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Partial Masquerading and Background Matching in Two Asian Box Turtle Species (*Cuora* spp.)

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Abstract Animals living in heterogeneous natural environments adopt different camouflage strategies against different backgrounds, and behavioral adaptation is crucial for their survival. However, studies of camouflage strategies have not always quantified the effect of multiple strategies used together. In the present study, we used a human visual model to quantify similarities in color and shape between the carapace patterns of two *Cuora* species and their preferred habitats. Our results showed that the color of the middle stripe on the carapace of *Cuora galbinifrons* (Indochinese box turtle) was significantly similar to the color of their preferred substrates. Meanwhile, the middle stripe on the carapace of *C. mouhotii* (keeled box turtle) contrasted more with their preferred substrates, and the side stripe matched most closely with the environment. Furthermore, the carapace side stripe of *C. galbinifrons* and the carapace middle stripe of *C. mouhotii* highly contrasted with their preferred substrates. We quantified the similarity in shape between the high-contrast stripes of both *Cuora* species and leaves from their habitats. The carapace middle stripe of *C. mouhotii* was most similar in shape to leaves from the broad-leaves substrate, and the carapace side stripe of *C. galbinifrons* was the most similar in shape to leaves from the bamboo-leaves substrate. We determined that these species adopt partial masquerading when their entire carapace is exposed and partially match their background when they semi-cover themselves in leaf litter. To the best of our knowledge, this is the first study to demonstrate that partial masquerading and background matching improve the camouflage effect of Asian box turtles in their preferred habitats. This is a novel study focusing on the influence of the shape and color of individual carapace segments on reducing detectability and recognition.

Keywords camouflage, *Cuora galbinifrons*, *Cuora mouhotii*, patterns, protective coloration

1. Introduction

Camouflage, the phenomenon in which the characteristics of an object or organism are arranged to prevent detection or recognition by an observer, is a common anti-predator strategy employed by many animals (Endler, 1978; Merilaita and Lind, 2005; Stevens and Merilaita, 2011) and is crucial for survival in many species (Cott, 1940; Ruxton *et al.*, 2004). Background matching, an important camouflaging strategy that helps an animal reduce detection by predators (Stevens *et al.*, 2015; Stevens and Merilaita, 2011), refers to the matching of

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the animals' color, lightness, orientation, and pattern with their background (Kang *et al.*, 2012; Marshall *et al.*, 2016; Troscianko *et al.*, 2016). Masquerading animals appear to closely resemble inedible or inanimate objects, such as twigs, leaves, stones, or bird droppings (Endler, 1981; Skelhorn *et al.*, 2010a), preventing predators from detecting or recognizing them.

However, it is difficult to determine whether a predator has detected and misidentified a camouflaged individual (due to masquerade) or entirely failed to detect the prey individual (due to background matching and disruptive coloration) (Skelhorn *et al.*, 2010a; Skelhorn and Ruxton, 2011). Thus, considering these two camouflage strategies separately is neither a requirement nor a realistic interpretation of the real world (Stevens *et al.*, 2011). Prey animals can exploit background matching and masquerading simultaneously to increase the anti-predation benefits of the strategies. Under these circumstances, the benefit and impact of each strategy may differ, as would the overall effect of the combined strategies (Hall *et al.*, 2017).

To maximize the effectiveness of the camouflage, animals likely choose microhabitats that closely complement their color and shape (Lovell *et al.*, 2013; Price *et al.*, 2019; Stevens and Ruxton, 2018). For example, the common potoo bird (*Nyctibius griseus*) selects perching sites that enhance its background matching and masquerading effects (Cestari *et al.*, 2018). Furthermore, many animals decorate themselves or modify their habitats to better conceal themselves (Gómez *et al.*, 2018; Marshall *et al.*, 2016; Suzuki and Sakurai, 2015).

Camouflage is likely the main defense mechanism for turtles, as they are slow moving and relatively easy to catch (Hall *et al.*, 2017). Thus, camouflage is an important anti-predation mechanism that improves their fitness. Some tortoise species have been described as having a likeness to stones (Willemsen and Hailey, 2003), and evidence suggests that desert tortoises (*Gopherus agassizii*) select habitats that contain stones similar in size to their own body size, which can reduce their detectability (Nafus *et al.*, 2015). There have been studies of color variation induced in turtles based on their habitats (McGaugh, 2008; Rowe *et al.*, 2014). However, few studies have explored color and shape similarities between turtles and habitats, or their models (see Xiao *et al.*, 2016, 2021). Moreover, these studies examined the animal as a whole rather than examining whether certain body parts of the animal impact camouflage.

In our long-term field studies on Indochinese box turtles (*C. galbinifrons*) and keeled box turtles (*C. mouhotii*) (Bu *et al.*, 2020; Xiao *et al.*, 2017), we discovered that the carapace roof of the former exhibits a bamboo-leaved pattern, specifically two yellow stripes (Figure 1A), while the carapace roof of the latter exhibits a broad-leaved pattern, created by two keels (Figure 1B). These patterns facilitate disruptive camouflage in their preferred substrates (Bu *et al.*, 2020). Both box turtle species bury their bodies in leaf litter when they are still, leaving the middle stripe exposed, which appears to match the surrounding substrate (Figure 2). Both species face predators in the wild, mainly mammals, such as the masked palm civet (*Paguma larvata*) and wild boar (*Sus scrofa*) (Xiao *et al.*, 2020).

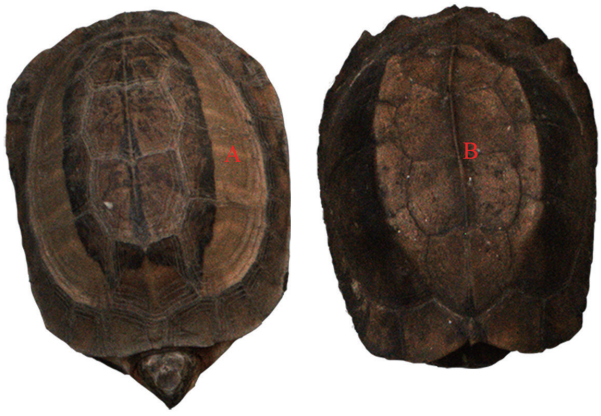

Figure 1 The carapace of an Indochinese box turtle (left) and a keeled box turtle (right). A indicates the yellow stripe of the Indochinese box turtle, and B is the middle stripe of the keeled box turtle.

In the present study, we predicted that the middle stripes on the carapace roof of these two *Cuora* species had evolved to match their preferred habitat, masquerading as leaves and serving an anti-predation purpose. To the best of our knowledge, this study is the first to focus on the anti-predation benefits associated with partial stripe color and shape variation in box turtles.

2. Materials and Methods

2.1. Ethics statement Fieldwork was carried out in strict accordance with the guidelines of the Animal Research Ethics Committee of Hainan Provincial Education Centre for Ecology and Environment, Hainan Normal University (HNECEE-2019-006), which conforms to the Law of the People's Republic of China. No turtles were euthanized for this study, and none were injured while in the traps. All turtles were released in the original capture area at the end of the experiment.

2.2. Microhabitat selection, photography, and calibration This study was conducted from September to December 2019. Field work was conducted in a rainforest in the Diaoluoshan National Nature Reserve (18°43′53″N, 109°52′10″E) in Hainan Province, People's Republic of China. In total, 12 adult Indochinese box turtles (carapace length ≥ 120 mm; seven females and five males) and 12 adult keeled box turtles

Figure 2 Both turtle species bury themselves in leaf litter, leaving the middle stripe exposed. Arrows indicate Indochinese box turtle (*Cuora galbinifrons*) (A) and keeled box turtle (*Cuora mouhotii*) (B).

Figure 3 Four kinds of backgrounds: A is an example of a broad-leaves substrate, B is a bare ground substrate, C is a bamboo-leaves substrate, and D is a grass substrate.

(carapace length ≥ 140 mm; eight females and four males) from the reserve were used for this study. The turtles did not exhibit obvious sexual dimorphism in terms of their carapace color. The preferred microhabitats of the keeled box turtle were broad leaves (Figure 3A) and bare ground (Figure 3B), and the preferred microhabitats of the Indochinese box turtle were broad leaves (Figure 3A) and bamboo leaves (Figure 3C), as determined using radio tracking data that recorded the turtles' movements from April 2015 to February 2016 (for detailed methodology see Xiao *et al.*, 2017). Grass microhabitats

were not favored by either species and served as the control (Figure 3D). Turtles and substrates were photographed using a Canon EOS 550D camera with a Canon lens (EF-S24–70 mm f/2.8, 8.2 megapixels). Photography in the field took place between 9:00 a.m. and 4:00 p.m., when natural light allowed turtles to be seen. A black umbrella was used to counter the direction of light entry and ensure uniform illumination of the photos. A tripod was used to ensure that the lens was perpendicular and placed at the same distance from the substrates, so that a constant image scale could be maintained. We collected 20 images of each substrate type and 24 images of turtles, totaling 104 images.

Images were saved in an uncompressed RAW format (Stevens *et al.*, 2007) for subsequent color measurements and analyses. The images were calibrated using the "Generate multispectral image" function in ImageJ 1.53e (Wayne Rasband and contributors, National Institutes of Health, USA) (Troscianko and Stevens, 2015). The images were ordered linearly based on radiance and standardized to control for differing light conditions by using an 18% standard gray card (Mennon, China, 14.5 cm × 9.5 cm) (Stevens *et al.*, 2007; Troscianko and Stevens, 2015).

2.3. Color measurement and calculation Standardized images were used to quantify the color contrast between the turtles' carapace and the substrates, following previously published protocols for images and survey methods (Bu *et al.*, 2020). A model of human discrimination was produced using MICA toolbox in ImageJ 1.53e (Wayne Rasband and contributors, National Institutes of Health, USA) (Troscianko and Stevens, 2015; Price *et al.*, 2019; van den Berg *et al.*, 2020). As visual searching is an important method that humans use to find turtles (Nafus *et al.*, 2015; Xiao *et al.*, 2016), we used human eyesight to evaluate whether color differences are distinguishable. According to the RNL model (Receptor Noise Limited Model) (Vorobyev and Osorio, 1998), the chromatic just-noticeable difference (CJND) and luminance just-noticeable difference (LJND) determine whether two objects can be differentiated, with increasing values suggesting decreased color similarity. In the present study, we evaluated the color contrast between both species and their substrates by eye. We used the elm 52-mm human D65 calibration profile as a cone model. We compared the entire carapace, the middle stripe, the side stripes (Figure 4), and five randomly selected areas of each substrate with the substrate color. In addition, we randomly selected three to five leaves from each substrate to represent the leaf color.

We converted the standard multispectral image to a cone catch, used the human visual system in sunlight (D65), and set the Weber coefficient as 0.02 (Wyszecki and Stiles, 1982) to analyze the chromaticity and luminance of each image (Troscianko and Stevens, 2015). The luminance channel was set to "lum" for the human vision model. We used pixels with a starting size of 2 and an ending size of 512. At each step, the pixels were multiplied by 1.414, to represent exponential growth. Luminance measurements ranged from 0 to 65,535 (Nokelainen *et al.*, 2020). These calculations were performed by a third party who was unaware of the aim of the study. The values of CJND ranged from three to eight, depending on the image content and on each individual subject (Lin *et al.*, 2015).

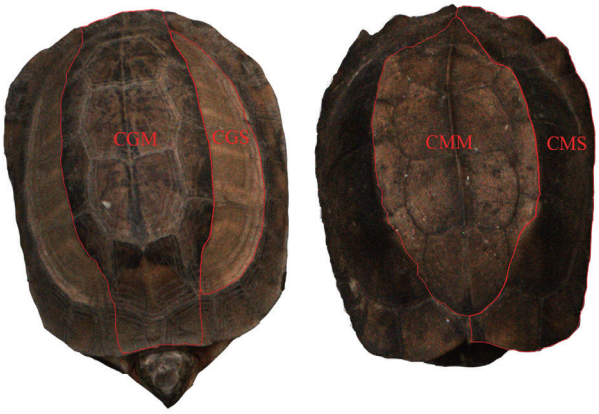

Figure 4 Color and structure segmentation diagram for a *Cuora galbinifrons* individual, with a carapace length of 176.64 mm, including CGM = middle stripe and CGS = side stripe, and a *Cuora mouhotii* individual, with a carapace length of 161.78 mm, with CMM = middle stripe and CMS = side stripe.

2.4. Shape measurement and similarity calculations Standardized images were imported into ImageJ 1.53e and converted into TIFF format. Scales with actual measurement values for area (A) and perimeter (P) were selected. In addition, four further descriptors were used: aspect ratio (AR), solidity (S), circularity (C), and roundness (R). These six shape descriptors were used to quantify the shapes of turtles' carapaces and the leaves in the substrates.

According to the similarity algorithm (Samal *et al.*, 2004), descriptor similarity is obtained by standardizing the differences between the prey and the model, as follows:

$$\mathrm{sim}(X, Y) = 1 - \mathrm{dis}(X, Y)/U \qquad \text{(Eq. 1)}$$

where sim (X, Y) is the similarity between objects X and Y; dis (X, Y) represents the Euclidean distance between objects X and Y; and U is the maximum distance between the corresponding features of the two elements.

The weighted average of similarity between shape descriptors X and Y was calculated as follows:

$$\varphi(X,Y) = \frac{\sum_{i=1}^{q} \omega_i \sigma_i}{\sum_{i=1}^{q} \omega_i} \qquad \text{(Eq. 2)}$$

where i is the ith (i = 1..., q) shape descriptor; σ_i is the similarity of the ith shape descriptor between X and Y; and

ω_i is a weighting factor for the similarity of the ith shape descriptor. ω_i was determined using the coefficient of variation (v) method:

$$\omega_i = \frac{v_i}{\sum_{i=1}^{q} v_i} \quad \text{(Eq. 3)}$$

where v_i is the ratio of the standard deviation of similarity between the ith shape descriptor and its mean.

The shape similarity between turtles' carapace and substrate leaves was then calculated based on Equations 2 and 3, with $q = 6$ representing the six shape descriptors. The values of shape similarity ranged from 0–1, with increasing values equating to higher misidentification rates by human predators (Xiao *et al.*, 2021). We calculated shape similarity for the entire carapace, the middle stripe, and the side stripes (Figure 4).

2.5. Statistical analysis All statistical analyses were performed using SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA), and all data are expressed as mean ± SE. A Kolmogorov-Smirnov test was used to test the normality of CJND, LJND, and the shape similarity data before analysis. Differences in CJND, LJND, and shape between each species, and similarity differences between each substrate type, were analyzed using the independent samples t-test when data were normally distributed; the rest of the data were analyzed using Mann-Whitney U-tests. The significance level was set at $P < 0.05$.

3. Results

3.1. Color contrast between turtles and substrates The CJND of the middle stripe and entire carapace of *C. galbinifrons* individuals were significantly lower in the turtles' preferred substrates (broad leaves and bamboo leaves) than in the grass substrate. Their side stripe had the lowest contrast with the bamboo-leaves substrate and contrasted more with the broad-leaves substrate than with the grass substrate. The CJND of the entire carapace of *C. mouhotii* individuals was higher on bare ground than on the grass substrate; however, it did not differ significantly between the grass and broad-leaves substrates. The CJND of their middle stripe was significantly higher in their preferred substrates than in the grass substrate, whereas the CJND of their side stripe was significantly lower in their preferred substrates than in the grass substrate (Figure 5, Tables 1–2).

The LJND of the middle stripe, side stripe, and entire carapace of *C. galbinifrons* individuals had the lowest contrast with the bamboo-leaves substrate; however, the LJND of their

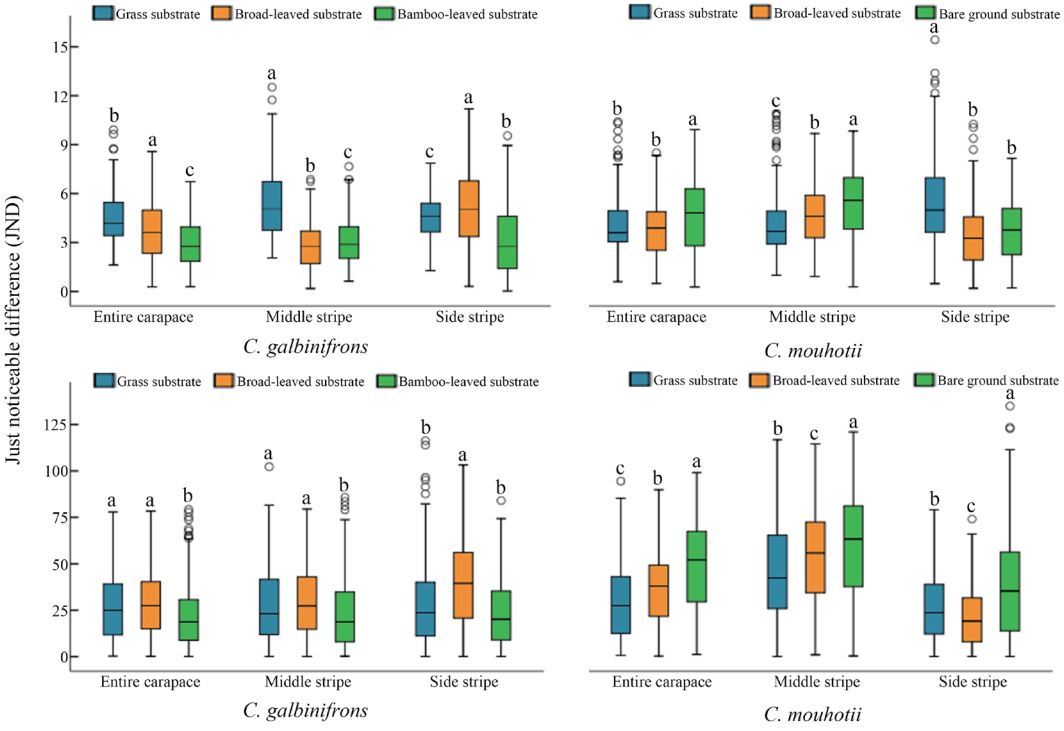

Figure 5 Vision modeling results of chromatic and luminance contrasts. The top panel shows the chromatic contrast values and the bottom panel shows the luminance contrast values. Different lowercase letters above the columns represent significant differences between each species' carapace pattern on different substrates (t-tests or Mann-Whitney U-tests; $P < 0.05$), with letter "o" indicating outliers in the data.

Table 1 Chromatic just-noticeable difference (CJND) and luminance just-noticeable difference (LJND) between both turtle species and the substrates.

	Body part	*Cuora galbinifrons*			*Cuora mouhotii*		
		Grass	Broad leaves	Bamboo leaves	Grass	Broad leaves	Bare ground
CJND	Whole carapace	4.533 ± 0.103	3.768 ± 0.113	2.943 ± 0.088	4.166 ± 0.119	3.825 ± 0.116	4.655 ± 0.141
	Middle stripe	5.457 ± 0.137	2.787 ± 0.087	3.101 ± 0.089	4.259 ± 0.132	4.626 ± 0.117	5.425 ± 0.143
	Side stripe	4.625 ± 0.078	5.190 ± 0.152	3.192 ± 0.140	5.575 ± 0.170	3.497 ± 0.128	3.804 ± 0.118
LJND	Whole carapace	26.978 ± 1.156	29.160 ± 1.129	22.514 ± 1.154	29.779 ± 1.259	36.512 ± 1.209	48.864 ± 1.593
	Middle stripe	26.958 ± 1.228	29.608 ± 1.180	23.824 ± 1.258	44.533 ± 1.649	52.816 ± 1.563	59.638 ± 1.809
	Side stripe	29.306 ± 1.524	39.406 ± 1.478	24.386 ± 1.177	26.689 ± 1.202	21.632 ± 1.056	38.328 ± 1.816

Table 2 Differences in chromatic just-noticeable difference (CJND) and luminance just-noticeable difference (LJND) between stripes of both turtle species and substrates.

Species	Body part	Substrate	CJND			LJND		
			t or *z*	*df*	*P*	*t* or *z*	*df*	*P*
Cuora galbinifrons	Entire carapace	Gr-Br	$t = 4.99$	478	< 0.001	$z = -1.452$		0.147
		Gr-Ba	$t = 11.706$	478	< 0.001	$z = -3.048$		0.002
		Br-Ba	$z = -5.047$		< 0.001	$z = -4.573$		< 0.001
	Middle stripe	Gr-Br	$z = -13.83$		< 0.001	$z = -1.824$		0.068
		Gr-Ba	$z = -12.44$		< 0.001	$z = -2.148$		0.032
		Br-Ba	$t = 2.522$	478	0.012	$z = -4.007$		< 0.001
	Side stripe	Gr-Br	$z = -2.542$		0.011	$z = -5.389$		< 0.001
		Gr-Ba	$t = 8.961$	375.104	< 0.001	$z = -1.948$		0.051
		Br-Ba	$t = 9.689$	478	< 0.001	$z = -7.341$		< 0.001
Cuora mouhotii	Entire carapace	Gr-Br	$z = -1.131$		0.258	$t = 3.857$	478	< 0.001
		Gr-Bg	$z = -2.813$		0.005	$z = -8.471$		< 0.001
		Br-Bg	$t = 4.546$	459.802	< 0.001	$t = 6.177$	445.721	< 0.001
	Middle stripe	Gr-Br	$z = -3.564$		< 0.001	$t = 3.646$	478	< 0.001
		Gr-Bg	$z = -6.977$		< 0.001	$t = 6.171$	478	< 0.001
		Br-Bg	$t = 4.325$	460.457	< 0.001	$t = 2.854$	468.129	0.005
	Side stripe	Gr-Br	$z = -9.072$		< 0.001	$z = -2.982$		0.003
		Gr-Bg	$z = -7.293$		< 0.001	$z = -4.218$		< 0.001
		Br-Bg	$t = 1.766$	478	0.078	$z = -6.594$		< 0.001

Gr = grass substrate, Br = broad-leaves substrate, Ba = bamboo-leaves substrate, Bg = bare ground substrate.

side stripe was higher in the broad-leaves substrate than it was in the grass substrate. The LJND of the middle stripe and the entire carapace of *C. mouhotii* individuals was significantly higher in their preferred substrates than in the grass substrate. However, the LJND of their side stripe was lowest in the broad-leaves substrate and greater on bare ground than in the grass substrate (Figure 5, Tables 1–2).

3.2. Shape similarity between turtles and leaves The entire carapace was the body part that had the lowest similarity between its patterns and the leaves from the three substrate types for both species. For *C. galbinifrons*, the middle stripe had the highest similarity with leaves from the broad-leaves substrate, whereas the side stripe had the highest similarity with leaves from the bamboo-leaves substrate. For *C. mouhotii*,

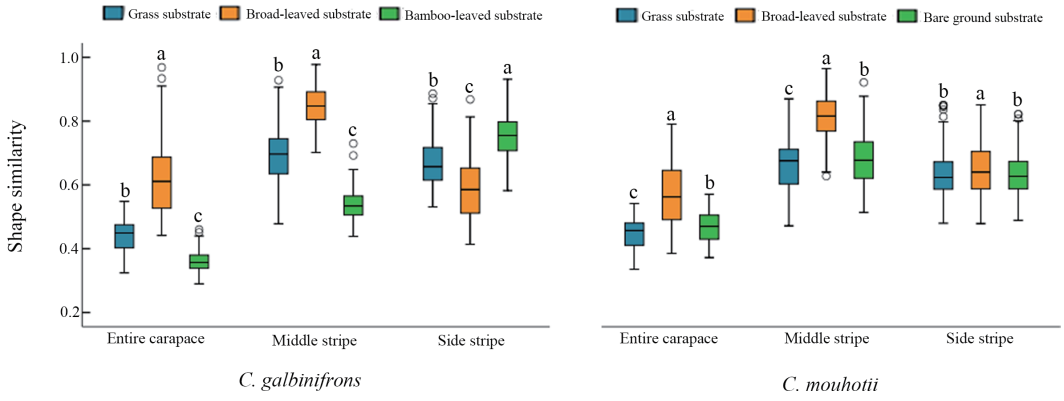

Figure 6 Shape similarity between each species and the leaves in the substrates. Different lowercase letters above the columns represent significant differences between each species' carapace pattern on different substrates (t-tests or Mann-Whitney U-tests; $P < 0.05$), with hollow cycle "o" indicating outliers in the data.

Table 3 Average shape similarity between both turtle species and leaves in the substrates.

Parts	*Cuora galbinifrons*			*Cuora mouhotii*		
	Grass	Broad leaves	Bamboo leaves	Grass	Broad leaves	Bare ground
Entire carapace	0.4374 ± 0.0032	0.6146 ± 0.0069	0.3601 ± 0.0020	0.4453 ± 0.0032	0.5670 ± 0.0063	0.4680 ± 0.0032
Middle stripe	0.6838 ± 0.0055	0.8473 ± 0.0039	0.5369 ± 0.0029	0.6585 ± 0.0054	0.8156 ± 0.0047	0.6795 ± 0.0050
Side stripe	0.6685 ± 0.0047	0.5882 ± 0.0059	0.7524 ± 0.0042	0.6319 ± 0.0046	0.6475 ± 0.0050	0.6332 ± 0.0042

Table 4 Shape similarity between stripes of both turtle species and leaves in different substrates.

Species	Part	Substrate	Shape similarity		
			t or *z*	*df*	*P*
Cuora galbinifrons	Entire carapace	Gr-Br	$z = -17.641$		< 0.001
		Gr-Ba	$z = -14.554$		< 0.001
		Br-Ba	$z = -18.949$		< 0.001
	Middle stripe	Gr-Br	$t = 24.390$	428.649	< 0.001
		Gr-Ba	$z = -15.824$		< 0.001
		Br-Ba	$z = -18.949$		< 0.001
	Side stripe	Gr-Br	$t = 10.650$	453.058	< 0.001
		Gr-Ba	$t = 13.319$	478	< 0.001
		Br-Ba	$z = -15.991$		< 0.001
Cuora mouhotii	Entire carapace	Gr-Br	$z = -13.555$		< 0.001
		Gr-Bg	$z = -4.504$		< 0.001
		Br-Bg	$z = -11.291$		< 0.001
	Middle stripe	Gr-Br	$t = 21.950$	466.933	< 0.001
		Gr-Bg	$z = -2.394$		0.017
		Br-Bg	$t = 19.881$	478	< 0.001
	Side stripe	Gr-Br	$t = 2.287$	475.465	0.023
		Gr-Bg	$t = -0.198$	478	0.843
		Br-Bg	$t = 2.191$	465.695	0.029

Gr = grass substrate, Br = broad-leaves substrate, Ba = bamboo-leaves substrate, Bg = bare ground substrate.

all carapace parts had the highest shape similarity with leaves from the broad-leaves substrate (Figure 6, Tables 3–4).

3.3. Color contrast between the contrast stripe of both turtle species and leaves The entire carapace of each turtle species was the body part with the lowest similarity in shape to the leaves of each substrate, meaning that the shape of the entire carapace differs greatly from that of the leaves. Moreover, the carapaces of both species were divided by color or structure, making it an unsuitable feature to analyze color similarity as a whole. It was thus unnecessary to analyze any color similarity between the turtles' entire carapace and the leaves.

The side stripe of *C. galbinifrons* had a lower CJND with leaves from the bamboo-leaves substrate than with leaves from the broad-leaves and grass substrates. Furthermore, there was no significant difference between the CJND of its side stripe with the grass substrate and that of the broad-leaves substrate. For *C. mouhotii*, the CJND between its middle stripe and leaves from its preferred substrates was higher than that between its middle stripe and the grass substrate.

For *C. galbinifrons*, there were no significant differences in LJND between its side stripes and the leaves from grass and broad-leaves substrates; however, the highest LJND value was observed between its side stripes and leaves from the bamboo-leaves substrate. For *C. mouhotii*, the highest LJND occurred between its middle stripe and leaves from the broad-leaves substrate (Figure 7, Tables 5–6).

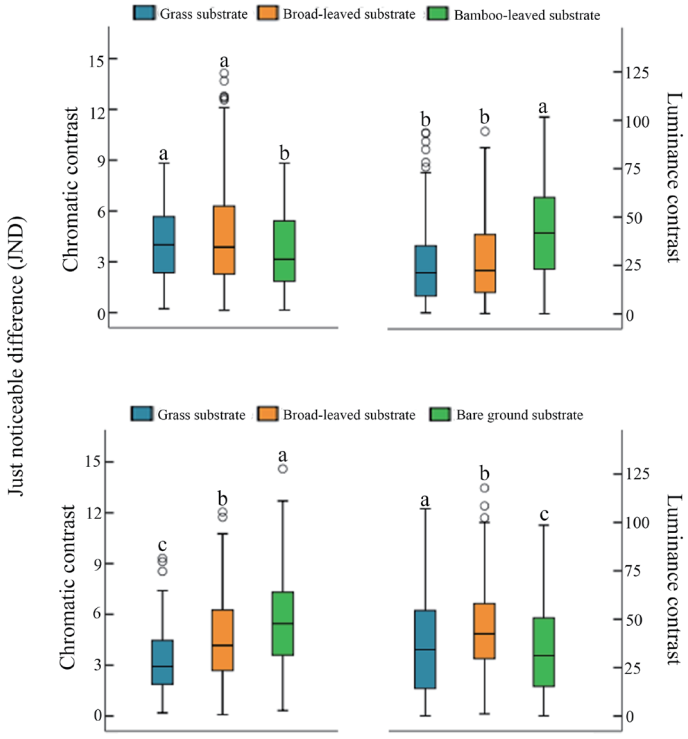

Figure 7 Vision modeling results of chromatic and luminance contrast between each species' stripe and leaves in the substrates. The top panel shows the chromatic just-noticeable difference (CJND) and luminance just-noticeable difference (LJND) values between the side stripe of *C. galbinifrons* and leaves. The bottom panel shows the CJND and LJND values between the middle stripe of *C. mouhotii* and leaves. Different lowercase letters above the columns represent significant differences between each species' carapace pattern on different substrates (t-tests or Mann-Whitney U-tests; $P < 0.05$), with hollow cycle "o" indicating outliers in the data.

Table 5 Chromatic just-noticeable difference (CJND) and luminance just-noticeable difference (LJND) between both species' contrast stripes and leaves in the substrate.

	Cuora galbinifrons			*Cuora mouhotii*		
	Grass	Broad leaves	Bamboo leaves	Grass	Broad leaves	Bare ground
CJND	4.058 ± 0.136	4.592 ± 0.197	3.609 ± 0.136	3.239 ± 0.118	4.472 ± 0.157	5.614 ± 0.179
LJND	24.802 ± 1.306	27.464 ± 1.294	41.539 ± 1.529	36.922 ± 1.653	44.972 ± 1.444	29.035 ± 1.473

Table 6 Differences in chromatic just-noticeable difference (CJND) and luminance just-noticeable difference (LJND) between contrast stripes in each species and leaves in different substrates.

Species	Part	Substrate	CJND			LJND		
			t or *z*	*df*	*P*	*t* or *z*	*df*	*P*
Cuora galbinifrons	Side stripe	Gr-Br	$z = -0.870$		0.384	$z = -1.770$		0.077
		Gr-Ba	$z = -2.388$		0.017	$z = -7.943$		< 0.001
		Br-Ba	$z = -3.072$		0.002	$z = -6.637$		< 0.001
Cuora mouhotii	Middle stripe	Gr-Br	$z = -5.706$		< 0.001	$t = 3.668$	469.568	< 0.001
		Gr-Bg	$z = -9.788$		< 0.001	$z = -3.441$		0.001
		Br-Bg	$t = 4.791$	478	< 0.001	$t = 7.724$	478	< 0.001

Gr = grass substrate, Br = broad-leaves substrate, Ba = bamboo-leaves substrate, Bg = bare ground substrate.

4. Discussion

We assessed the camouflage abilities of two *Cuora* species in broad-leaves, bamboo-leaves, bare ground, and grass substrates; in all substrates, the CJND was within the human visual discrimination threshold. The color contrast analysis showed that the side stripe of *C. mouhotii* individuals on their preferred substrates had the lowest CJND and LJND, whereas their middle stripe had the highest CJND and LJND. Conversely, the middle stripe of *C. galbinifrons* individuals on their preferred substrates had the lowest CJND and LJND, whereas their side stripe had the highest CJND and LJND. LJND is not only affected by color but also by the distance from the observer. The part closer to the observer is brighter, while the part further away is darker (Nakayama and Silverman, 1986), resulting in a greater LJND in the stripes in different planes and the substrates. The high contrast between the middle and side stripes of each species also indicates that the partial stripes are highlighted in the substrate, which increases the disruptive camouflage for each species in their preferred substrate (Bu *et al.*, 2020).

Our previous long-term studies found that both *Cuora* species prefer deciduous leaf substrates (Lian, 2009; Wang, 2007; Xiao *et al.*, 2017) and habitually semi-cover themselves in leaf litter. The middle stripe of *C. galbinifrons* matched the preferred substrates; however, the greater color difference observed between the middle stripes of *C. mouhotii* and the substrate may be because they are not in the same horizontal plane. When they semi-cover themselves, the middle stripes are in the same plane as the substrate, and the difference is expected to be much smaller. Both box turtle species adopt this strategy of covering themselves with leaves to better match their preferred substrate. Previous studies have demonstrated that some birds, crabs, and insects that cover themselves with decorations can reduce their detectability by predators (Holveck *et al.*, 2017; Ruxton and Stevens, 2015). This suggests that potential predators, such as humans, cannot discriminate visually between certain species and the substrate within which they are camouflaged. This strategy adds more noise to object discrimination at an early stage (Stevens and Cuthill, 2006) and makes it more difficult for a predator to group the same features together visually (Espinosa and Cuthill, 2014; Farmer and Taylor, 1980). Our results indicate that both box turtle species may adopt a partial background matching camouflage strategy that reduces the contrast in their predator's visual field, and they achieve this by semi-covering themselves in leaf litter.

The middle stripe on the carapace of *C. mouhotii* and the side stripe on the carapace of *C. galbinifrons* did not match their preferred substrates. A high-contrast pattern that does not match the background can impose a high survival cost (Cott, 1940). In visual search experiments with specific targets, humans quickly learned that an unusual color is a clue that their target is present (Fraser *et al.*, 2007). In many cases, the patterns on animals appear to be stand out from their background, which may make them vulnerable to predation (Cott, 1940; Stevens and Merilaita, 2011). However, in some cases a predator may detect prominent features of a prey individual but does not recognize them as part of an object that is worth examining (Cott, 1940), an effect that has been demonstrated in humans through eye-tracking experiments (Webster *et al.*, 2013). The middle stripe of *C. mouhotii* and the side stripe of *C. galbinifrons* may be interpreted by predators as leaves rather than as recognizable parts of turtles. Studies have shown that internal contrast markers can in fact provide protection, as predators treat them as separate objects by dividing the entire object into visually disconnected parts (Cuthill *et al.*, 2005; Osorio and Srinivasan, 1991; Schaefer and Stobbe, 2006; Stevens *et al.*, 2009).

The middle stripe of both *Cuora* species shared the highest shape and color similarity levels with leaves from the broad-leaves substrate, whereas the side stripe of *C. galbinifrons* had the highest shape similarity with the bamboo-leaves substrate.

When a predator has a short time frame within which to make a decision, the similarity between the species' patterns and the background can provide protection (Hall *et al.*, 2017). Several studies have demonstrated that patterned objects on a patterned background result in the slowest identification by predators. When objects are very similar in appearance, a predator's tracking performance is impeded (Feria, 2012; Hall *et al.*, 2017; Howe and Holcombe, 2012). The most promising evidence comes from a study on the four-eyed turtle (*Sacalia quadriocellata*), which showed that higher shape similarity resulted in a higher recognition error rate, indicating more effective camouflage (Xiao *et al.*, 2021). Interestingly, the middle stripe of *C. mouhotii* had greater chromatic contrast with their preferred substrates than with the grass substrate, meaning that the middle stripe in the preferred substrate can be easily detected by a predator. Despite contrasting in color with their preferred substrates, the middle stripe of *C. mouhotii* and the side stripe of *C. galbinifrons* did have a similar shape to that of the leaves. Previous studies have found that masquerading animals are usually similar in size, color, and shape to their model objects or species (Karpestam *et al.*, 2014; Panetta *et al.*, 2017; Skelhorn *et al.*, 2010b; Suzuki and Sakurai, 2015). Furthermore, they frequently select the appropriate microhabitats to increase their similarity to the model (Nafus *et al.*, 2015; Skelhorn and Ruxton, 2013; Xiao *et al.*, 2021). For example, the caterpillars of the early thorn moth (*Selenia dentaria*) masquerade better when the density of a similar model increases (Skelhorn *et al.*, 2011). The common potoo bird selects perches on tree stumps that will effectively enhance their masquerading effect (Cestari *et al.*, 2018). We believe that both *Cuora* species adopt a partial masquerading strategy, rather than using their entire carapace as a means of camouflage. This allows them to increase their masquerading efficiency in varied substrates.

Our results showed that area accounted for the largest amount of similarity; however, perimeter, AR, S, C, and R (except for the roundness of the side stripe for *C. galbinifrons*) had high similarity among the four substrate types. Shape and size also have an important influence on the efficiency of camouflage through masquerading. Chiao and Hanlon (2001) found that cuttlefish (*Sepia pharaonis*) adjust their body markings based on the area of the objects against which they need to camouflage. Skelhorn and Ruxton (2013) found that the size of the moth larvae determines the efficiency of masquerading; however, Suzuki and Sakurai (2015) confirmed that, despite having the same size and area, caterpillars can affect their masquerading efficiency by changing their shape.

In the present study, area, perimeter, and the other four shape descriptors all had high similarity across habitats. This suggests that the similarity in shape between the turtle species' contrast stripes and the leaves of the habitat types is also a crucial aspect of their masquerading. Furthermore, human trichromatic vision is more sensitive to visual signals than the dichromatic vision of some other mammals (Caves *et al.*, 2018; Troscianko *et al.*, 2017). Therefore, both turtle species would be camouflaged against the vision of these non-human mammals due to their shape similarity.

In conclusion, this is the first study to demonstrate that partial masquerading and background matching have a significant impact on turtles' camouflage efficiency and habitat choice. *C. galbinifrons* and *C. mouhotii* both exhibited two types of camouflage strategies in their preferred substrates; partial masquerading was apparent when the entire carapace was exposed, and background matching was evident when the turtles were semi-covered in leaf litter. This study introduces a novel approach to camouflage studies that focuses on the influence of shape and color of parts of the body to reduce detectability or recognition. This study evaluated the camouflage effect for human vision, but did not analyze its effect on the turtles' actual predators in the wild. Future research should explore the effects of turtles' camouflage strategies on real predators in the wild.

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References

Bu R., Xiao F., Lovell P. G., Ye Z., Shi H. 2020. Structural and colored disruption as camouflage strategies in two sympatric Asian box turtle species (*Cuora* spp.). Glob Ecol Conserv, 24: e01361

Caves E. M., Brandley N. C., Johnsen S. 2018. Visual acuity and the evolution of signals. Trends Ecol Evol, 33: 358–372

Cestari C., Goncalves C. D., Sazima I. 2018. Use flexibility of perch types by the branch-camouflaged Common Potoo (*Nyctibius griseus*): Why this bird may occasionally dare to perch on artificial substrates. Wilson J Ornithol, 130: 191–199

Chiao C. C., Hanlon R. T. 2001. Cuttlefish cue visually on area — Not shape or aspect ratio — of light objects in the substrate to produce disruptive body patterns for camouflage. Biol Bull, 201: 269–270

Cott H. B. 1940. Adaptive Coloration in Animals. London, UK: Methuen and Co. Ltd

Cuthill I. C., Stevens M., Sheppard J., Maddocks T., Parraga C. A., Troscianko T. 2005. Disruptive coloration and background pattern matching. Nature, 434: 72–77

Endler J. A. 1978. A predator's view of animal color patterns. Evol Biol, 11: 319–364

Endler J. A. 1981. An overview of the relationships between mimicry and crypsis. Biol J Linn Soc, 16: 25–31

Espinosa I., Cuthill I. C. 2014. Disruptive colouration and perceptual

grouping. PLoS ONE, 9: e87153
Farmer E. W., Taylor R. M. 1980. Visual search through color displays: Effects of target-background similarity and background uniformity. Percept Psycho, 27: 267–272
Feria C. S. 2012. The effects of distractors in multiple object tracking are modulated by the similarity of distractor and target features. Perception, 41: 287–304
Ferreira T., Rasband W. 2011. The ImageJ User Guide. Bethesda, MD, USA: National Institutes of Health, http://rsb.info.nih.gov/ij/docs/user-guide.pdf
Gómez J., Ramo C., Troscianko J. Stevens M., Castro M., Pérez-Hurtado A., Liñán-Cembrano G., Amat J. A. 2018. Individual egg camouflage is influenced by microhabitat selection and use of nest materials in ground-nesting birds. Behav Ecol Sociobiol, 72: 142
Hall J. R., Baddeley R. J., Scottsamuel N. E., Shohet A., Cuthill I. C. 2017. Camouflaging moving objects: Crypsis And masquerade. Behav Ecol, 28: 1248–1255
Holveck M. J., Grégoire A., Guerreiro R., Staszewski V., Boulinier T., Gomez D., Doutrelant C. 2017. Kittiwake eggs viewed by conspecifics and predators: Implications for colour signal evolution. Biol J Linn Soc, 122: 301–312
Howe P. D., Holcombe A. O. 2012. The effect of visual distinctiveness on multiple object tracking performance. Front Psychol, 3: 307
Kang C. K., Moon J. Y., Lee S. I., Jablonski P. G. 2012. Camouflage through an active choice of a resting spot and body orientation in moths. J Evolution Biol, 25: 1695–1702
Karpestam E., Merilaita S., Forsman A. 2014. Body size influences differently the detectabilities of colour morphs of cryptic prey. Biol J Linn Soc, 113: 112–122
Lin J. Y., Jin L., Hu S., Katsavounidis I., Li Z., Aaron A., Kuo C. C. J. 2015. Experimental design and analysis of JND test on coded image/video. Applications of Digital Image Processing XXXVIII. International Society for Optics and Photonics
Lovell P. G., Ruxton G. D., Langridge K. V., Spencer K. A. 2013. Egg-laying substrate selection for optimal camouflage by quail. Curr Biol, 23: 260–264
Marshall K. L. A., Philpot K. E., Stevens M. 2016. Microhabitat choice in island lizards enhances camouflage against avian predators. Sci Rep, 6: 19815
McGaugh E. S. 2008. Color variation among habitat types in the spiny softshell turtles (Trionychidae: Apalone) of Cuatrociénegas, Coahuila, Mexico. J Herpetol, 42: 347–353
Merilaita S., Lind J. 2005. Background-matching and disruptive coloration, and the evolution of cryptic coloration. P Roy Soc Lond B Bio, 272: 665–670
Nafus M. G., Germano J. M., Perry J. A., Todd B. D., Walsh A., Swaisgood R. R. 2015. Hiding in plain sight: A study on camouflage and habitat selection in a slow-moving desert herbivore. Behav Ecol, 26: 1389–1394
Nakayama K., Silverman G. H., 1986. Serial and parallel processing of visual feature conjunctions. Nature, 320: 264–265
Nokelainen O., Brito J. C., Scott-Samuel N. E., Valkonen J. K., Boratyński Z. 2020. Camouflage accuracy in Sahara — Sahel desert rodents. J Anim Ecol, 89: 1658–1669
Panetta D., Buresch K., Hanlon R. T. 2017. Dynamic masquerade with morphing three-dimensional skin in cuttlefish. Biol Letters, 13: 20170070
Price N., Green S., Troscianko J., Tregenza T., Stevens M. 2019. Background matching and disruptive coloration as habitat-specific strategies for camouflage. Sci Rep, 9: 7840
Rowe J. W., Miller B. J., Stuart M. A., Snyder C., Tucker J. K., Clark D. L., Wittle L. W., Lamer J. T. 2014. Substrate color-induced melanization in eight turtle species from four chelonian groups. Zoology, 117: 245–252
Ruxton G. D., Sherratt T. N., Speed M. P. 2004. Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals, and Mimicry. Oxford: Oxford University Press, 23–25
Ruxton G. D., Stevens M. 2015. The evolutionary ecology of decorating behaviour. Biol Letters, 11: 20150325
Samal A., Seth S., Cueto K. 2004. A feature-based approach to conflation of geospatial sources. Int J Geogr Inf Sci, 18: 459–489
Schaefer H. M., Stobbe N. 2006. Disruptive coloration provides camouflage independent of background matching. P Roy Soc Lond B Bio, 273: 2427–2432
Skelhorn J., Rowland H. M., Speed M. P., Ruxton G. D. 2010a. Masquerade: Camouflage without crypsis. Science, 327: 51
Skelhorn J., Rowland H. M., Speed M. P., Wert L. D. W., Quinn L., Delf J., Ruxton G. D. 2010b. Size-dependent misclassification of masquerading prey. Behav Ecol, 21: 1344–1348
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. P Natl Acad Sci USA, 108: 6532–6536
Skelhorn J., Ruxton G. D. 2011. Mimicking multiple models: Polyphenetic masqueraders gain additional benefits from crypsis. Behav Ecol, 22: 60–65
Skelhorn J., Ruxton G. D. 2013. Size-dependent microhabitat selection by masquerading prey. Behav Ecol, 24: 89–97
Stevens M., Cuthill I. C. 2006. Disruptive coloration, crypsis and edge detection in early visual processing. P Roy Soc Lond B Bio, 273: 2141–2147
Stevens M., Parraga C. A., Cuthill I. C., Partridge J. C, Troscianko T. 2007. Using digital photography to study animal coloration. Biol J Linn Soc, 90: 211–237
Stevens M., Winney I. S., Cantor A., Graham J. 2009. Outline and surface disruption in animal camouflage. Philos T R Soc B, 276: 781–786
Stevens M., Merilaita S. 2011. Animal Camouflage: Mechanisms and Function. Cambridge: Cambridge University Press
Stevens M., Searle W. T., Seymour J. E., Marshall K. L., Ruxton G. D. 2011. Motion dazzle and camouflage as distinct anti-predator defenses. BMC Biol, 9: 81
Stevens M., Broderick A. C., Godley B. J., Lown A. E., Troscianko J., Weber N., Weber S. B. 2015. Phenotype-environment matching in sand fleas. Biol Letters, 11: 20150494
Stevens M., Ruxton G. D. 2018. The key role of behaviour in animal camouflage. Biol Rev, 94: 116–134
Suzuki T. N., Sakurai R. 2015. Bent posture improves the protective value of bird dropping masquerading by caterpillars. Anim Behav, 105: 79–84
Troscianko J., Stevens M. 2015. Image calibration and analysis toolbox — A free software suite for objectively measuring reflectance, colour and pattern. Methods in Ecol Evol, 6: 1320–1331
Troscianko J., Wilson-Aggarwal J., Stevens M., Spottiswoode C. N. 2016. Camouflage predicts survival in ground-nesting birds. Sci Rep, 6: 19966
Troscianko J., Wilson-Aggarwal J., Griffiths D., Spottiswoode C. N., Stevens M. 2017. Relative advantages of dichromatic and trichromatic color vision in camouflage breaking. Behav Ecol, 28: 556–564
van den Berg C. P., Troscianko J., Endler J. A., Marshall N. J., Cheney K. L. 2020. Quantitative colour pattern analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature. Methods in Ecol Evol, 11: 316–332
Vorobyev M., Osorio D. 1998. Receptor noise as a determinant of colour thresholds. P Roy Soc Lond B Bio, 265: 351–358
Webster R. J., Hassall C., Herdman C. M., Godin J. J., Sherratt T. N. 2013. Disruptive camouflage impairs object recognition. Biol Letters, 9: 20130501
Willemsen R. E., Hailey A. 2003. Sexual dimorphism of body size and shell shape in European tortoises. J Zool, 260: 353–365
Wyszecki G., Stiles W. S. 1982. Color Science: Concepts and Methods, Quantitative Data and Formulae. New York: Wiley-Interscience
Xiao F., Yang C., Shi H., Wang J., Sun L., Lin L. 2016. Background matching

and camouflage efficiency predict population density in four-eyed turtle (*Sacalia quadriocellata*). Behav Process, 131: 40–46
Xiao F., Wang J., Shi H., Long Z., Lin L., Wang W. 2017. Ecomorphological correlates of microhabitat selection in two sympatric Asian Box Turtle species (Geoemydidae: *Cuora*). Can J Zool, 95: 753–758
Xiao F., Hong Z., Wang J., Butterfield T., Shi H. 2020. The relationship between shell morphology and crevice size affecting retreat selection of the keeled box turtle (*Cuora mouhotii*). Asian Herpetol Res, 11: 342–349
Xiao F., Bu R., Shi H. 2021. Quantifying shape similarity between prey and uninteresting models to study animal masquerade. Behaviour, 158: 1–18

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