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Camouflaged objects are harder to detect if the background itself is more heterogeneous, and search becomes increasingly inefficient when the scene contains multiple items resembling the target. Some adult leaf beetles (Coleoptera: Chrysomelidae) with highly specialized habits make holes on host plant leaves while feeding. We propose that leaf beetles camouflage themselves with their feeding holes. The presence of holes makes predators' visual search harder, thus giving beetles more time to escape from the leaf surface either by jumping (Galerucinae: Alticini) or rolling (rest of Chrysomelidae). Based on behavioral observations and analysis of 25 photographs of feeding leaf beetles (15 species), we demonstrate that adult leaf beetles camouflage themselves by creating holes of uniform size, approximately half of the beetle body size. Observation of the feeding behavior and anatomy of a typical hole-feeding beetle (*Altica cirsicola*) showed that the foregut volume and head-prothorax mobility of beetles are the two major factors that constrain the hole size. A computer-simulated visual search test showed that the greater the number of holes, and the more each hole approached beetle body size, the longer it took humans (as models) to locate a beetle on a leaf. This study reports a newly discovered kind of camouflage, hole-feeding camouflage, in leaf beetles, which makes visual detection or recognition more difficult by changing the environmental background. This type of camouflage may open up a range of new possibilities for studies in animal cognition analysis and evolution of anti-predation defenses.

Key words: camouflage, feeding damage, visual search, holes, leaf beetle

INTRODUCTION

Camouflage is a method of concealment used throughout evolution by plants (Lev-Yadun and Ne'eman, 2013; Soltau et al., 2009; Wiens, 1978), animals (Kettlewell, 1965; Stevens and Merilaita, 2011), and, of course, the military (Cuthill et al., 2005; Stevens and Merilaita, 2009). The most prototypical examples of camouflage occur in animals, especially in insects (Kerr, 1941). Camouflage and its necessary morphological and behavioral adaptations in insects evolved in the late Middle Jurassic; for example, the mimicry between a hangingfly and a ginkgo (Wang et al., 2012). Camouflage is one of the most widespread forms of anti-predator defense, because it helps prevent prey from being detected or correctly recognized by potential predators (Skelhorn and Rowe, 2016; Stevens and Merilaita, 2009).

Camouflaged prey use body coloration (Cuthill et al., 2005; Stevens and Cuthill, 2006; Stevens et al., 2009), morphological structures (Liu et al., 2014; Skelhorn, 2015; Skelhorn et al., 2010), materials found in the environment (Hultgren and Stachowicz, 2009) or even patterns of movement (Anderson and McOwan, 2003; Srinivasan and Davey, 1995; Stevens and Merilaita, 2009) to make detection or recognition more difficult to better conceal themselves (Anderson and McOwan, 2003; Skelhorn and Rowe, 2016; Stevens and Merilaita, 2009). Stevens and Merilaita (2009) and Skelhorn and Rowe (2016) defined certain common camouflage forms. The most general forms are visual camouflage, such as crypsis or masquerade (Skelhorn and Rowe, 2016; Stevens and Merilaita, 2009). In addition, it was shown that camouflaged objects are more difficult to find if the background itself is more heterogeneous (Duncan and Humphreys, 1989; Lovell et al., 2008; Merilaita, 2003; Troscianko et al., 2009). Visual complexity of the habitat, and visual background complexity usually increase prey detection time (Dimitrova and Merilaita, 2009; Merilaita,

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2003; Xiao and Cuthill, 2016). Visual search efficiency could vary as a function of both target-distractor and distractor-distractor similarity (Duncan and Humphreys, 1989; Lovell et al., 2008).

One common feature of visual camouflage strategies is that they all focus on adapting the camouflage subject to its environment in order to hinder predator detection or recognition. These forms of camouflage force the camouflage subject to make a compromise by body color, shape adaption, or "decorating" itself, etc. Here, we define a newly discovered form of visual camouflage: hole-feeding camouflage of adult leaf beetles, which makes visual detection or recognition more difficult by changing or "decorating" the environmental background rather than the camouflaging subject itself.

While studying flea beetles (Chrysomelidae: Galerucinae: Alticini) in the field, we observed that many, including some other leaf beetles (Galerucini), make numerous holes that are similar in size while feeding on leaves of their host plants. The presence of the holes made it difficult to identify the specific position of a beetle on a leaf (Fig. 1). The beetle in Fig. 1B with many holes in the background is harder for a human observer to detect than the beetle in Fig. 1A without any holes. In nature, leaf beetles as well as many other insect groups (Blough, 1977) are preyed upon by numerous visual predators (Jolivet and Petitpierre, 1981), including birds (Lindroth, 1971). Based on visual search theories (Blough, 1977; Troscianko et al., 2009), we propose that these small beetles camouflage themselves with their feeding holes and that these holes make predators' visual search harder. This delay of an attack due to misidentification or lack of detection would give the beetles more time to escape.

Leaf beetles (Chrysomelidae) are one of the most species-rich family of beetles with about 38,000 species (Wilf et al., 2000) with the greatest diversity of 9900 species in flea beetles (Galerucinae: Alticini) (Konstantinov, 2016). The family arose during the Jurassic period nearly 150 million years ago (Engel, 2015) evolving highly specialized feeding habits that, according to Termonia et al. (2001), do not necessarily lead to "evolutionary dead ends". Most leaf beetles feed externally on leaves of their host plants, particularly on the upper, sunlit, leaf surface which is a more nutritious and easier to consume than the shaded surface (Łukowski et al., 2015). Openly feeding insects suffer greater mortality from natural enemies and predators than internal-feeding insects, such as leaf miners (Connor and Taverner, 1997). Hole-making is a common external feeding pattern in adult leaf beetles, particularly in taxa that harbor beetles of smaller body size such as Alticini and some Galerucini. Other leaf beetles (Chrysomelinae, Cryptocephalinae and Eumolpinae) feed on leaf margins or lower side of the leaves. Leaf beetle larvae often live and feed inside of various substrates: leaf litter, plant tissues etc.

Here, we describe how hole-feeding beetles harvest resources from a self-modified environment using a subtle but functional camouflaging activity to reduce predation risk. We verified that feeding holes provide functional camouflage effectiveness for adult leaf beetles against a predator's visual search.

MATERIALS AND METHODS

Leaf hole and beetle body measurements

Photographs of adult leaf beetles and their feeding damage (Fig. 2A) were taken with digital camera (Olympus E-PL1) from 2012 to 2013 in China and Bolivia. A total of 25 beetles representing 15 species were photographed, captured, and identified. Voucher specimens are deposited in the collection of the National Museum of Natural History, Smithsonian Institution, Washington, DC. Based on the 25 photographs, we calculated the hole areas and beetle body areas using Photoshop CS6. We measured the average areas of 2–3 randomly selected holes and the average body area of 1–2 randomly selected beetles from each photograph. For more sample and measurement information, see Supplementary Table S1.

Computer-simulated predation experiment

Based on the theories of visual search efficiency, which predicted that visual search getting more difficult when the background containing more items that resemble the target, and search efficiency varying with target-distractor and with distractor-distractor similarity (Dimitrova and Merilaita, 2009; Duncan and Humphreys, 1989; Lovell et al., 2008; Merilaita, 2003; Merilaita et al., 2001; Troscianko et al., 2009; Xiao and Cuthill, 2016), we assumed that a small leaf beetle represented a visual search "target" and the holes represented "distractors". To test whether the hole-feeding pattern decreased the efficiency of the visual search, we wrote a computer program in the Java programming language to simulate the responses of a visually-oriented predator.

In this program, we used computerized images rather than photographs taken in the field, because it was difficult to keep shooting conditions constant in nature. Computerized images were easy to edit and standardize. The simulation experiment used 32 adult human participants as the visually-oriented predators. Each participant was presented with 29 different computerized leaf images: 1 with no holes as a control, and 28 leaf images with hole number and size combinations varying according to four levels for hole number (n = 5, 10, 50, 100) and seven levels for hole-to-body-area ratio (r = 1/8, 1/4, 1/2, 1, 2, 4, 8). Images were presented in random order to each observer.

All images were normalized for color, intensity, contrast, and size. Each image had a background of a green leaf with three computerized adult beetles. In all images, leaf background, beetle number and size are constant, while beetle position was constrained randomly (a beetle and a hole could not be in the same place); hole number and size are varying as (n, r), where n was the number of holes, and r was the hole-to-body-area ratio. Each hole position was also assigned randomly.

Fig. 1. A beetle (*Eumolpinae*) on a leaf **(A)** without holes **(B)** with many holes.
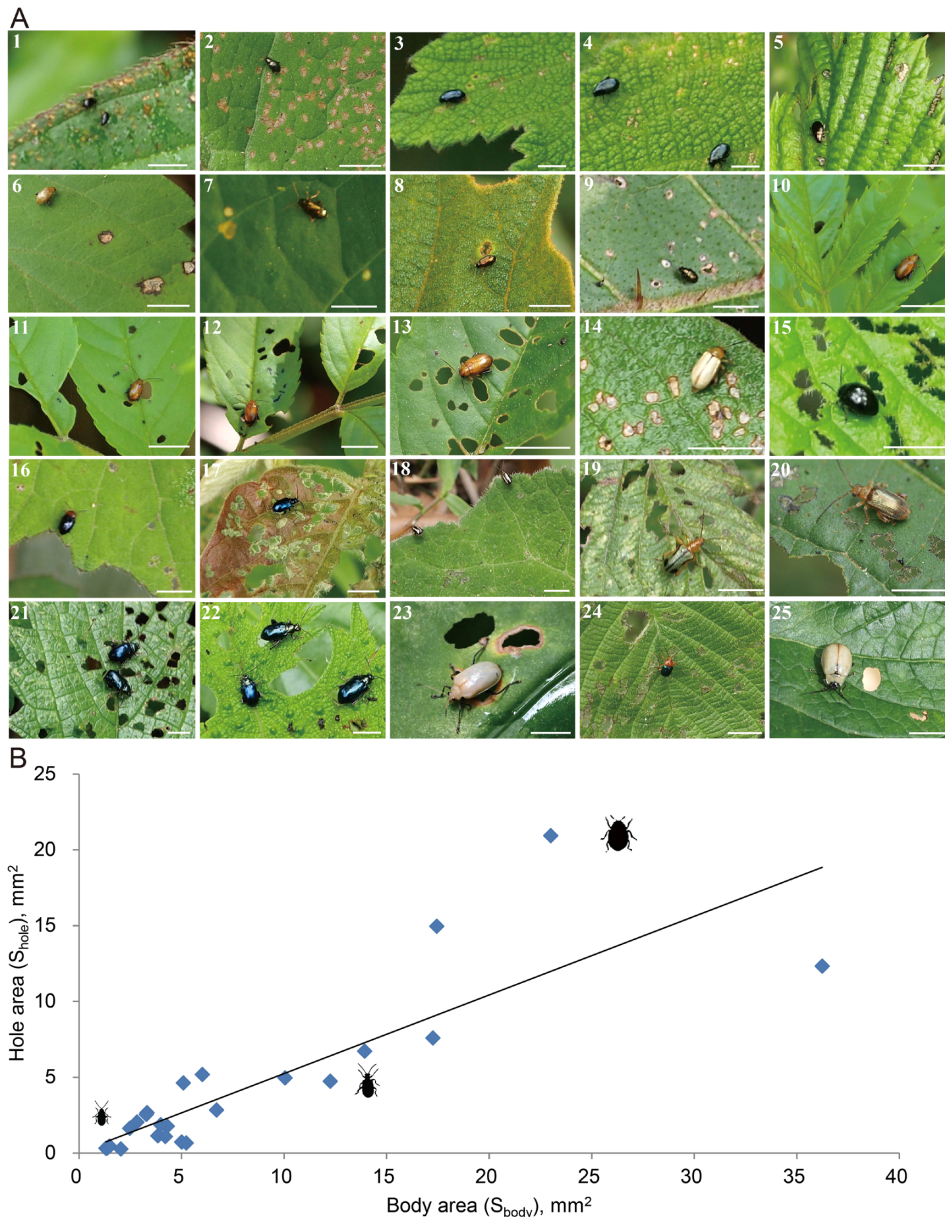
Fig. 2. Leaf beetles chew holes proportional to their body area. **(A)** Photographs of leaf beetles feeding on their host plants. The images are sorted according to body size (25 individual leaf beetles, 15 species). Scale: 5 mm. **(B)** The relationship between beetle body area S_{beetle} and hole area S_{hole}: the solid line is a best fit of linear regression prediction ($S_{hole} = 0.52 S_{beetle} + 0.06$, $R^2 = 0.71$, $F_{1,24} = 55.51$, $P < 0.001$).

This research complied with all of the human ethical research requirements at the Georgia Institute of Technology under IRB H16037.

Time-lapse videography

The leaf beetle, *Altica cirsicola* Ohno (Chrysomelidae: Galerucinae: Alticini), and its host plant (*Cirsium setosum*), were used as a model system of hole-feeding behavior. Beetles and their host plant were captured in Beijing, China, and taken to the laboratory. We placed a single beetle and a single leaf together into a glass cuvette. The leaf's stalk extended out of the cuvette and was wrapped in wet cotton to plug the open end of the cuvette and keep the leaf fresh. The feeding video was recorded for 8 hr at 30 frames/sec using software AMCAP-Direct Show 9.013 connected to a USB pen-type high-definition electronic microscope (ANDONSTAR A1) in the laboratory at 25°C.

The feeding path was tracked using the software Tracker 4.91 based on the feeding videography (Supplementary Movie S1). The mouthpart of *A. cirsicola* was marked as the origin of the coordinates, and the long body axis was the X-direction. The position of the tracked mouthpart was marked by hand every 100 video frames.

Micro-CT

To measure foregut volume, *A. cirsicola* that had just finished a feeding bout that produced a hole was immediately frozen. After dehydration and critical point drying, the beetle was put into X-radio 400 (beams strength: 60 keV; optical magnification: 4X) for the micro-CT images. Based on the micro-CT images, the foregut of the beetle was reconstructed and the foregut volume was measured by 3D software Amira 5.4.

RESULTS

Hole size is proportional to beetle size

Most of the beetles presented in Fig. 2A are very small, with body lengths ranging from 1 to 8 mm. The chewing damage of some adult leaf beetles resembles shotgun-like punctures of various depths. In some areas of damage, only the top layers of the leaf are eaten, leaving brown or white patterns from the remaining layers and forming a hole rimmed with necrotic damage (Fig. 2A, photographs 1–6). In some damaged areas, the leaf is eaten through, forming a hole that assumes the darkness of the shadows below the leaf (Fig. 2A, photographs 11–13).

Before each test, participants were verbally instructed to click on all the beetles as soon as possible. The computer timer started when an image was displayed on the screen. The participants then moved the mouse cursor to click on each of the beetles they detected. The timer was stopped once the last of three beetle targets was clicked. The time was recorded and displayed to the participant. The screen also showed a button for participants to click to continue to the next image. This process continued until all 29 images were shown to the participant. Once the final image was displayed, all the recorded time data were saved for analysis. Each participant took 1–2 min to complete the computer-simulated program. The data of computer-simulated predation experiment based on 32 human observers see Supplementary Table S2.

The relationship between the beetle body area (S_{beetle}) and hole area (S_{hole}) is shown in Fig. 2B (for area measurements see Supplementary Table S1). The solid line shows the linear regression prediction that best fit the data, which is described by the following equation:

$$S_{hole} = 0.52S_{beetle} + 0.06, \qquad (1)$$

Equation (1) fits the data well ($R^2 = 0.71$, $F_{1, 24} = 55.51$, $P < 0.001$). The hole area S_{hole} to beetle area S_{beetle} ratio was $r = 0.52$ (< 0.001, 95% confidence intervals [0.37, 0.66]), indicating that holes were about half of the beetle's body area. These leaf beetles created holes of a relatively uniform size, which was about 1/2 the size of their body areas.

Two major factors constrain the feeding holes size

Based on extensive observation, we found the beetle chewed a single hole for every bout of feeding. A feeding time sequence over 24 seconds is shown in Fig. 3A. During 8 hr video recording period, the beetle created 10 holes, averaging (±SD) in area was 3.07 mm^2 ± 0.99, which was approximately half the beetle's body area. For *A. cirsicola*, the hole area-to-body area ratio of was $r = 0.56$ (Table 1), which is very close to the predicted ratio 0.52 (Equation 1, Fig. 2).

Figure 3B shows the duration of feeding and resting events for each of the 10 holes recorded. Each hole was created in approximately 12 min. After making a hole, the beetle rested for approximately 45 min, presumably to digest. This rest time corresponds roughly to that of other insects; caterpillars rest 15–30 min, and grasshoppers rest for 1–2 hr (Chapman, 1998). Rest time activities involve defecation, self-cleaning, and very little locomotion. Then, the beetle initiated a new hole.

We found two factors of *A. cirsicola* anatomy to directly determine hole size: (1) the flexibility of the head-prothorax, and (2) the size of the beetle's digestive system. We discuss each in turn.

Factor 1: Head-prothorax mobility of the leaf beetle limits hole width

We observed that the beetle kept its legs mostly stationary when chewing holes, presumably to reduce energy consumption. We also found that the beetles fed by moving their head and prothorax from the middle of body to the side as shown in Fig. 3A. Considering a coordinate axis with the origin at the mouthparts (Fig. 3C), we tracked the feeding path of *A. cirsicola* based on an additional movie file (see Supplementary

Movie S1). Figure 3D, E shows the motion along the X-direction, the long axis of the body, and the Y-direction, the short axis of the body, respectively. The motion in the X-direction is relatively conservative. The amplitude of Y-direction motion is approximately twice that of the X-direction. This type of middle-to-side feeding pattern differs from other insects, such as grasshoppers, which move their head primarily up and down (Chapman, 1998) in the X-direction. Middle-to-side feeding pattern depends on the motion range of the mouthpart, which is restricted by the head-prothorax
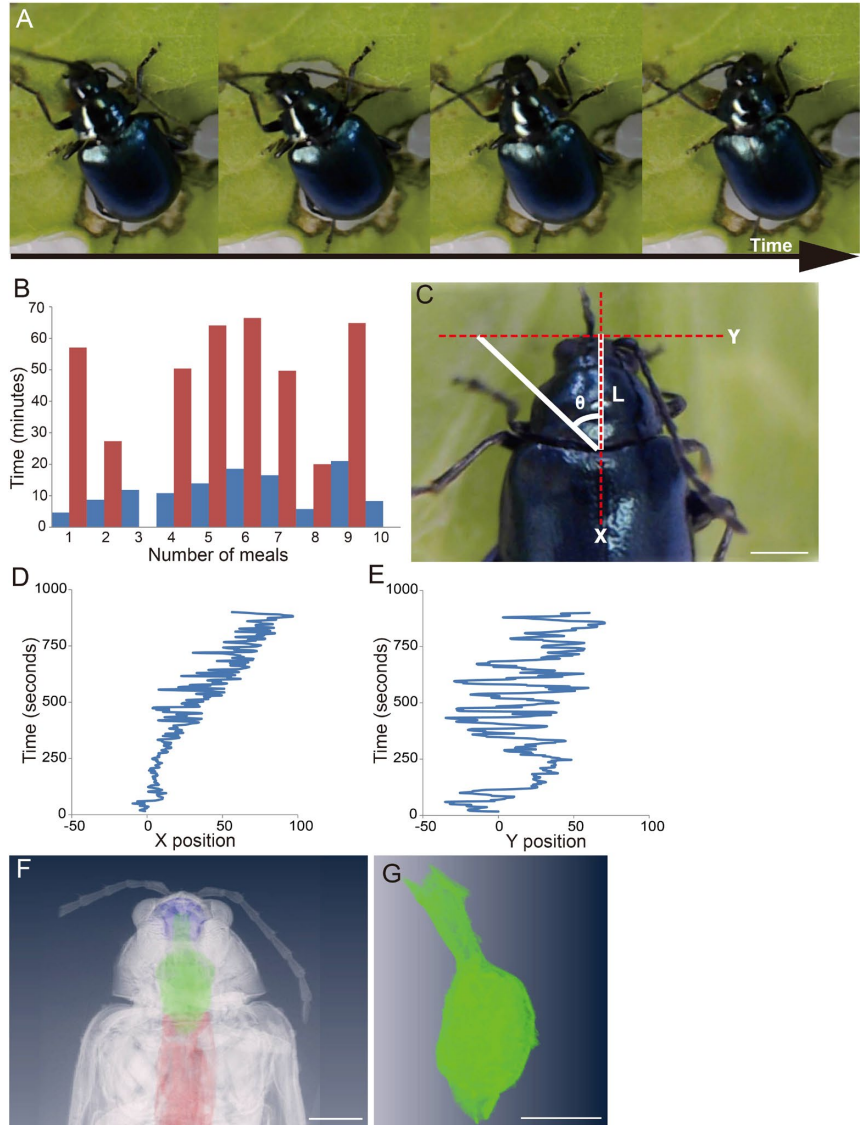

Fig. 3. Feeding kinematics of *Altica cirsicola*. **(A)** The image sequence of the beetle chewing over 24 seconds. Frames are separated by 6 sec. **(B)** Histogram of chewing time (red) and resting time (blue) over 8 hr of leaf feeding. **(C)** Definition of coordinate axes, where the X-direction denotes the long body axis, the Y-direction denotes the short body axis, and X and Y intersect at the mouthpart; *L* is the length of the head and prothorax; θ is the maximum angle that the head and prothorax can move to one side. **(D)** The moving track of the mouthpart in the X-direction during beetle chewing. **(E)** The moving track of the mouthpart in the Y-direction. **(F)** The 3D-reconstruction of *A. cirsicola*: the white part is the body, the blue part is the mouthpart, the green part is the foregut, and the red parts are the mid- and hindguts (dorsal view). **(G)** The 3D-reconstruction of the lateral view of the foregut of *A. cirsicola*. **(C)**, **(F)** and **(G)** scale: 0.5 mm.

Table 1. Feeding measurements of the beetle Altica cirsicola in 8 hr.

Measurements	N	Average	Stdev.S
Hole number, n	10	–	–
Feeding time per hole, $T_{feeding}$ (min)	10	12.01	5.46
Interval time between meals, T_{rest} (min)	9	44.45	23.35
The maximum amplitude of the leaf beetle rotating head and prothorax to one body side, θ (°)	10	51.51	11.83
The beetle head-prothorax length, L (mm)	10	0.95	0.05
The beetle body surface area, s (mm^2)	3	5.46	0.09
The foregut volume of the beetle, $V_{foregut}$ (mm^3) [a]	1	3.42	–
The leaf thickness of the host plant, h (mm)	3	0.42	0.04
Hole width, $W_{experiment}$ (mm)	10	2.23	0.36
Hole area, S_{hole} (mm^2)	10	3.07	0.99
Hole volume, V_{hole} (mm^3) [b]	10	1.31	0.45
Ratio of hole area to beetle body area, r	10	0.56	0.18

[a] Foregut volume of the leaf beetle measured based on micro-CT data. [b] Hole volume was calculated by the product of hole area and leaf thickness: $V_{hole} = hS_{hole}$.

mobility.

Our observations showed that the beetle kept their legs stationary while feeding, and the feeding pattern was from the middle to side by moving the head and prothorax. Consequently, the width of the feeding hole could be estimated by the ranges of motion of the mouth and prothorax. We observed that the leaf beetles rotated their head and prothorax a maximum amplitude of $\theta = 53°$, and their head-prothorax length was $L = 0.95$ mm (Fig. 3C; Table 1). Based on the geometry of the head and prothorax, we predicted the hole width $W_{prediction}$ using the following equation:

$$W_{prediction} = 2 L \tan \theta, \qquad (2)$$

where the values for θ and L yield $W_{prediction} = 2.60$ mm, which is comparable with the experimental hole width ($W_{experiment} = 2.23$ mm; Table 1) which was measured as the average width of the hole in the Y-direction based on the 10 holes $A.$ $cirsicola$ made over 8 hr of feeding. These similar values indicate that the leaf beetle's head-prothorax anatomy and flexibility are responsible for hole width.

Factor 2: The foregut volume of the leaf beetle limits hole size

What determines the size of the hole? Our videos show that the beetle $A.$ $cirsicola$ defecated between making holes. Thus, we assume that leaf beetles eat a hole until satiated. The foregut of the insect's digestive system is responsible for the temporary storage of food; the midgut enables digestion; and the hindgut is responsible for defecation (Chapman, 1998). We found that leaf beetles do not take breaks when creating a single hole. We assume that the leaf contents are stored in the foregut. In turn, the volume of the foregut influences the volume of the hole. We used micro-CT to visualize and estimate the volume of the $A.$ $cirsicola$ foregut. Figure 3F, G shows the 3D reconstruction of the foregut, which is shaded green. We used the 3D-software Amira to measure the foregut volume of $A.$ $cirsicola$ as $V_{foregut} = 3.4$ mm^3, which is nearly three times larger than the average hole volume $V_{hole} = hS_{hole} = 1.3$ mm^3 found based on the product of the leaf thickness $h = 0.42$ mm and the average hole area

$S_{hole} = 3.1$ mm^2 based on 8 hr of feeding observation (Table 1). Our measurements indicate that the leaf beetle eats to approximately 1/3 of the full capacity of the foregut. This proportion is similar to human feeding. The contents of the average adult human stomach after a meal is 900 mL (Ferrua and Singh, 2010), which is roughly 1/4–1/3 of the stomach's maximum capacity of 2000–4000 mL (Wenzel et al., 1998).

The micro-CT images revealed that the entire foregut was full of leaf materials; because we obtained these images after the specimens $A.$ $cirsicola$ was fed. We assumed that the beetle fed until its foregut was full as possible in a feeding bout when food is available. We also assumed that the time and rate of digestion are constant. In our study, the beetle rested for approximately 45 min (Fig. 3C) to await digestion in the midgut and excretion in the hindgut. During this process, the food stored in the foregut was then transferred to the midgut, making approximately 1/3 of the foregut available for another meal. Thus, foregut volume and digestion rate constrain meal consumption, which in turn limits the hole size.

The more holes and hole size approaching beetle body size, the better the camouflage effect

Figure 4A–E shows five example images in the computer program. In these images, the beetles body size is equal to hole size, and the holes are of different numbers, ranging from 0, 5, 10, 50, and 100. Based on test results from 32 participants, we analyzed the averaged search time t of the human "predator" to locate a beetle (Fig. 4F–I).

Figure 4F shows the relationship between search time t and hole number n under different hole sizes r, where r refers to the ratio of hole area to beetle body area, as defined in Table 1. As holes increase in number, search time increases—up to a point: in particular, when the hole is as large as the beetle ($r = 1$), the search time is the longest as shown in Fig. 4F. Figure 4G shows the averaged data of the relationship between t and n. For example, an image with no holes took the human predator 0.79 seconds to locate a beetle, whereas one with five holes took 0.86 seconds to locate, an increase of 9%. Adding an additional 5–50 holes increased the search time more slowly. However, at 100 holes, the search time increases to 0.91 seconds, an increase of nearly 15% compared to a leaf without holes. We conclude that the visual search of predators becomes more inefficient as the number of holes increases.

Figure 4H shows the relationship between search time t and hole size under different hole numbers n. Search efficiency is significantly reduced when the target and distractors resemble each other (Rosenholtz et al., 2012). Our experiments support this statement. There is an obvious peak where $r = 1$, which means when hole size approached beetle body size, it took more time to locate. Fig. 4H also confirms that as the hole number increases, the search time is getting longer. Fig. 4I shows the averaged data for the relationship between t and r. A sharp peak in search time occurs at 0.90 sec when $r = 1$ (holes equal to beetle body
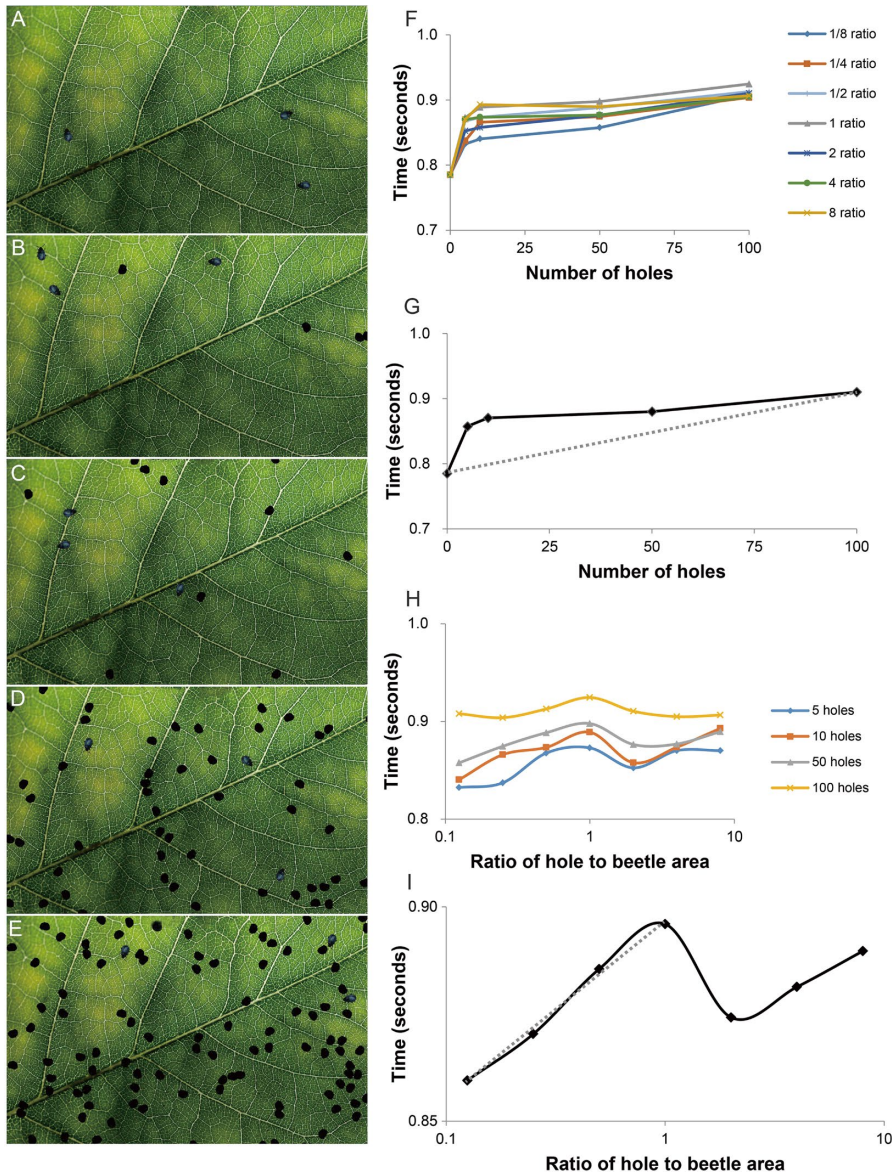
Fig. 4. Computer program for testing the hole camouflage effectiveness of the leaf beetles. **(A–E)** Images sequence examples from the computer program with hole area equal to beetle body area ($r = 1$), and numbers of holes varying from 0, 5, 10, 50, and 100. **(F)** The relationship between search time t and the number of holes n; different colors indicate different beetle sizes. **(G)** Averaged data regarding the relationship between t and n; the dashed line indicates the measured slope ($\alpha = 0.0013$ sec). **(H)** The relationship between search time t and hole size r; different colors indicate different hole numbers. **(I)** Averaged data regarding the relationship between t and r; the dashed line indicates the slope ($\beta = 0.018$ sec).

Leaf beetles may make the optimal number and size of holes to maximize camouflage effectiveness

During evolution, the body geometry of leaf beetles adapted to create feeding holes of constant size – about 1/2 their body size. However, our computer simulations showed that the beetles are in fact best camouflaged when the holes are twice as large. We now present a mathematical model (Equation 3) that explains this seeming contradiction.

We assume that hole number n and hole-to-body area ratio r each influence search time t independently. The search time t needed to find the beetle is therefore written:

$$t = \alpha n + \beta r, \qquad (3)$$

where α and β are constants from the estimation based on Fig. 4G and I. We used a linear best fit between the n values of 0 and 100 (the dashed line in Fig. 4G) to estimate the constant α ($\alpha = 0.0013$ sec per hole). In other words, every 10 holes add approximately 0.01 sec to the search time. In nature, beetles do not usually bite holes larger than their body size. We also used a linear best fit as the slope between r values of 1/8 to 1 (the dashed line in Fig. 4I) to estimate the constant β ($\beta = 0.018$ sec); in a certain number of holes, as the hole size doubled, search time increases about by 0.02 sec. To gain the best camouflage effectiveness, the beetle should try to make the search time t as long as possible with an appropriate choice of hole numbers n and size of hole r.

Based on feeding video data, we let b denote the time a leaf beetle stays on a leaf, c was the feeding time proportion during b, and d was the feeding rate of the beetle. According to the observation of *Altica cirsicola*, the beetle stayed on a leaf for $b = 8$ hr and fed for proportion of $c = 0.25$ (Table 1). The leaf beetle ate at a constant rate of $d = 0.0018$ mm^3/s, which was calculated using the hole volume divided by the time needed to complete each hole (Table 1). The beetle's feeding consumption in time b is bcd. The total volume of the leaf matter eaten is nv, where n is the number of feeding holes, and v is the average volume of holes during staying time b. Ignoring water loss and other uncontrolled factors, we used the conservation of mass to equate the leaf material eaten to calculate the beetle's feeding consumption in time b as

size; see Fig. 4I). This search time results in a 5% increase over the smallest hole sizes studied, 1/8 beetle body size, and a 3% increase over two times the beetle's body size (Fig. 4I). But when holes are larger than twice beetle size ($r > 2$), the search time increaes again. When the holes are 8 times beetle body size ($r = 8$), the average search time $t = 0.89$ s, which is close to the time associated when $r = 1$. In nature, beetles do not usually bite holes larger than their body size. So, the analysis still could be concluded that as the hole size close to the beetle body size, the search time increases. We conclude that the visual search of predators becomes more inefficient as hole size approaching the beetle body size.

$$nv = bcd. \tag{4}$$

Equation 4 provides a useful physical intuition: hole size is inversely proportional to hole number. Thus, the beetle faces a tradeoff. It can choose to create many small holes or a few large holes. Hereon, and without a loss of generality, we use $r = v/sh$, the hole-to-beetle area ratio in place of hole volume v, where s is the *Altica cirsicola* body area ($s = 5.46$ mm^2; Table 1), and h is the leaf thickness of the host plant ($h = 0.42$ mm; Table 1). Substituting $r = v/sh$ into Equation 4, we determined the hole-to-body area ratio r in terms of the hole number: $r = bcd/shn$. Substituting this expression into Equation 3, we have $t = \alpha n + \beta bcd/(shn)$. Taking the first derivative of t with respect to n, we optimize t according to hole number n. We find that the optimal search time t occurs at

$$n^* = \left(\frac{\beta}{\alpha} \frac{bcd}{sh} \right)^{1/2}, \quad r^* = \frac{bcd}{shn}. \tag{5}$$

Based on the computer visual test (Fig. 4) and the observation of *Altica cirsicola* (Fig. 3, Table 1), replacing the values of α, β, b, c, d, h, s into Equation 5, we find that the optimal number of holes for camouflage effectiveness maximization of *Altica cirsicola* during an 8 hr feeding is $n^* = 13.8$ holes, which approximates the observed value of $n = 10$ holes from the 8 hours of experimental observation; the optimal hole-to-body area ratio is $r^* = 0.41$, which is also match for $r = 0.56$ based on the 8 hr of experimental observation, and remarkably similar to the value of $r = 0.52$ found based on the 25 feeding photographs of the leaf beetles in nature (see the solid line in Fig. 2B). The flea beetle, *Altica cirsicola* could make the optimal number and size of holes in a period of time to maximize camouflage effectiveness.

DISCUSSION

The study of camouflage has progressed considerably over the past 70 years. Since Cott's landmark study (1940) there has been an explosion of camouflage studies, especially over the past decade, that has explored the evolution of camouflage patterns and predator recognition in an ecological context, producing new links among biology, visual psychology, computer science, and art (Anderson and McOwan, 2003; Cuthill et al., 2005; Duncan and Humphreys, 1989; Kettlewell, 1965; Lovell et al., 2008; Rosenholtz et al., 2012; Rowland et al., 2008; Rowland et al., 2007; Skelhorn and Rowe, 2016; Skelhorn et al., 2009; Skelhorn and Ruxton, 2013; Stevens and Merilaita, 2009; Stevens et al., 2009; Troscianko et al., 2009). Following this historical trend, our investigation, which uniquely combined field and laboratory observations with computer-simulated predation experiments and quantification of feeding behavior, confirmed the existence of a previously unknown form of hole-feeding camouflage produced by adult leaf beetles (Kanstantinov et al., 2018).

We found that some small adult leaf beetles camouflage themselves by chewing feeding holes of a species-specific size approximately 1/2 of their body size. We believe that the foregut volume and head-prothorax ranges of motion constrain the generation of hole size patterns. A computer-simulated visual search experiment using humans as model predators demonstrated that the more holes in the visual field and the closer hole size approaches beetle body size, the more time human subjects took to locate target beetles. Our findings support the basic tenets of predator search theory, which posits that increasing background heterogeneity and increasing similarity between the target (beetles) and distractors (feeding holes) reduce search efficiency (Dimitrova and Merilaita, 2009; Duncan and Humphreys, 1989; Lovell et al., 2008; Merilaita, 2003; Xiao and Cuthill, 2016). Based on the computer predator simulations, we concluded that visual searches by human and probably natural visually-orienting predators becomes more inefficient as feeding hole number increases and as hole size approaches beetle body size.

We suspect that birds are the principle agents driving the evolution of beetle feeding-hole camouflage. Consider 10–14 g, insectivorous birds foraging in an entangled bank of sun-dappled foliage, wherein prey is likely to be feeding. In stressed habitats, such as lowland tropical rainforests and higher elevations, birds are always near starvation, all available habitats are fully-booked by competing conspecifics, which live a relatively long time, often more than 15 years. As endotherms, these small, agile birds maintain high metabolic rates to support flight, constant foraging, and the thermal exigency of their small size (i.e., high surface-to-volume ratio). Thus, bird caloric budgets are nearly always in the red. On the plus side, birds are intelligent and capable of learning quickly and retaining information, especially in the visual mode. However, their insect prey is generally poisonous, poisonous-looking, small, fast, agile, inedible, inedible-looking, or invisible, or some combination of these features. Several studies identify birds as active visual predators of leaf beetles (Blough, 1977; Jolivet and Petitpierre, 1981; Lindroth, 1971). Based on this, we suggest that all these factors have conspired to drive the evolution of feeding hole camouflage as an adaptation to reduce avian predator detection; leaf-feeding beetles have evolved bodies to more closely resemble their feeding damage and their feeding habits to produce damage that resembles their own bodies.

This study establishes the possibility that feeding hole mimicry provides functional camouflage that effectively serves as an anti-predation defense. We still need empirical data that demonstrates that birds actually suffer reduced search efficiency when confronted with feeding damage camouflage. In our computer simulation, we tested non-intersecting hole patterns to ensure repeatability between experiments. In nature, birds are the major predators that use visual searching to hunt small insect prey. But there are many previous cases of using human to do visual search, and a body of visual search evidence from studies of humans or species in which vision approximates to that of humans (Anderson and McOwan, 2003; Biederman, 1987; Hiris, 2007; Mendola et al., 1999; Párraga et al., 2002; Troscianko et al., 2009). For example, Xiao and Cuthill (2016) noted common features of background complexity that affect visual searches in both birds and in human. For these reasons, we feel justified using humans as model predators.

In nature, the background on which beetles feed or rest is more heterogeneous and complicated. Different leaf level and leaf shadow could provide good shelter for small insects. Small body size itself has advantages in evolution. It's really

difficult to discover a small insect in the field. At the same time visually oriented predators use leaf damage as a search cue (Heinrich and Collins, 1983), so the feeding holes could expose beetle positions. However, at the same time, holes also provide visual interference, giving beetles more time to escape. Comparing the success of individual organisms, it is often in practice more useful to compare the success of "strategies" (Dawkins, 1982). In the 25 feeding photographs (Fig. 2A), some beetles feed on the top layer of a leaf to form hole-like remains whose color approximates the beetle's body color (Fig. 2A6, 14). Intuitively, this technique partially increases the difficulty of detection and visual search, although we have not verified this with experimental data.

Our experiments represent a "best case scenario" for the hole feeding damage camouflage of leaf beetles. Although we did not consider plant defensive strategies, such as induction or compartmentalization of secondary compound reactants as found in cyanogenesis. There is no data from plants at present to explain why beetles make damage sites that consistently similar to their own body shapes and colors when other herbivorous insects on the same host plants consume the entire leaf. Feeding habits are known to be influenced by the diversity of plants and their chemistry, and by natural enemies of the herbivores (Bernays, 1998). Camouflage damage feeding that creates a constant hole size is an adaption in the long coevolution with the host plant and natural predators. We have shown the following morphological and physiological adaptions of leaf beetle for the way of creating constant size of holes: the head-prothorax mobility and foregut volume limit the size of the hole that a beetle could make (Fig. 3). The hole-feeding pattern from middle to body side was also found in the other typical hole-feeding flea beetle (*Sphaeroderma* sp.). It seems that all the hole-feeding beetles make holes by the aforementioned movement.

As feeding progresses, or when there are multiple beetles, holes are made so close together that the holes may coalesce. Such events can increase hole size. We observe in our computer program, when holes are bigger than beetle body size (for example, hole size is 2 times of beetle body size), the search time decreases (Fig. 4H and I). But when the hole size approaches 4–8 times the beetle body size, the search time increases again (Fig. 4H and I). In nature, the hole size caused by leaf beetle in one meal is no bigger than the beetle body size. Due to the volumetric limits to the digestive system we observed in the CT scan experiments, it is functionally not possible for an insect to consume more than its body size in a single feeding bout. However, in some cases, hole coalescence increases hole size. When several small holes combine, it is still helpful as camouflage for small leaf beetles.

This study reports a newly discovered form of camouflage, hole-feeding camouflage of leaf beetles, which makes visual detection or recognition more difficult by changing or "decorating" the environmental background rather than the camouflaging subject itself. The results obtained in this study might lead to additional quantifications of small animal behavior. In a broader context, this type of camouflage may open up a range of new possibilities for studies of animal cognition analysis and evolution of anti-predation defenses.

COMPETING INTERESTS

No competing interests declared: the authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

SG, DH and JR conceived and designed the study; JR and NG performed the experiments; AK conceived of the idea, conducted original field observations and provided the experimental photographic materials; JR, DH and SG wrote the manuscript; FV edited the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online (URL: http://www.bioone.org/doi/suppl/10.2108/zs170136).

Supplementary Table S1. Body area of leaf beetles and hole area.

Supplementary Table S2. Computer-simulated predation experiment data.

Supplementary Movie S1. Feeding time lapse video of *Altica cirsicola* in 2 hr, time speed up 350 times.

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