Journal of Zoology. Print ISSN 0952-8369

Cellular basis of anti-predator adaptation in a lizard with autotomizable blue tail against specific predators with different colour vision

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Keywords
anti-predator adaptation; autotomy; colour vision; iridophore; transmission electron microscopy; TEM; UV; *Plestiodon latiscutatus*.

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Editor: Mark-Oliver Rödel

Received 20 April 2015; revised 29 April 2016; accepted 29 April 2016

doi:10.1111/jzo.12361

Abstract

Juveniles of numerous lizard species have a vividly blue-coloured tail that likely serves to deflect predator attacks toward the autotomizable tail rather than the lizard's body. The shades of blue colour in the tails of juvenile *Plestiodon latiscutatus* lizards vary across populations, most notably among those island populations with different predator assemblages. Here, we determine if this intraspecific variation is associated with the differences in colour vision capabilities of lizard predator species. If associated, it would be evidence for local adaptation of tail colour phenotype – natural selection is maximizing the conspicuousness of the tail to the dominant predator species to increase the chance of successfully deflecting attacks. We also use transmission electron microscopy (TEM) to determine the proximate cellular mechanisms that produce the shades of blue in different populations. We revealed that lizard tails with vivid blue reflectance evolved in communities with either weasel or snake predators, two groups of animals with the ability to detect blue wavelengths. However, lizard tail UV reflectance was much higher in populations with only snake predators; that snakes can detect UV, yet weasels cannot, suggests that high UV reflectance is an adaptation to increase tail conspicuousness specifically to snake predators. Finally, a cryptic brown tail evolved independently on the islands where birds are the primary lizard predator. We suggest that because birds have keen visual acuity; a brown, camouflaged phenotype is more advantageous. We also determined through TEM that the thickness of light reflecting platelets in iridophores, and densities of iridophores and xanthophores, predicted the wavelengths and intensity of light reflected by the lizard tail. For example, blue coloration was produced by selective reflection of short wavelengths of light by the thin light reflecting platelets of the iridophore. Greater iridophore density increased light reflectance, while greater xanthophore density decreased light reflectance.

Introduction

The colour pattern of animals is a traditional yet important topic in modern evolutionary biology. It provides us with rich opportunities to study functionally convergent characteristics among distantly related lineages, independently acquired parallel evolution of traits in related species, and intraspecific divergence of traits among ecologically different populations of the same species (e.g. McKinnon & Pierotti, 2010; Kronforst *et al.*, 2012; Olsson, Stuart-Fox & Ballen, 2013). Elucidating the spatial and vertical arrangements of pigment cells within skin tissue is fundamental to the understanding of the genetic and developmental mechanisms that control colour pattern formation (e.g. Kuriyama *et al.*, 2013). However, few histological studies of pigment cells have been conducted to determine the pigmentation mechanisms of particular colour patterns among different species or among different populations of the same species (Morrison, 1995; Morrison, Rand & Frost-Mason, 1995; Saenko *et al.*, 2013).

Lizards with blue-coloured tails are notable candidates for such studies because blue tails have independently evolved several times amongst several different lizard families (Pianka & Vitt, 2003). A conspicuous blue tail, in conjunction with a contrasting striped body pattern, enhances escape from predation by distracting the predator's attention away from the more vulnerable parts of the body (head and trunk) at the cost of losing their autotomizable tails that will later regenerate (e.g. Clark & Hall, 1970; Cooper & Vitt, 1985; Castilla *et al.*,

1999; Bateman & Fleming, 2009). Laboratory and field-based studies have widely recognized that the predator distraction is the primary natural selection pressure that drives repeated independent evolution of a blue tail in lizards (Hawlena *et al.*, 2006; Hawlena, 2009; Watson *et al.*, 2012; Bateman, Fleming & Rolek, 2014; Ortega, López & Martín, 2014). Nevertheless, these studies usually used qualitative classification of colour to define 'blue', and very few integrative studies have been performed to understand the proximate factors producing different or similar colouration amongst different populations by considering variation in light reflectance and pigment cell architectures; nor did these studies specifically integrate information about variation in predators' ability to perceive specific colour (Stuart-Fox, Moussalli & Whiting, 2008). Moreover, very few studies have focused on the cellular basis of different colouration, although it is essential for exploring the genetic mechanisms of colour pattern formation. We have already found that blue colouration is produced by the multiple thin-layer interference of thinner reflecting platelets in iridophore pigment cells (Kuriyama *et al.*, 2006).

Populations of the Okada's five-lined skink, *Plestiodon latiscutatus* (formerly *P. okadae*; Okamoto *et al.*, 2006) are notable for the geographical variation in juvenile tail colouration on the Japanese Izu Peninsula the major Izu Islands; moreover, these different phenotypes are associated with the islands' predator assemblages that vary in the relative abundance of weasels, snakes, and birds (Fig. 1; Brandley, Kuriyama & Hasegawa, 2014). In the northern Izu Islands and Izu Peninsula (Fig. 1) where diurnal snakes (Japanese four-lined snake *Elaphe quadrivirgata*) and carnivorous mammals (Japanese weasel *Mustela itatsi*) are the major predators (Hasegawa, 1994), juvenile lizards exhibit yellowish white-striped body and blue tail, although the shade of blue may vary across populations. However, juvenile *P. latiscutatus* from the southern Izu Islands of Japan, where birds (especially the Izu Islands thrush *Turdus celaenops*) are the major predators (Hasegawa, 1990), are almost uniformly brown except for a blue tip of the tail (Fig. 1). Phylogenetic evidence has shown that either a vivid blue or drab brown tail colouration has independently evolved at least twice in *P. latiscutatus*, thereby supporting the hypothesis that tail colouration has evolved via strong natural selection (Brandley *et al.*, 2014).

In this study, we determine the geographical variation in the light reflectance profile of *P. latiscutatus* tails from populations that vary in phenotype and predator assemblage (Fig. 1). We then compare this variation in tail colour to the different wavelengths visible to different species of sympatric predators on each island. Finally, to attain a mechanistic understanding of tail colour adaptation, we examined histological characteristics of pigment cells responsible for variation in the light reflectance profiles of tail colour across lizard populations. On the basis of our previous finding that blue colouration is produced by the multiple thin-layer interference of thinner reflecting platelets in iridophores (Kuriyama *et al.*, 2006), we discuss whether positional changes in iridophores with thin or thick light-reflecting platelets along the longitudinal axis of the tail correspond to geographic variation in the extent and brightness of blue tail colouration. Additionally, we review and discuss potential intracellular mechanisms controlling the thickness of light-reflecting platelets as a foundation for studying their genetic mechanisms.

Materials and methods

Study system

We collected data from a total of three island populations that each differs in the abundance of lizard predators: Kozu island (snake predators only), Hachijokojima island (birds only), and the mainland Izu Peninsula (primarily the weasel). We examined only lizards with complete tails not previously autotomized. The proportion of the length of the blue coloured portion to the total tail length was the shortest in Hachijokojima, the medium in Kozu and the longest in Izu Peninsula (Fig. 1; Brandley *et al.*, 2014). Light reflectance of tail colouration was measured for a total of 15 juvenile lizards

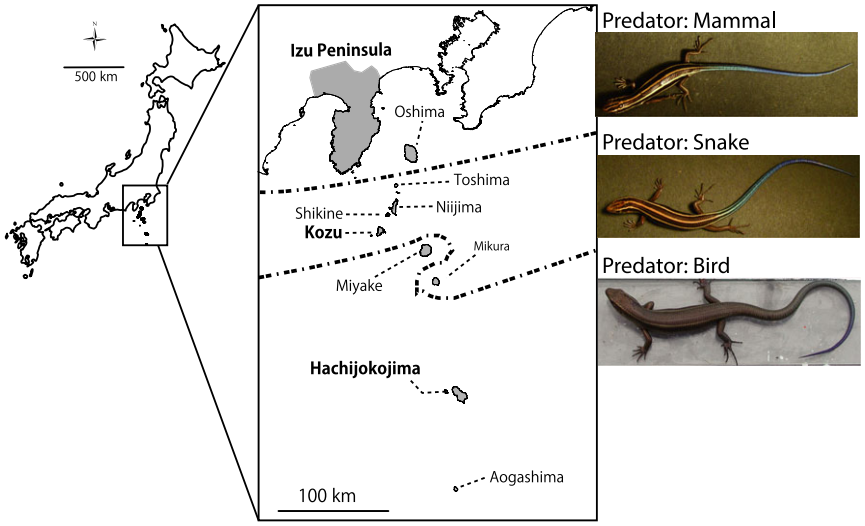


Figure 1 Map of the study area and geographical colour variation in *Plestiodon latiscutatus* with its main sympatric predator. Geographic range of *P. latiscutatus* in the mainland is shown in gray (after Okamoto *et al.*, 2006).

younger than 1-year post-hatching, with typical juvenile body colouration and unbroken tail [Peninsula, $n = 3$, snout-vent length (SVL), $\bar{x} \pm$ SD (mm) = 42.7 ± 4.0, TL (tail length) 70.3 ± 8.1; Kozu, $n = 7$, SVL 46.6 ± 2.4, TL 80.6 ± 4.8; Hachijokojima, $n = 5$, SVL 46.6 ± 5.9, TL 76.0 ± 10.8]. We compared the light reflectance profile to the vertical architecture of pigment cell in three additional individuals per each population ($n = 3$ for each population). We measured light reflectance of the dorsal surface of tail scales and observed vertical architecture of pigment cells at 10 different positions from the base (T1) just behind the hind leg to the tip of the tail (T10) (Fig. 2a).

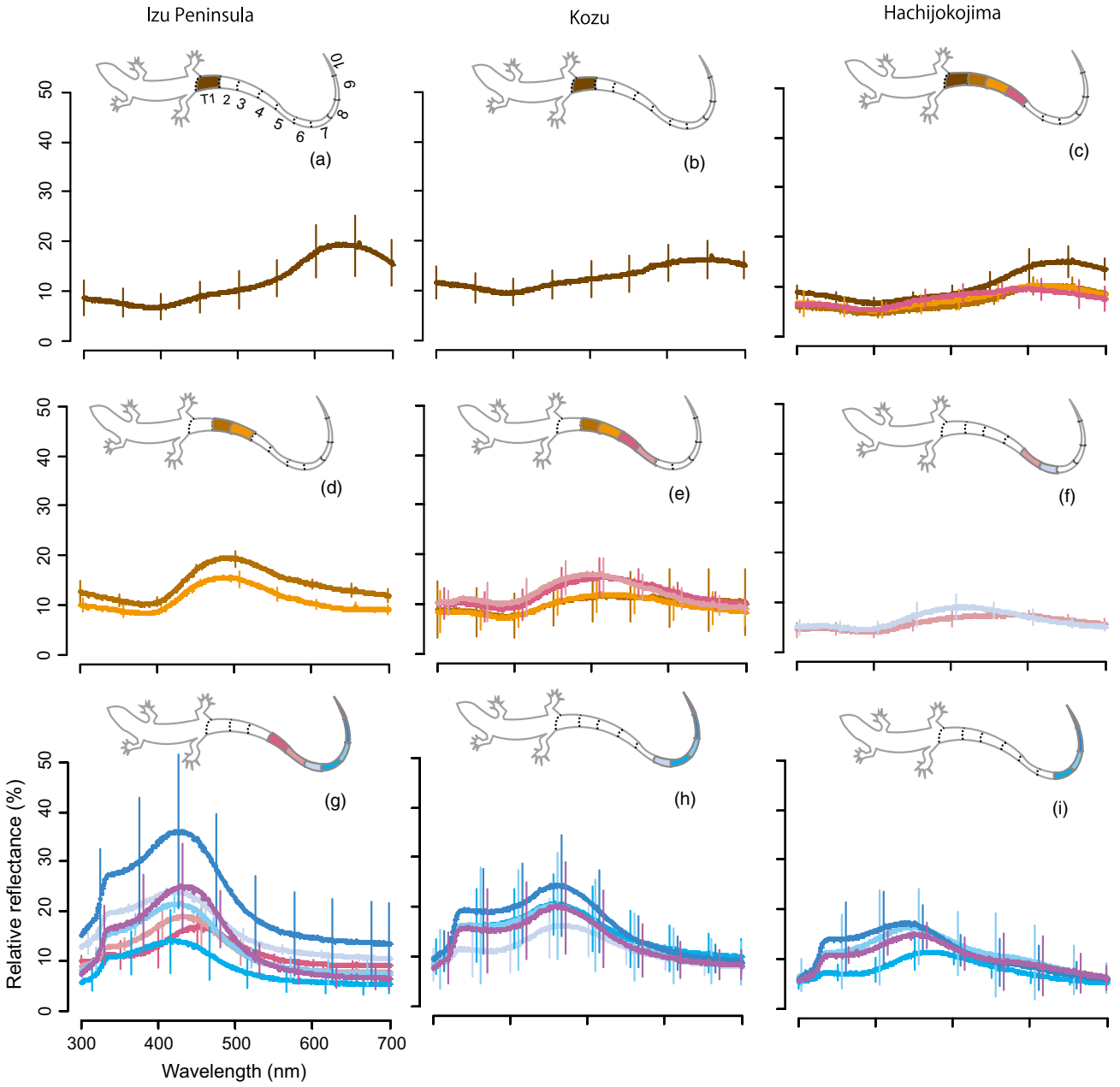


Figure 2 The mean and standard deviation of light reflectance profiles of the tails of juvenile *Plestiodon latiscutatus* from different populations measured at ten equal positions from the base to the tip of the tail. The colour profiles (wavelengths) coincide with the section of the tail indicated by the lizard schematic drawing above. (a)–(c) The light reflectance profile with peak wavelength of *c.* 650 nm reflected brown colouration. (d)–(f) Green coloured skin reflected the light with peak wavelength of *c.* 550 nm. (g)–(i) Blue coloured tail reflected light with two peaks of wavelength both at *c.* 450 and 350 nm. (a), (d) and (g) show reflectance of Izu Peninsula population. (b), (e) and (h) show reflectance of Kozu. (c), (f) and (i) show reflectance of Hachijokojima populations.

Light reflectance profiles

Following euthanizing, each lizard by inhalation of 100% diethyl ether, the light reflectance profiles of tail scales were measured at 10 equal positions from the basal tail to the tip at wavelengths ranging from ultraviolet (300 nm) to red (700 nm) with a USB-2000 spectrometer and DT-MINI-2-GS light source (Ocean Optics, Inc., Dunedin, FL, USA) in a completely dark room. The measurements were taken at a 90° angle to the samples. For standardization of the measurements, we used a WS-1 diffuse white standard (Ocean Optics, Inc.).

Histological examination

Cellular mechanisms producing different tail colourations were studied by examining spatial variation in the vertical architectures of pigment cells for the three populations with different predator regimes, using transmission electron microscopy (TEM). We measured the relative densities of xanthophores, iridophores and melanophores. The thickness and number of light reflecting platelets in iridophores were also measured because Kuriyama *et al.* (2006) showed that these variables played important roles in determining the peak wavelength and the relative reflectance of the skin.

Nine *P. latiscutatus* juveniles were euthanized, using 100% diethyl ether and skin tissues were dissected for sectioning and TEM observation, using a pair of scissors and two stainless-steel knives. Pieces of skin tissue ~2 mm^3 were fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) overnight at 4°C. The tissues were rinsed with 0.25 M sucrose in 0.1 M phosphate buffer (pH 7.3) and were subsequently fixed in 0.1 M buffer containing 1% osmium tetroxide for 2 h at room temperature. The tissues were then sufficiently rinsed with the same buffer solution and dehydrated by increasing concentrations of ethanol. The specimens were embedded in epoxy resin (Quetol 651; Okenshoji Co., Ltd., Tokyo, Japan). Ultra-thin sections were cut with a diamond knife on a Porter Blum MT-1 ultra-microtome (Ivan Sorvall, Inc., Norwalk, CT, USA). Ultra-thin sections were double stained with uranyl acetate and alkaline lead citrate and then viewed with a JEM-1210 transmission electron microscope at 80 kV.

The area of each pigment cell that occupied the dermal skin, 10 μm in width × the depth of the dermal layer, was measured with ImageJ using three electron micrographs taken at 4000× magnification per tail parts of individuals. The thickness of the reflecting platelets and cytoplasmic spaces between upper and lower platelets in the iridophores was measured to estimate the peak wavelength of reflectance from three electron micrographs for each part of the tail taken at 15 000× magnifications (details in Kuriyama *et al.*, 2006). The peak reflecting wavelength (λ_{max}) was predicted, using the following formula, originally proposed by Huxley (1968) and Land (1972):

$$\lambda_{max} = 2(n_a d_a + n_b d_b), \quad (1)$$

where n_a and n_b are the refractive indices of the platelet (n_a = 1.83) and cytoplasm (n_b = 1.37), and d_a and d_b are the actual thickness of the platelet and cytoplasmic space. To correlate the relative amount of light reflected by the tail skin with the microstructure of iridophores, we counted the number of platelets along the vertical axis of iridophores at 10 points (with 1-μm intervals between points) along a horizontal transect; we reviewed three micrographs of each part of the tail at 4000× magnification. All animal work was reviewed and approved by the Toho University Bioethics and Animal Ethics Committee (approval number 12-51-242).

Predator colour vision

To evaluate how predators perceived and recognized prey using visual signals, we compared the wavelengths reflected by the lizard tail skin to the colour wavelengths detected by different mammal, snake, and bird predators. Vertebrates generally recognize colour signals using photoreceptor cells, e.g. cone cells in the retina of the eye. The cone cell type determines the ability of the animal to discriminate colour, because one cone cell type absorbs a specific peak wavelength of light. The relative abundance of cone cell types varies from only two types in terrestrial vertebrate predators to as many as four types in other predators (Yokoyama, 2000; Osorio & Vorobyev, 2008). We were unable to collect cone-cell data for the specific weasel, snake, and bird predators of *P. latiscutatus.* We therefore used published cone cell data sets for species that are phylogenetically related and ecologically similar to these predators. As a proxy for the Japanese weasel, we used data from another mustelid mammal, the ferret (*Mustela putorius furo*, Calderone & Jacobs, 2003; Table 1). We used data from the diurnal garter snake (*Thamnophis sirtalis*, Sillman *et al.*, 1997), and diurnal insectivorous bird (blackbird *Turdus merula*, Hart *et al.*, 2000) as proxies for the Izu Island snake (*Elaphe quadrivirgata*) and bird (*Turdus caelenops*) predators (Table 1).

Statistical analysis

To clarify factors that affected the peak wavelength and peak reflectance of the skin-reflectance profile, we used a generalized linear mixed model with Poison distribution and log link function (lme4 package in R ver. 3.0.1; R Core Team, 2013). The full model for peak wavelength included five independent variables: thickness of iridophore platelets; thickness of

Table 1 Wavelength of maximum spectral sensitivity (λ_{max}) of proxy predators

Group	Species	Reference	UVS	SWS	MWS	LWS
Mammal	*Mustela putorius furo*	Calderone & Jacobs, 2003	–	430	558	–
Snake	*Thamnophis sirtalis*	Sillman *et al.*, 1997	360	482	554	–
Bird	*Turdus melura*	Hart *et al.*, 2000	373	453	504	557

Table 2 Morphological characteristics of reflecting platelets in iridophores and densities of pigment cells in the three island populations of *Plestiodon latiscutatus*. Each value indicated mean (min-max)

Population	Tail Position	Colour	Plate width (nm)	Cytoplasmic (nm)	Predicted wavelength (nm)	Number of Platelet	Density of xanthophore (μm^2/10 μm)	Density of iridophore (μm^2/10 μm)	Density of melanophore (μm^2/10 μm)
Peninsula	T1	Brown	82.0 (76.8–87.6)	111.6 (98.2–122.4)	605.7	6.3 (4.5–7.7)	16.2 (14.3–17.2)	20.4 (12.7–27.1)	13.5 (10.1–15.3)
	T2	Green	57.0 (52.1–63.8)	91.0 (77.2–103.7)	457.9	14.8 (10.8–17.6)	24.2 (17.7–34.1)	35.7 (29.6–42.5)	17.7 (13.2–23.7)
	T3	Green	43.7 (35.6–57.9)	100.9 (88.2–112.3)	436.4	11.6 (7.7–13.7)	17.3 (12.3–22.1)	26.3 (22.9–32.0)	21.0 (17.2–24.4)
	T4	Blue	40.5 (41.3–47.2)	102.2 (81.5–117.6)	438.4	12.4 (10.0–14.4)	5.9 (0.9–9.0)	35.8 (20.3–57.3)	25.1 (21.7–29.2)
	T5	Blue	33.2 (33.5–46.3)	99.5 (94.3–105.8)	421.1	14.2 (11.3–20.0)	0	29.4 (19.2–49.2)	32.4 (22.3–51.6)
	T6	Blue	33.2 (32.0–34.0)	102.1 (93.5–111.6)	401.0	16.3 (11.1–19.8)	0	37.1 (32.2–40.2)	19.9 (13.2–30.7)
	T7	Blue	34.6 (29.4–43.6)	97.7 (84.9–110.5)	392.9	13.8 (12.1–16.5)	0	25.5 (23.8–26.9)	22.8 (15.1–35.0)
	T8	Blue	37.0 (30.1–42.9)	104.8 (97.8–112.5)	422.6	15.0 (12.7–16.5)	0	34.8 (23.5–41.5)	14.6 (5.3–23.7)
	T9	Blue	34.9 (32.2–39.8)	94.4 (87.9–104.4)	386.6	15.7 (10.2–21.3)	0	30.8 (12.4–54.2)	22.0 (10.3 -34.7)
	T10	Blue	38.8 (35.0–44.0)	98.7 (78.1–117.6)	412.3	18.7 (16.2–22.6)	0	43.0 (31.3–55.0)	18.1 (4.8–35.1)
Kozu	T1	Brown	65.7 (58.0–75.8)	93.5 (88.6–97.9)	496.7	9.1 (6.6–13.6)	11.3 (7.4–15.2)	26.3 (22.3–32.3)	20.1 (8.2–35.0)
	T2	Green	51.9 (40.4–61.1)	85.9 (77.4–100.0)	425.2	12.3 (8.7–15.9)	14.7 (8.7–18.4)	26.7 (18.4–31.5)	11.9 (5.8–18.9)
	T3	Green	53.0 (50.2–54.7)	95.3 (89.1–100.0)	454.9	10.7 (8.9–12.2)	14.2 (12.0–16.4)	26.2 (20.8–33.8)	29.9 (9.3–44.3)
	T4	Green	54.3 (48.9–57.9)	101.0 (97.4–104.7)	475.4	11.3 (9.7–13.9)	8.1 (4.2–10.8)	25.7 (18.1–34.7)	28.2 (23.2–36.9)
	T5	Green	52.7 (43.6–62.6)	97.0 (90.3–102.4)	458.6	13.9 (10.4–16.0)	9.7 (6.3–13.2)	33.9 (24.1–41.1)	31.8 (21.8–43.5)
	T6	Blue	47.2 (38.3–57.4)	99.5 (88.9–109.5)	445.6	14.9 (13.0–16.9)	4.7 (2.6–8.9)	43.1 (34.7–50.7)	20.8 (12.6–35.1)
	T7	Blue	46.0 (42.6–49.7)	89.8 (81.7–99.7)	414.3	14.0 (11.6–17.4)	0.6 (0–1.8)	32.2 (19.7–49.4)	29.2 (23.9–27.8)
	T8	Blue	41.9 (26.8–58.1)	96.3 (94.1–98.5)	417.3	17.9 (16.4–18.9)	2.0 (0–6.1)	49.1 (46.2–54.2)	25.8 (23.9–27.8)
	T9	Blue	42.5 (31.8–54.5)	89.9 (85.0–93.5)	401.7	18.3 (16.0–21.4)	0.6 (0–1.8)	42.1 (31.4–55.0)	16.7 (9.7–21.6)
	T10	Blue	32.8 (28.5–36.3)	94.3 (88.8–101.0)	378.4	19.6 (11.8–32.2)	0	48.6 (24.0–68.6)	20.4 (15.5–29.1)
Hachijokojima	T1	Brown	72.4 (66.9–82.9)	106.0 (103.2–109.7)	555.2	8.3 (5.0–10.4)	19.1 (9.8–24.1)	20.9 (15.4–25.2)	22.2 (13.7–29.5)
	T2	Brown	76.8 (73.1–79.2)	111.7 (101.0–119.0)	587.0	8.1 (4.2–10.6)	14.7 (6.2–24.8)	20.2 (7.1–29.3)	20.8 (18.6–22.5)
	T3	Brown	66.9 (65.0–69.9)	101.4 (91.7–112.9)	522.5	7.1 (7.1–7.2)	11.7 (2.9–19.6)	15.7 (7.6–20.5)	24.1 (13.1–32.6)
	T4	Brown	73.4 (60.4–80.4)	107.9 (98.4–126.3)	564.3	7.3 (3.8–9.1)	17.3 (9.6–24.1)	21.4 (9.0–29.0)	24.8 (18.3–31.4)
	T5	Green	63.4 (56.7–76.5)	104.7 (101.8–106.3)	518.8	9.5 (5.4–11.5)	17.2 (3.9–32.8)	17.5 (8.7–25.9)	17.2 (11.7–25.2)
	T6	Green	50.8 (39.7–58.1)	87.0 (82.8–91.8)	424.1	8.0 (7.5–8.3)	4.4 (3.9–4.7)	13.8 (10.5–17.2)	19.1 (12.9–28.3)
	T7	Blue	47.9 (38.3–56.4)	87.2 (85.3–89.4)	414.4	11.4 (8.8–12.7)	5.7 (0–14.5)	24.7 (11.7–34.7)	18.3 (13.9–26.0)
	T8	Blue	43.0 (28.4–50.7)	90.8 (75.2–104.8)	406.1	13.2 (11.3–14.1)	1.5 (0–4.6)	28.9 (18.5–39.2)	27.0 (21.4–32.1)
	T9	Blue	40.0 (36.7–45.3)	84.8 (72.7–96.0)	378.6	9.4 (9.0–10.1)	0	24.6 (9.2–50.4)	20.4 (10.3–30.5)
	T10	Blue	43.4 (31.6–56.8)	96.3 (87.5–101.0)	422.6	13.5 (6.9–16.8)	0	33.8 (12.1–46.3)	16.2 (12.2–21.2)

cytoplasmic spacing between platelets; and areas of iridophores, xanthophores, and melanophores. The full model for peak reflectance included three independent factors: areas of iridophores, xanthophores and melanophores. The number of platelets along the vertical axis of iridophores was excluded from the model because platelet number was positively correlated with iridophore area (Spearman's rank correlation coefficient; see Results). For the comparison of influence among independent variables, we modified the standardized value with the average 0 and the standard deviation 1 before the estimation. The random effect was the individual. We ranked competing models based on AIC (Akaike's Informative Criterion), using the MuMIn package.

Results

Colouration and light wavelength reflectance of lizard tails in relation to predation regime

Blue, green and brown colourations of juvenile tails from different populations of *Plestiodon latiscutatus* were quantitatively described by light reflectance profiles (Fig. 2). Blue colouration reflected light with two wavelength peaks, *c*. 450 and 350 nm (Fig. 2g–i), and appeared in the most distal part of tail from T4 to T10 in the Izu Peninsula, T6 to T10 in the Kozu, and T7 to T10 in the Hachijokojima populations. Green-coloured skin reflected light with a peak wavelength of *c*. 550 nm (Fig. 2d–f) and appeared at the central part of the tail, from T2 to T3 in the Izu Peninsula, T2 to T5 in the Kozu, and T5 to T6 in the Hachijokojima populations. The light reflectance profile with a peak wavelength of *c*. 650 nm gave brown colouration (Fig. 2a–c). Brown colouration generally occupied the anterior part of the tail, and the lizards from Hachijokojima exhibited a greater proportion of brown colouration in the tail position from T1 to T4 than did lizards from the Izu Peninsula and Kozu, where the brown colouration occupied only the tail base T1.

Pigment cell architecture

Fundamental histological structures of epidermal and dermal skin layers, fine morphological features of pigment cells, and their vertical arrangement and relative densities were observed using TEM (Table 2). All three types of pigment cells (xanthophores, iridophores, and melanophores) were found in the dermal layer, but only melanophores were found in the epidermal layer (Fig. 3).

Yellow xanthophores, containing pterinosomes approximately 0.3 μm in diameter, were detected in the uppermost layer of the dermis in the region of the tail that exhibited brown and green, but were not observed in the blue tail (Table 2, Fig. 3). Iridophores containing many horizontally oriented reflecting platelets (Table 2, Fig. 3) occupied dermal spaces (10 μm in width $\times$ depth of dermal layer) 1.5 times greater in the region with blue colouration than in regions with either brown or green colouration (Table 2, Fig. 3). The platelets within iridophores exhibiting brown colouration at the tail base (*c*. 80–60 nm) were 1.5 times thicker than iridophores exhibiting blue colouration at the distal end of the tail (*c*. 40 nm) in each of the three populations (Table 2). However, the cytoplasmic spaces between platelets were not different among the skin exhibiting different colouration (*c*. 100 nm, Table 2). The peak wavelength predicted by the formula using the thickness of the platelets and cytoplasmic spaces roughly coincided with the actual reflecting wavelength in blue skin. By contrast, in brown and green skin, the predicted wavelength was less than the actual wavelength reflecting the skin surface (Table 2). The number of platelets along the vertical axis of iridophores was correlated with iridophore density (Spearman's rank correlation coefficient; Izu Peninsula: $S = 1120.4$, $P < 0.001$, Spearman correlation = 0.75; Kozu: $S = 489.6$, $P < 0.001$, Spearman correlation = 0.89; Hachijokojima: $S = 1212.7$, $P < 0.001$, Spearman correlation = 0.73). Dermal melanophores (containing highly electron-dense oval granules) *c*. 0.7 μm along the long axis and 0.4 μm along the short axis

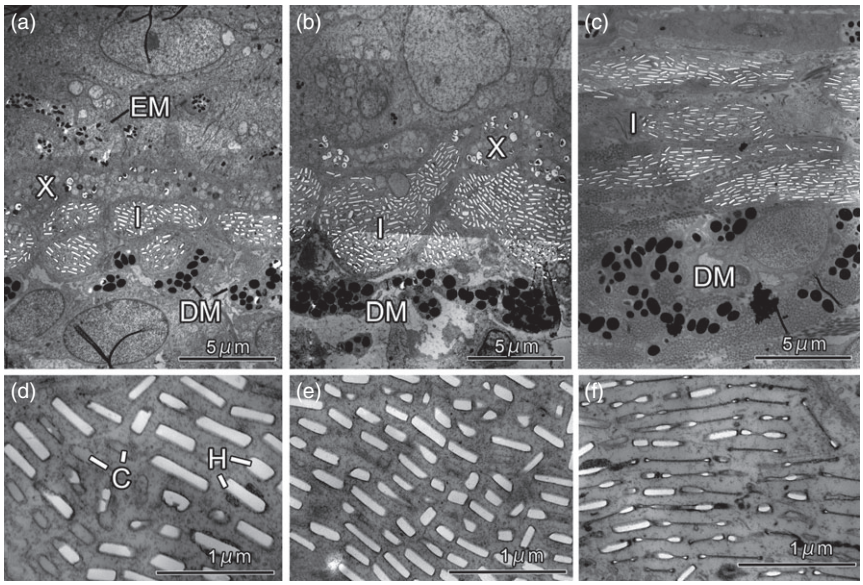


Figure 3 Electron photomicrograph showing the vertical combination of pigment cells and light reflecting platelets of brown, green and blue coloured tail skin of *Plestiodon latiscutatus*. Tail skin with xanthophores, iridophores, and melanophores appear either brown (a) or green (b), whereas only iridophore and melanophpore were observed in blue skin (c). Thickness of the light reflecting platelets decreased from brown (d), through green (e), to blue (f). EM, epidermal melanophore; X, xanthophore; I, iridophore; DM, dermal melanophores; C, light reflecting platelets with crystals; H, platelet without crystal (detail see Kuriyama *et al.*, 2006).

(Fig. 3) were detected in all types of tail skin, and their densities were not significantly different among tail positions in each of the three populations (Table 2).

Cellular basis of factors influencing light reflectance profiles

The generalized linear mixed model for predicting peak wavelength selected platelet widths and iridophore and xanthophore densities in all competition models (Table 3). This model predicted that greater thickness of platelet and increased xanthophore density produced a longer wavelength and that increased iridophore density produced a shorter wavelength. Cytoplasmic spacing and melanophore density were selected in two models; however, *z* values (Estimate/SE) were less than two and were considered inconsequential.

The generalized linear mixed model for predicting maximum reflectance selected two variables (iridophore and xanthophore density) in all competition models (Table 4). The model predicted that greater iridophore density increased light reflectance but that greater xanthophore density decreased light reflectance. Melanophore density was selected in the best model; however, *z* values were less than two.

Discussion

The primary goal of this study was to elucidate the proximate cellular mechanism of intraspecific tail colour variation in *Plestiodon latiscutatus* lizards that evolved in response to predators with different colour vision. We first discuss the association between geographical variations in the profiles of reflecting light wavelength and colour vision of predators. Second, we correlate the light reflectance profiles of lizard tails with histological characteristics of pigment cells that produce different colourations in the three populations under different predation regimes.

Light reflectance of lizard tails in relation to colour vision of predators

Differences in blue and UV light reflectance among the lizard populations imply that lizard tail colouration is an anti-predator adaptation to predators with different colour vision capabilities. The weasel *Mustela putorius furo* has one cone (SWS) covering the short-wavelength region, and can detect blue colour, but not UV (Table 1). On the mainland Izu Peninsula, lizards are exposed to high mammal (especially weasel) predation, and consequently, the light reflectance of blue (450 nm) in the lizard tails was approximately 1.4 times higher than that of UV (350 nm).

Snakes have two cones, SWS like the weasel, and an additional UVS cone that detects UV wavelengths (Table 1). Both blue and UV are reflected by the tails of the Kozu population where snakes are the most dominant predator (Hasegawa, 1994). However, unlike the Izu Peninsula, the UV reflectance of the Kozu *P. latiscutatus* was more pronounced than that of blue. Thus, lizards with weasel predators exhibit blue

Table 3 Factors affecting peak wavelength of the tail skin-reflecting profile in *Plestiodon latiscutatus*

		Thickness of platelets		Cytoplasmic spacing		Iridophore		Xanthophore		Melanophore	
AIC	ΔAIC	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
211.9	0.0	5.03×10^{-3}	6.22×10^{-4}	1.06×10^{-3}	7.07×10^{-4}	-1.64×10^{-3}	4.98×10^{-4}	3.05×10^{-3}	8.37×10^{-4}	-0.97×10^{-3}	5.98×10^{-4}
212.2	0.3	5.44×10^{-3}	5.58×10^{-4}			-1.54×10^{-3}	4.93×10^{-4}	2.89×10^{-3}	8.30×10^{-4}	-0.84×10^{-3}	5.91×10^{-4}
212.2	0.3	5.49×10^{-3}	5.58×10^{-4}			-1.42×10^{-3}	4.87×10^{-4}	3.03×10^{-3}	8.25×10^{-4}		
212.6	0.7	5.15×10^{-3}	6.19×10^{-4}	0.87×10^{-3}	6.98×10^{-4}	-1.49×10^{-3}	4.90×10^{-4}	3.18×10^{-3}	8.33×10^{-4}		
650.4	438.5	–	–	–	–	–	–	–	–	–	–

Table 4 Factors affecting relative reflectance the tail skin-reflecting profile in *Plestiodon latiscutatus*

		Iridophore		Xanthophore		Melanophore	
AIC	ΔAIC	Estimate	SE	Estimate	SE	Estimate	SE
109.4	0.0	9.05×10^{-3}	2.43×10^{-3}	-7.47×10^{-3}	3.33×10^{-3}	-5.89×10^{-3}	3.10×10^{-3}
111.0	1.6	9.65×10^{-3}	2.40×10^{-3}	-6.20×10^{-3}	3.28×10^{-3}	–	–
135.4	26.0	–	–	–	–	–	–

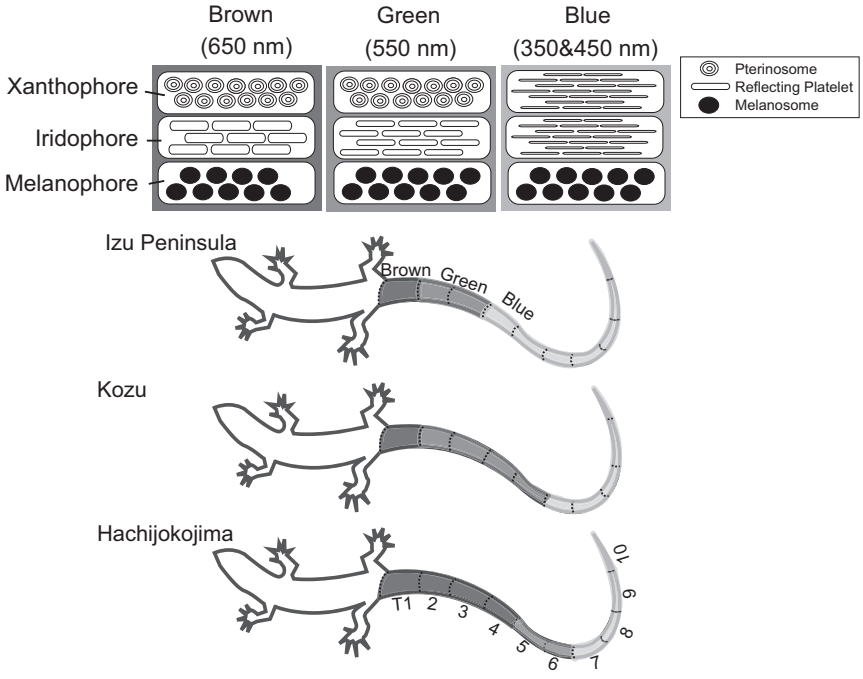


Figure 4 A schematic drawing of the vertical combination and relative thickness of dermal pigment cells producing brown, green and blue skin colouration in *Plestiodon latiscutatus*. Number, shape and size of pigment cells and organelles are simplified and exaggerated.

colouration more than UV colouration, but lizards with snake predators exhibit both blue and UV colouration. Because most natural backgrounds (e.g. leaves, bark, and soil) do not reflect UV (Endler, 1993; Finger & Burkhardt, 1994), the sharp contrast between the UV reflecting tail and its background (i.e. conspicuousness) increases the deflective function (Ruxton, Sherratt & Speed, 2004) of a blue tail to snake predators.

Birds have well-developed colour vision, including a greater sensitivity in the short-wavelength regions, UV and blue, than in the other wavelength regions (Maier, 1993). Classical decoy hypothesis predict that increased bird predation promote evolution of blue tail (Bateman *et al.*, 2014). However, lizards of Hachijokojima, Miyake and Aogashima where the thrush *Turdus celaenops*, a primary predator on juvenile lizard is abundant (Brandley *et al.*, 2014) showed the lowest reflectance of either blue or UV compared to the Kozu and Izu Peninsula populations (Fig. 2). We argue that, because the keen visual acuity of birds may be able to distinguish between the tail and body, the benefit of potentially deflecting bird attacks to a conspicuous blue tail would be outweighed by increasing risk of being detected by birds in the islands where birds are abundant but native snakes and weasel are absent (Hasegawa, 1994). To test our hypothesis, however, we should do a field experiment directly comparing attack rate by birds on the brown and blue tailed lizards in the islands with different predator fauna and abundance.

Pigmentary mechanism underlying geographical colour variation

Geographical variation in tail colouration was interpretable according to pigment cell type and the reflecting light of iridophores (Fig. 4). Tail skin with xanthophores, iridophores, and melanophores looks either brown or green, depending on the iridophores that are reflecting different wavelengths of light. The skin reflecting 650 nm looks brown, and that reflecting 550 nm looks green, whereas the skin reflecting 350 and 450 nm looks blue only if iridophores and melanophores are involved (Kuriyama *et al.*, 2006). We revealed that the three tail colourations were a combination of light reflectance from iridophores and the yellow filtre of xanthophores located above iridophores.

Iridophores contain highly reflective platelets that produce structural colours (Fujii, 1993; Bagnara, 1998), and this optical phenomenon is the most important mechanism for producing the tail colourations observed in the geographically different populations of *P. latiscutatus*. Structural colours are produced by multiple thin-layer interference of reflecting platelets in

fishes and reptiles (Fujii, 1993; Morrison, 1995; Morrison *et al.*, 1995; Kuriyama *et al.*, 2006; Bagnara, Fernandez & Fujii, 2007).

Reflecting light of iridophores differs according to the tail colouration of *Plestiodon* lizards. The thickness of reflecting platelets decreased from about 30 to 80 nm for the skin reflecting 650 nm to the skin reflecting 350 and 450 nm, but cytoplasm spacing between platelets was nearly constant, around 100 nm. Estimated peak reflectance wavelengths coincided with actual tail reflectance measured by the spectrometer (Table 2, Fig. 2). The brown skin actually reflecting 650 nm of peak wavelength had iridophores with a platelet thickness (*c*. 70–80 nm) that was predicted to reflect wavelengths ranging from about 500 to 600 nm. Similarly, green skin actually reflecting 550 nm of peak wavelength had platelets that were *c*. 50–60-nm thick, and the predicted range of wavelengths (450–500 nm) was lower than the actual values observed. Yellow filtering of xanthophores would adjust discrepancy between the structural colour of iridophores itself and the actual light reflected by the skin. Blue skin reflecting two peaks of light, 350 and 450 nm, had platelets that were thin (30–50 nm), and the predicted range of reflecting wavelengths (350–450 nm) coincided well with actually measured values. Theoretically, one iridophore reflects one peak wavelength, and skin reflecting two peaks would contain two types of reflecting iridophores, one reflecting 350 nm and the other reflecting 450 nm.

Although the cellular mechanisms determining platelet thickness remain unknown, the endoplasmic reticulum and Golgi apparatus, which produce platelets in iridophores, are possible candidates (Bagnara *et al.*, 1979). Morrison & Frost-Mason (1991) later proposed three possible pathways of platelet formation in iridophores in *Sceloporus* lizards based on the observation that reflecting platelets were produced from double-membrane vesicles budded from the endoplasmic reticulum and Golgi apparatus. Therefore, studies to identify gene mechanisms for tail colouration require a basic understanding of organellogenesis in iridophores with different platelet thicknesses.

In conclusion, this study attained a general understanding of proximate cellular mechanisms of a classic anti-predator adaptation by the autotomizable blue tail against specific predators with different colour vision. Our focus on cellular mechanisms producing different colouration is an initial but important step to identify genetic mechanism of adaptive colouration. This research strategy is indispensable for the study aiming to reveal genetic mechanisms of colouration in the lower vertebrates where spatial and vertical combination of multiple pigment cells determines skin colouration (Kuriyama *et al.*, 2006, 2013; Saenko *et al.*, 2013; Murakami, Hasegawa & Kuriyama, 2016), because candidate genes for colour pattern formation would not be a single but multiple genes responsible for cell differentiation, proliferation, death and localization (Chang *et al.*, 2009; Olsson *et al.*, 2013; Parichy & Spiewak, 2014).

Acknowledgements

We thank H. Abe, S. Fukasawa, K. Furuse, T. Mizuta, K. Osawa, T. Okamoto, H. Takahashi, H. Takeda, K. Tomizawa, H. Sanayama, H. Usuda, members of the Laboratory of Geographical Ecology of Toho University for their field assistance. We also thank M.C. Brandley for language assistance in writing this manuscript. We especially thank M. Ohata and M. Sugimoto for help in observing and discussing pigment cells. This research was partially supported by the Japan Society for the Promotion of Science to M. Hasegawa (19570026, 21570024, 24570031 and 15H04426) and G. Morimoto (20770018), and the Sasakawa Scientific Research Grant from The Japan Science Society and the Fujiwara Natural History Foundation to T. Kuriyama.

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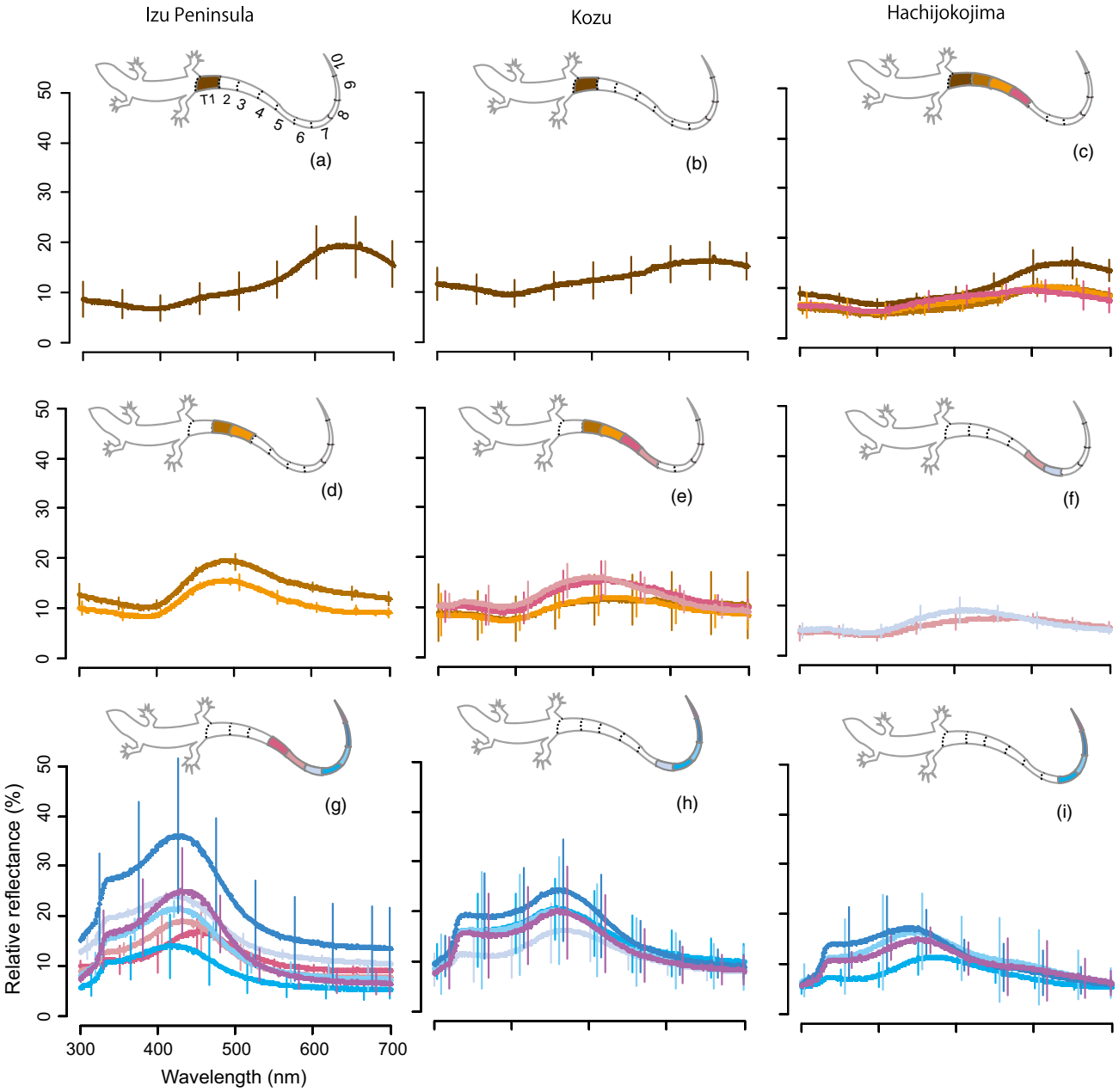
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Graphical Abstract

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The shades of blue colour in the tails of juvenile lizards, which likely serve to deflect predator attacks toward the autotomizable tail rather than the lizard's body, vary across populations, most notably among those island populations with different predator assemblages. Lizard tails with vivid blue reflectance evolved in communities with either weasel or snake predators, which have the ability to detect blue wavelengths. However, a cryptic brown tail evolved independently on the islands where birds are the primary lizard predator. We also determined through TEM that thickness of light reflecting platelets in iridophores, and densities of iridophores and xanthophores, predicted the wavelengths and intensity of light reflected by the lizard tail.