

# Combination of local selection pressures drives diversity in aposematic signals

Koji Mochida

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**Abstract** The diversity of aposematic signals is one of the most difficult phenomena for understanding the evolution of such signals because aposematic animals are most effectively protected when they are common. Theoretical and experimental studies predict that a combination of local selection pressures could maintain variation in aposematic signals. However, the application of this hypothesis to large-scale geographic variation in aposematic signals, other than mimicry systems, is yet to be tested empirically. I investigated geographic variation in morphological and behavioural aposematic signals of the newts, *Cynops pyrrhogaster*, and in predation pressures on them in populations ranging over 800 km of latitude. Field experiments demonstrated that local differences in predation pressures explain well the island-mainland variation in the aposematic colouration and behaviour of newts. Furthermore, I found a latitudinal gradient in aposematic colouration but not in behaviour, independent of predation pressures. The results suggested that island-mainland variation in aposematic signals resulting from local differences in predation pressures might also be shaped by several factors, such as temperature, body size variation, and genetic differences, and such factors might act on each aposematic trait differently.

**Keywords** Aposematism · Latitudinal gradient · Correlated evolution · Carotenoid colouration · Tetrodotoxin

## Introduction

Aposematic animals advertise their unprofitability to potential predators by conspicuous colouration, occasionally in combination with other aposematic traits. These animals are most effectively protected when they are common because of purifying frequency-dependent

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K. Mochida (✉)  
Department of Zoology, Graduate School of Science, Kyoto University,  
Sakyo-Ku, Kyoto 606-8502, Japan  
e-mail: spike@ethol.zool.kyoto-u.ac.jp

selection (Endler 1988). The benefit of close resemblance, through sharing predator education, should drive unpalatable animals to display monomorphic aposematic signals (Speed 1999; Rowland et al. 2007). Because this prediction has been generally accepted, diversity of aposematic colouration is one of the most difficult phenomena for understanding the evolution of such colouration (Endler and Mappes 2004; Mappes et al. 2005).

Recent studies have suggested that phenotypic diversification, including diversification of such organismal colouration, results from localized interactions that vary in outcome depending on local conditions (Stachowicz and Hay 2000; Brodie et al. 2002). For example, in aposematic species, some populations might exhibit specific aposematic colouration, depending on whether they fall inside the geographic range of another aposematic species, a phenomenon called “Müllerian mimicry” (Müller 1879). Müllerian mimicry might occur simultaneously among multiple species and traits, called “mimicry rings” (Brown and Benson 1974; Mallet and Joron 1999). Furthermore, such mimetic relationships might also be influenced by activity rhythms, abiotic factors, or genetic dynamics because they change the detectability or costs of visual signals (Brakefield 1985; Harper and Pfennig 2008). Thus, both local community and environmental compositions would mediate mimetic relationships, creating different aposematic colouration at different places. However, except in the case of mimicry systems, empirical studies have not examined whether this hypothesis applies to large-scale geographic variation in aposematic colouration, although theoretical studies suggested that several aspects of local community compositions might drive such spatial variation in aposematic colouration (Thompson 1984; Mallet and Joron 1999; Endler and Mappes 2004; Mappes et al. 2005; Speed et al. 2010).

Aposematic studies have shown variation in aposematic colouration on various scales from intra-individual to inter-regional, and demonstrated that such variation might be caused by both biotic and abiotic factors (Sword 1999; Ojala et al. 2007; Lindstedt et al. 2008). Regarding large-scale geographic variation in aposematic signals, studies of poison-dart frogs suggested that island-variation in aposematic colouration might be caused by sexual selection (ex. Maan and Cummings 2008), while local differences in predator community might also explain local variation in aposematic signals of tiger moths (Ratcliffe and Nydam 2008). Mochida (2009) found island-mainland variation in aposematic colouration and behaviour of the Japanese fire-bellied newt, *Cynops pyrrhogaster*, which appears to be related to local differences in the predator community. This species releases tetrodotoxin (TTX) from its skin glands (Tsuruda et al. 2002). These newts perform an aposematic behaviour (Johnson and Brodie 1974; Brodie 1977) by displaying their complex of black and red ventral aposematic colouration (Mochida 2010; see photographs in SM). A larger red area on the ventral surface is presumed to work more efficiently against visual hunting predators due to increased conspicuousness. However, a greater extent of the red area is thought to incur higher costs because the red colouration is derived from carotenoid pigments (see “Materials and methods”). Mochida (2009) suggested that island populations of newts might exhibit conspicuous aposematic colouration and behaviour because they fall outside the geographic range of potential mammalian predators. Most of the mammalian predators were nocturnal species and thought to lack colour vision (Jacobs and Rowe 2004). Although these animals might be able to identify prey items using monochromatic vision, smell, taste, and tactile sense would be important cue to search and identify them (Ewer 1973). Therefore, such visual signals might be less effective against them. Namely, it may be advantageous for newts on islands to devote their resources to producing conspicuous aposematic colouration and their escape time to displaying aposematic behaviour more often against predator stimuli to avoid signal misrecognition by avian predators, which might be the main predators of newts on islands

and the primary target of these signals. On the other hand, newts on the mainland, where mammals are the main predators, might devote their resources and time to other traits and demands because of the costs and risks of using such aposematic defenses.

In this study, I focus on the diversity of aposematic signals of newts to test the hypothesis predicting that (1) potential predation pressures by birds and mammals on newts vary depending on geographical regions that differ in the abundance of these two classes of predator, and such differences in local predation pressures might drive island-mainland variation in aposematic signals of newts. I also tested the hypothesis predicting that (2) island-mainland variation in aposematic signals of newts resulting from local predation pressures might be shaped by other factors, such as abiotic and genetic factors. I investigated inter and intrapopulational variation in morphological and behavioural traits of newts from 46 populations and local differences in predation pressures on the newts in populations ranging over 800 km of latitude. I also examined the influence of abiotic and genetic factors on the variation in aposematic traits of newts. My results suggest that local community and environmental compositions mediate predator–prey relationships, creating different aposematic colouration pattern in different places. This may assist in our understanding why aposematic animals have not maximized the effectiveness of their defense.

## Materials and methods

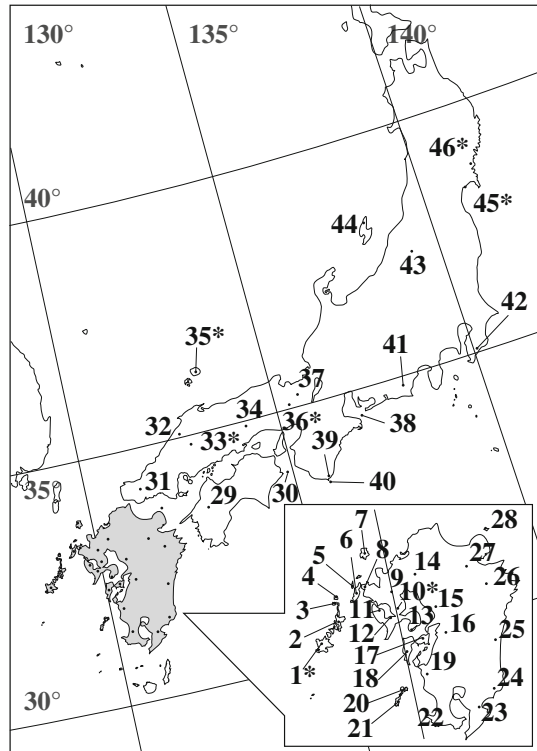
### Animal sampling

*Cynops pyrrhogaster* is distributed on mainland Japan and several adjacent islands. I collected adult newts from 46 populations, including “all local races of this species” (Tohoku, Kanto, Chukan, Nishi, and Minami), which are divided based on genetic variation (Hayashi 1993), between 2003 and 2010 (Fig. 1). A part of the data of 21 populations (ID 1–20 and 22) is same as that in Mochida (2009). Geographic variations in aposematic colouration, aposematic behaviour, and SVL (the length from the tip of the snout to the tip of the vent) as body size were examined in 1,226 animals from 46 populations, 862 animals from 38 populations, and 1,632 animals from 46 populations, respectively.

### Aposematic traits of the newt

*Cynops pyrrhogaster* shows intra- and inter-populational variation in the relative extent of the red area on its ventral surface (Mochida 2009). The ratio of red to black area on its ventral surface is largely heritable and varies very little with growth after sexual maturity, and the hue of the red area changes according to the amount of red pigments (Matsui et al. 2002, 2003a; Mochida unpublished data). If the newt has an almost entirely red area on its ventral surface, the colour pattern is presumed to work best against visual hunting predators as an aposematic signal because of its conspicuousness, at least for human vision (see photographs in SM). However, a greater extent of the red area has been considered to incur higher costs because the red colouration is derived from carotenoid pigments (Matsui et al. 2002, 2003b). Animals cannot synthesize carotenoids by themselves and must obtain carotenoids from carotenoid-containing diets (Goodwin 1984). Because the hue of the red area is related to the content of carotenoids, if the amount of carotenoids in a newt is low, the ventral colouration, consisting of black and off-white or dull yellow regions, becomes inconspicuous (Matsui et al. 2002). Therefore, a greater extent of area displaying

**Fig. 1** A map of the sampling sites ranging over 800 km of latitude. The sites included 18 islands and 28 localities on the mainland. ID numbers of sites in the figure correspond to those in SM. Asterisks indicate sites where the plasticine replica experiments were conducted (sites 1, 10, 33, 35, 36, 45, and 46)



conspicuous red colour requires a larger dietary content of carotenoids. Aside from their use as signals, carotenoids have been demonstrated to function in general immunity in various organisms (Olson and Owens 1998). The immunological utilization of carotenoids causes a scarcity of carotenoids as a signal resource in an animal's body. Therefore, a trade-off between the benefits of conspicuousness against visually hunting predators and costs to use carotenoids as a signal resource might be expected in their colouration (Mochida 2009). To investigate geographic variation in aposematic colouration of newts, I took photographs of the ventral surface of newts using a Nikon digital camera (E2100), and measured the relative extent of the red area (relative colour signal size) on the ventral surface, ranging from the snout to the vent and excluding the limbs, using NIH Image 1.63 (available online from the U. S. National Institutes of Health). I used this relative colour signal size as the degree of conspicuousness of the aposematic colouration.

A rigid immobile posture of the newt is a reflex movement that enhances the effectiveness of aposematic colouration (Johnson and Brodie 1974). Newts should perform this behaviour more often against visually hunting predators to avoid signal misrecognition. On the other hand, the immobile posture might result in death for newts if they perform it against predators such as nocturnal mammals. Such a fatal mistake is often observed in laboratory experiments in which newts that reacted to mammalian predators with the immobile posture lost the opportunity to escape and were killed (Mochida and Matsui, unpublished data). To examine geographic variation in aposematic behaviour of newts, I began behavioural trials 1 week after each newt was captured. Two trials were conducted for each newt, with an interval of 1 week between trials. Before a trial, the newt was kept at a constant temperature (4°C) for 48 h. In each trial, I administered a predator stimulus

by grasping a newt around the midtrunk with forceps, lifting it 15 cm, and then dropping it onto wet paper. I repeated this process three times at 15-s intervals. The induced reaction was considered to be the immobile posture if the newt maintained a rigid posture exposing the ventral surface between the chin and tail for more than 5 s. The tendency to perform the immobile posture was recorded as a score: if a newt showed the posture for the first time against the first, the second, or the third stimulus, the score was ranked three, two, or one, respectively. If a newt did not respond to the three stimuli, the score was zero. In the statistical analyses, I used the total score of the two trials for each animal.

### Predation pressures

I examined the prediction that potential predation pressures by birds and mammals on newts might vary depending on geographical regions (Mochida 2009). I specifically asked whether newts on the mainland are more likely to be attacked by mammals than those on islands where there is less potential mammalian predator fauna. To assess potential predation pressures on newts, I used plasticine newt replicas, largely following the methods of previous studies that investigated natural predation pressures on aposematic prey (Brodie 1993). I created newt replicas, which ranged in SVL from 50.00 to 60.00 mm, using non-toxic plasticine clay precoloured with red paint (Sculpey III, serial colour number 1685). Then the dorsal surface of the replicas was painted with black colour, leaving their ventral surface almost entirely red. I verified that the colouration of *C. pyrrhogaster* and the replicas did not exhibit appreciable levels of UV reflectance using a portable spectrometer (Ocean Optics S2000; see reflectance curves in SM). Finally, I sprayed the replicas with water in a case containing many newts to put the odour of newts on them. To conduct control experiments, I also created plasticine cube models (20 mm × 20 mm × 10 mm) that were painted red, black, blue, and green without adding the odour of newts, because predators might have bitten plasticine replicas due to curiosity or fat- and mineral-seeking.

I conducted plasticine replica experiments at three island sites (in June 2008 at site 1, July 2010 at site 35, and August 2010 at site 45) and at four sites on the mainland (in June 2008 at site 10, June 2009 at site 33 and at site 36, and August 2010 at site 46). In each experimental period, newts aggregated in water and the ground surrounding paddy fields for mating and egg-laying. In each site, I placed 200 newt replicas lying on their ventral surface on the ground along the edges of paddy fields, whose total area was approximately one hectare. The distance between each replica was 5 meters. I put the odour of newts on the replicas every day during the experimental period. I collected all replicas 3 days after placement and recorded the number of attacks. I assessed each replica for the presence of attacks and assigned each attack mark to a predator type, which included birds and mammals, and unidentified predators, using beak and teeth marks on the replicas (multiple marks on the same replica were recorded as one attack; Brodie 1993). I recorded replicas that could not be found after 3 days as “missing”. I also conducted control experiments in August 2010 at sites 45 and 46, and placed 100 cube models along the edges of paddy fields, whose total area was approximately 0.5 hectare, at each site. Different coloured replicas were placed alternately. Other procedures of control experiments followed the methods of the newt replica experiments.

### Statistics

I used R 2.5 for all statistical analyses. Using null-hypothesis testing (NHT; Stephans et al. 2005), I examined geographical patterns of variation in following traits: relative colour signal size, behavioural score, and SVL. In general linear models (GLMs), I fitted latitude

**Table 1** Geographic variation in morphological and behavioural traits of the newt

Response terms	Explanatory terms	Coefficient	SE	<i>t</i>	<i>P</i>
Relative colour	Latitude	−5.29	0.74	−7.109	<0.0001*
Signal size	Island	2.7	0.65	4.135	0.0002*
Behavioural score	Latitude	−0.05	0.07	0.626	0.5355
	Island	0.2	0.07	2.922	0.0061*
Standardized SVL	Latitude	0.88	0.4	2.194	0.0337*
	Island	−0.35	0.35	−0.995	0.3253

In each GLM, explanatory terms included latitude (°N) and distance from the mainland to the sampling island (log km). Asterisks indicate significant terms ( $P < 0.05$ )

and distance from the mainland to the sampling island as explanatory terms against each response term (Table 1). Distance was converted to a logarithm. If a site was located on the mainland, I gave it a distance of zero. In these analyses, I used the population averages of each trait as response terms. I used standardized SVL for statistical analyses (measured values/mean values of all males or females) because in a previous study (Mochida 2009), I detected a sexual difference in body size.

I analyzed differences in the number of attacks on newt replicas between island and mainland sites for each potential predator group, mammals and birds, separately, using the Mann–Whitney test. I also analyzed the correlation between the number of attacks on replicas by predators and relative colour signal size and behavioural score, using Spearman’s coefficient of concordance (Fig. 3).

Finally, I examined whether island-mainland variation in aposematic signals of newts resulting from local predation pressures was also shaped by other factors such, as abiotic factors, SVL, sex and genetic differences. In the analyses, I used information theoretic model comparisons (ITMC), based on the Akaike Information Criterion (AIC; Stephens et al. 2005). In generalized linear mixed models (GLMMs) to fit relative colour signal size and behavioural score of each individual as response terms, I treated identity of the local race to imply genetic differences, abiotic factors including annual mean temperature, annual precipitation, and annual time of solar radiation (in year 2006: available online from the Japan Meteorological Agency), SVL, sex, and distance from the mainland to the sampling island as explanatory terms of models (Table 2). The identity of the population was also fitted as a random term. If observatories of the Japan Meteorological Agency were not located at sampling sites, I excluded the data of these sampling sites from the analyses (see SM). I used the restricted maximum likelihood model (REML) to decompose the variances and to derive parameter estimates with a package ‘lme4’ using Gaussian error and a normal-link function in the models of relative colour signal size and a package ‘glmmML’ using Poisson errors and a log-link function in the models of behavioural score.

## Results

### Geographic variation in aposematic traits

Interpopulational variation in the relative colour signal size on the ventral surface, behavioural score, and standardized SVL were explained by latitude and distance from the mainland to the sampling island (Table 1). The newts on islands displayed significantly

**Table 2** Biotic and abiotic variables contributing to variation in morphological and behavioural traits of the newt

Response terms	Explanatory terms		Coeff.	SE	
Relative colour	Island		1.38	0.79	
Signal size	Local race	Tohoku	4.10	10.65	
		Kanto	0	0	
		Chukan	-4.43	9.42	
		Nishi	9.13	8.63	
		Minami	5.62	10.41	
	SVL		-0.44	0.07	
	Sex	Male	-	-	
		Female	-	-	
		Temperature		4.31	0.96
		Precipitation		-	-
	Solar radiation		-	-	
Behavioural score	Island		0.09	0.03	
	Local race	Tohoku	-	-	
		Kanto	-	-	
		Chukan	-	-	
		Nishi	-	-	
		Minami	-	-	
	SVL		-0.02	0.00	
	Sex	Male	-	-	
		Female	-	-	
		Temperature		-	-
	Precipitation		-	-	
	Solar radiation		-	-	

In each GLMM, explanatory terms included annual mean temperature, annual precipitation (mm), annual time of solar radiation (hours), ID of local races (Tohoku, Kanto, Chukan, Nishi, or Minami), SVL (mm), sex (male or female), and distance from the mainland to the sampling islands, and a random term was ID of populations. Based on the AIC, a best-fitted model for each response term was selected. In each trait, the best five models that explain their variation and AIC of models are shown in SM

larger relative signal size on their ventral surface than those on the mainland ( $t = 4.135$ ,  $P = 0.0002$ , Table 1). This tendency was more marked when the populations were farther from the mainland. The newts showed a significantly larger relative signal size with decreasing latitude ( $t = -7.109$ ,  $P < 0.0001$ , Table 1, Fig. 2a). Newts on islands showed a significantly stronger tendency to perform the aposematic behaviour than those on the mainland, but there was not a latitudinal difference in this behaviour (Fig. 2b). The tendency was more pronounced when the populations were farther from the mainland ( $t = 2.922$ ,  $P = 0.0061$ , Table 1). The body size of the newts significantly increased with latitude ( $t = 2.194$ ,  $P = 0.0337$ , Table 1).

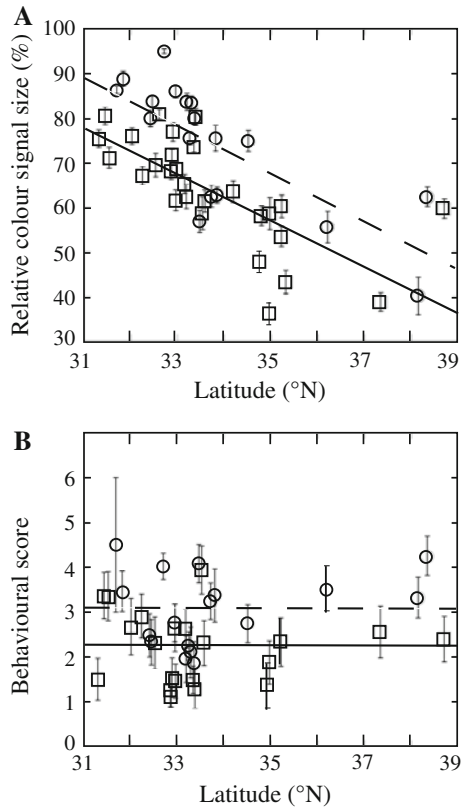
#### Potential predation pressures

Of the 1,400 replicas, 46, 84, and 52 were attacked by birds, mammals, and unidentified predators, respectively, and 85 replicas were “missing”. Most of the teeth marks of

**Fig. 2** Gradients in aposematic colouration and behaviour of the newts along latitude. *Circles* and *squares* indicate populations on islands and the mainland, respectively. Significant regression lines are shown as *dotted lines* for populations on the islands and *solid lines* for populations on the mainland. *Error bars* indicate SE.

**a** Relative colour =  $247.43 - 5.45 \times \text{Latitude} + 10.90$  (if site = Island; Adjusted  $R^2 = 0.59$ ,  $F_{2, 43} = 33.91$ ,  $P < 0.0001$ ).

**b** Behaviour =  $2.20 + 0.87$  (if site = Island; Adjusted  $R^2 = 0.20$ ,  $F_{1, 36} = 10.51$ ,  $P = 0.0023$ )



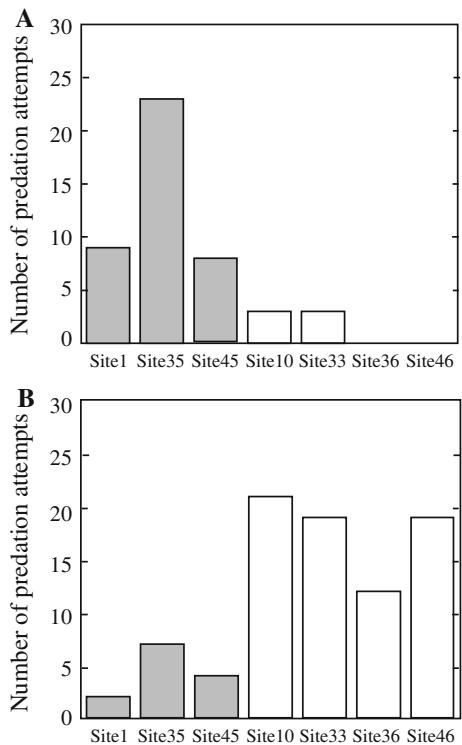
mammals on replicas were thought to be carnivorous, as indicated by the size of the marks, and rodents, shrews, and boars probably attacked a few replicas. Newt replicas on islands were attacked at higher frequency by birds and lower frequency by mammals than those on the mainland ( $P < 0.05$ , Mann–Whitney test; Fig. 3). A significant correlation was found only between the number of attacks by mammals and behavioural score ( $r = -0.8857$ ,  $P = 0.0188$ ). In control experiments at two sites, I did not find any beak or teeth marks on the 200 cube models, although 12 and 19 replicas were attacked at sites 45 and 46, respectively ( $P < 0.05$ , Fisher's exact probability test with Bonferroni correction). This supported the idea that predators did not attack replicas only out of curiosity or because of fat- or mineral-seeking in plasticine objects.

#### Biotic and abiotic factors contributing to geographic variation in aposematic traits

GLMMs showed that explanatory terms of models adequately explained the variation in relative colour signal size and behavioural score (Table 2). Among the abiotic explanatory terms, annual mean temperature was included in a best-fitted model that explained the variation in relative colour signal size (AIC = 7,399). Relative colour signal size was larger when newts inhabited environments with higher temperature (Coef.  $\pm$  SE =  $4.31 \pm 0.96$ ). In the best-fitted model, genetic differences among five local races and SVL were also included as explanatory terms. Relative colour signal size was larger when the body size of newts was smaller (Coef.  $\pm$  SE =  $-0.44 \pm 0.07$ ). On the other hand, in a best-fitted model



**Fig. 3** Geographic variation in predation attempts on newts. Grey and white columns indicate the number of predation attempts on newt replicas at island and mainland sites, respectively. **a** There were a significant difference in the number of attack attempts by birds between the island and mainland sites ( $P < 0.05$ , Mann–Whitney test), but no correlation between the number of attack attempts and behavioural score ( $r = 0.5789$ ,  $P = 0.2278$ ) or relative colour signal size of newts ( $r = 0.1835$ ,  $P = 0.6937$ ). **b** There were a significant difference in the number of attack attempts by mammals between the island and mainland sites ( $P < 0.05$ , Mann–Whitney test) and a correlation between the number of attack attempts and behavioural score ( $r = -0.8857$ ,  $P = 0.0188$ ) but not relative colour signal size of newts ( $r = -0.3717$ ,  $P = 0.4116$ )



explaining the variation in aposematic behaviour, only SVL was included as an explanatory term, besides distance from the mainland to the sampling island (AIC = 1,629; Table 2). Newts performed the aposematic behaviour at higher frequency when the body size of newts was smaller (Coef.  $\pm$  SE =  $-0.02 \pm 0.00$ ).

## Discussion

The present study found island-mainland variation in aposematic colouration and behaviour and predation pressures on newts. Previously, it was predicted that variation in a predator's ability to recognize and handle aposematic prey might be an important driver of microevolution of aposematic colouration (Mallet and Joron 1999; Endler and Mappes 2004). Ratcliffe and Nydam (2008) also found temporal correlation between local predator communities consisting of mixed species with different predator abilities and variation in aposematic colouration. The present study suggested that differences in predator communities between islands and the mainland might act as different selection pressures driving local variation in aposematic signals of newts. The plasticine replica experiments showed that newts on the mainland were more likely to be attacked by mammals, against which such visual signals might be less effective, than those on islands. These results suggested that geographic variation in predation by mammals might alter the strength of aposematic signals.

The experiments also suggested a different hypothesis predicting that local differences in only avian predation pressures, independent of mammalian pressures, might cause

island-mainland variation in aposematic signals, because newts on islands were more likely to be attacked by avian predators than newts on mainland sites. However, there was no correlation between the variation in the two aposematic signals and the number of predation attempts on replicas by birds, and therefore this hypothesis seemed not to be supported. Rather, the number of predation attempts on replicas by mammals was correlated with the tendency of newts to perform the aposematic behaviour among sites. I explain later in this discussion the reason why the relative colour signal size of newts was not correlated with the number of predation attempts on replicas by mammals. My results supported the hypothesis of the previous study (Mochida 2009) predicting that newts on the mainland, where mammals are the main predators, devote their carotenoid resources and escape time to other traits and demands because of the costs and risks of using aposematic defenses, while newts on islands where main predators are birds, but not mammals, more use the resources and time as aposematic defenses, and there might be island-mainland variation in aposematic signals of newts.

The present study also showed that the geographical patterns of variation in two aposematic traits do not coincide with each other in part. Aposematic colouration varied along latitude, but the intensity of performing the behaviour did not change along latitude. The results of field experiments showed that predation pressures by both predator groups were not correlated with variation in aposematic colouration of newts, in contrast to the variation in behaviour. These findings imply that different selection pressures might act on a colour signal, or an aposematic trait might be constrained environmentally, genetically, or developmentally in evolutionary response to selection pressures along latitude. GLMM analyses found that genetic differences could explain the variation in aposematic colouration. Such genetic background might be an influential factor affecting the expression of aposematic signals (Holloway et al. 1995; Brodie and Agrawal 2001; Ojala et al. 2007). It might be able to cause phenotypic clines, independently of selection pressures, such as those found in the present study (Mallet and Turner 1998). The body size of aposematic animals might also affect the efficacy and expression of their signals (Gamberale and Tullberg 1996; Mänd et al. 2007; Ojala et al. 2007). GLMM analyses unexpectedly found that the body size variation might influence the variation in aposematic colouration and behaviour and might explain inconsistent geographic variation between colour and behaviour, if body size differently affects the efficacy and expression between them.

In aposematic animals, diet might influence the signal size (Grill 1999; Ojala et al. 2007; Lindstedt et al. 2010). Only two studies investigated the diets of *C. pyrrhogaster* in its terrestrial periods (when the newt acquires carotenoid-based aposematic colouration; Tsuruda 2001; Matsui et al. 2003a). These studies suggested that newts had similar food habits in the two places, such as dominant prey items. However, there might be differences in the annual amount of prey animals on which newts can feed and in their quality as carotenoid resources among local places. Newts at high latitude might have more difficulty in accessing a sufficient amount of carotenoid-containing prey animals compared to those at low latitude because of stronger limitation of their annual activity period (the activity period per year is more limited in cold areas than temperate areas in newt species Miaud et al. 2000). Therefore, in *C. pyrrhogaster*, a short annual activity period at high latitude might limit the time and chances to acquire and consume carotenoid-prey. Also, there might be an environmental gradient in the carotenoid resources used for aposematic signals, which could be caused by local differences in abiotic factors that influence the production of carotenoids. Carotenoids are produced by plants, algae, and bacteria, whose carotenoid productivity depends on abiotic variables such as temperature (Goodwin 1981). Although there is little direct evidence supporting geographic variability in the

environmental productivity of carotenoid resources used as signals (Grether et al. 1999), many agricultural studies have shown latitudinal differences in carotenoid productivity by plants depending on ambient temperature (e.g., Assunção and Mercadante 2003; Zanatta and Mercadante 2007). Therefore, there might be local differences in the carotenoid productivity in different environments, depending on temperature. If temperature causes a latitudinal difference in the size of the aposematic colour signal, it might not directly influence the extent to which newts perform aposematic behaviour (Mochida 2010). In conclusion, my results suggest that island-mainland variation in aposematic signals resulting from local differences in predation pressures might also be shaped by several factors, such as temperature, body size variation, and genetic differences, and such factors might act on each trait differently.

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