* is not the head but the pronotum and the blackish band is moderately dotted in larval *B. crini* (Fig. 4-1). These body marking patterns are kept through the larval development except for earlier (1st- and/or early 2nd-) instars of larvae, and do not differ between the photographs taken under visible and ultraviolet light conditions (Fig. 4-1). The body length of the last instar larvae was 27.0 mm (SD = 6.0, *n* = 24) in *A. plana*, 27.9 mm (SD = 4.1, *n* = 37) in *B. crini*, and 26.0 mm (SD = 2.9, *n* = 11) in *C. benjaminii*. Their body width was 4.9 mm (SD = 1.0, *n* = 24), 5.9 mm (SD = 1.0, *n* = 37), and 4.0 mm (SD = 0.6, *n* = 11), respectively. Larvae of *A. plana* and *B. crini* were generally found on leaf surface of the host plants. However, larvae of *C. benjaminii* made a curled leaf nest with a dozen of small holes and they moved far (occasionally over 1 m) from their nests to feed on the host leaves (Fig. 4-1C; Sagami Butterfly Investigators' Society 2016).

Pupae and adults of the three species are different in color marking patterns (Fig. 4-1). In *A. plana* and *B. crini*, pupae are dark brown; the former being cryptic under the substrates on the ground and the latter being cryptic between the leaves near the host plant stems (Hayashi *et al*. 1971) or in the soil (Morishita 1994), but in *C. benjaminii*, the pupae are covered with white wax in the rolled leaves or substances on the ground (Sagami Butterfly Investigators' Society 2016). Adults have species specific color marking patterns in the body and wings; colorful in *A. plana*, cryptic in *B. crini*, and slightly pale reddish in the posterior margin of hindwings in *C. benjaminii* (Fig. 4-1).

Palatability

All mealworms given at the 1st- and 5th-days were eaten by the lizards. Although one larva of *A. plana* was bitten but immediately released at the first trial of the lizard #1, all other larvae were never bitten (Table 4-1A). In *B. crini*, one lizard released the biting larva and two lizards regurgitated the swallowing larvae at their first tries, but thereafter the lizards never swallow the larvae, which suggesting learning by the repeated experience of unprofitable prey (Table 4-1B). However, the most larvae of *C. benjaminii* were normally eaten by the lizards, although the lizards #7 and #8 tended to decrease their willingness of eating it (Table 4-1C). Thus, the larvae of *A. plana* and *B. crini* are unpalatable and those of *C. benjaminii* are generally palatable although less palatable for some lizards in repeated feeding.

Prey learning and discrimination

In Control, all types of prey were completely eaten by the lizards without feeding experience of *A. plana* and/or *B. crini* (Fig. 4-2A, Table S1).

In Experiment 1, all lizards did not eat the unpalatable larvae of *A. plana* in the 3-day experience period, although they regurgitated or released the larvae in 5 early trials of total 60 trials (Table S4-1). After experience of larval *A. plana*, 4 of 7 *C. benjaminii* larvae, 4 of 7 color-banded silkworms were not eaten, whereas all of grey and intact silkworms were eaten (Fig. 4-2B, Table S2). When the larvae were eaten, the time taken to bite the larvae of *C. benjaminii* and color-banded silkworms became longer by the experienced lizards than by the unexperienced lizards, whereas that time is not different between the experienced and unexperienced lizards in grey and intact silkworms (Fig. 4-2D, Table S2). These results suggest that the lizards cannot well discriminate the larvae among *A. plana*, *C. benjaminii*, and color-banded silkworms.

In Experiment 2, All lizards did not eat the unpalatable larvae of *B. crini* in the 3-day experience period, but they regurgitated or released the larvae in 23 early trials of total 60 trials (Table S4-3). After experience of larval *B. crini*, 5 of 7 *C. benjaminii* larvae, 5 of 7 color-banded silkworms were not eaten, whereas all of grey and intact silkworms were eaten (Fig. 4-2C, Table S3). When the larvae were eaten, the time taken to bite the larval*C. benjaminii* and color-banded silkworms became longer by the experienced lizards than by unexperienced lizards, whereas that time is not different between the experienced and unexperienced lizards in grey and intact silkworms (Fig. 4-2D, Table S3). As in Experiment 1, the lizards cannot well discriminate the larvae among *B. crini*, *C. benjaminii*, and color-banded silkworms.

In the pooled data of Experiments 1 and 2 because of small sample size, the feeding rates (Fig. 4-2A–C) differed between the control and learned lizards to both unpalatable *A. plana* and *B. crini* larvae in larval *C. benjaminii* (5/5 and 5/14, respectively; Fisher's exact test, $P = 0.033$) and in color-banded silkworms (7/7 and 5/14, respectively; $P = 0.007$), but did not differ in brown silkworms (7/7 and 6/6, respectively; $P = 1.00$) and in intact silkworms (7/7 and 6/6, respectively; $P = 1.00$). When the prey was eaten, the duration from heading to the prey to biting it (Fig. 4-2B) also differed between the control and learned lizards in larval *C. benjaminii* (Mann-Whitney U-test; $n_1 = 5$, $n_2 = 5$,

$z = 2.20$, $P = 0.028$) and in color-banded silkworms ($n_1 = 7$, $n_2 = 5$, $z = 2.70$, $P = 0.007$), but did not differ in brown silkworms ($n_1 = 7$, $n_2 = 6$, $z = 1.60$, $P = 0.11$) and in intact silkworms ($n_1 = 7$, $n_2 = 6$, $z = 0.65$, $P = 0.51$).

Discriminative predation in the field

In the field, the elongated clay models attached to the twigs were pecked by wild birds because V-shaped or holed marks on the surface of models caused by the beak of birds (Fig. 4-3). In some cases, the wires of the models were released partially or entirely from the twigs (Fig. 4-3C, D, H). In all study occasions, all models were recovered and such conspicuous pecking marks were checked. In Yonaguni where all species of caterpillars are distributed (Fig. 4-4A), the color-banded models were pecked less frequently than the control gray models (Fig. 4-4B), suggesting that such a color pattern is avoided by birds' learning. In fact, the observed avian fauna consisted of the resident and winter birds staying in this island (Table S4-4). These birds may experience the unpalatable caterpillars there.

In Ueda where the palatable *C. benjaminii* is only distributed (Fig. 4-4A), there was no significant difference in frequency of pecking between the color-banded and grey models in April, but marginally significant difference in June (Fig. 4-4B). In this sites, little birds experienced the unpalatable color pattern in April, but most summer birds migrated from the southern area to breed (but not still fledged) experienced such unpalatable color patterns (Table S4-4), resulting in different pecking frequencies between the two models. In Tomakomai where the three species of caterpillars are not distributed (Fig. 4-4A), the frequency of pecking did not differ between the two models (Fig. 4-4B). In this site, birds were fewer although the possibly learned summer bird *Turdus cardis* was observed (Table S4-4).

4-4. Discussion

Aposematic mimicry rings

Mimicry rings are communities containing at least one well-defended prey species that has a warning signal and experiences reduced predation pressure owing to its aposematism and at least one more associated prey species that derives a fitness benefit from mimicking the well-established aposematic signal (Kunte et al. 2021). In this ring, dynamic prey-predator interactions are included (Kunte et al. 2021). Aposematic mimicry rings can be also defined as two or more species that evolved independently to have a similar warning color pattern and interact for each other to escape attacks by the predators that search prey visually and learn unprofitable prey by experience. What evidences do demonstrate the mimicry rings?

First, one of the prey in the mimicry rings need to be unprofitable for the predators. A powerful sting of the hymenopteran insects is apparently harmful for the predators and therefore the mimicry rings regionally with various warning color patterns are reported in wasps and bees (e.g., Willadsen 2022; Hlaváček *et al*. 2022) and ants (e.g., Wilson *et al*. 2012). Poisonous snakes are also apparently harmful for the predators and their mimicry rings are well studied (e.g., Pfennig & Mullen 2010; Banci 2020; Goiran *et al*. 2022). However, it is usually difficult in insect communities to define the mimicry rings including such insects unpalatable or palatable and Batesian or Müllerian mimicries because there was no feeding examination and/or chemical analysis of defensive toxin except for some insects such as adult *Heliconius* and *Danaus* butterflies (Mattila *et al*. 2022; Blount *et al*. 2023). In our study, the larvae of the three species in the different families, *A. plana*, *B. crini*, and *C. benjaminii* are suggested to form the mimicry rings consisting of the two former unpalatable species and the latter palatable species from the feeding experiments by the lizards as potential predators. The larvae of *B. crini* are known to include alkaloids derived from the host plant of Amaryllidaceae (Nair *et al*. 2020).

The second problem to define the mimicry rings is how different the color patterns of prey that predators can no longer discriminate them (e.g., McLean *et al*. 2021). Although ontogenetic color change from cryptic (green) to aposematic (orange) is reported at the later stages of the caterpillars *Saucrobotys futilalis* (Lederer, 1863) (Grant 2007), the red/black/white color patterns of larval *A. plana*, *B. crini*, and *C. benjaminii* resemble to each other during the development except for much smaller 1st- and early 2nd-instars. In our study, the lizards that experienced unpalatable larvae of *A. plana* and *B. crini* failed mostly in discrimination of palatable larvae of *C. benjaminii* and also artificially color-banded silkworms from unpalatable species, and even if eaten, the lizards took more time to discriminate palatable and unpalatable species. Therefore, the mimetic relationship between *A. plana* and *B. crini* is Müllerian mimicry and *B. benjaminii* is Batesian mimicry to the former two species in this mimicry ring with a similar red/black/white color banding that the lizards cannot discriminate them perfectly.

Mimicry rings including allopatric species

The species of the same mimicry ring should be distributed sympatrically as the basic assumption (Wallace 1867; Poulton 1890; Wickler 1968; Pfennig & Mullen 2010). For example, the ground beetles of *Ceroglossus* exhibit a high degree of color matching among sympatric species, but lack color matching in the allopatric regions by different selective pressures of Müllerian mimicry among these regions (Muñoz-Ramírez 2016). Selection against mimics is stronger in sympatric region with the model than the allopatric region (Pfennig et al. 2007). Thus, incomplete mimicry occurs outside the overlapped area of two mimetic species. In other cases, intraspecific color polymorphism occurs because the mimetic species (usually distributed widely) resembles the regionally specific model species with different color patterns (Wallace 1865; Basu et al. 2023).

On the other hand, recent studies suggest the hypothesis that aposematic mimicries are maintained across the ranges of the regularly migrating birds because they learn prey unpalatability and remember it for a long time (Poulton 1908; Wickler, 1968; Waldbauer 1988; Pfennig & Mullen 2010). Differences in experience affect the great tit feeding avoidance of aposematic bug adults; more experienced southern individuals (mostly resident) show less frequent attacks and longer latency to peck prey than rarely experienced northern individuals (Exnerová *et al*. 2015). In our study, the three species are not distributed in the same ranges; the unpalatable *A. plana* and *B. crini* are southern species and palatable *C. benjaminii* expands the distribution range to northern Japan. The field experiments using artificial clay models of caterpillars showed that the unpalatable color-banded models are pecked less frequently by the wild birds than the control grey models in March in southern Japan and in June in central Japan in which the resident and summer birds (just begin breeding) that learned unpalatable caterpillar were abundant. However, the frequencies of pecking did not differ between the color-banded and control grey models in April in central Japan and in July in northern Japan in which probably unlearned resident and winter birds were abundant. The memory of the migratory birds from the south where they have chance to learn unpalatable models to the north where lacking unpalatable models plays an important role to maintain Batesian mimicry. Thus, the mimicry rings among the aposematic but geographically asynchronous species might be more common across the temperate region via the memory of the regularly migrating birds.

It is difficult to measure the predation pressures in the field. Recently we can use artificial plasticine models of insects to assess the predation pressures in the field and their special and temporal dynamics were examined (Mappes *et al*. 2014; Roslin *et al*. 2017; Aslam *et al*. 2020; Hernández-Agüero 2020; Zvereva & Kozlov 2021). The trend of higher predation risk at lower latitudes is reported because the clay caterpillar models were attacked more frequently at lower latitudes (Roslin *et al*. 2017). In the temperate zone, mimetic species may be selected to avoid the midsummer season when newly fledged insectivorous birds are abundant and they have not yet learned to shun the models which the mimics resemble (Waldbauer *et al*. 1977). In fact, the survival of the caterpillar models with conspicuous colors is lower than the cryptic ones in early summer before fledging, but higher in summer when naïve fledglings appear synchronously in the temperate region (Mappes *et al*. 2014; Hernández-Agüero *et al*. 2020; Zvereva & Kozlov 2021). This method will be more applied to assess avian predation pressures to the specific type of color patterns of arthropods in the field as used in our study.

Table 4-1. Feeding responses of lizards to larval *Asota plana* (A), *Brithys crini* (B), and *Choaspe benjaminii* (C) in the five-day feeding trials, using a total of nine lizards (#1–9). Feeding responses are categorized into the following six sequences: no response (0), heading toward prey (1), licking prey (2), pecking prey with their mouthparts (3), biting prey but immediately releasing it (4), swallowing prey but regurgitating it (5), and complete feeding (6). Categories (4) and (5) are shaded with orange as active responses and category (6) was shaded with green as complete feeding. SVL = lizard snout to vent length in mm.

(A) Feeding response to laval *A. plana*			
Lizaed ID	#1	#2	#3
Lizaed SVL (mm)	70	60	56
1st day	4	2	2
2nd day	1	2	1
3rd day	1	1	2
4th day	1	1	0
5th day	1	1	0

(B) Feeding response to laval *B. crini*			
Lizaed ID	#4	#5	#6
Lizaed SVL (mm)	57	65	58
1st day	5	4	5
2nd day	1	4	2
3rd day	2	2	2
4th day	2	4	2
5th day	1	4	1

(C) Feeding response to laval *C. benjaminii*			
Lizaed ID	#7	#8	#9
Lizaed SVL (mm)	62	66	63
1st day	6	6	6
2nd day	6	4	6
3rd day	6	4	6
4th day	4	4	6
5th day	2	1	6

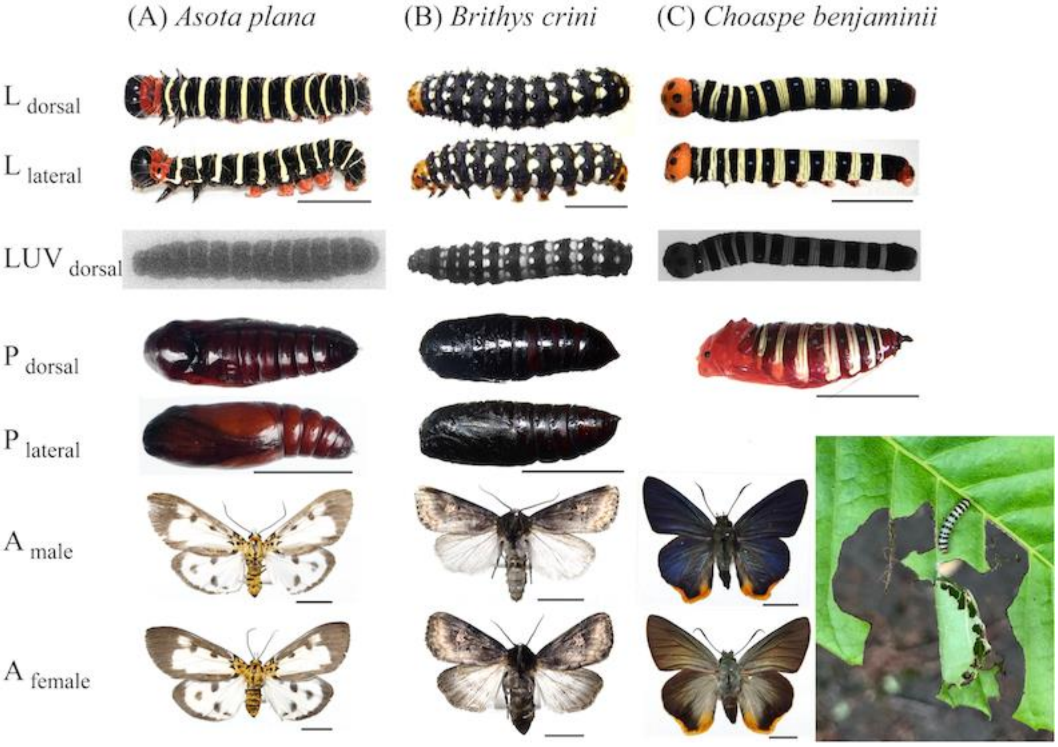

Figure 4-1. Dorsal, lateral or ventral view of larvae (L), pupae (P) and adults (A) of *Asota plana*, *Brithys crini* and *Choaspe benjaminii*. In larvae, the views under ultraviolet light (UV) are also shown. Bars indicate 10 mm. Larval *C. benjaminii* make a rolled leaf nest with several small holes and walk out of it to feed.

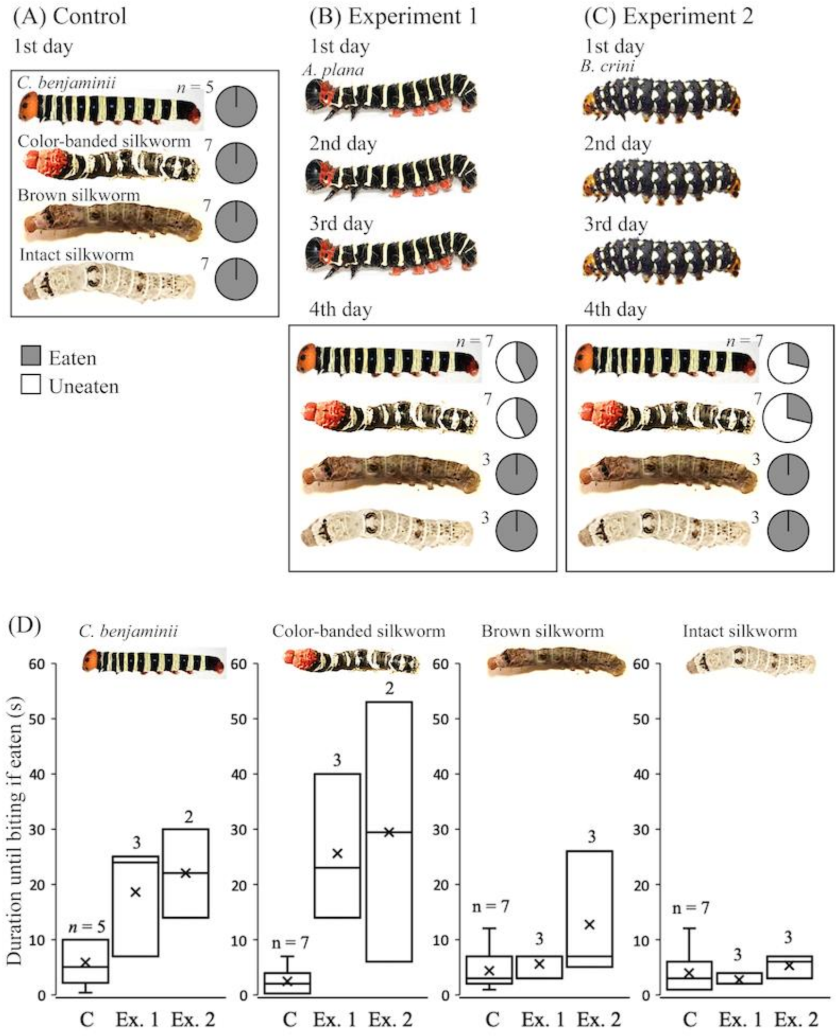

Figure 4-2. Lizard's learning and discrimination abilities of four types of unparatable prey, larval *Choaspe benjaminii*, color-banded silkworms painted with red and black similarly to *C. benjaminii*, brown silkworms painted with mixed red and black, intact unpainted silkworms. In Control (A), these larvae were given to the lizards unexperienced to unpalatable larvae of *Asota plana* and *Brithys crini*. These larvae were also given to the lizards experienced to unpalatable *A. plana* for 3 days in Experiment 1 (B) and to unpalatable *B. cryni* for three days in Experiment 2 (C). In percent of the eaten larvae of each type was shown by pi graphs (*n*=number of lizards examined). If eaten, the duration from heading toward prey to biting it of the lizards were measured and compared among Control, Experiments 1 and 2 in each type of prey (D; *n*=number of lizards eaten, close=the mean duration). For the statistical tests, see the text.

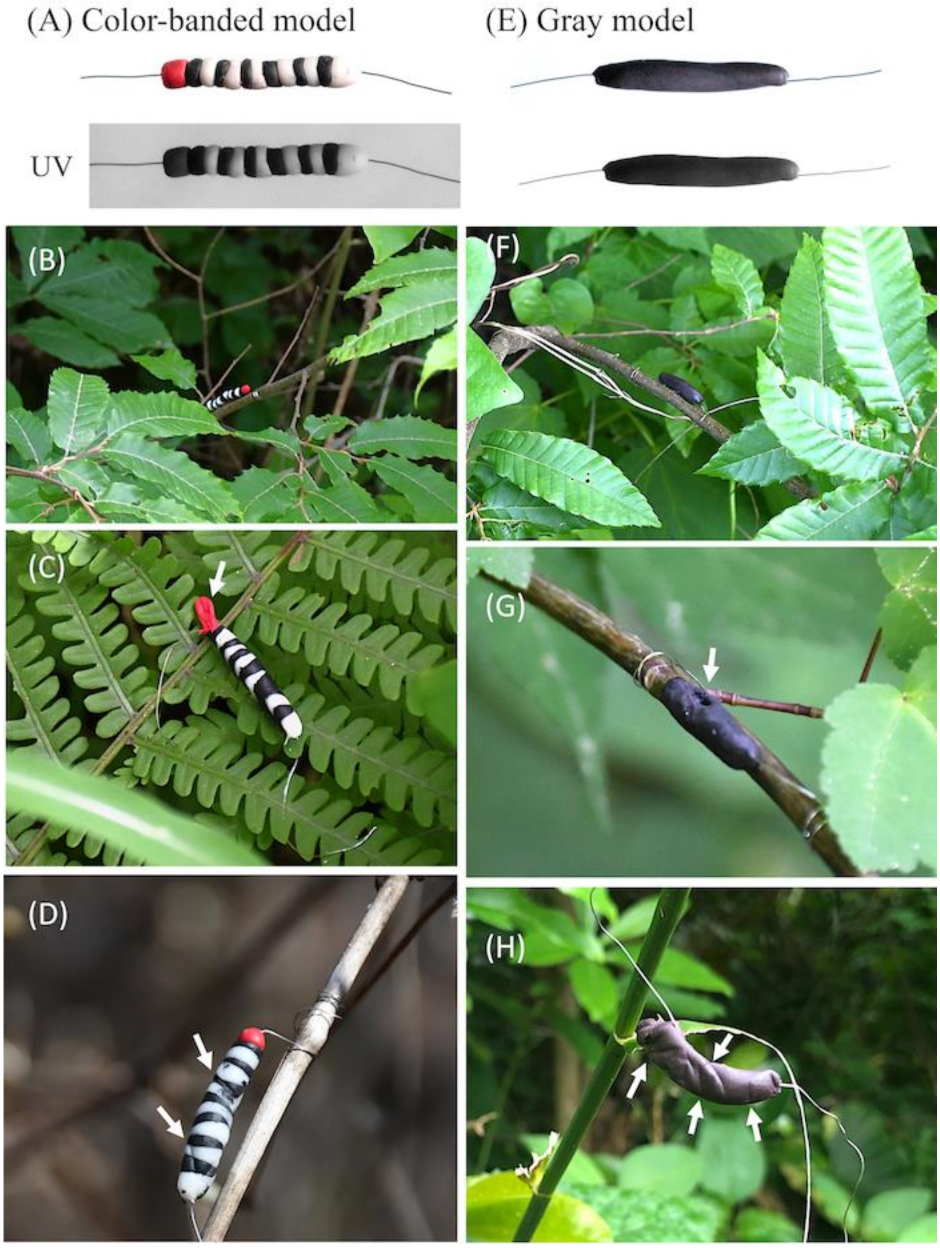

Figure 4-3. The field experiments using two types of artificial clay models, color-banded model made by red, black and white plastic clays (A) and gray models made by mixed of three-color clays (B). The photographs taken under ultraviolet light (UV) are also shown. These models were set on the surface of twigs using a fine stainless wire (B, F), and the pecking marks (arrows) on the models by the wild birds were checked for three days (C, D, G, H).

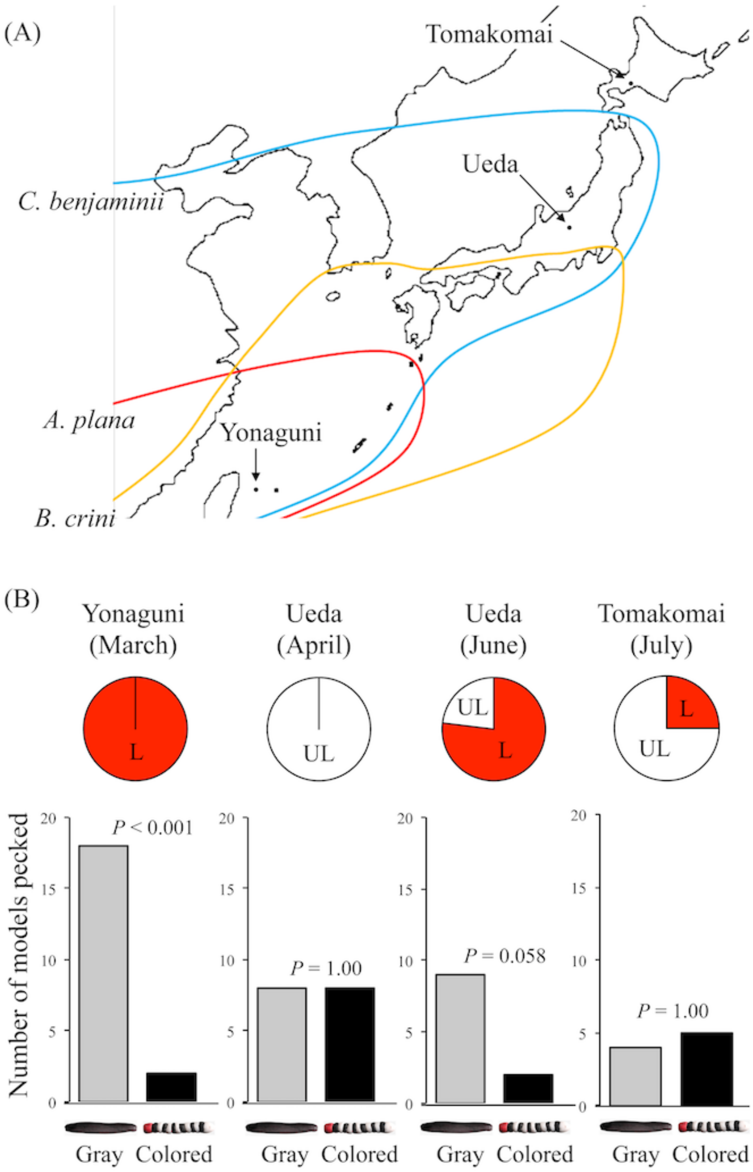

Figure 4-4. The distribution ranges of *Asota plana*, *Brithys crini*, and *Choaspe benjaminii* in East Asia and the three study sites, Yonaguni in March, Ueda in April and June, and Tomakomai in July, of avian predation rates using the grey and color-banded artificial clay models in the field (A). For two types models and the method, see Fig. 4-3 and the text. Species compositions of insectivorous birds possibly learned unpalatable prey (L) and possibly unlearned it (UL) are shown in pi graphs on each study occasion (B). The number of pecked grey and color-banded models per 100 models for 3 days is also shown on each study occasion (*P* in Fisher's probability tests).

Table S4-1. Feeding responses of lizards to larval *Choaspe benjaminii* (A), artificially color-banded silkworms (B), artificially colored brown silkworms (C), and unpainted intact silkworms (D) in the first encounter, using a total of the lizards #50–54, #55–61, #62–68, and #69–75, respectively. Individual prey are given after measuring the body length. Feeding responses are categorized into the following six sequences: no response (0), heading toward prey (1), licking prey (2), pecking prey with their mouthparts (3), biting prey but immediately releasing it (4), swallowing prey but regurgitating it (5), and complete feeding (6, shaded with green). All lizards fed these prey items normally. The duration from heading toward prey to bite it is also shown. SVL = lizard snout to vent length in mm.

(A) Feeding response to a larval *C. benjaminii*											
Lizaed ID	#50	#51	#52	#53	#54				*n*	Mean	SD
Lizaed SVL (mm)	62	66	63	64	63			Lizard SVL (mm)	5	63.6	1.5
Response at the 1st day	6	6	6	6	6			Larval length (mm)	5	28.0	3.9
Duration until biting (s)	4.0	10.0	5.0	0.4	10.0			Duration (s)	5	5.9	4.1
(B) Feeding response to a color-banded silkworm											
Lizaed ID	#55	#56	#57	#58	#59	#60	#61		*n*	Mean	SD
Lizaed SVL (mm)	57	59	58	59	65	66	60	Lizard SVL (mm)	7	60.6	3.5
Response at the 1st day	6	6	6	6	6	6	6	Larval length (mm)	7	27.5	0.7
Duration until biting (s)	0.2	0.3	2.0	7.0	2.0	2.0	4.0	Duration (s)	7	2.5	2.7
(C) Feeding responce to a brown silkworm											
Lizaed ID	#62	#63	#64	#65	#66	#67	#68		*n*	Mean	SD
Lizaed SVL (mm)	55	63	52	57	50	62	60	Lizard SVL (mm)	7	57.0	5.0
Response at the 1st day	6	6	6	6	6	6	6	Larval length (mm)	7	26.9	0.9
Duration until biting (s)	7.0	12.0	4.0	2.0	2.0	3.0	1.0	Duration (s)	7	4.4	3.6
(D) Feeding response to an intact silkworm											
Lizaed ID	#69	#70	#71	#72	#73	#74	#75		*n*	Mean	SD
Lizaed SVL (mm)	65	59	57	54	54	63	68	Lizard SVL (mm)	7	60.0	5.5
Response at the 1st day	6	6	6	6	6	6	6	Larval length (mm)	7	23.6	1.3
Duration until biting (s)	3.0	3.0	2.0	12.0	1.0	6.0	1.0	Duration (s)	7	4.0	3.9

Table S4-2. Feeding responses of lizards to larval *Choaspe benjaminii* (A), artificially color-banded silkworms (B), artificially colored brown silkworms (C), and unpainted intact silkworms (D) after the #10–29 lizards that experienced the unpalatability of larval *Asota plana* for three days. Individual prey are given after measuring the body length. For the categories of feeding responses (1–6), see Table S4-1. Categories (4) and (5) are shaded with orange as active responses and category (6) was shaded with green as complete feeding. If eaten as in the categories (5–6), the duration from heading toward prey to biting of it is also shown. SVL = lizard snout to vent length in mm.

(A) Feeding response to a larval *C. benjaminii* after 3-day experience of larval *A. plana*

Lizaed ID	#10	#11	#12	#13	#14	#15	#16		*n*	Mean	SD
Lizaed SVL (mm)	63	51	55	67	60	62	58	Lizard SVL (mm)	7	59.4	5.4
Response to *A. plana* at the 1st day	4	2	2	2	1	2	2	Larval length (mm)	21	26.9	1.7
Response to *A. plana* at the 2nd day	4	1	3	2	1	2	1				
Response to *A. plana* at the 3rd day	1	1	1	1	1	1	1				
Response to *C. benjaminii* at the 4th day	1	1	6	6	1	2	6	Larval length (mm)	7	27.3	1.8
Duration until biting (s)			25.0	7.0			24.0	Duration (s)	3	10.1	18.7

(B) Feeding response to a color-banded silkworm after 3-day experience of larval *A. plana*

Lizaed ID	#17	#18	#19	#20	#21	#22	#23		*n*	Mean	SD
Lizaed SVL (mm)	60	58	62	65	57	65	52	Lizard SVL (mm)	7	59.9	4.7
Response to *A. plana* at the 1st day	2	2	2	2	2	1	2	Larval length (mm)	21	26.8	0.1
Response to *A. plana* at the 2nd day	1	2	2	0	2	1	2				
Response to *A. plana* at the 3rd day	2	1	2	1	1	2	1				
Response to a silkworm at the 4th day	6	6	4	1	6	2	0	Larval length (mm)	7	28.4	2.3
Duration until biting (s)	40.0	14.0			23.0			Duration (s)	3	25.7	13.2

(C) Feeding responce to a brown silkworm after 3-day experience of larval *A. plana*

Lizaed ID	#24	#25	#26						*n*	Mean	SD
Lizaed SVL (mm)	64	60	63					Lizard SVL (mm)	3	62.3	2.1
Response to *A. plana* at the 1st day	5	5	4					Larval length (mm)	9	24.6	0.1
Response to *A. plana* at the 2nd day	4	2	2								
Response to *A. plana* at the 3rd day	1	0	2								
Response to a silkworm at the 4th day	6	6	6					Larval length (mm)	3	23.7	0.6
Duration until biting (s)	3.0	7.0	7.0					Duration (s)	3	5.7	2.3

(D) Feeding response to an intact silkworm after 3-day experience of larval *A. plana*

Lizaed ID	#27	#28	#29						*n*	Mean	SD
Lizaed SVL (mm)	56	59	58					Lizard SVL (mm)	3	57.6	1.9
Response to *A. plana* at the 1st day	2	2	1					Larval length (mm)	9	27.8	0.2
Response to *A. plana* at the 2nd day	2	2	1								
Response to *A. plana* at the 3rd day	2	2	1								
Response to a silkworm at the 4th day	6	6	6					Larval length (mm)	3	27.7	3.6
Duration until biting (s)	2.0	2.0	4.0					Duration (s)	3	2.7	1.2

Table S4-3. Feeding responses of lizards to larval *Choaspe benjaminii* (A), artificially color-banded silkworms (B), artificially colored brown silkworms (C), and unpainted intact silkworms (D) after the #10–29 lizards that experienced the unpalatability of larval *Brithys crini* for three days. Individual prey are given after measuring the body length. For the categories of feeding responses (1–6), see Table S4-1. Categories (4) and (5) are shaded with orange as active responses and category (6) was shaded with green as complete feeding. If eaten as in the categories (5–6), the duration from heading toward prey to biting of it is also shown. SVL = lizard snout to vent length in mm.

(A) Feeding response to a larval *C. benjaminii* after 3-day experience of larval *B. crini*											
Lizaed ID	#30	#31	#32	#33	#34	#35	#36		*n*	Mean	SD
Lizaed SVL (mm)	52	59	63	52	63	57	55	Lizard SVL (mm)	7	57.3	4.6
Response to *B. crini* at the 1st day	5	5	5	2	5	5	5	Larval length (mm)	21	27.5	3.2
Response to *B. crini* at the 2nd day	2	2	2	5	0	2	1				
Response to *B. crini* at the 3rd day	5	2	1	2	0	5	1				
Response to *C. benjaminii* at the 4th day	1	6	4	1	2	0	6	Larval length (mm)	7	27.3	1.7
Duration until biting (s)		14.0					30.0	Duration (s)	2	22.0	
(B) Feeding response to a color-banded silkworm after 3-day experience of larval *B. crini*											
Lizaed ID	#37	#38	#39	#40	#41	#42	#43		*n*	Mean	SD
Lizaed SVL (mm)	68	60	61	63	66	58	64	Lizard SVL (mm)	7	62.8	3.4
Response to *B. crini* at the 1st day	5	5	1	4	5	4	5	Larval length (mm)	21	28.9	2.7
Response to *B. crini* at the 2nd day	5	1	5	2	0	5	2				
Response to B. *crini* at the 3rd day	1	1	1	2	1	1	2				
Response to a silkworm at the 4th day	6	0	1	4	2	0	6	Larval length (mm)	7	28.4	1.6
Duration until biting (s)	6.0						53.0	Duration (s)	2	29.5	
(C) Feeding responce to a brown silkworm after 3-day experience of larval *B. crini*											
Lizaed ID	#44	#45	#46						*n*	Mean	SD
Lizaed SVL (mm)	63	66	54					Lizard SVL (mm)	3	61.3	6.3
Response to *B. crini* at the 1st day	5	5	5					Larval length (mm)	9	27.3	3.8
Response to *B. crini* at the 2nd day	2	0	1								
Response to *B. crini* at the 3rd day	2	0	1								
Response to a silkworm at the 4th day	6	6	6					Larval length (mm)	3	28.7	1.1
Duration until biting (s)	5.0	26.0	7.0					Duration (s)	3	12.7	11.6
(D) Feeding response to an intact silkworm after 3-day experience of larval *B. crini*											
Lizaed ID	#47	#48	#49						*n*	Mean	SD
Lizaed SVL (mm)	57	65	58					Lizard SVL (mm)	3	60.0	4.3
Response to *B. crini* at the 1st day	5	4	5					Larval length (mm)	9	26.9	3.6
Response to *B. crini* at the 2nd day	1	4	2								
Response to *B. crini* at the 3rd day	2	2	2								
Response to a silkworm at the 4th day	6	6	6					Larval length (mm)	3	26.6	0.6
Duration until biting (s)	3.0	6.0	7.0					Duration (s)	3	5.3	2.1

Table S4-4. Insectivorous birds observed in the field experiments (Yonaguni in March, Ueda in April and June, and Tomakomai in July) to examine the bird pecking rates on the color-banded and uniformly gray models made by clay (see Fig. 4-3). Birds observed visually and their songs are listed categorizing them into the resident (cross), winter (open circle), and summer birds (closed circle). The summer birds possibly learn (L) the unpalatable caterpillars as in the color-banded model in the southern area that they spent in cold seasons, but the winter birds possibly unlearned (UL) because they use only the northern area except for those found in southern area as in *Phoenicurus auroreus*.

Family	Species	Yonaguni	Ueda	Ueda	Tomakomai
		(March)	(April)	(June)	(July)
Aegithalidae	*Aegithalos caudatus*		×UL		
Campephagidae	*Pericrocotus divaricatus*			●L	
Cettiidae	*Horornis diphone*		×UL		
Cettiidae	*Urosphena squameiceps*			●L	
Cuculidae	*Cuculus poliocephalus*			●L	
Cuculidae	*Cuculus saturatus*			●L	
Emberizidae	*Emberiza cioides*			×UL	×UL
Fringillidae	*Fringilla montifringilla*		○UL		
Monarchidae	*Terpsiphone atrocaudata*			●L	
Muscicapidae	*Cyanoptila cyanomelana*			●L	
Muscicapidae	*Ficedula narcissina*			●L	
Muscicapidae	*Phoenicurus auroreus*	○L	○UL		
Muscicapidae	*Turdus eunomus*		○UL		
Paridae	*Parus minor*		×UL		×UL
Phylloscopidae	*Phylloscopus coronatus*			●L	
Picidae	*Dendrocopos kizuki*			●L	
Picidae	*Picus awokera*			●L	
Pycnonotidae	*Hypsipetes amaurotis*	×L	×UL	×UL	×UL
Pycnonotidae	*Pycnonotus sinensis*	×L			
Timaliidae	*Garrulax canorus*		×UL	×UL	
Turdidae	*Turdus cardis*				●L
Zosteropidae	*Zosterops japonicus*	×L	×UL		
Possibly learned (L) species		4	0	10	1
Possibly unlearned (UL) species		0	9	3	3
Total number of species		4	9	13	4
× Resident birds, ○ Winter birds, ● Summer birds at each study occasion					

5. General discussion

5-1. Mimicry rings with the dotted blackish lines on the yellow body

Pupae of most lepidopteran species are cryptic in color and hide under the substrates or in inconspicuous cocoons (Lindstedt *et al*. 2019; Wiklund & Sillen-Tullberg 2019; Edmunds 1990; Janzen *et al*. 2010). Some lepidopterans have the pupae with a uniformly green color. The brown and green pupae above the ground are also cryptic to the background of brown twigs and green leaves, respectively (Wiklund & Sillen-Tullberg 2019). In contrast, the pupal color of some Lepidoptera is prominent and seems to be warning coloration as seen in *Delias* and *Aporia* of butterflies (Braby & Nishida 2010) and *Abraxas grossulariata* (Linnaeus, 1758) of moths (Nishida *et al*. 1994; Sourakov 2016). However, prominent pupal colors are not always warning colors.

Only a few lepidopterans have been proven to be warning colors. In our previous study (Yazaki *et al*. 2019), unpalatable moth, *Ivela auripes* (Butler, 1877), which pupates at the exposed sites such as tree trunk and rock surface above the ground and displays the conspicuous yellow pupal body with seven longitudinal stripes of blackish dots (Fig. 5-1). This color pattern is kept during the pupal stage and similar to those pupae studied in Chapters 2 and 3 in this thesis. As shown in Chapter 2, the pupae of *Amata germana* and *A. flava* are similar in shape, size and color. These pupae have the color patterns of the dotted blackish lines longitudinally on the pale-yellowish body surface during the pupal stage except for the blackish wing pads just before adult emergence (Fig. 5-1). In Chapter 3, the pupae of the three species *Cystidia*, *C. couaggaria*, *C. truncangulata*, and *C. stratonice*, are also similar to each other in shape, size, color, and marking pattern, which also resemble the pupae of *I. auripes*, *Amata germana*, and *A. flava* (Fig. 5-1). In these *Cystidia*, the pupae of *C. couaggaria* and *C. truncangulata* are kept in their colors during the pupal stage except for the blackish wing pads just before adult emergence, but those of *C. stratonice* are slightly different in the blackish wing pads during the pupal stage.

Thus, the pupae of these moths may form the mimicry ring with the same signal of aposematic color pattern. All these pupae belonging to the families, Lymantriidae, Erebidae, and Geometridae, are unpalatable (Predation rates 0%) for the lizards (Yazaki *et al*. 2019, Chapters 1, 2) (Fig. 5-2). In addition, the unpalatable last-instar larvae of *Pryeria sinica* Moore, 1877 (family Zygaenidae) have the dotted blackish longitudinal lines on yellow body surface (Fig. 5-2), which is considered to be Müllerian mimicry with pupal *I. auripes* in the rare case of mimicry between the different developmental stages of co-occurring insects (Yazaki *et al*. 2019). Their distributions overlap widely. The shape of all species is oval and the size is also similar; 13.5 mm in pupal *A. germana*, 14.7 mm in pupal *A. flava*, 21.0 mm in pupal *C. couaggaria*, 17.7 mm in pupal *C. truncangulata*, 18.2 mm in pupal *C. stratonice*, 21.7 mm in pupal *I. auripes*, and 13.8 mm in larval *P. sinica* (Fig. 5-2).

This type of marking pattern is further seen in the larvae of *Elcysma westwoodii* (Vollenhoven, 1863) (Zygaenidae), the pupae of *Naxa seriaria* (Motschulsky, 1866) (Geometridae), the pupae of the *Aporia crataegi* (Linnaeus, 1758) (Pieridae), and pupae of the *A. hippia* (Bremer, 1861) (Perridae). *Elcysma westwoodii* is distributed widely in western Japan and the larvae are seen in late spring to early summer (Kishida 2011). *Naxa seriaria* is distributed widely in Japan and the pupae are seen in late spring to early summer (Kishida 2011). *Aporia crataegi* is distributed in Hokkaido in Japan and the pupae are seen in late spring to early summer, and the closely related *A. hippia* is endemic to central Japan and the pupae are seen in late spring to early summer (Shirôzu 2006). These larvae and pupae are oval and similar in size to those studied in Chapters 2 and 3 (Fig. 5-2). Their distributions and season that they appear overlap widely. They may be included in the mimicry ring with the dotted blackish longitudinal lines on yellow body surface, and therefore, future works will be needed for gathering information of the similarity in body color under visible and ultraviolet lights, their pupation sites (exposed or hidden), palatability, and learning based on their color patterns to know this aposematic mimicry ring is more complex or not.

5-2. Mimicry rings with the red/black/white marking

Another aposematic mimicry ring is the communities of lepidopteran larvae with a reddish tip and repeated black/white bands (Fig. 5-3). As shown in Chapter 4, larvae of *Asota plana* (family Erebidae), *Brithys crini* (Noctuidae) and *Choaspe benjaminii* (Hesperiidae) have similar size and body color pattern of the red tip and repeated black/white bands. Palatability tests using lizards suggested that the former two species are unpalatable but the latter is palatable. The lizards that learned unpalatability of *A. plana* or *B. crini* failed to discriminate the palatable *C. benjaminii* and also the silkworms painted with red and black as similar to *C. benjaminii*, although they discriminated perfectly the silkworms painted entirely with brown (a mix of red and black) and without painting. Thus, the potential predators can learn and indiscriminate such a conspicuous color pattern of these three species of larvae.

In the field experiments using artificial clay models of caterpillars, the red/black/white color-banded models were pecked less frequently by the wild birds than the control grey models in March at the southern Japan, and also in June at central Japan in which the resident and summer birds that learned unpalatable caterpillars were abundant. In contrast, the frequencies of pecking did not differ between the color-banded and control grey models in April at central Japan and in July at northern Japan in which unlearned resident and winter birds were abundant. In palatable *C. benjaminii* and unpalatable *A. plana* and *B. crini* are distributed sympatrically in southern Japan where the aposematic Batesian and Müllerian mimicries occur among the three species of larvae. In most part of central Japan, only palatable *C. benjaminii* is distributed. However, Batesian mimicry is probably maintained there by predation avoidance for *C. benjaminii* via the memory of the migratory birds from the south where they have a chance to learn unpalatable *A. plana* and/or *B. crini*. Thus, this aposematic mimicry ring with red/black/white color marking seems to be maintained by the migrate birds, particularly by the summer bird migration from south where they have a chance to learn unpalatable species and remember it for longtime.

Larvae of another Japanese moth *Orthosia carnipennis* (Butler, 1878) are somewhat similar to this mimicry ring species (Fig. 5-3). This species is distributed from Hokkaido to Kyusyu in Japan, Taiwan, Korea, Northern China, and Far East Russia (Kishida 2011). The preliminary study was conducted for this species based on the larvae collected at Ueda, Nagano, central Japan, on 14–17 June 2021, giving the leaves of the tree *Juglans mandshurica* (Juglandaceae) at the room temperature and natural day length. The body length and width of the last-instar larvae were 24.0 mm (SD = 4.9, *n* = 12) and 3.7 mm (SD = 0.7, *n* = 12). The lizards ate all mealworms and larvae. The used lizards were three individuals with 59, 57, and 54 mm in SVL (4-day, 4-day, and 3-day feeding trials, respectively). There was no significant difference in marking patterns under visible and ultraviolet lights. Larvae hide within the rolled leaf but moved out of it to eat the leaves as well as in *C. benjaminii*. Pupae are entirely brown and hide in the soil. These results suggest that this larva is palatable and if the lizards cannot discriminate it from unpalatable *A. plana* and *B. crini* larvae, this is also the member of Batesian mimicry in this mimicry ring. However, it is unclear the potential predators can discriminate this larva from others, and we need information on such a discrimination assessment to define the community of each type of aposematic mimicry rings.

There is an intraspecific mimicry, or automimicry, when the model and mimic are different individuals within a species (Pasteur 1982; Maran 2017). Sexual mimicry is a specific resemblance between members of different sexes that has functionality in intraspecific communication. It is also called self-mimicry when the mimetic individual mimics itself (Pasteur 1982). In plants, the self-mimicry occurs mainly in flower where a part of petal (pollen-like patch) resembles another part of it (stamen anther) (Maran 2017). A similar concept may be applied to the mimicry between the different stages of the same individual. In a few species of moths, the larval body color patterns are suggested to be similar to adult wing color patterns (adult-caterpillar mimicry) (Sourakov 2016). The similarity between the larval body color pattern and the adult color patterns may occur in *C. benjaminii* (Fig. 5-4). The posterior reddish part with some small black spots of the hind wings and the abdomen banded by black and white colors seems to be the self-mimicry to larval body color pattern. In this case, a dark green wing with dark venations might be camouflaged in the background leaves. If this color pattern functions in self-mimicry, the adult *C. benjaminii* must be added in this mimicry ring. This is an interesting phenomenon to be resolved and we must pay more attention to such a specific case to understand the dynamic interactions of prey and predators in the mimicry ring.

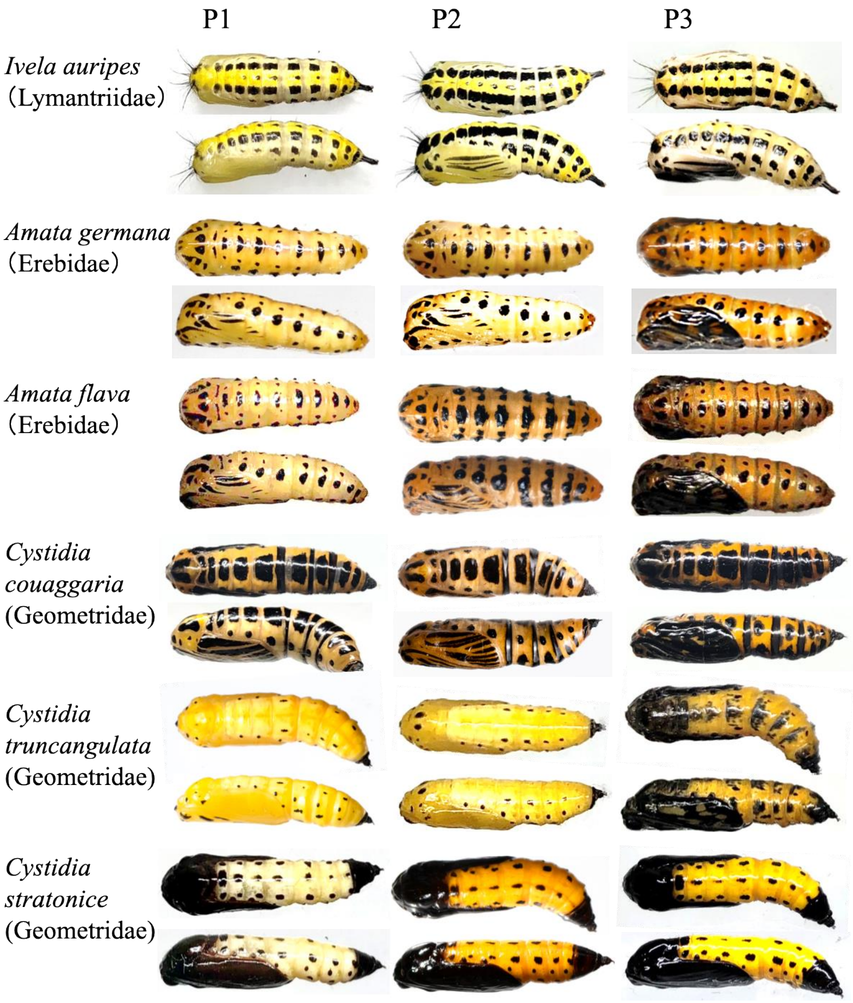

Figure 5-1. Dorsal and lateral view of pupae at the 1st day of pupation (P1), at the middle (P2), and just before adult emergence (P3) of *Ivela auripes*, *Amata germana*, *A. flava*, *Cystidia couaggaria*, *C. truncangulata* and *C. stratonice* whose pupae are similar color marking patterns with the dotted blackish longitudinal lines on the yellow body surface.

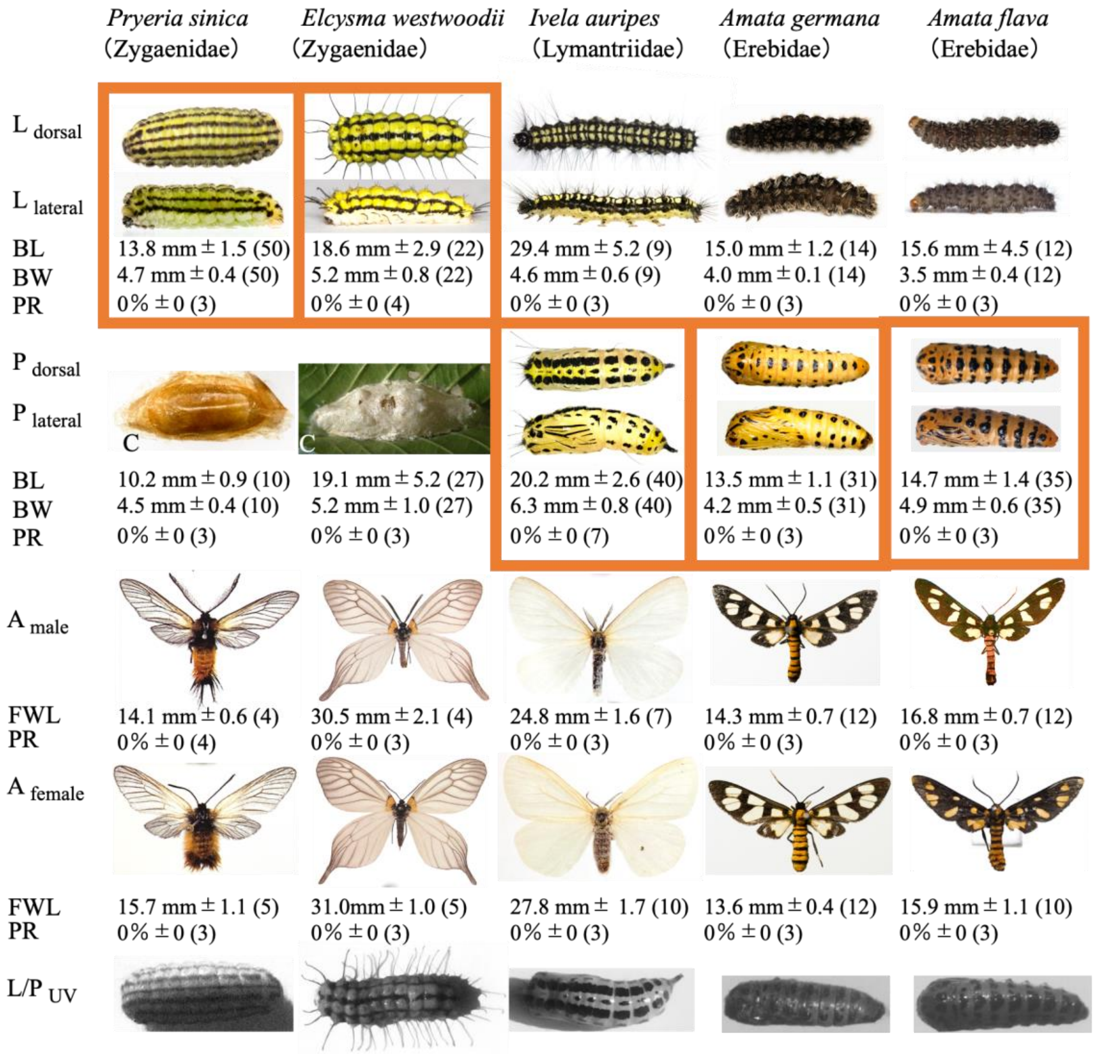

Figure 5-2. The possible mimicry ring with the dotted blackish longitudinal lines on the yellow body in the lepidopteran larvae and pupae as marked with orange lines. Dorsal and lateral view of larvae (L) and pupae (P), and adults (A) of *Pryeria sinica*, *Elcysma westwoodii*, *Ivela auripes*, *Amata germana*, *A. flava*, *Cystidia couaggaria*, *C. truncangulata*, *C. stratonice*, *Naxa seriaria*, and *Aporia crataegi*. In *P. sinica* and *E. westwoodii*, they pupate in the cocoons (C). The views under ultraviolet light (UV) are also shown for the larvae of *P. sinica* and *E. westwoodii* and the pupae of the other species. The mean body length (BL) and body width (BW) of the larvae and pupae, and the mean forewing length (FWL) of the adults are shown with SD (*n*). The predation rates (PR) of larvae, pupae and adults by the lizards are also shown with SD (*n*).

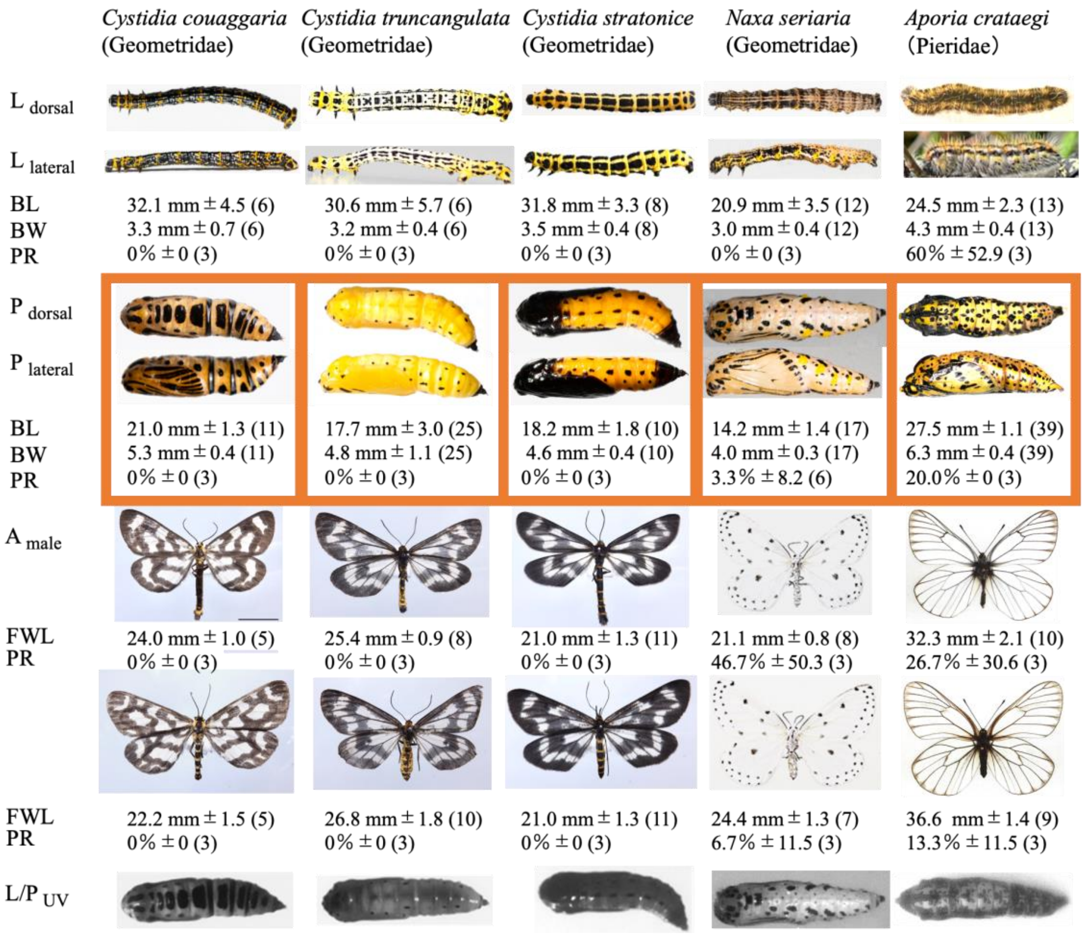

Figure 5-2 (continued).

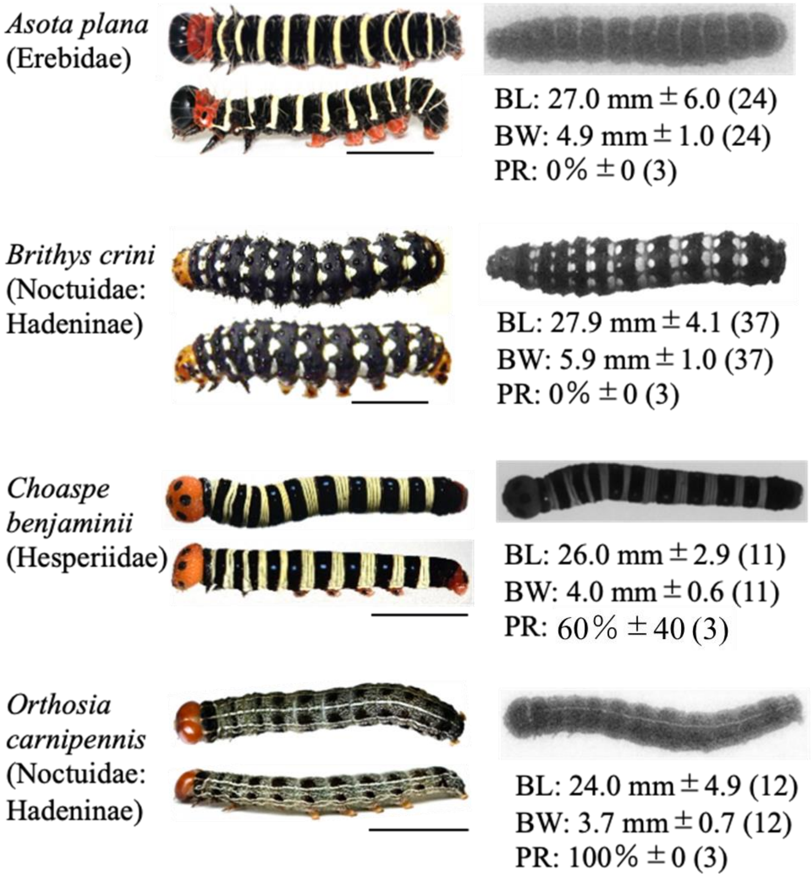

Figure 5-3. Similarity among the three species of larvae in the aposematic mimicry ring with red/black/white color banding patterns and the possible one of *Orthosia carnipennis*. The photographs were taken under visible (left) and ultraviolet (right) lights. The mean body length (BL), body width (BW), and predation rates by the lizards (PR) are shown with SD (*n*). A scale bar is 10 mm. For more explanation, see the text of Chapter 5 General discussion.

Figure 5-4. Adult and larval *Choaspe benjaminii* with similar color patterns to each other. The posterior reddish part with some small black spots of the hind wings and the abdomen banded by black and white colors seems the self-mimicry to larval body color pattern. In this case, a dark green wing with dark venations might be camouflaged in the background leaves. A scale bar is 10 mm.

6. Acknowledgments

I would like to Fumio Hayashi, Yasukazu Okada, and Katsuyuki Eguchi for their useful comments and help in all aspects of this study, Hirotaka Sugawara for help in DNA sequence and phylogenetic analysis, and Hidemori Yazaki, Hideshi Naka, and Masaaki Kimura for providing materials. I also thank Sakie Kodama, Honami Kawaguchi and Rira Ozawa for help in the field study, and Naomi Nakamura and Yoshihiro Suzuki for providing the pupal photograph of *C. benjaminii*. All experiments using the lizards were conducted under the permission by the Tokyo Metropolitan University Animal Ethics Committee (A31-7 in 2019, A2-25 in 2020, A3-3 in 2021, A4-14 in 2022). This work was partly supported by JST SPRING, Grant Number JPMJSP2156, to me. The field study at Tomakomai was partly supported by Ministry of Education, Culture, Sports, Science and Technology - Japan project on "Joint usage/Education Center" in Forest Research Station, Field Science Center for Northern Biosphere, Hokkaido University.

7. References

Anikin VV, Baryshnikova SV, Beljaev EA, Dubatolov VV, Efetov KA, Zolotuhin VV, Kovtunovich VN, Kozlov MV, Kononenko VS, Lvovsky AL, Nedoshivina SV, Ponomarenko MG, Sinev SY, Streltsov AN, Ustjuzhanin PY, Chistyakov YA, Yakovlev RV (2016) *Annotated catalogue of the insects of Russian Far East. Volume II. Lepidoptera*. Dalnauka, Vladivostok. pp 812. (In Russian)

Arenas LM, Stevens M (2017) Diversity in warning coloration is easily recognized by avian predators. *Journal of Evolutionary Biology* **30**, 1288–1302.

Aslam M, Nedvěd O, Sam K (2020) Attacks by predators on artificial cryptic and aposematic insect larvae. *Entomologia Experimentalis et Applicata* **168**, 184–190.

Banci KR, Eterovic A, Marinho PS, Marques OA (2020) Being a bright snake: Testing aposematism and mimicry in a neotropical forest. *Biotropica* **52**, 1229–1241.

Basu DN, Bhaumik V, Kunte K (2023) The tempo and mode of character evolution in the assembly of mimetic communities. *Proceedings of the National Academy of Sciences* **120**, e2203724120.

Bayarsaikhan U, Na SM, Bae YS (2016) Review of the subfamily Aganainae (Lepidoptera, Erebidae) from Cambodia. *Journal of Asia-Pacific Biodiversity* **9**, 219–229.

Blount JD, Rowland HM, Mitchell C, Speed MP, Ruxton GD, Endler JA, Brower LP (2023) The price of defence: toxins, visual signals and oxidative state in an aposematic butterfly. *Proceedings of the Royal Society B* **290**, 20222068.

Blum CR, Fitch WT, Bugnyar T (2020) Rapid learning and long-term memory for dangerous humans in ravens (*Corvus corax*). *Frontiers in Psychology* **11**, 581794.

Boos S, Meunier J, Pichon S, Kölliker M (2014) Maternal care provides antifungal protection to eggs in the European earwig. *Behavioral Ecology* **25**, 754–761

Boyden TC (1976) Butterfly palatability and mimicry: experiments with *Ameiva* lizards. *Evolution* **30**, 73–81.

Brodie Jr ED (1981) Phenological relationships of model and mimic salamanders. *Evolution* **35**, 988–994.

Caro T, Koneru M (2021) Towards an ecology of protective coloration. *Biological Reviews* **96**, 611–641.

Chen DM, Collins JS, Goldsmith TH (1984) The ultraviolet receptor of bird retinas. *Science* **225**, 337–340.

Chiba H (2009) A revision of the subfamily Coeliadinae (Lepidoptera: Hesperiidae). *Bulletin of the Kitakyushu Museum of Natural History & Human History, Series A (Natural History)* **7**, 1–102.

Cooper TL, Zabinski CL, Adams EJ, Berry SM, Pardo-Sanchez J, Reinhardt EM, Roberts KM, Watzek L, Brosnan SF, Hill RL, Weigel EG, Mendelson III JR (2020) Long-term memory of a complex foraging task in monitor lizards (Reptilia: Squamata: Varanidae). *Journal of Herpetology* **54**, 378–383.

Dixon MM, Jones PL, Ryan MJ, Carter GG, Page RA (2022) Long-term memory in frog-eating bats. *Current Biology* **32**, R557–R558.

Dossey AT (2011) Chemical defenses of insects: a rich resource for chemical biology in the tropics. In: Vivanco J, Weir T (eds) *Chemical Biology of the Tropics. Signaling and Communication in Plants Vol 8*, pp 27–57. Springer, Berlin, Heidelberg.

Edmunds M (1990) The evolution of cryptic coloration. In: Evans DL, Schmidt JO (eds) *Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*, pp 3–21. State University of New York Press, Albany.

Endler JA (1981) An overview of the relationships between mimicry and crypsis. *Biological Journal of the Linnean Society* **16**, 25–31.

Exnerová A, Ježová D, Štys P, Doktorovová L, Rojas B, Mappes J (2015) Different reactions to aposematic prey in 2 geographically distant populations of great tits. *Behavioral Ecology* **26**, 1361–1370.

Fleishman LJ, Loew ER, Leal M (1993) Ultraviolet vision in lizards. *Nature* **365**, 397.

Goiran C, Shine T, Shine R (2022) The banded colour patterns of sea snakes discourage attack by predatory fishes, enabling Batesian mimicry by harmless species. *Proceedings of the Royal Society B*, **289**, 20221759.

Grant JB (2007) Ontogenetic colour change and the evolution of aposematism: a case study in panic moth caterpillars. *Journal of Animal Ecology* **76**, 439–447.

Hasegawa M (1985) Effect of brooding on egg mortality in the lizard *Eumeces okadae* on Miyake-jima, Izu Islands, Japan. *Copeia* **1985**, 497–500.

Hasegawa M, Taniguchi Y (1994) Visual avoidance of a conspicuously colored carabid beetle *Dischissus mirandus* by the lizard *Eumeces okadae*. *Journal of Ethology* **12**, 9–14.

Hausmann A, Haszprunar G, Segerer AH, Speidel W, Behounek G, Hebert PD (2011) Now DNA-barcoded: the butterflies and larger moths of Germany. *Spixiana* **34**, 47–58.

Hayashi T, Kamiyama Y, Iida T, Iwata M (1971) Damage by the moth *Brithys crini* (Fabricius, 1775) and its control in Izu Peninsula. *Annual Report of the Kanto-Tosan Plant Protection Society* **18**, 100. (In Japanese)

Hernández-Agüero JA, Polo V, García M, Simón D, Ruiz-Tapiador I, Cayuela L (2020) Effects of prey colour on bird predation: an experiment in Mediterranean woodlands. *Animal Behaviour* **170**, 89–97.

Hlaváček A, Daňková K, Benda D, Bogusch P, Hadrava J (2022) Batesian-Müllerian mimicry ring around the Oriental hornet (*Vespa orientalis*). *Journal of Hymenoptera Research* **92**, 211–228.

Hotta M, Nakasone H, Watanabe K, Uematsu A, Miyazawa T, Matsubara H, Yoshida Y, Saitou A, Saitou A (2012) Survey for first arrival and first song of summer birds by members of eight citizen's groups concerned with birds in Nagano Prefecture, 2011. *Bulletin of Nagano Environmental Conservation Research Institute* **8**, 43–47. (In Japanese)

Huheey JE (1976) Studies in warning coloration and mimicry. VII. Evolutionary consequences of a Batesian-Müllerian spectrum: a model for Müllerian mimicry. *Evolution* **30**, 86–93.

Huheey JE (1980) The question of synchrony or "temporal sympatry" in mimicry. *Evolution* **34**, 614–616.

Igarashi S, Nagato M, Hosoi T, Matsui S (2015) The forest bird fauna of Fukushima City 2003–2013 and its ecological traits. *Japanese Journal of Ornithology* **64**, 147–160. (In Japanese)

Issiki S (1969) Early Stages of Japanese Moths in Colour Volume 2. Hoikusha Publishing Co. Ltd., Osaka.

Janzen DH, Hallwachs W, Burns JM (2010) A tropical horde of counterfeit predator eyes. *Proceedings of the National Academy of Science, USA* **107**, 11659–11665.

Johki Y, Hidaka T (1979) Function of the "warning coloration" in larvae of a diurnal moth, *Pryeria sinica* Moore (Lepidoptera: Zygaenidae). *Applied Entomology and*

Zoology **14**, 164–172.

Joron M, Mallet JL (1998) Diversity in mimicry: paradox or paradigm? *Trends in Ecology & Evolution* **13**, 461–466.

Kamata N, Sato H, Shimazu M (1997) Seasonal changes in the infection of pupae of the beech caterpillar, *Quadricalcarifera punctatella* (Motsch.) (Lep., Notodontidae), by *Cordyceps militaris* Link (Clavicipitales, Clavicipitaceae) in the soil of the Japanese beech forest. *Journal of Applied Entomology* **121**, 17–21.

Kashetsky T, Avgar T, Dukas R (2021) The cognitive ecology of animal movement: evidence from birds and mammals. *Frontiers in Ecology and Evolution* **9**, 724887.

Kawaji N, Kawaji K (2015) The first record of reddish-brown egg parasitism by the Oriental Cuckoo *Cuculus optatus* upon the Black-faced Bunting *Emberiza spodocephala* in western Hokkaido. *Journal of the Yamashina Institute for Ornithology* **46**, 119–126. (In Japanese)

Kawaji N, Kawaji K, Hirokawa J (1996) Breeding ecology of the Short-tailed Bush Warbler *Cettia squameiceps* in western Hokkaido. *Japanese Journal of Ornithology* **45**, 1–15.

Kishida Y (2011) *The Standard of Moths in Japan II*. Gakken Education Publishing, Tokyo, Japan. (In Japanese)

Knowlton GF (1938) Lizards in insect control. *Ohio Journal of Science* **38**, 235–238.

KonDo Y, Naka H, Tsuchida K (2011) Artificial diet for the Japanese nine-spotted moth *Amata fortunei fortunei* (Arctiidae: Syntominae). *Entomological Science* **14**, 387-391.

KonDo Y, Naka H, Tsuchida K (2012a) A wasp moth Syntomoides imaon invaded and established in Ishigaki island.*Yadoriga* **232**, 50-52. (In Japanese)

KonDo Y, Naka H, Tsuchida K (2012b) Pheromones and body coloration affect mate recognition in the Japanese nine-spotted moth *Amata fortunei* (Lepidoptera: Arctiidae). *Journal of Ethology* **30**, 301-308.

Konno Y, Matsuda K, Konishi K (2002) Hymenopterous parasitoids of *Cystidia couggaria* (Guenee) (Lepidoptera: Geometridae). Japanese Journal of Applied *Entomology and Zoology* **46**, 182–184.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology* *and Evolution* **35**, 1547‑1549.

Kunte K, Kizhakke AG, Nawge V (2021) Evolution of mimicry rings as a window into community dynamics. *Annual Review of Ecology, Evolution, and Systematics* **52**, 315–341.

Landová E, Hotová Svádová K, Fuchs R, Štys P, Exnerová A (2017) The effect of social learning on avoidance of aposematic prey in juvenile great tits (*Parus major*). *Animal Cognition* **20**, 855–866.

Lee W, Koh SH, Choi WI, Jung CS, Kim IK, Byun BK, Lee BW, Kim YS, Lim J, Kim S, Akimoto S, Lee S (2012) Barcoding forest insect pests in South Korea: constructing a basic endemic species dataset. *Journal of Asia-Pacific Entomology* **15**, 363‑368.

Liebhold AM, Higashiura Y, Unno A (1998) Forest type affects predation on gypsy moth (Lepidoptera: Lymantriidae) pupae in Japan. *Environmental Entomology* **27**, 858–862.

Liebhold A, Elkinton J, Williams D, Muzika RM (2000) What causes outbreaks of the gypsy moth in North America? *Population Ecology* **42**, 257–266.

Lindstedt C, Murphy L, Mappes J (2019) Antipredator strategies of pupae: how to avoid predation in an immobile life stage? *Philosophical Transactions of the Royal Society B* **374**, 20190069.

Machado G (2000) Maternal care in neotropical centipede *Otostigmus scabricauda* (Chilopoda: Scolopendromorpha). *Entomological News* **111**, 250–254.

Mallet J, Gilbert Jr LE (1995) Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. *Biological Journal of the Linnean Society* **55**, 159–180.

Mallet J, Mathieu J (1999) Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. *Annual Review of Ecology and Systematics* **30**, 201–233.

Mappes J, Marples N, Endler JA (2005) The complex business of survival by aposematism. *Trends in Ecology & Evolution* **20**, 598–603.

Mappes J, Kokko H, Ojala K, Lindström L (2014) Seasonal changes in predator community switch the direction of selection for prey defences. *Nature Communications* **5**, 5016.

Maran T (2017) *Mimicry and Meaning: Structure and Semiotics of Biological Mimicry*. Springer, Berlin.

Mattila AL, Jiggins CD, Saastamoinen M (2022) Condition dependence in biosynthesized chemical defenses of an aposematic and mimetic *Heliconius* butterfly. *Ecology and Evolution* **12**, e9041.

Mayekar HV, Kodandaramaiah U (2017) Pupal colour plasticity in a tropical butterfly, *Mycalesis mineus* (Nymphalidae: Satyrinae). *PloS One* **12**, e0171482.

McLain DK (1984) Coevolution: Müllerian mimicry between a plant bug (Miridae) and a seed bug (Lygaeidae) and the relationship between host plant choice and unpalatability. *Oikos* **43**, 143–148.

McLean DJ, Herberstein ME (2021) Mimicry in motion and morphology: do information limitation, trade-offs or compensation relax selection for mimetic accuracy? *Proceedings of the Royal Society B* **288**, 20210815.

Mizutani T, Kanouchi T (2020) *Field Guide to the Birds of Japan, 2nd Edition*. Bun-ichi Sôgô Press, Tokyo, Japan. pp 431. (In Japanese)

Morishita K (1994) *Brithys crini* (Fabricius) (Noctuidae) found in Zushi City, Kanagawa-ken, Japan. *Tyô to Ga* **45**, 113–114. (In Japanese)

Mount RH (1963) The natural history of the red-tailed skink, *Eumeces egregius* Baird. *American Midland Naturalist* **70**, 356–385.

Muñoz-Ramírez CP, Bitton PP, Doucet SM, Knowles LL (2016) Mimics here and there, but not everywhere: Müllerian mimicry in *Ceroglossus* ground beetles? *Biology Letters* **12**, 20160429.

Nair JJ, Andresen AL, Papenfus HB, Staerk D, van Staden J (2020) Identification of Amaryllidaceae alkaloids in larvae of the ‘lily borer’moth *Brithys crini*. *South African Journal of Botany* **131**, 351–359.

Naka H (2018) Female sex pheromones and mating behavior in diurnal moths: implications for conservation biology. In Tabata J (ed) *Chemical Ecology of Insects: Applications and Associations with Plants and Microbes*, pp 170–196. CRC Press, Florida, USA.

Nakamura M (2004) A morphological and phylogenetic study on the pupae of Geometridae (Insecta: Lepidoptera) from Japan. *Tinea* **18** (Supplement 1), 1–227.

Nakamura M (2007) Pupa of Japanese Arctiidae (Lepidoptera). Part I Syntominae, Nyctemerinae and Lithosiinae. *Transactions of the Lepidopterological Society of Japan* **58**, 25–39.

Nishida R (1994) Sequestration of plant secondary compounds by butterflies and moths. *Chemoecology* **5**, 127–138.

Nishida R, Fukami H, Iriye R, Kumazawa Z (1990) Accumulation of highly toxic ericaceous diterpenoids by the geometrid moth, *Arichanna gaschkevitchii*. *Agricultural and Biological Chemistry* **54**, 2347–2352.

Okagaki H (1956) Larva and pupa of *Amata germana* Felder. *Transactions of the Lepidopterological Society of Japan* **7**, 29. (In Japanese)

Pasteur G (1982) A classificatory review of mimicry systems. *Annual Review of Ecology and Systematics* **13**, 169–199.

Paul SC, Stevens M, Pell JK, Birkett MA, Blount JD (2018) Parental phenotype not predator cues influence egg warning coloration and defence levels. *Animal Behaviour* **140**, 177–186.

Pfennig DW, Harper GR, Brumo AF, Harcombe WR, Pfennig KS (2007) Population differences in predation on Batesian mimics in allopatry with their model: selection against mimics is strongest when they are common. *Behavioral Ecology and Sociobiology* **61**, 505–511.

Pfennig DW, Mullen SP (2010) Mimics without models: causes and consequences of allopatry in Batesian mimicry complexes. *Proceedings of the Royal Society B* **277**, 2577–2585.

Pinheiro CE (2007) Asynchrony in daily activity patterns of butterfly models and mimics. *Journal of Tropical Ecology* **23**, 119–123.

Poulton EB (1890) *The Colours of Animals:Ttheir Meaning and use, Especially Considered in the Case of Insects*. 2nd edition. Kegan Paul, Trench Trübner, & Co., Ltd., Lomdon.

Poulton EB (1908) *Essays on Evolution 1889–1907*. Claredon Press, Oxford, pp 479.

Prusa LA, Hill RI (2021) Umbrella of protection: spatial and temporal dynamics in a temperate butterfly Batesian mimicry system. *Biological Journal of the Linnean Society* **133**, 685–703.

Przybylowicz L, Morek W, Byun B-K (2016) Taxonomic review of the tribe Syntomini (Lepidoptera: Erebidae: Arctiinae) in Korea. *Journal of Asia-Pacific Entomology*

19, 785–791.

Rammert U (1992) The reaction of birds to the larval defensive system of *Zygaena trifolii* (Esper 1783) (Lepidoptera Zygaenidae). In: Dutreix C, Naumann CM, Tremewan WG (eds) *4th Symposium on Zygaenidae – Recent advances in burnet moth research (Lepidoptera Zygaenidae),* pp 38–52. Koeltz Scientific Books, Koenigstein.

Rettenmeyer CW (1970) Insect mimicry. *Annual Review of Entomology* **15**, 43–74.

Robinson ML, Weber MG, Freedman MG, Jordan E, Ashlock SR, Yonenaga J, Strauss SY (2023) Macroevolution of protective coloration across caterpillars reflects relationships with host plants. *Proceedings of the Royal Society B*, **290**, 20222293.

Roslin T, Hardwick B, Novotny V, Petry WK, Andrew NR, Asmus A, Barrio IC, Basset Y, Boesing AL, Bonebrake1 TC, Cameron EK, Dáttilo W, Donoso DA, Drozd P, Gray CL, Hik DS, Hill SJ, Hopkins T, Huang S, Koane B, Laird-Hopkins B, Laukkanen L, Lewis OT, Milne S, Mwesige I, Nakamura A, Nell CS, Nichols E, Prokurat A, Sam K, Schmidt NM, Slade A, Slade V, Suchanková A, Teder T, van Nouhuys S, Vandvik V, Weissflog A, Zhukovich V, Slade EM (2017) Higher predation risk for insect prey at low latitudes and elevations. *Science* **356**, 742–744.

Rothschild M (1963) Is the buff ermine (*Spilosoma lutea* (Huf.)) a mimic of the white ermine (*Spilosoma lvbricipeda* (L.))? *Proceedings of the Royal Entomological Society of London. Series A, General Entomology* **38**, 159–164.

Rothschild M, Euw JV, Reichstein T (1973) Cardiac glycosides (heart poisons) in the polka-dot moth *Syntomeida epilais* Walk. (Ctenuchidae: Lep.) with some observations on the toxic qualities of *Amata* (= *Syntomis*) *phegea* (L.). *Proceedings of the Royal Society of London. Series B. Biological Sciences* **183**, 227–247.

Rothschild M, Moore BP, Brown WV (1984) Pyrazines as warning odour components in the Monarch butterfly, *Danaus plexippus*, and in moths of the genera *Zygaena* and *Amata* (Lepidoptera). *Biological Journal of the Linnean Society* **23**, 375–380.

Ruxton GD, Allen WL, Sherratt TN, Speed MP (2018) *Avoiding attack: the evolutionary ecology of crypsis, aposematism, and mimicry. Second edition*. Oxford University Press, Oxford, pp 278.

Sagami Butterfly Investigators' Society (2016) *Searching of Butterfly Larvae in and around Kanagawa Prefecture*. Sasshi-Insatsu-Sha Co., Ltd., Chiba, Japan. pp 296. (In Japanese)

Saliveros AM, Blyth EC, Easter C, Hume GV, McAusland F, Hoppitt W, Boogert NJ (2020) Learning strategies and long-term memory in Asian short-clawed otters (*Aonyx cinereus*). *Royal Society Open Science* **7**, 201215.

Sato R, Nakajima H (1975) Larvae of Japanese *Cystidia* and *Planociampa* (Lepidoptera: Geometriidae; Ennominae). *Tyô to Ga* **26**, 14–28. (In Japanese)

Sexton OJ (1960) Experimental studies of artificial Batesian mimics. *Behaviour* **15**, 244–252.

Sherratt TN (2008) The evolution of Müllerian mimicry. *Naturwissenschaften* **95**, 681–695.

Shirôzu T (2006) *The Standard of Butterflies in Japan*. Gakken Education Press, Tokyo, Japan, pp 336. (In Japanese)

Sourakov A (2016) On adult-caterpillar mimicry: Cases from the moth world. *News of the Lepidopterists' Society* **58**, 97–99.

Sugiura N (1978) Further analysts of the data by Akaike' s information criterion and the finite corrections. *Communications in Statistics, Theory and Methods* **7**, 13–26.

Sugiura S (2020) Predators as drivers of insect defenses. *Entomological Science* **23**, 316–337.

Suzuki H, Ajioka Y, Takahashi K, Kurosawa T (2010) The breeding biology and habitat of the Black Paradise Flycatcher *Terpsiphone atrocaudata* at Kotorinomori, Fukushima Prefecture. *Japanese Journal of Ornithology* **59**, 168–173. (In Japanese)

Szabo B, Noble DW, Whiting MJ (2021) Learning in non-avian reptiles 40 years on: advances and promising new directions. *Biological Reviews* **96**, 331–356.

Takagawa S, Ueta M, Amano T, Okahisa Y, Kamioki M, Takagi K, Takahashi M, Hayama S, Hirano T, Mikami OK, Mori S, Morimoto G, Yamaura Y (2011) JAVIAN Database: a species-level database of life history, ecology and morphology of bird species in Japan. *Bird Research* **7**, R9–R12. (In Japanese)

Tamada K (2013) Breeding records of Asian Stubtail and Narcissus Flycatcher in Nopporo Forest Park 2012. *Report of Institute of Environmental Sciences*, **3**, 53–

56. (In Japanese)

Tanhuanpää M, Ruohomäki K, Kaitaniemi P, Klemola T (1999) Different impact of pupal predation on populations of *Epirrita autumnata* (Lepidoptera; Geometridae) within and outside the outbreak range. *Journal of Animal Ecology* **68**, 562–570.

Thery M, Gomez D (2010) Insect colours and visual appearance in the eyes of their predators. *Advances in Insect Physiology* **38**, 267–353.

Van Meyel S, Devers S, Meunier J (2019) Love them all: mothers provide care to foreign eggs in the European earwig *Forficula auricularia*. *Behavioral Ecology* **30**, 756–762.

Waldbauer GP (1988) Asynchrony between Batesian mimics and their models. *American Naturalist* **131**, S103–S121.

Waldbauer GP, Sternburg JG (1967) Differential predation on cocoons of *Hyalophora cecropia* (Lepidoptera: Saturniidae) spun on shrubs and trees. *Ecology* **48**, 312–315.

Waldbauer GP, Sternburg JG, Maier CT (1977) Phenological relationships of wasps, bumblebees, their mimics, and insectivorous birds in an Illinois sand area. *Ecology* **58**, 583–591.

Wallace AR (1865) I. On the phenomena of variation and geographical distribution as illustrated by the Papilionidae of the Malayan Region. *Transactions of the Linnean Society of London* **25**, 1–71.

Wallace AR (1867) Mimicry and other protective resemblances among animals. *Westminster and Foreign Quarterly Review* **32**, 1–43.

Wickler W (1968) *Mimicry in Plants and Animals*. (R. D. Martin, transl.) McGraw-Hill, New York.

Wiklund C, Sillen-Tullberg B (1985) Why distasteful butterflies have aposematic larvae and adults, but cryptic pupae: evidence from predation experiments on the monarch and the European swallowtail. *Evolution* **39**, 1155–1158.

Willadsen PC (2022) Aculeate hymenopterans as aposematic and mimetic models. *Frontiers in Ecology and Evolution* **10**, 827319.

Willmott KR, Elias M, Sourakov A (2011) Two possible caterpillar mimicry complexes in Neotropical danaine butterflies (Lepidoptera: Nymphalidae). *Annals of the Entomological Society of America* **104**, 1108–1118.

Wilson JS, Williams KA, Forister ML, von Dohlen CD, Pitts JP (2012) Repeated evolution in overlapping mimicry rings among North American velvet ants. *Nature Communications* **3**, 1272.

Yagi S (2020) Observation record of breeding Ryukyu Ashy Minivet *Pericrocotus divaricatus tegimae* in Hadano City, Kanagawa Prefecture. *BINOS*, **27**, 1–10. (In Japanese)

Yamakawa R, Takubo Y, Ohbayashi K, Naka H, Ando T (2012) Female sex pheromone of *Cystidia couaggaria couaggaria* (Lepidoptera: Geometridae): identification and field attraction. *Bioscience, Biotechnology, and Biochemistry* **76**, 1303–1307.

Yamamoto S, Sota T (2009) Incipient allochronic speciation by climatic disruption of the reproductive period. *Proceedings of the Royal Society B* **276**, 2711–2719.

Yazaki H, Kishimura M, Tsubuki M, Hayashi F (2019) Müllerian mimicry between cohabiting final-instar larval *Pryeria sinica* Moore, 1877 (Lepidoptera: Zygaenidae) and pupal *Ivela auripes* (Butler, 1877) (Lepidoptera: Lymantriidae). *The Pan-Pacific Entomologist* **95**, 83–91.

Yumnam T, Banerjee B, Kodandaramaiah U (2021) Pupal colour plasticity in the butterfly *Catopsilia pomona* (Lepidoptera: Pieridae). *Biological Journal of the Linnean Society* **134**, 331–341.

Zheng X, Zhang R, Yue B, Wu Y, Yang N, Zhou C (2022) Enhanced resolution of evolution and phylogeny of the moths inferred from nineteen mitochondrial genomes. *Genes* **13**, 1634.

Zvereva EL, Kozlov MV (2021) Seasonal variations in bird selection pressure on prey colouration. *Oecologia* **196**, 1017–1026.

8. Appendices

8-1. Publications

Tsubuki, M. and Hayashi, F. (2023) Pupal warning coloration of three species of *Cystidia* (Lepidoptera: Geometridae: Ennominae) in relation to their pupation sites. Insects, 14: 38.

8-2. Titles and summary in Japanese

学位論文要旨（博士（理学））

津吹 真

論文題名：Two types of mimicry rings at the immature stages of Lepidoptera with black spots on the yellow body and the red/black/white marking

（邦題）：鱗翅目の幼虫および蛹に見られる２つの擬態環：黄色地に黒すじ模様の警告色種群と赤/黒/白模様の警告色種群（英文）

多くの昆虫では, 視覚で餌を探す捕食者から身を守るため, 隠蔽色（カモフラージュ）を発達させている．一方，昆虫が捕食者に対する毒物や忌避物質を獲得すると，それに対する捕食者の学習を促進させる警告色（目立つ色彩）が有利となる．2 種間でその収斂進化が起こると警告色擬態となる. さらに多くの系統群から構成されるとそれは擬態環（mimicry ring）となる．昆虫の擬態の研究はこれまで主に成虫の色彩について研究されてきたが，本研究では，鱗翅目の幼虫や蛹にも２つの擬態環があることを発見し，その構成種と擬態としての機能を明らかにした.

第一の擬態環は, 幼虫および蛹の色彩が黄色地に黒すじ模様となっている種群である. まず，カノコガ類 4 種において，地上の枝や葉の間のよく目立つ場所で蛹化する 2 種の蛹がこの色彩をもち, 石や落葉の下などの地表で蛹化する 2 種の蛹は一様に暗色であった．蛾の捕食者の 1 種であるヒガシニホントカゲ（以下トカゲ）を用いてこれら 4 種の

蛹の毒性試験を行った結果，地上蛹化の2種と地表蛹化の1種は忌避されたが，地表蛹化の別の1種は捕食された．つまり，無毒で暗色の蛹（地表）→有毒で暗色の蛹（地表）→有毒で警告色の蛹（地上で目立つ）の順で蛹化場所の違いが警告色への変化を生じさせたのではないかと考えられた．こうした蛹化場所と警告色の関係はトンボエダシャク類3種においても認められた．これら3種はいずれもトカゲに忌避され，黄色地に黒すじ模様をもつ（ただし，葉を糸で紡いで袋状にし，その中で蛹化する種では，この色彩が半減していた）．一方，これらに近縁のエダシャク類では，蛹は暗色で地表において蛹化する．黄色地に黒すじ模様という警告色は，カノコガ類とトンボエダシャク類だけでなく，他の蛾類や蝶類にも認められた（ミノウスバ幼虫，ウスバツバメガ幼虫，キアシドクガ蛹，ホシシャク蛹，エゾシロチョウ蛹）．これらの種の分布・生活史・形態・捕食者への忌避効果を比較した結果，互いの分布が重複しており，同時期に幼虫および蛹が見られ，大きさも大きく違わず，可視光および紫外光下でも斑紋に差はなく，いずれも地上のよく目立つところで見られた．以上の結果から，鱗翅目の幼虫および蛹に共通のこの警告色は複数の系統からなる擬態環を形成していると考えられた．トカゲを用いた忌避性の判定から，この擬態環の中で，エゾシロチョウの蛹はベイツ型擬態（無毒な種が有毒な種に擬態），他の種はミュラー型擬態（有毒な種どうしが擬態）の関係をもつことが明らかとなった．

第二の擬態環は，幼虫の色彩が赤，黒，白の縞模様となっている種群である．そのうち，ホシヒトリモドキ，ハマオモトヨトウ，アカバキリガ，アオバセセリの幼虫を用いてその擬態関係を調査した．これら4種の幼虫は体の大きさと可視光および紫外光下での斑紋が酷似していたが，トカゲを用いた捕食実験では，ホシヒトリモドキとハマオモトヨトウの幼虫は捕食されず忌避され，アカバキリガとアオバセセリの幼虫は捕食された．トカゲの学習実験を行った結果，これらの幼虫の斑紋を識別することなく忌避した．さらに，4種の分布の組み合わせや鳥類群集が異なる日本の3地点において，警告色を模した幼虫モデルに対する野外での捕食率を比較した結果，鳥類の捕食忌避の経験の有無がこの警告色の学習に関わっており，その結果として，ホシヒトリモドキとハマオモトヨトウの幼虫がミュラー型擬態，アカバキリガとアオバセセリの幼虫がベイツ型擬態として組み込まれているこが明らかとなった．