DOES APOSEMATIC COLORATION REDUCE PREDATION RISK IN SNAKES? A SHORT PERIOD EXPERIMENT USING PLASTICINE SNAKE MODELS

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ABSTRACT
Aposematism in an anti-predation mechanism that occurs when animals exhibit conspicuous signals, which are often of a contrasting color patterns, to alert potential predators of their unpalatability or toxicity. This study aims to test (in a short period) the effectiveness of aposematic coloration by comparing the predatory attack upon snakes models with and without an alert coloration on the body. To simulate snakes, we made 80 greenish plasticine snake models. Half of the models had a red strip on the dorsal part of the body, imitating an aposematic coloration. The other half of the models had only a greenish tint. The models were exposed to predators for 12 hours in an area with countryside vegetation. Among the 20 models showing signs of predation, 65% were purely greenish models and 35% were models with red coloration on the back. Attacks at extremities (head and tail) were meaningly more frequent on models with red coloration. Our results suggest the efficiency of red coloration as a warning sign and anti-predation mechanism, since the models with red coloration, imitating aposematic preys, were less preyed and were attacked preferentially at the extremities, which suggests caution by the predator.

Keywords: Aposematism; Artificial Models; Defense Strategies.

RESUMO
A coloração aposemática reduz o risco de predação em serpentes? Um experimento de curto prazo, usando modelos de serpentes de plasticina. Aposematismo é um mecanismo anti-predação que ocorre quando os animais exibem sinais conspicuos, que são frequentemente padrões de coloração chamativa, para alertar predadores potenciais da sua impalatabilidade ou toxicidade. O objetivo deste trabalho foi testar (num curto periodo) a eficiência da coloração aposemática, através da comparação da predação por vertebrados em modelos de serpente com e sem coloração de alerta no corpo. Para simular serpentes, nós confeccionamos 80 modelos de serpente utilizando massa modelável de coloração esverdeada. Metade dos

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modelos possuíam uma listra vermelha na parte dorsal do corpo, imitando uma coloração aposematônica. A outra metade dos modelos possuía apenas a coloração esverdeada. Os modelos ficaram expostos aos predadores por 12 horas em uma área com vegetação campestre. Entre os 20 modelos predados, 65% foram modelos que possuíam apenas a coloração esverdeada e 35% foram modelos com a listra vermelha no dorso. Os ataques nas extremidades (cabeça e cauda) foram significativamente mais frequentes nos modelos com coloração vermelha. Nossos resultados sugerem a eficiência da coloração vermelha como sinal de alerta e mecanismo anti-predação, visto que os modelos que imitavam presas aposematônicas foram menos predados e atacados preferencialmente nas extremidades, o que sugere precaução por parte do predador.

**Palavras-chave:** Aposematismo; Modelos Artificiais; Estratégias de Defesa.

**INTRODUCTION**

Aposematism is a defense strategy in which animals display conspicuous signs which can be morphological, physiological, or behavioural to warn potential predators of their toxicity or unpalatability (Poulton, 1890; Caro and Ruxton, 2019). These signals are important for defense against visually oriented predators (Exnerová et al., 2006; Fabricant and Smith, 2014) and their effectiveness has been suggested and tested by several authors (e.g., Brodie, 1993; Saporito et al., 2007; Valkonen, 2011; Paluhet al., 2014). The warning signs, as mentioned by Caro and Ruxton (2019) can be honest or dishonest and animals which display warning signs may benefit from the ability of predators to generalize their experiences with other aposmatic prey with similar signs (Gamberalle-Stille and Tullberg, 1999). This generalization process is considered important for the evolution of aposmatism and mimetic associations (Ten Cate and Rowe, 2007). Red, orange, yellow and white, often combined with black and forming patterns, are predominant colors in aposmatic signals (Poulton, 1890), being red the most efficient aposmatic color for warning signs (e.g., Exnerová et al., 2006), and in the generalization of learning by predators (e.g., Gamberale-Stille and Tullberg, 1999; Svádová et al., 2009).

Nevertheless, some studies address the paradoxical question that involves the development of a differentiated and contrasting coloration that, on one hand serves as a warning signal to predators, and on the other makes the prey more obvious in the environment and may facilitate their finding by predators (Speed and Ruxton, 2005; Halpin et al., 2008). The range of efficiency of contrasting coloration as a warning signal may be related to the abundance of the pattern in a given area, and also to the presence of mimetic palatable species co-occurring with the model (Pffening et al., 2006), factors that can cause different predator responses.

Among vertebrates, reptiles are characterized by a huge variety of anti-predation tactics and mechanisms, including the display of aposmatic coloring (Greene, 1988). Several reptile species have red as aposmatic coloration, including many snake species (Tozetti et al., 2004; 2009). Despite the warning signal, there are some records of predation of aposmatic snakes (Oliveira et al., 2004; Tozetti et al., 2004) and salamanders (Kuchta, 2005). A common behavior observed in these cases is the guidance of the attack to the head region (Kuchta, 2005) or extremities of the prey (Brodie, 1993; Wuster et al., 2004; Niskanen and Mappes, 2005; Buasso et al., 2006). Several studies about predation use artificial models of non-toxic modeling plasticine (Brodie, 1993; Guimarães and Sawaya, 2011; Dell’Aglio et al., 2012) because they facilitate the study of predation events and the observation and quantification of the predator’s attacks by easily registering pecking marks or bites.
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In this study, we tested the efficiency of aposematic coloration as an anti-predation mechanism, comparing predation by vertebrates in models that simulate snakes with and without red color on the back. Whereas the presence of red color is related to warning signals, indicating toxicity or unpalatability in aposematic animals, it was expected that the models with red coloration would be less attacked than models without the red coloration. Still, we sought to determine whether the predation event would take place in different regions of the body of the models with and without red coloration. It was expected that the models with red coloration on the body were attacked preferentially at the head and tail, since individuals recognized as dangerous are attacked preferentially at the ends.

MATERIAL AND METHODS

Study Area

We performed this study in the Atlantic Forest biome, in a region of highlands, known as Campos de Cima da Serra. The study site was the city of São Francisco de Paula, in Rio Grande do Sul State, Brazil (29°27’S; 50°08’W), with grassland vegetation and Araucaria forest (IBGE, 2004). The climate is temperate (Cf) mesothermal and super humid, with precipitation evenly distributed throughout the annual period. The annual average temperature is 14.5°C, with mild summers and cold winters with frequent frost formation and eventually snowfall in the cooler months (Maluf, 2000). According to long-term local meteorological data, the annual sum of precipitation varies between 1.750 and 2.500 mm and the mean temperature is 16 °C. Frosts are common for at least 30 days in winter.

Data Sampling

Sampling was performed two days in January 2015. In order to simulate snakes with and without aposematic coloration, we made snake models (N = 80) using colored modeling plasticine with cornflour base. Each model was 25 cm long and had 1 cm of diameter (Figure 1c); 40 units were greenish and without red color on the body surface (non aposematic model), and 40 had the same greenish tinge, but with red lines along the body, mimicking an aposematic pattern (aposematic model). The models’ length was based on the approximate size of the most abundant regional snake species, and the greenish color of the body was chosen because it is a common color in snakes as in *Liophis poecilogyrus* (Wied-Neuwied 1825) and *Liophis jaegeri* (Günther 1858). The red pattern was chosen because in the study site there are some non-venomous snake species with red pattern coloration as it is the case of *L. jaegeri* and of *Oxyrhopus rhombifer* (Duméril, Bibron & Duméril 1854). Also, venomous aposematic snake species, with red pattern coloration, such as *Micrurus altirostris* (Cope 1860) and *Micrurus silviae* (Di-Bernardo, Borges-Martins and Silva, 2007) are abundant in southern Brazil and occur in the region of Campos de Cima da Serra.

We distributed the models in a field along an unpaved road (Figure 1a). This site was chosen because of the lack of vegetation, which contributes to the visibility of the models, increasing its chance of being found by predators. We distributed four transects with a distance of 1 km from each other, reducing the possibility of the same predator meeting all the snakes. We distributed 20 models in each transect, those being 10 m apart from each other, and alternated models with and without aposematic coloration (Figure
1b). We placed the models at dusk (22 pm) and held the observation and data collection the next day, at 10 am, totaling 12 hours of exposure. We evaluated the presence or absence of attack and the location of attack (body and/or extremities). We considered as an attack event the pecking marks left by a predator in the snakes’ models (Figure 1d). The disappearance or displacement of models were considered attacks as well, since snakes can be carried by hawks and no evidence of human action or domestic animals was found during the period of activity of the present study in the area (Mateus de Oliveira, personal observation).

Statistical Analysis

To compare the number of predated models between the treatments, data were analyzed by Mann-Whitney test, since the Levene test (22.95, p<0.001) e Shapiro-Wilk (0.843, p=0.004) revealed the data were non-parametric. Assessment of relationship between the color of models and the part of body attacked was performed by Pearson’s chi-square test. Data were analyzed with Bioestat 5.3 and Systat 12.0, considering a 5% significance level.

RESULTS AND DISCUSSION

During the 12 hours of sampling, 20 models were attacked. Among these, 65% (13 models) were models without red coloration and 35% (7 models) were models with red coloration on the body. When compared statistically, the Models without red coloration were more attacked than those that had such color (U1 = 1.732, p = 0.042). Models with red coloration were significantly more attacked at the extremities (χ² 1 = 4.432, p = 0.035). Models without red coloration had 23.07% of attacks directed to the extremities (head
and tail) and 76.92% directed to the rest of the body. In models with red coloration, 71.42% of attacks were directed to the extremities and 28.57% were directed to the rest of the body.

The results support the hypothesis that snakes with red coloration are less susceptible to predation (predatory attacks) and that snakes with red coloration attacked predominantly at body extremities. Considering that species with red coloration are common at the experiment’s site, and this color occurs in both poisonous/unpalatable and palatable mimetic species, the result is an interesting indicator of the efficiency of red as a warning sign and anti-predation mechanism. The mechanisms that lead predators to avoid potentially aposematic prey are controversial and include the possibility of learning from previous experiences with unpalatable preys which show a conspicuous sign (Brodie, 1993; Svadová et al., 2009; Aronsson and Gamberalle-Stille, 2012) and generalization of this experience for other preys with similar marks (Kuchta, 2005; Ham et al., 2006; Dell’Aglio et al., 2012). Moreover, the avoidance of preys that show conspicuous warning signs may be an innate response of predators, resulting from an adaptive pressure generated by aposematism (Smith, 1975; Sherratt and Beatty, 2003). Pegram and Rutowsky (2014) demonstrated that native predators are more likely to avoid preys that display warning colours than to avoid cryptics preys.

Potential predators of snakes at the study site are raptors with diurnal and nocturnal predation habits, visually oriented (e.g., *Athene cunicularia* Molina 1782, *Rupornis magnirostris* Gmelin 1788, *Urubitinga coronate* Vieillot 1817, *Falco femoralis* Temminck 1822, *Heterospizias meridionalis* Latham 1790, *Geranospiza caerulescens* Vieillot 1817, *Bubo virginianus* Gmelin 1788). Despite the possibility of the colors used in the models not being perceived in the same way by all potential predators, there is evidence that birds are able to discriminate between colours (Kraemer and Adams, 2013). It is also possible that the models with contrasting reddish coloration have been avoided because predators often prefer common prey and avoid different phenotypes, a known phenomenon named as neophobia, already demonstrated by Exnerová et al. (2006) and Pfenning et al. (2006).

Regarding the location of attack events at the body, the fact that models with red coloration were attacked predominantly at the extremities indicates that predators may have recognized these models as dangerous. The attacks on the head possibly intended to cause rapid death of a prey that offers danger. Moreover, according to Brodie (1993), attacks are common in both extremities and the attacks on the tail may represent caution by the predator (Wuster et al., 2004; Niskanen and Mappes, 2005; Buasso et al., 2006).

The results of this study show the efficiency of displaying red coloration as an anti-predation mechanism in snakes. Nevertheless more studies are necessary in order to better understand the underlying mechanisms of the observed pattern. It is also important to highlight the efficiency of modeling plasticine models in studies of predation. The findings of this study may serve as a basis for other studies involving aposematic coloration as anti-predation mechanism, considering that the present study has a short sampling period. In addition, this methodology may be used to test other hypothesis, such as the investigation of the relationship between the predation rate and the functionality and level of preservation of habitats, as seen in Santos et al. (2013).
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