Aposematism and crypsis are not enough to explain dorsal polymorphism in the Iberian adder

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ABSTRACT

Aposematic organisms can show phenotypic variability across their distributional ranges. The ecological advantages of this variability have been scarcely studied in vipers. We explored this issue in *Vipera seoanei*, a species that exhibits five geographically structured dorsal colour phenotypes across Northern Iberia: two zigzag patterned (Classic and Cantabrica), one dorsal-strip patterned (Bilineata), one even grey (Uniform), and one melanistic (Melanistic). We compared predation rates (raptors and mammals) on plasticine models resembling each colour phenotype in three localities. Visual modelling techniques were used to infer detectability (i.e. conspicuousness) of each model type for visually guided predators (i.e. diurnal raptors). We hypothesize that predation rates will be lower for the two zigzag models (aposematism hypothesis) and that models with higher detectability would show higher predation rates (detectability hypothesis). Classic and Bilineata models were the most conspicuous, while Cantabrica and Uniform were the less. Melanistic presented an intermediate conspicuousness. Predation rate was low (3.24% of models) although there was variation in attack frequency among models. Zigzag models were scarcely predated supporting the aposematic role of the zigzag pattern in European vipers to reduce predation (aposematism hypothesis). From the non-zigzag models, high predation occurred on Bilineata and Melanistic models, and low on Uniform models, partially supporting our detectability hypothesis. These results suggest particular evolutionary advantages for non-zigzag phenotypes such as better performance of Melanistic phenotypes in cold environments or better crypsis of Uniform phenotypes. Polymorphism in *V. seoanei* may respond to a complex number of forces acting differentially across an environmental gradient.

1. Introduction

Two evolutionary strategies derived from antagonistic predator-prey interactions are aposematism, i.e. showing warning signals to inform predators that the individual may be venomous or unpalatable, and camouflage, i.e. preventing detection and recognition (e.g. crypsis; Endler, 1991; Ruxton et al., 2004; Stevens and Merilaita, 2009). Aposematism presupposes that predators "learn" toxicity and/or danger of particular prey's colouration (Ruxton et al., 2004). Experimental studies have shown that phenotypes deviated from the aposematic signal such as those exotic to a particular locality can be more predated (Santos et al., 2017) and thus, negatively selected, promoting phenotypic uniformity in populations (Noonan and Comeault, 2009; Chouteau and Angers, 2011). Nevertheless, aposematic organisms can show phenotypic variability in their aposematic traits across their

distributional ranges (e.g. Noonan and Comeault, 2009; Chouteau and Angers, 2012) and thus, it is questionable whether phenotypic variants will maintain the aposematic role, and more importantly, which are the ecological advantages of such phenotypic variability. We have explored these questions using a European viper as model.

European vipers (genus *Vipera*) often have an enigmatic dorsal zigzag pattern that has been studied several times due to its apparently not mutually exclusive cryptic and aposematic functions (Andrén and Nilson, 1981; Wüster et al., 2004; Niskanen and Mappes, 2005; Valkonen et al., 2011a, 2011b). Some species exhibit a striking polymorphism in the dorsal colour pattern (e.g. the Iberian *V. latastei*, Santos et al., 2014), which has been barely addressed in such studies (e.g. Valkonen et al., 2011a). In addition, populations at high altitude and/or latitude often show high frequencies of melanistic individuals (e.g. *V. aspis* and *V. berus* in the Swiss Alps, Broennimann et al., 2014).

Melanism in vipers follows the "thermal melanism hypothesis" that predicts thermoregulatory advantages (heat faster and reach higher equilibrium temperatures) for specimens with dark pigmentation (low skin reflectance) in areas with low levels of solar radiation (Clusella-Trullas et al., 2007). Melanistic vipers exhibit increased growth rate, better body condition and locomotor performances, longer activity periods, and higher fertility in females than non-melanistic ones (Luiselli, 1992; Capula and Luiselli, 1994; Castella et al., 2013). Andrén and Nilson (1981) showed that melanistic vipers have higher predation risk than zigzag patterned vipers; however, whether this is due to an increase of detectability by predators or lack of the aposematic zigzag signal remains unclear.

Vipera seoanei (Lataste 1879) is a Euro-Siberian viper species (*Pelias* clade), nearly-endemic to northern Iberia (Martínez-Freiría and Brito, 2014). It exhibits low intraspecific phylogenetic variability but high polymorphism in pholidothic and colouration traits, being both types of traits geographically structured (Bea et al., 1984; Saint-Girons et al., 1986; Martínez-Freiría and Brito, 2013; Martínez-Freiría et al., 2015). Pholidotic trait variation presents a clinal pattern from Cantabrian Mountains to coastal regions and it is correlated to environmental gradients, being likely subject of local adaptation (Martínez-Freiría and Brito, 2013). Colouration variability encompasses five colour pattern phenotypes with different geographic distributions (Martínez-Freiría and Brito, 2013), and is not concordant to mitochondrial (neutral) haplotypes (Martínez-Freiría et al., 2015). Therefore, ecological rather than historical pressures might be determinant in the current geographical colour pattern variability displayed by *V. seoanei*.

The main goal of this study was to examine the aposematic function of the dorsal morphs shown by *V. seoanei* in a scenario of geographical phenotypic variability, and specifically to infer the role of predation as selective pressure on *V. seoanei* colouration phenotypes. To attain this objective, we recorded attacks caused by diurnal avian predators on distinct coloured snake plasticine models resembling the existent colour phenotypes in *V. seoanei*. Given that predation is likely related to prey detectability, we used visual modelling techniques to determine how conspicuous the distinct models are for diurnal raptors. Although attacks caused by nocturnal mammals are not presumably driven by visual cues, we also recorded nocturnal attacks with the aim to compare the incidence of visual and not visual-mediated attacks on each plasticine model. Experiments were done in three localities with different frequencies of colouration phenotypes. The specific aims of this study are expressed as the following questions: (1) do distinct coloured snake plasticine models present different rates of attacks? (2) Do rates of attacks over distinct coloured snake plasticine models relate to their chromatic and achromatic conspicuousness? and (3) Do rates of attacks over distinct coloured snake plasticine models vary between localities according to the frequency of each colour phenotype?

We hypothesized that, irrespective of the locality, (i) predation rates would be lower for the two zigzag models according to the aposematic role demonstrated in previous studies, i.e. the aposematism hypothesis (e.g. Valkonen et al., 2011a), and (ii) models with higher detectability (i.e. conspicuousness) would show higher predation rates, i.e. the detectability hypothesis. Moreover, if predators recognize colouration phenotypes found at each locality (e.g. Noonan and Comeault, 2009; Chouteau and Angers, 2011), (iii) we would expect higher predation on models never recorded to a specific locality (i.e. exotic hypothesis).

2. Material and methods

2.1. Colour variability within Vipera seoanei

Colour variability within *V. seoanei* encompasses five phenotypes (Fig. 1; Braña and Bas, 1983; Bea et al., 1984; Saint-Girons et al., 1986; Martínez-Freiría and Brito, 2013). There is no information on ontogenic changes in colouration for this species and other *Vipera* species; thus, we assume that the five colouration phenotypes remain unalterable

along animals' life. Furthermore, no biometric differences were reported among phenotypes (Bea et al., 1984; Saint-Girons et al., 1986), and, in our dataset (see below), both sexes were presented in a similar proportion in the five phenotypes.

We examined 534 georeferenced specimens, from museum collections (n = 346; Martínez-Freiría and Brito, 2013), field work carried out until 2015 (n = 122) and pictures provided by collaborators (n = 66; see Acknowledgements), to infer the frequency and distribution patterns of these colouration types. The most abundant colour types were two zigzag patterned phenotypes that show widely complementary distributions (Figs. 1 and 2): (1) the Classic type (brownish or reddish, with a darker wide dorsal line and a rounded zigzag) represents 48.5% of specimens (n = 259) and occurs all around the Cantabrian region; and (2) the Cantabrica type (greyish, with a narrow dorsal line with triangular zigzag or transversal bars) accounts for 28.5% of the specimens (n = 152) and is mostly located in the Cantabrian Mountains. A third type with dorsal design but lack of zigzag pattern is the Bilineata type (brownish or reddish, with a wide dorsal line), which represents 7.68% of the specimens (n = 41) and it is mostly located in the northern coastal region. Finally, there are two alternative concolor types: (1) the Melanistic type (black specimens), which accounts for 11.61% of the specimens (n = 61) and it is frequent at high altitudinal ranges; and (2) the Uniform type (grey or light brownish), which sums up to 3.74% of the specimens (n = 20) and it is mostly restricted to the eastern Cantabrian region.

2.2. Study areas

For testing our hypotheses, we selected three areas with distinct frequencies of colour phenotypes. We estimated the frequency of each colour phenotype by quantifying the number of phenotypes from specimens located within a 25 km buffer around each study area. This area size was selected as a compromise between local scale distribution of specimens (i.e. selecting specimens within coherent geographic regions) and home range sizes of viper predators (e.g. 92 km² in *Circaetus gallicus*; Meyburg et al., 1996). The three study areas were (Fig. 2): (1) Castro Laboreiro plateau (hereafter Castro-Laboreiro), within Peneda-Gerês National Park, in Northern Portugal, where the colour phenotypes are Melanistic (67%, n = 18) and Classic (33%, n = 9); (2) Western Ancares Mountains (hereafter Ancares) located in the Western Cantabrian Mountains in North-western Spain, presenting Melanistic (43%, n = 12) and Cantabrica phenotypes (57%; n = 16); and (3) Covadonga Mountains (hereafter Covadonga), located in the North Eastern Cantabrian Mountains, within Picos de Europa National Park in Northern Spain, where the five colour phenotypes are represented, i.e. Bilineata (37.5%, n = 15), Cantabrica (10%, n = 4), Classic (20%, n = 8), Melanistic (20%, n = 8) and Uniform (12.5%, n = 5). Detailed descriptions of topographic, climatic, lithological and habitat characteristics for each study area are provided in Appendix 1 in Supplementary Material.

2.3. Plasticine models

Our experimental method largely follows Valkonen et al. (2011a), performing field experiments with plasticine models to estimate rates of attacks on the phenotypic variability within *V. seoanei*. Models were constructed to resemble the five described colouration phenotypes within the species (Fig. 1). Additionally, we used a sixth model type (i.e. White) to simulate a non-natural colouration that would hamper identification as a potential prey. The six model types were constructed by melting non-toxic plasticine (Van Aken, http://www.vanaken.com/plastalina.html) with black, beige, grey and white colours, in a silicone mould obtained from a real viper preserved in ethanol (with 450 mm of body length). Unpainted black, grey and white plasticine were used directly for making Melanistic, Uniform and White model types, respectively. Bilineata, Cantabrica and Classic models were painted with