EUROPEAN JOURNAL OF ENTOMOLOGY

ISSN (online): 1802-8829
http://www.eje.cz

Eur. J. Entomol. **121**: 296–302, 2024
doi: 10.14411/eje.2024.031

ORIGINAL ARTICLE

Experimental and comparative analysis of masquerade in flea beetles (Coleoptera: Chrysomelidae)

Tadashi SHINOHARA[1,2] and Alexander S. KONSTANTINOV[3]

[1] Graduate School of Human Development & Environment, Kobe University, 3–11 Tsurukabuto, Kobe 657-8501, Japan; e-mail: shinohara014@gmail.com

[2] Department of Biological Science, Faculty of Science, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

[3] Systematic Entomology Laboratory, Agricultural Research Service, Unites States Department of Agriculture, c/o National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA; e-mail: alex.konstantinov@usda.gov

Key words. Alticini, body colour, camouflage, feeding damage, predator-prey interaction

Abstract. Prey animals employ masquerade to avoid recognition by visual predators by developing a resemblance to inedible objects in their environment. Phytophagous flea beetles seem to resemble models of their own manufacture. While feeding they cause light- or dark-coloured hole-like damage on the leaves of their host plants that resembles the beetle's body in colour and size. Resemblance to the model and the frequency of the model can influence the efficiency of masquerade. To examine masquerade efficiency in light- and dark-coloured beetles, we evaluated their survival benefits from resembling feeding damage in the field. This was done by using two species of beetle of different colour as prey and a jumping spider as the predator. Dark-coloured species were more likely to avoid predation when they were placed on a background with damage similar in colour to their body, whereas increased survival was not recorded for light-coloured species. The extent of the feeding damage of 34 light- and dark-coloured species of beetle was compared. Variation in the extent of the damage was associated more with host plant taxa than beetle body colour. These results indicate that the efficiency of masquerade can vary among beetle species and/or phenotypes.

INTRODUCTION

Animals use various forms of camouflage to avoid detection or recognition by visual predators (Merilaita et al., 2017; Ruxton et al., 2018; Cuthill, 2019). Masquerade is a form of camouflage in which the prey mimics innocuous and inedible objects in the environment (e.g., twigs, leaves, stones or bird droppings) and their predators do not identify them as prey (Skelhorn et al., 2010a; 2015). The similarity of prey to these models (e.g., in colour, size, and shape) is an important factor in the efficiency of masquerade (Skelhorn et al., 2010b; Skelhorn & Ruxton, 2013). In addition, the frequency of the occurrence of model and mimic also influences predation risk for mimics (Skelhorn et al., 2011; Ren et al., 2018). Therefore, the efficiency of masquerade can be improved in at least two ways, by enhancing resemblance to models and/or increasing relative frequency of the model. Feeding damage to plant leaves is a common feature in the environment of animals living on leaf surfaces. Phytophagous insects may create or modify the appearance and frequency of feeding damage, so they are an ideal model system for examining the above-mentioned processes determining the efficiency of masquerade.

Flea beetles (Coleoptera: Chrysomelidae: Galerucinae: Alticini) (600 genera and 9900 species) are the most species-rich group within the Chrysomelidae (Konstantinov, 2016). Adult flea beetles are vulnerable to various visual predators including jumping spiders (Freed, 1984; Riechert & Bishop, 1990), frogs (Turner, 1959; Freed, 1982), and birds (Lack & Owen, 1955; Owen, 1955; Campbell, 1998; Whitehead, 1991). The feeding damage of flea beetles looks like holes, scrapes or trenches in the leaf surface. It can be classified into two ways: light-coloured damage, which are scrapes in the upper epidermis and only a part of the mesophyll and are a pale colour when dry; and dark-coloured damage, which are holes in a leaf and appear dark because of the dark background. A resemblance between the flea beetles and their feeding damage was recently reported: light-coloured and dark-coloured beetles tend to make light-coloured and dark-coloured damage, respectively (Konstantinov et al., 2018, Folgar-Cameán et al., 2021). The similarity between the beetle's body and feeding damage is interpreted as an example of masquerade in which beetles mimic the damage they cause to leaves (Konstantinov et al., 2018).

Covariation in the appearance of the beetles and their feeding damage may result from two masquerade-dependent processes (Folgar-Cameán et al., 2021). In most examples of masquerade, there are morphological and/or physi-

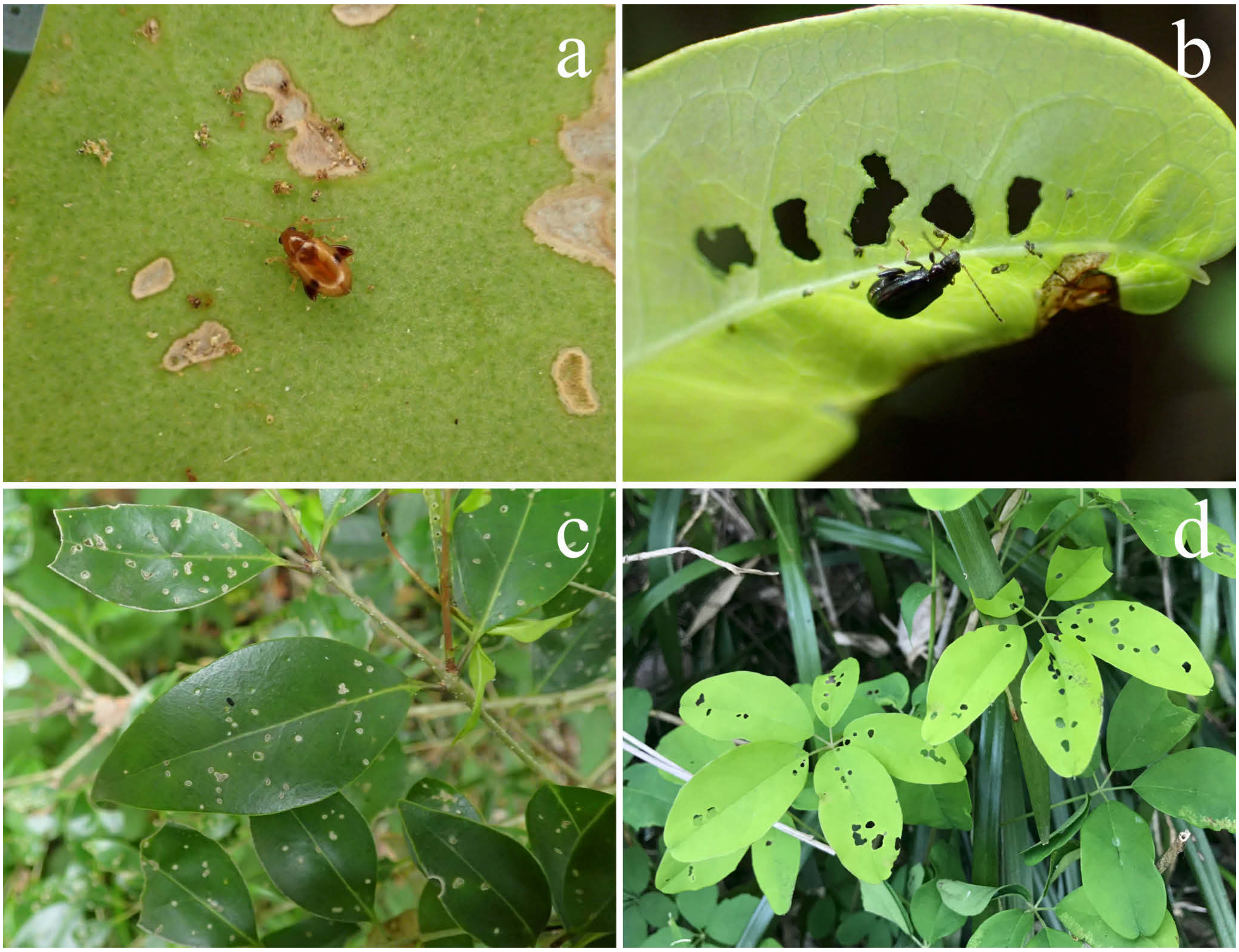

Fig. 1. Body colour and feeding patterns of adult flea beetles. Light- or dark-coloured spots on leaves are the feeding damage caused by flea beetles. (a) Light-coloured species *Longitarsus bimaculatus* on the lower surface of a leaf of its host plant. (b) Dark-coloured species *Aphthonaltica angustata* on the lower surface of a leaf of its host plant. (c) *Ligustrum japonicum* leaves (upper surface) with light-coloured feeding damage created by *L. bimaculatus*. (d) *Akebia quinata* leaves (upper surface) with dark-coloured feeding damage caused by *A. angustata*.

ological constraints exerting a selective pressure for the beetle to damage leaves in a specific way (Folgar-Cameán et al., 2021). In addition, the tissue characteristics of the leaves of their host plants may constrain the appearance of feeding damage, exerting a selection favouring the survival of beetles that are of similar colour and size to the feeding damage (Folgar-Cameán et al., 2021). Evolutionary interaction between phytophagous beetles and their host plants may drive and/or constrain the evolution of body colour and feeding behaviour of the beetles, generating variation in the efficiency of masquerade in different species and/or phenotypes of beetles. For example, light- and dark-coloured species of beetles may differ in the efficiency of masquerade. Species with relatively low efficiency of masquerade due to low similarity with their feeding damage may offset this by increasing the frequency of the damage they do to host plant leaves. In summary, masquerade in flea beetles is hypothesized to (1) increase the survival of beetles on leaves with similar feeding damage, and possibly (2) cause a quantitative difference in the frequency of feeding damage between beetle phenotypes.

In this study we test two hypotheses. First, we test if the colour resemblance between the beetle and feeding damage improves the survival of the beetle, based on a predation experiment using an artificial leaf background and a jumping spider as a predator. Second, we examined if the frequency of feeding damage differs between light- and dark-coloured beetles, by a comparative analysis of the feeding damage of 34 species. This is followed by a discussion on the variation in the efficiency of masquerade in the different beetle species and phenotypes.

MATERIALS AND METHODS

Choice of prey and predators for predation experiment

Adults of two species of flea beetles, *Longitarsus bimaculatus* (Baly) and *Aphthonaltica angustata* (Baly), were used in the predation experiments as representatives of light- and dark-coloured prey, respectively. *Longitarsus bimaculatus* is 2.0 ± 0.1 mm (mean ± SD, N = 10) in length (from the tip of head to the end of elytra), with the dorsal surface of the head, pronotum, and elytra light brown with a pair of small dark spots on the elytra in some individuals (Fig. 1a). In contrast, the dorsal surface of *A. angustata* is dark blue and its length is 2.5 ± 0.2 mm (N = 10) (Fig.

1b). Feeding damage of *L. bimaculatus* tends to consist of light-coloured scrapes on leaves of *Ligustrum* spp. (Oleaceae) (Fig. 1c), whereas *A. angustata* almost always creates dark-coloured feeding damage on leaves of its host plant, *Akebia* spp. (Lardizabalaceae) (Fig. 1d). The feeding damage in this case consists of relatively small, isolated holes, but with some holes merging with one other and occasionally including a larger area of leaf, resulting in a feeding damage similar in size to that of the beetle. Both species were observed in the field on both the upper and lower surface of leaves.

Jumping spiders of the family Salticidae are visual predators that feed on flea beetles (Freed, 1984; Riechert & Bishop, 1990). They can perceive a wide range of colours. The anterior median eye of *Menemerus confusus* has four types of visual cells with different maximum sensitivity to wavelengths ranging from 360 to 580 nm (Yamashita & Tateda, 1976), and *Hasarius adansoni* is able to discriminate between blue, green, yellow and red papers mainly by their hue (Nakamura & Yamashita, 2000). Adult females and males of the jumping spider *Evarcha albaria*, a very common species in Japan (about 7 mm in body length; Suguro, 2017), was used as the predator in our experiments. *Evarcha albaria* inhabits almost the same strata of vegetation as the flea beetles. Jumping spiders can attack immobile prey (Jackson & Tarsitano, 1993). In the laboratory *E. albaria* attacked living and dead adults of *L. bimaculatus* and *A. angustata*, but did not consume them if they were dead.

Flea beetles and jumping spiders were collected from May to June 2021 in Nishinomiya and Kobe, Hyogo Prefecture, Japan. Collected beetles were frozen at –20°C until the experiment to keep them fresh. Collected spiders were kept in an incubator at 20°C with adults of small Heteroptera as food, and starved for at least two days before the experiment.

Preparation of the visual background

To standardize the visual background in the predation experiment, the visual patterns of leaf surfaces were artificially reproduced based on the features of real leaves and feeding damage. Feeding-damaged leaves of the host plants *Ligustrum japonicum* and *Akebia quinata* were collected from the same localities as the beetles and scanned, with their colour measured using Natsumushi software (Tanahashi & Fukatsu, 2018). RGB values (i.e., red, green, and blue) for leaf colour were obtained from 20 scanned leaf images for each species of plant, 10 of which were of the upper surface and 10 of the lower surface. Mean RGB values based on 40 leaf images, excluding damaged areas (R = 94, G = 111, B = 66), were used for the common background leaf colour in order to exclude the effect of differences in leaf colour on beetle survival. The colour of light-coloured damage was measured in the same way using 20 scanned images of *L. japonicum* leaves, 10 of which were of the upper surface and 10 of the lower surface (mean RGB values, R = 155, G = 137, B = 108). Although the colour of dark-coloured damage is dependent on environmental factors such as exposure to the sun and the colour of ground surface, most of them appear black (Fig. 1b, d). Therefore, the colour of dark-coloured damage was assumed to be black (R = 0, G = 0, B = 0) as seen in the field. To determine the shape of feeding damage four samples of each type were selected and the outlines recorded in the form of digital images. The size of feeding damage was adjusted to 1.5–2.5 mm, which is close to or slightly smaller than the body size of the beetles recorded in the field. Percentage of the area damaged by feeding was calculated using the mobile application LeafByte (Getman-Pickering et al., 2019) (2.6 ± 2.1% for light-coloured damage on leaves of *L. japonicum*, N = 10; 3.1 ± 1.6% for dark-coloured damage on leaves of *A. quinata*, N = 10). Based on these measurements, outlines of feeding damage were coloured light or dark and scattered arbitrarily on about 3% of the area of a background of the common leaf colour. Some of the outlines of feeding damage partially overlapped as observed on leaves in the field. Background images were printed on high-quality mat paper using a printer (Canon; PIXUS MG5130).

Predation experiment

The survival of beetles on different backgrounds were recorded. Four experimental treatments, consisting of two species of beetle (light- and dark-coloured species) and two types of background (light- and dark-coloured damage on leaves of the same colour) were used, in a total of 162 trials (light-coloured beetle on light-coloured damage, N = 41; light-coloured beetle on dark-coloured damage, N = 38; dark-coloured beetle on light-coloured damage, N = 41; dark-coloured beetle on dark-coloured damage, N = 42). Thirty-two jumping spiders were used as predators, and 31 and 30 individuals of the light- and dark-coloured frozen prey, respectively, were used in these experiments. The combination of spider and beetle was fixed for each individual beetle, used up to 4 times, with an interval of at least 24 h between trials. Spiders were arbitrarily assigned to different treatments. Repeated use of a combination of beetle and spider was taken into account in the analysis (see below).

Petri dishes (85 mm in diameter and 20 mm in height) with in each case a particular background paper on the bottom were used as the experimental arenas. The frozen beetles were thawed immediately before the experiment, and one beetle was placed on the background paper with its dorsal surface facing up, not overlapping with the damage areas, together with one spider under laboratory conditions (20–25°C and fluorescent light). Petri dishes were placed within a cylinder of white paper (90 mm in diameter and 25 mm in height) to reduce the effect of external visual stimuli. Because beetles were dead, spiders could not recognize a beetle as prey by taking a cue from its motion. Spiders could walk freely on the bottom (i.e., at the same level as the prey), sides and top of the arena, and therefore they could visually inspect prey from various angles. This experiment was carried out during daytime and was recorded for 60 min (starting within 1 min of the introduction of a spider) using a digital video recorder (DMX-CA100; SANYO). Since the spider started walking immediately after being introduced, the spider's placement in the arena at the start of the experiment was random. The time from the start of the experiment to the first attack by the spider was recorded. Position of the spider in the arena just before attacks was also recorded in order to determine whether the angle at which a spider views a beetle affects its survival. An attack was defined as the spider jumping and holding the beetle.

Our hypothesis that the efficiency of masquerade in terms of the frequency of attacks and the time to the attack by a spider is lower and longer, respectively, for beetles on a leaf with feeding damage similar to their body colour was tested using a generalized linear mixed model (GLMM) with a binomial distribution, which was fitted for attack frequencies using the *glmer* function in the *lme4* package (Bates et al., 2015) in R 3.6.3 (R Core Team, 2020). Prey survival may also be influenced by the sex of the spider because in some species of jumping spiders prey size preference differs between sexes (Givens, 1978; Li & Jackson, 1996; Li et al., 1997). Therefore, type of background (i.e., light- or dark-coloured damage) and spider sex were used as independent variables, and prey survival (i.e., attacked or not) was the dependent variable. The ID of the combination of a predator and a prey was included as a random effect to correct for the repeated use of a particular combination. In addition, for the time to attack, the survival analysis was done using the *survreg* function in the R package *survival* (Therneau, 2015). Background type and spider sex

Table 1. Flea beetles and host plants used to compare the frequency of the damage caused by different types of feeding. * Beetle species whose colour does not closely resemble the colour of their feeding damage. † Beetle species that were included in the small dataset for model 2 used in the comparative analysis.

Beetle colour	Beetle species	Region	Host plant species	No. of leaves examined
Light	*Aphthona bombayensis*	India	*Phyllanthus* sp. (Phyllanthaceae)	2
	*Chanealtica cuevas**†	Bolivia	*Tecoma stans* (Bignoniaceae)	3
	Chilocoristes sp.	India	*Smilax* sp. (Smilacaceae)	1
	Epitrix sp.1	Jamaica	not known	1
	Gioia sp.1	Panama	not known	2
	Gioia sp.2	Panama	not known	1
	Haemaltica sp.	India	*Memecylon* sp. (Melastomataceae)	1
	Heikertingerella sp.1†	Dominican Republic	*Tecoma stans* (Bignoniaceae)	1
	Hyphasis sp.†	India	*Thunbergia* sp. (Acanthaceae)	1
	Letzuella ornata†	China	Scrophulariaceae gen. et sp.	1
	Letzuella sp.†	China	*Buddleja* sp. (Scrophulariaceae)	3
	Longitarsus bimaculatus†	Japan	*Ligustrum japonicum* (Oleaceae)	22
	Longitarsus nr. *pratensis*	Turkey	*Plantago* sp. (Plantaginaceae)	1
	Nesaecrepida rufomarginata	Jamaica	*Psidium guajava* (Myrtaceae)	1
	Nesaecrepida sp.	Puerto Rico	*Prosobis* sp. or *Vachellia* sp. (Fabaceae)	3
	Philopona decemaculata†	India	*Chionanthus* sp. (Oleaceae)	1
	Yingaresca varicornis†	Puerto Rico	*Cordia* poss. *sulcata* (Boraginaceae)	2
Dark	*Acallepitrix* sp.1*	Panama	not known	1
	Acallepitrix sp.2	Panama	not known	3
	Altica sp.1	USA	*Epilobium* sp. (Onagraceae)	2
	Altica sp.2	Turkey	*Rumex scutatus* (Polygonaceae)	4
	Aphthonaltica angustata	Japan	*Akebia quinata* (Lardizabalaceae)	30
	Batophila olexai	Turkey	*Alchemilla* sp. (Rosaceae)	1
	Diphaulaca sp.†	Bolivia	Urticaceae gen. et sp.	3
	Epitrix sp.2	Panama	not known	1
	Heikertingerella sp.2*	Panama	not known	1
	Heikertingerella sp.3	Panama	not known	1
	Hemiglyptus basalis†	USA	*Hydrophyllum* sp. (Boraginaceae)	1
	Hemipyxis sp.†	India	*Andrographus tropurpurea* (Acanthaceae)	1
	Longitarsus audisioi†	Turkey	*Symphytum asperum* (Boraginaceae)	2
	Longitarsus sp.†	USA	*Urtica* sp. (Urticaceae)	1
	*Normaltica obrieni**	Puerto Rico	poss. *Thelypterys* (Thelypteridaceae)	1
	Sphaeroderma sp.†	China	*Urtica* sp. (Urticaceae)	2
	Xuthea yunnanica†	China	*Girardinia* sp. (Urticaceae)	1

were independent variables, and time to attack was the dependent variable. The ID of the combination of predator and prey was included as a random effect. Log-normal distribution was chosen based on the Akaike information criterion (AIC).

Comparative analysis of the extent of feeding damage recorded in the field

To assess the variation in the extent of feeding damage caused by species of beetles with differently coloured bodies, the feeding damage on leaves of the host plant of 34 species of beetle were compared (Table 1). Beetles were identified using standard references and the collection housed at the National Museum of Natural History, Smithsonian Institution. Host plant leaves from the same locality for each beetle species were used. Except for scanned images of *Ligustrum japonicum* (30 leaves) and *Akebia quinata* (22 leaves) used in the predation experiment, images of damaged foliage on which beetles were present (one to four leaves of mature individuals for each plant species) were taken in the field from above at the time of observation. This treatment did not include leaves without feeding damage. In total, images of 103 leaves (47 for light-coloured beetles and 56 for dark-coloured beetles) were used. Total leaf area including damaged areas was calculated using ImageJ version 1.53a (National Institute of Health, available at https://imagej.nih.gov/ij). Body length of a beetle was used as a scale in the field images. If a whole leaf did not fit in an image, the number of holes and leaf area was measured within the image.

To determine whether the extent of feeding damage differed between beetle species with different body colours, GLMM (model 1) with a Poisson distribution was fitted to the results for feeding damage using the *glmer* function in the R package *lme4* (Bates et al., 2015). Beetle body colour (i.e., light or dark) and the number of feeding damages on a leaf were used as independent and dependent variables, respectively. Log-transformed leaf area was included as an offset term to control for the variation in leaf size. To address phylogenetic constraints, beetle species and genera were used as nested random effects because a phylogeny including all beetle species used in this study was unavailable. In addition, the possibility that the property of leaves may differ among host plant taxa and influence the evolution of masquerade was determined. To do this, host plant family was added to model 1 as an independent variable (model 2). To improve the fit of the model, singleton plant families corresponding to only one species of beetle and unknown host plants were excluded, resulting in a relatively small dataset for model 2 (45 leaves from 6 plant families: 34 for light-coloured beetles and 11 for dark-coloured beetles, as shown in Table 1).

RESULTS

Predation experiment

Contrary to the prediction, light-coloured beetles were attacked by spiders at similar rates on the different backgrounds: 31.7% (N = 41) on the background with light-

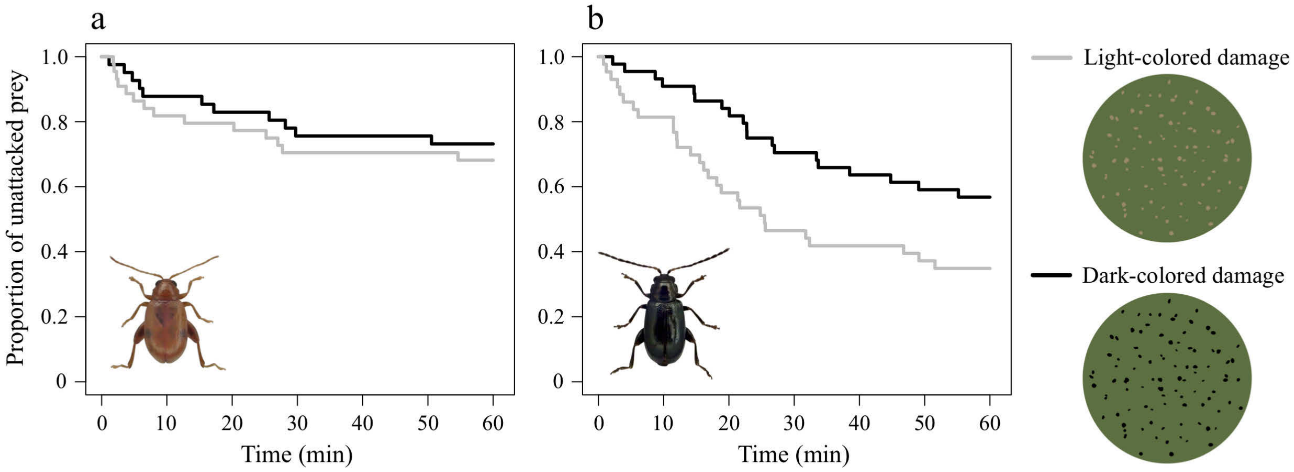

Fig. 2. Survival curves of light-coloured prey (a) and dark-coloured prey (b) on two types of background. Gray and black lines indicate backgrounds with light-coloured damage and dark-coloured damage, respectively.

coloured damage and 26.3% (N = 38) on the background with dark-coloured damage (likelihood ratio test: χ^2 = 0.736, df = 1, P = 0.391). No significant difference in the attack frequency between spider sexes was recorded for light coloured beetles (likelihood ratio test: χ^2 = 3.179, df = 1, P = 0.075). There was also no significant difference in the time to attack on different types of background for light-coloured beetles (survival model: z = 1.35, P = 0.179, Fig. 2a), whereas they were attacked by male spiders in a significantly shorter time (survival model: z = –2.31, P = 0.021). In contrast, as expected, dark-coloured beetles were less frequently attacked by spiders on the background most similar to their body colour: 65.9% (N = 41) on the background with light-coloured damage and 42.9% (N = 42) on the background with dark-coloured damage (likelihood ratio test: χ^2 = 4.359, df = 1, P = 0.037). Dark-coloured beetles were attacked significantly more frequently by male spiders (likelihood ratio test: χ^2 = 10.258, df = 1, P = 0.001). Time to attack was longer on the background with dark-coloured damage than that with light-coloured damage (survival model: z = 3.80, P < 0.001, Fig. 2b), and they were attacked by male spiders in a significantly shorter time (survival model: z = –2.51, P = 0.012).

Many spiders attacked prey when they were on the same plane as the beetle (i.e., the bottom of the experimental arena), whereas some attacked from above (i.e., the top of the experimental arena). The incidence of spiders attacking light-coloured beetles on the bottom of the arena with a background of light-coloured damage was 76.9% (N = 13) and on a background with dark-coloured damage was 100% (N = 10). Similarly, the incidence of spiders attacking dark-coloured beetles on the bottom of the arena on a background of light-coloured damage was 88.9% (N = 27) and on a background with dark-coloured damage was 77.8% (N = 18).

Comparative analysis of feeding damage

The density of feeding damage varied between species of beetles. For most species it was low to moderate (< 10 cm^{-2}), whereas some species, such as *Letzuella* sp., it was relatively high (> 15 cm^{-2}) (Fig. 3). The analysis of data for 103 leaves, using model 1 that included beetle body colour as the independent variable, revealed that the number of feeding damages caused by light-coloured beetles was greater than that by dark-coloured beetles (likelihood ratio test: χ^2 = 12.670, df = 1, P < 0.001). However, the analysis of the data from 45 leaves using model 2 that included beetle body colour and host plant family as independent variables, revealed that the effect of beetle body colour was not significant (likelihood ratio test: χ^2 = 2.999, df = 1, P = 0.083), whereas there was a significant effect of host plant family (likelihood ratio test, χ^2 = 16.690, df = 5, P = 0.005).

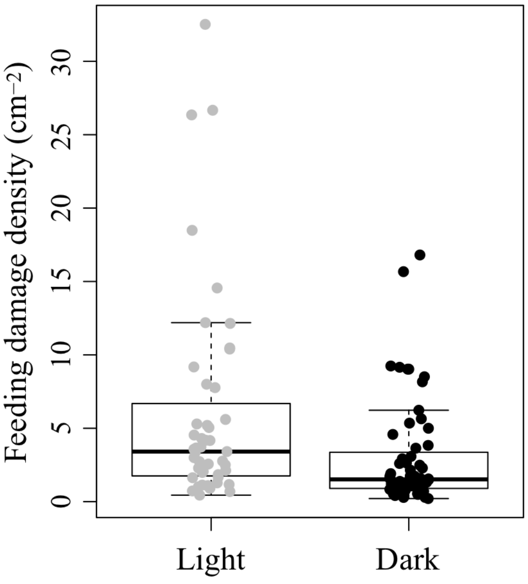

Fig. 3. Number of per leaf area (density) for light- and dark coloured beetles. Gray and black points indicate leaves damaged by light-coloured beetles (N = 47) and dark-coloured beetles (N = 56), respectively. The bold line in the middle of the box, the line above the box, and the line below the box indicate the median, third quartile point, and the first quartile point, respectively. Beetle and plant species used are listed in Table 1.

DISCUSSION

The result of the predation experiment showed that dark-coloured beetles were more likely to be attacked when they are placed on a background with light-coloured damage than with dark-coloured damage (Fig. 2b), as predicted by the hypothesis. However, no significant difference in the efficiency of masquerade was recorded for light-coloured species (Fig. 2a). This could be because the light-coloured species *L. bimaculatus* does not closely resemble its feeding damage, as its feeding damage appears to be slightly lighter than their bodies (Fig. 1a). There are possible reasons for poor resemblance: body colour similar to feeding damage is unlikely due to developmental and/or genetic constraints, or light-coloured species resemble multiple models (Kikuchi & Pfennig, 2013). For example, brown spots on diseased leaves of *Ligustrum* plants (e.g., Yoshikawa & Yokoyama, 1992; Liao et al., 2023) may be another model. However, some light-coloured species (e.g., *Letzuella* sp. and *Nesaecrepida rufomarginata*) are likely to resemble their own feeding damage more than others (cf., photographs in Konstantinov et al., 2018), at least to the human eye, which could hinder predation.

Despite the fact that in the current experiments the dark-coloured beetles survived probably due to masquerade, nevertheless they are vulnerable to attack by the predator under certain conditions. Jumping spiders attacked light- and dark-coloured beetles mainly from the side in this study, even though they could freely walk around inside the experimental arena. This suggests that the angle at which the predator views a beetle may be one of the factors influencing the beetle survival. Whereas jumping spiders search for close by prey on the same plane, large predators, such as birds, usually search for distant prey from above. Although masquerade is generally effective even at close range (Skelhorn et al., 2010b), flea beetles that mimic flat models are likely to be recognized by predators when they are viewed from the side due to their convex shape. Therefore, beetles may be more easily recognized as prey by spiders than birds. Further research is needed to determine whether flea beetle masquerade is as effective when they are confronted by other predators.

In the comparative analysis, model 1 indicated that the feeding by light-coloured beetles resulted in a greater number of damages than that by dark-coloured beetles. However, model 2, which took host plant taxa into account revealed no difference. Taking these results into consideration, the variation in the extent of feeding damage caused by the different species of beetle was more likely to be influenced by host plant taxa than beetle body colour. A possible reason for this may lie in the plant defences that have evolved in the arms race between herbivores and their host plants (Ehrlich & Raven, 1964). Specialized leaf morphology or chemical compounds as a defence against herbivores may influence the extent of feeding damage, resulting in the variation in the efficiency of masquerade in different species of beetles.

This study provides evidence that the efficiency of masquerade in flea beetles as defence against jumping spiders varies depending on the species and/or phenotype of flea beetles. However, many other factors could influence the efficiency of masquerade. For example, the specific feeding style of herbivorous insects may induce plant structural defences (Traw & Dawson, 2002) or their feeding damage is a cue for predators searching for prey (Heinrich & Collins, 1983), which may result in trade-offs in terms of the efficiency of masquerade. Moreover, there is no evidence that predators misidentify beetles as their feeding damage. To demonstrate that beetle avoids predation through misidentification by predators, further experiments, in which the predator's experience with models is manipulated, are needed. There is also insufficient evidence that a similar appearance of a beetle to its feeding damage is an adaptation. Phylogenetic comparative analyses of masquerade based on several flea beetle lineages are needed in order to confirm that it is an adaptation. These studies will improve our understanding of the diversity of animal traits related to masquerade.

ACKNOWLEDGEMENTS. We are grateful to Y. Takami, N. Woodley, and anonymous reviewers for reading the manuscript and providing helpful comments. K.D. Prathapan kindly provided data on beetles and their host plants collected in India. We also thank P. Acevedo-Rodriguez, V. Dorofeyev, N. Gültekin and J.R.I. Wood for identifying the plants used in this paper and H. Shinohara for help with collecting beetles in Japan. Beetle vouchers used in this study are kept at: Department of Biological Science, Shizuoka University – vouchers from Japan; Entomology Department, Kerala Agricultural University – vouchers from India; National Museum of Natural History, Smithsonian Institution – vouchers from other countries. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA; USDA is an equal opportunity provider and employer.

AUTHOR CONTRIBUTIONS. TS and ASK collected the data. ASK identified flea beetle species. TS conducted experiments and statistical analyses. Both authors contributed to the writing of the manuscript.

CONFLICT OF INTEREST. The authors declare that they have no conflict of interest.

REFERENCES

Bates D., Maechler M., Bolker B. & Walker S.C. 2015: Fitting linear mixed-effects models using lme4. — *J. Stat. Softw.* **67**: 1–48.

Campbell J.M. 1998: *An Atlas of Oxfordshire Coleoptera, Chrysomelidae.* Technical paper/occasional paper No. 13, Oxfordshire Museums, Woodstock, 100 pp.

Cuthill I.C. 2019: Camouflage. — *J. Zool.* **308**: 75–92.

Ehrlich P.R. & Raven P.H. 1964: Butterflies and plants: a study in coevolution. — *Evolution* **18**: 586–608.

Folgar-Cameán Y., Gómez-Rodríguez C., Konstantinov A.S. & Baselga A. 2021: Hiding among holes: mechanisms underlying the evolution of masquerade in flea beetles (Chrysomelidae). — *Ecol. Entomol.* **47**: 137–145.

Freed A.N. 1982: A treefrog's menu: selection for an evening's meal. — *Oecologia* **53**: 20–26.

Freed A.N. 1984: Foraging behaviour in the jumping spider *Phidippus audax*: bases for selectivity. — *J. Zool.* **203**: 49–61.

Getman-Pickering Z.L., Campbell A., Aflitto N., Grele A., Davis J.K. & Ugine T.A. 2019: LeafByte: A mobile application that measures leaf area and herbivory quickly and accurately. — *Methods Ecol. Evol.* **11**: 215–221.

Givens R.P. 1978: Dimorphic foraging strategies of a salticid spider (*Phidippus audax*). — *Ecology* **59**: 309–321.

Heinrich B. & Collins S.L. 1983: Caterpillar leaf damage, and the game of hide-and-seek with birds. — *Ecology* **64**: 592–602.

Jackson R.R. & Tarsitano M.S. 1993: Responses of jumping spiders to motionless prey. — *Bull. Br. Arachnol. Soc.* **9**: 105–109.

Kikuchi D.W. & Pfennig D.W. 2013: Imperfect masquerade and the limits of natural selection. — *Quart. Rev. Biol.* **88**: 297–315.

Konstantinov A.S. 2016: Possible living fossil in Bolivia: a new genus of flea beetles with modified hind legs (Coleoptera, Chrysomelidae, Galerucinae, Alticini). — *Zookeys* **592**: 103–120.

Konstantinov A.S., Prathapan K.D. & Vencl F.V. 2018: Hiding in plain sight: leaf beetles (Chrysomelidae: Galerucinae) use feeding damage as a masquerade decoy. — *Biol. J. Linn. Soc.* **123**: 311–320.

Lack D. & Owen D.F. 1955: The food of the swift. — *J. Anim. Ecol.* **24**: 120–136.

Li D. & Jackson R.R. 1996: Prey preferences of *Portia fimbriata*, an araneophagic, web-building jumping spider (Araneae: Salticidae) from Queensland. — *J. Insect Behav.* **9**: 613–642.

Li D., Jackson R.R. & Barrion A. 1997: Prey preferences of *Portia labiata*, *P. africana*, and *P. schultzi*, araneophagic jumping spiders (Araneae: Salticidae) from the Philippines, Sri Lanka, Kenya, and Uganda. — *N. Z. J. Zool.* **24**: 333–349.

Liao Y., Wan Y., Si Y., Geng J., Li D. & Zhu L. 2023: Leaf spot of *Ligustrum japonicum* caused by *Diaporthe eres* newly reported in China. — *Crop. Prot.* **164**: 106139, 5 pp.

Merilaita S., Scott-Samuel N.E. & Cuthill I.C. 2017: How camouflage works. — *Philos. Trans. R. Soc. Lond. (B)* **372**: 20160341, 9 pp.

Nakamura T. & Yamashita S. 2000: Learning and discrimination of colored papers in jumping spiders (Araneae, Salticidae). — *J. Comp. Physiol. (A)* **186**: 897–901.

Owen D.F. 1955: Coleoptera taken by swifts (*Apus apus* L.). — *J. Soc. Brit. Entomol.* **5**: 105–109.

R Core Team 2020: *R: A Language and Environment for Statistical Computing. Vienna.* R Foundation for Statistical Computing, URL: https://www.R-project.org/

Ren J., de Gunten N., Konstantinov A.S., Vencl F.V., Ge S. & Hu D.L. 2018: Chewing holes for camouflage. — *Zool. Sci.* **35**: 199–207.

Riechert S.E. & Bishop L. 1990: Prey control by an assemblage of generalist predators: spiders in garden test systems. — *Ecology* **71**: 1441–1450.

Ruxton G.D., Allen W.L., Sherratt T.N. & Speed M.P. 2018: *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Masquerade*. Oxford University Press, New York, 304 pp.

Skelhorn J. 2015: Masquerade. — *Curr. Biol.* **25**: R643–R644.

Skelhorn J. & Ruxton G.D. 2013: Size-dependent microhabitat selection by masquerading prey. — *Behav. Ecol.* **24**: 89–97.

Skelhorn J., Rowland H.M., Delf J., Speed M.P. & Ruxton G.D. 2011: Density-dependent predation influences the evolution and behavior of masquerading prey. — *Proc. Natl. Acad. Sci.* **108**: 6532–6536.

Skelhorn J., Rowland H.M. & Ruxton G.D. 2010a: The evolution and ecology of masquerade. — *Biol. J. Linn. Soc.* **99**: 1–8.

Skelhorn J., Rowland H.M., Speed M.P. & Ruxton G.D. 2010b: Masquerade: camouflage without crypsis. — *Science* **327**: 51.

Suguro T. 2017: *Handbook of Jumping Spiders*. Bun-ichi Sōgō Shuppan, Tokyo, 152 pp. [in Japanese].

Tanahashi M. & Fukatsu T. 2018: Natsumushi: Image measuring software for entomological studies. — *Entomol. Sci.* **21**: 347–360.

Therneau T. 2015: *A Package for Survival Analysis in S. Version 2.38.* URL: https://CRAN.R-project.org/package=survival

Traw B.M. & Dawson T.E. 2002: Differential induction of trichomes by three herbivores of black mustard. — *Oecologia* **131**: 526–532.

Turner F.B. 1959: An analysis of the feeding habits of *Rana p. pretiosa* in Yellowstone Park, Wyoming. — *Am. Midl. Nat.* **61**: 403–413.

Yamashita S. & Tateda H. 1976: Spectral sensitivities of jumping spider eyes. — *J. Comp. Physiol.* **105**: 29–41.

Yoshikawa M. & Yokoyama T. 1992: *Thedgonia ligustrina* on *Ligustrum japonicum* and *Cercospora kyotensis* sp. nov. on *Hydrangea serrata* var. *thunbergii*. — *Trans. Mycol. Soc. Japan* **33**: 177–184.

Whitehead P.F. 1991: Observations on Coleoptera in the diet of two bird species in Worcestershire. — *Entomol. Rec. J. Var.* **103**: 81–82.

Received July 26, 2023; revised and accepted July 9, 2024