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Janne K. Valkonen

Warning Signalling in European Vipers and Their Mimics

Implications for Conservation of the Smooth Snake

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ABSTRACT

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Predation is expected to drive visual warning signals of defended prey towards conspicuousness because of enhanced recognition and avoidance learning by predators. However, specialist predators that can cope with prey defence may select for reduced conspicuousness of warning signals. European vipers (genus *Vipera*) are seemingly inconspicuous, venomous and share a characteristic dorsal zigzag pattern. This pattern has been shown to act as a warning signal, but also is suggested to camouflage snakes from predators due to its disruptive effect. In addition to mimicking the zigzag pattern of vipers, some non-venomous snakes triangulate their head when threatened, which further enhances their resemblance to vipers. In a series of field experiments I expose artificial snakes to free ranging birds of prey to test predator-mediated selection on the characteristic zigzag pattern and triangular head shape of European vipers. Results show that these features are recognised and avoided by avian predators as warning signals without additional benefits of disruptiveness. My findings also reveal that specialist predators, such as buzzards, that are capable of killing vipers without injuring themselves can select for reduced conspicuousness of warning signals. Theories of warning signalling and mimicry can be also applied cost-effectively to conservation actions in order to protect endangered species. Such a case can be found in Åland where the endangered, non-venomous smooth snake (*Coronella austrica*) mimics the adder (*Vipera berus*). My study in Åland revealed that the effective population size of smooth snakes is small and fragmented into sub-populations. Because the effectiveness of (Batesian) mimicry is dependent on the number of model species, viable populations of adders are crucial for the survival of smooth snakes. Adders however, are found in alarmingly low numbers in Åland, which means that the anti-predator strategy (mimicry) of smooth snakes may cease to work unless the populations of adders are secured first. I suggest that conservation planning for the protection of endangered mimics should not ignore the model species even if not itself endangered.

Keywords: Aposematism; conservation; mimicry; smooth snake; viper.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-V.

I Janne Valkonen, Martti Niskanen, Mats Björklund & Johanna Mappes 2011. Disruption or aposematism? Significance of dorsal zigzag pattern of European vipers. *Evolutionary Ecology* 25: 1047–1063.

II Janne K. Valkonen, Ossi Nokelainen, Martti Niskanen, Janne Kilpimaa, Mats Björklund & Johanna Mappes 2012. Variation in predator species abundance can cause variable selection pressure on warning signaling prey. *Ecology and Evolution* 2: 1971–1976.

III Janne K. Valkonen, Ossi Nokelainen & Johanna Mappes 2011. Antipredatory function of head shape for vipers and their mimics. *PLoSONE* 6(7): e22272.

IV Juan A. Galarza, Johanna Mappes & Janne K. Valkonen 2014. Biogeography of the smooth snake (*Coronella austriaca*): origin and conservation of the northernmost population. Manuscript.

V Janne K. Valkonen & Johanna Mappes 2014. Resembling a viper: implications for the conservation of an endangered mimic, *Coronella austriaca*. Manuscript is provisionally accepted for Conservation Biology.

The table shows author contributions to original papers. JV = Janne Valkonen, MN = Martti Niskanen, MB = Mats Björklund, JM = Johanna Mappes, ON = Ossi Nokelainen, JG = Juan Galarza and JK = Janne Kilpimaa.

Contribution	I	II	III	IV	V
Original idea	JM, MN	JM	JV, JM	JV	JM, JV
Design	JM, MN, JV	JM, JV	JV	JV, JG, JM	
Data	JV, MN, MB, JM	JV, ON, MN, JK, MB, JM	JV, ON, JM	JV, JG	
Analysis	JV	JV	JV	JG	JV
Writing	JV, MN, MB, JM	JV, ON, JM	JV, ON, JM	JG, JM, JV	JV, JM

1 INTRODUCTION

1.1 Protective coloration

Animal coloration has raised great interest among evolutionary biologists over the last 150 years (Darwin 1859, Bates 1862), provoking a vast body of studies on its adaptive significance. One of the most vital functions of animal coloration is to provide protection from predation. This can be gained via two distinctive but not mutually exclusive ways; avoiding detection or recognition, and signalling unprofitability to predators (Thayer 1909, Cott 1940, Ruxton *et al*. 2004).

Several animals have highly detectable warning colour patterns that inform predators that a given individual is toxic, unpalatable, or otherwise unprofitable. Anti-predator strategies whereby prey signal their unprofitability to potential predators by warning signals (e.g. coloration, behaviour, odour, sound) is termed aposematism (Poulton 1890). Predators learn to associate the warning signals with the defence of aposematic prey, allowing them to switch to more palatable prey (Poulton 1890, Ruxton *et al*. 2004). Visual warning signals are thus expected to evolve towards conspicuousness because it ensures that the message is effectively delivered to predators (Poulton 1890, Cott 1940, Edmunds 1974, Evans and Schmidt 1990, Endler 1991). Predators also learn to avoid unpalatable prey more quickly when they are conspicuous than when their coloration is cryptic (see below) (Gittleman *et al*. 1980, Gittleman and Harvey 1980, Roper and Wistow 1986, Lindström *et al*. 1999). Increasing signal size (Lindström et al. 1999, Lindstedt *et al*. 2008) and colour pattern symmetry has also been shown to increase signal efficiency by increasing predator's avoidance (Forsman and Merilaita 1999, but see Stevens et al. 2008a).

Unlike conspicuous advertisement, a more common anti-predator strategy is to hide from the predator's eyes by matching the coloration of the background. Cryptic animals achieve protection from predation by colour pattern resembling a random sample of background coloration (Cott 1940, Endler 1981, 1991). Background coloration varies in nature though, so the colour pattern of a cryptic prey that perfectly resembles the coloration in one habitat patch can greatly deviate from the coloration in the next habitat patch. This can set limitations in habitat use and movement of cryptic animals. Most animals are, however, forced to move and use various habitat patches for foraging, breeding or thermoregulation purposes (reviewed in Ruxton *et al*. 2004). Furthermore, background matching alone may not provide optimal concealment because the animals' outlines form boundaries between their body and the background (Thayer 1909, Cott 1940, Stevens and Cuthill 2006). Concealment of cryptic coloration can hence be further enhanced, by adjacent or marginal disruptive markings that hinder detectability of the body parts and outline (Cot 1940, Stevens and Merilaita 2009b).

Disruptive coloration is a form of crypsis that can decrease detectability even in backgrounds that do not perfectly match the coloration of an animal (Thayer 1909, Cott 1940, Stevens *et al*. 2006, Stevens and Merilaita 2009a). Disruptive coloration often contains both highly conspicuous and cryptic elements, so that the signal-to-noise ratio can be higher than in background matching, even though disruptive camouflage might work best when some background matching elements are involved (Stevens and Merilaita 2009b). The theory of disruptive coloration predicts that elements of coloration used to break up body outlines should be located more peripherally than elements of background matching coloration. The edge-breaking elements are expected to make the body shape of an animal more difficult to detect than randomly placed elements, and disruptive coloration can be effective even when background matching is reduced (Cott 1940, Cuthill *et al*. 2005, Merilaita and Lind 2005, Schaefer and Stobbe 2006, Stevens and Cuthill 2006, Stevens *et al*. 2006). Disruptive coloration, containing some contrasting markings, may serve both cryptic and signalling functions. For example, aposematic wood tiger moths (*Parasemia plantaginis*) appear conspicuous when resting on the vegetation but are difficult to detect when they drop down on the leaf litter. This can allow the moths to behaviourally switch the function of their colour from signalling to camouflage (Honma et al. unpublished).

Crypsis and aposematism appear to be intuitively incompatible anti-predator strategies because the function of the former is to hinder detectability whereas the latter is evolved to advertise preys secondary defence to predator. However, not all aposematic species maximise the conspicuousness of their signals and instead express only a moderate or weak signal. In this respect, warning signals do not necessarily need to be conspicuous as long as they are distinctive from profitable alternatives (Sherrat and Beatty 2003, Merilaita and Ruxton 2007). For example, venomous adders (*Vipera berus*) are rather inconspicuously coloured, and can even appear cryptic against their natural background, although they are suggested to be aposematic (Wüster *et al*. 2004). Thus it seems that classifying protective colorations into different anti-predator strategies likely represent a non-mutually exclusive continuum from crypsis to aposematism rather than two extremes of strategies (Marshall 2000, Sherratt and Beatty 2003). In this regard, coloration of the adder (*Vipera berus*) is suggested to protect them from predation both with its cryptic (Andrén and

Nilson 1981) and warning signalling (Wüster *et al*. 2004) function. Furthermore, their zigzag pattern may also be used as an example of disruptive protective coloration (Cott 1940).

1.2 Variation in predators

In general, predators are expected to avoid aposematic prey and shift toward more profitable prey after they have learned to associate the prey signal with its defence. This avoidance learning is often considered an individual process based on unpleasant encountering with signalling prey, or socially learned based on other individual's experiences. However, avoidance of aposematic prey can also evolve without individual learning. For example, if prey cannot be encountered or consumed without fatal injury, strong selection against the individuals attacking defended prey can lead to inherent avoidance of the aposematic prey. Whether the avoidance of aposematic prey is inherited or based on individual learning, predators are variable and do not unequivocally refuse to attack aposematic prey (reviewed in Brodie and Brodie 2004, Ruxton *et al*. 2004).

Similarly to aposematism and crypsis, prey palatability (i.e. acceptability) is likely to be a continuum rather than two extremes (Brower et al. 1968, Speed 1993). Palatability of prey is not only dependent on the prey itself, but also related to the hunger level and nutrition needs of a predator, as well as the availability of alternative prey. If alternative food sources or the nutrition level of predator are limited predators can be forced to consume less profitable prey (Barnett *et al*. 2012). Furthermore, palatability of defended prey also depends on the predator's ability to cope with the prey´s defences and nutrition level of the toxic prey. Despite their toxicity defended prey also contain nutrients, and predators can trade-off benefits of nutrition intake with costs of toxin ingestion (Endler and Mappes 2004, Sherratt *et al*. 2004, Mappes *et al*. 2005, Barnett *et al*. 2007, Skelhorn and Rowe 2007, Remmel and Tammaru 2009, Sandre *et al*. 2010, Halpin *et al*. 2012, 2014). For example, European starlings (*Sturnus vulgaris*) have been shown to increase consumption of mildly toxic aposematic prey when the preys' nutrition level was increased (Barnett *et al*. 2012).

Whereas some predators tend to avoid aposematic prey, some predators can become specialized to prey on them by overcoming their secondary defence. For example, the diet of bee-eaters (Meropidae) consists of a large proportion of hymenopterans. In order to overcome their preys' venomous sting the bee-eaters force the venom out by rubbing the insects against a hard surface before consuming them (Fry *et al*. 1992). Similarly, the short-toed eagle (*Circaetus gallicus*) is highly specialized in preying upon snakes and lizards including venomous ones, which they are able to kill without getting injured (Forsman 2007). Also the common buzzard (*Buteo buteo*) is known to commonly consume venomous vipers (*Vipera sp*.) (Selas 2001, Forsman 2007). These specialist predators that are capable of handling their aposematic prey are not expected to hesitate when attacking them. If the predator community consists of a high proportion of predators that do not hesitate to attack signalling prey; the cost of conspicuous warning signalling may outweigh its benefits (i.e. protection from generalist predators that do avoid aposematic prey) and thereby reduced signal conspicuousness can be selected (Endler and Mappes 2004).

It is hence possible that specialist predators select for coloration of aposematic prey to be as hard to detect as possible, but easily recognizable if detected by a generalist predator. Even though, the rather well known theoretical work by Endler and Mappes (2004) suggested a decade ago that variation in predator community might influence the direction and strength of selection on warning signal conspicuousness, this hypothesis has not yet been experimentally tested (but see Nokelainen *et al*. 2014).

1.3 Batesian mimicry

When predator avoidance towards warning signals evolves it also enables mimicry to evolve. In Batesian mimicry, palatable species mimic the appearance of unpalatable or dangerous model species, and gain protection from predators who may misclassify them as a model species (Bates 1862). Predators generalize the warning signal of the model with the similar signal of the mimic thus avoiding both model and mimic. As predators do not unequivocally refuse to attack the warning signalling prey (see above), and encounters with the palatable mimic are rewarding, predator avoidance on warning signals are likely to degrade when mimics are present. Models should thus be relatively common, and usually more common than mimics, to induce efficient maintenance of the predator’s aversion towards the warning signal (Cott 1940, Huheey 1964, Lindström *et al*. 1997, Joron and Mallet 1998, Mallet 1999, Honma *et al*. 2008). However, determining how abundant the model should be to sufficiently protect the Batesian mimicry is not so simple because it may dependent on several other factors (e.g. accuracy of the mimicry or potency of the model defence and the abundance of alternative prey) (Lindström et al. 1997, Mappes and Alatalo 1997, Kokko *et al*. 2003, Rowland *et al*. 2010, Ihalainen *et al*. 2012). What is clear though is that if the relative abundance of the mimic increases excessively compared to the model, then the benefits of the model warning signal can also be diluted; again because predator avoidance learning and memory may be weakened by rewarding encounters with the mimic (Huheey 1964). Mimicry is common among snakes (reviewed in Brodie and Brodie 2004). Several snake populations are also alarmingly declining (Reading *et al*. 2010) and many are in need of conservation. This raises the interesting aspect on conservation of endangered snake populations highlighting the importance of understanding how relative abundance of models and mimics affect the effectiveness of their natural anti-predator strategy (see chapter 1.4.3 and V).

1.4 Study system

1.4.1 Warning signal of European vipers

Protective coloration plays an important role in the survival of many snake species. They are important prey for many different predator species such as vision-orientated raptors (Cramp 1985, Selas 2001). As ectotherms, snakes need to seek out thermally variable microhabitat patches for thermoregulation purposes and they are often exposed to predation during basking.

European vipers (genus *Vipera*) are venomous, widely distributed and many of them exhibit a typical dorsal zigzag pattern. Several previous studies have shown that the zigzag pattern provides protection against predation (Andrén and Nilson 1981, Forsman 1995a, b, Lindell and Forsman 1996, Wüster *et al*. 2004, Niskanen and Mappes 2005). The protective effect of the dorsal zigzag pattern was first described by Andrén and Nilson (1981), when they compared the predation pressure of natural predators on adders (*Vipera berus*) with melanistic (i.e. black) versus zigzag-patterned coloration. They found that melanistic individuals were attacked more often than those with a zigzag pattern and suggested that the zigzag pattern makes the snakes more cryptic than the melanistic coloration. However, they presented artificial snakes only on natural backgrounds without controlling for the effect of background matching. Controlling for background matching would be crucial for separating the effect of crypsis from warning signalling, and thereby they could not rule out the possibility of an aposematic function of the zigzag pattern. In later experiments, Wüster *et al*. (2004) revealed that zigzag-patterned snakes were attacked less frequently by avian predators also in the situation where conspicuousness and background matching was controlled for. They placed brown and grey snake replicas both on the natural background and on white paper sheets. Zigzag-patterned snake replicas with both colours on both backgrounds were attacked less than snake replicas without the pattern suggesting that the pattern had a warning function. Later, Niskanen and Mappes (2005) repeated the experiment in southern Spain with grey snake models and confirmed the results of Wüster *et al*. (2004).

The zigzag pattern of vipers is also used as an example of disruptive coloration (Cott 1940, Edmunds 1974) and the experiments by Wüster *et al*. (2004) and Niskanen and Mappes (2005) did not control for the possibility of disruptiveness in the pattern. They used only one type of zigzag pattern without manipulating its signal function or disruptiveness. Manipulating signalling and disruptive function in addition to background matching would be crucial to differentiate these effects because, as stated previously, disruptive coloration can hinder detection of prey even if background matching is decreased (Schaefer and Stobbe 2006). Thus, it is possible that the higher survival of zigzag patterned snakes in those previous experiments was influenced by disruptive coloration and not by aposematism; although these two may also interact and are thus not necessarily mutually exclusive.

Furthermore, those experiments used patternless snake replicas as controls for the zigzag-patterned snakes and thus, a potential effect of any colour pattern *per se* could have potentially caused an aversion effect of predators. Therefore, signalling function of the predation risk on zigzag patterned snakes should be compared with snakes that are patterned but without any anti-predator function.

1.4.2 Mimics of vipers

Vipers (Viperidae) are widely distributed, inhabiting all the continents excluding Australia and Antarctica. In addition to being venomous and capable of harming or even killing potential predators they are also important predators for several species. This makes them suitable models for mimicry to evolve. Venom glands of vipers are located in the anterior part of their head creating their typical triangular head shape (Fig.1). Several organisms other than snakes also mimic vipers. For example, several lepidopteran larvae mimic vipers by concealing their heads and inflating their thorax or abdomen to express a false head (Bates 1862, Berenbaum 1995, Hossie and Sherrat 2013, 2014). Non-venomous snakes from the Colubridae family (Lawson *et al*. 2005) often have narrower heads and some of them actively flatten their head if threatened by potential predators enhancing their resemblance to vipers (Greene 1977, Brodie and Brodie 2004). For example viper-like appearance in the non-venomous viperine snake (*Natrix maura*) can be impressive when the mimicry of its zigzag pattern is reinforced with a defensive head flattening behaviour (Arnold and Burton 1978, Fig. 1).

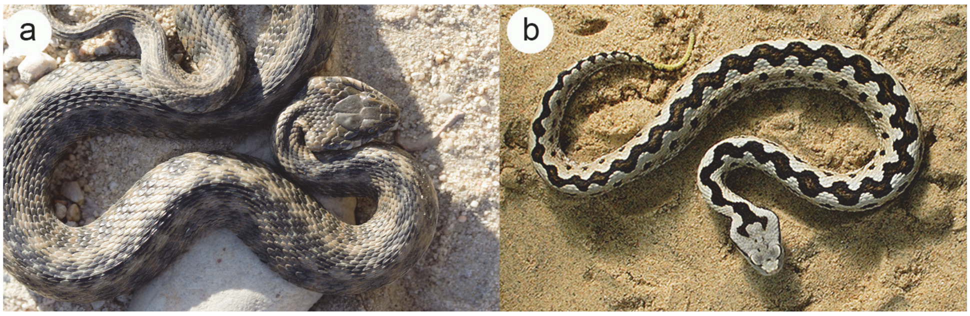

FIGURE 1 Non-venomous viperine snake (*Natrix maura*) (a) flattens its head if threatened by potential predator. Head flattening is suggested to increase similarity with vipers (b).

Potential head shape mimicry has independently evolved several times among snakes. Horizontal head display is known to exist at least among 13 Old World and nine New World genera of the families Colubridae, Elapidae, and Viperidae (Greene 1977). Snakes commonly flatten or inflate their bodies as a defensive behaviour to make themselves appear bigger than they are. Enlargement may occur over the whole body or it may be concentrated in a particular area, such as the neck or head (Mattison 1995). While head triangulation in snakes is often combined with body flattening/inflating behaviour, it also occurs among species that do not exhibit body-flattening behaviour. Smooth snakes (*Coronella austriaca*), for example, flatten (triangulate) their heads when disturbed but do not flatten their bodies (personal observation). Given the taxonomic and geographic diversity of these genera, much of the similarity in defensive behaviour must be due to convergence (Greene 1977), which may have been driven by defensive mimicry. Further support for these behavioural observations comes from Young *et al*. (2006) who described the mechanical basis of head triangulation in distantly-related colubrid snakes. They found that the morphological mechanisms required to triangulate the head differ greatly between the genera *Hetrodon* and *Dasypeltis*.

1.4.3 Mimicry and conservation of the smooth snake in Åland

Mimicry seems to be common among snakes including the famous mimicry ring example of venomous coral snakes and their mimics (reviewed in Brodie and Brodie 2004). Mimicking highly toxic or dangerous models is particularly beneficial when unsuccessful attacks can produce serious injury, which can explain why venomous snakes are often mimicked (e.g. Pfennig *et al*. 2001). Snake populations are recently found to be in decline and more and more of them are in need of conservation actions. This alarming trend of globally declining snake species requires re-consideration of conservation actions for this group (Reading *et al*. 2010). Recognizing and considering anti-predator strategies of the target species as part of a conservation issue can lead to significant improvement of conservation management. Vast theoretical and experimental knowledge on the evolution of mimicry argues that co-evolutionary arms races should be carefully considered during conservation actions. Such a case can be found in the Åland Islands where the smooth snake (*Coronella austriaca*) mimics the adder (*Vipera berus*) (III, V).

The smooth snake (Fig. 2) is a non-venomous, medium-sized (up to 80 cm) and relatively slender Colubrid species, which is widely distributed across Europe. Its geographic range covers the Iberian Peninsula to the Ural Mountains, with the Northernmost populations reaching up to 60° N latitudes in Sweden and Åland (Arnold and Burton 1978). Despite the wide distribution populations are scattered and rarely abundant. This diurnal highly secretive species feeds mainly on lizards, but larger individuals also consume smaller snakes and mammals, whereas smaller ones prey on invertebrates (Arnold and Burton 1978, Goddard 1984, Luisielli *et al*. 1996). Females reach maturity in their fourth or fifth summer, approximately longer than 40 cm, and the majority of females reproduce every second or third year and gives birth to two to eleven live young at a time (Spelerberg 1977, Luisielli *et al*. 1996, Reading 2004). Smooth snakes can be found in various habitats, but in the northern part of its range (Sweden and Åland) its typical habitat is relatively dry, open and rocky hillsides. The smooth snake is included in the list of specially protected species in the European Union (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora, annex IV), and has been recently classified as vulnerable in Finland (Rassi *et al*. 2010). The only known populations of smooth snakes in Finland are located on the Åland islands (Rassi *et al*. 2010).

Terrestrial snakes are sensitive to habitat fragmentation, ecotype differences and anthropogenic landscape disturbances (Goldingay and Newell 2000, Manier *et al*. 2007, DiLeo *et al*. 2010). The landscape in Åland is naturally fragmented by numerous islets and inlets, but also by urbanisation, road networks and crop fields. Despite the smooth snake's special status in the European Union and Finnish legislation, knowledge on its population size and structure in Åland is missing. Furthermore the biogeography of the species is only partly known (Santos *et al*. 2008). Understanding the interplay between environmental factors and biological processes in shaping a species' distribution is of primary importance when devising conservation-management strategies. By doing so, valuable information can be obtained such as the identification of isolated populations, associations to particular habitats, distribution range shifts and biological invasions (Araújo *et al*. 2008, Tolley *et al*. 2008 Long *et al*. 2009, Rohr and Raffel 2010).

FIGURE 2 Smooth snake (*Coronella austriaca*) female from Åland.

1.5 Aims of study

The model-mimic relationship is particularly important if a given species of the mimicry ring are rare or endangered, because mimicry works only if the population densities are sufficiently high. The overall aim of my thesis is to examine the nature of that relationship using European vipers and their mimics, and to provide knowledge for the more suitable conservation management of snakes. The first part of my thesis is concentrated around the seemingly inconspicuous warning signalling of European vipers (I–III). In the first study (I) I examine if the dorsal zigzag pattern of European vipers acts as disruptive coloration in addition to its suggested warning signalling function (Wüster *et al*. 2004, Niskanen and Mappes 2005). The aim of the second study (II) is to test experimentally if variation in the composition of predatory community structure affects predation on warningly and non-warningly coloured snakes as suggested by theory (Endler and Mappes 2004). As several species have been suggested to mimic venomous snakes via head triangulation and displaying false heads (e.g. Bates 1862, Greene 1977, Arnold and Burton 1978), but the adaptive significance of this behavioural mimicry has never been tested, in my third (III) study I empirically test whether triangular head shape acts as a warning signal.

The second part of my thesis (IV, V) is aimed at demonstrating the importance of the population consequences of mimetic species in order to successfully manage endangered smooth snakes in Åland. Study (IV) will provide crucial information on colonization routes and population structure of endangered smooth snake populations in Åland. In the last chapter (V) of my thesis I discuss how theoretical knowledge about frequency dependent predation of mimicry systems can be used for conservation managements of endangered mimic. I use snake populations in Åland as an example, where the non-venomous smooth snake mimics the venomous adder by triangulating its head if threatened by a predator.

2 METHODS

2.1 Predation experiments (I–III)

Direct observations of predation in nature can be challenging because of the rarity and sparse spatial distribution of predation events. Artificial prey items, made of soft clay or plasticine therefore provide an effective method to estimate attack rates by wild predators on snakes and several other organisms (e.g. Wüster *et al*. 2004, Hegna *et al*. 2011, Nokelainen *et al*. 2014). This is due to the fact that a large number of replicas can be used, and the coloration of both the models and their background can be easily manipulated. The use of artificial prey items also enables control of behavioural variation, spatial distribution and microhabitat use of the prey. Predators such as raptors and mammals can be distinguished from the bite, beak and claw marks left on the soft clay model surface (see Andrén and Nilson 1981, Brodie and Janzen 1995, Wüster *et al*. 2004, Niskanen and Mappes 2005, Fig. 3). Telling apart raptor and mammal attacks is crucial when plasticine prey items are used because of their different predation behaviour. Raptors are largely visual predators whereas mammals generally rely on olfactory cues. Plasticine has a strong odour that could even attract mammal models (Rangen *et al*. 2000), and it is thus likely that mammalian attacks on plasticine replicas do not reflect real predation behaviour but rather curiosity or mineral seeking (Valkonen and Mappes 2012). During these studies I did not find any evidence that mammalian predators selectively avoid any of the pattern types of model snakes (I, III).

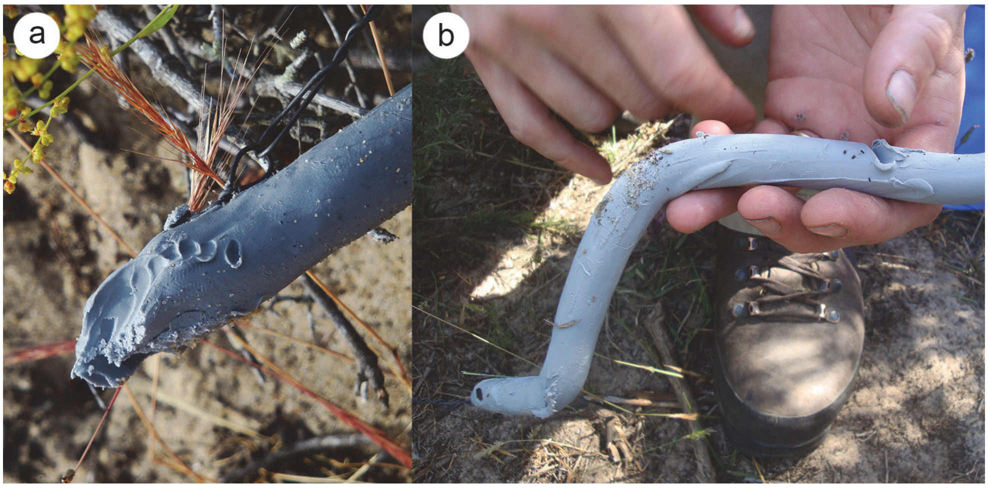

FIGURE 3 Attacks of mammalian (a) and avian (b) predators can be separated from characteristic imprints left on soft clay models during the attacks.

Following a previously employed method (see Andrén and Nilson 1981, Brodie and Janzen 1995, Pfennig *et al*. 2001, Wüster *et al*. 2004, Niskanen and Mappes 2005) snake replicas for predation experiments (I–III) were fabricated with non-toxic pre-coloured plasticine. The different colour patterns used as treatments (see below) were painted on the models with black acryl paint. Approximately 100 g plasticine was used in each snake replica, whose length varied from 36 cm to 45.5 cm, sufficiently representing sub-adult/adult sized Lataste's viper (*Vipera latastei*). A piece of built-in iron wire enabled the attachment of the snake models to the vegetation, preventing predators from carrying them away during attack. To evaluate the predation pressure on differently colored models, plasticine snakes were exposed to natural predators in transect lines (see below and I–III). All the snake replicas were placed in an s-shape posture to make their appearance more natural.

To test if the zigzag pattern of European vipers provides protection against predation due to either its disruptive or aposematic function (I), snake replicas with five different pattern types were used in the experiment (Fig. 4). With these pattern types and by controlling the background matching with paper sheets I was able to separate the aposematic from the disruptive function of the zigzag pattern of European vipers, and to compare the effects of different colour patterns on the probability of predator attack (I).

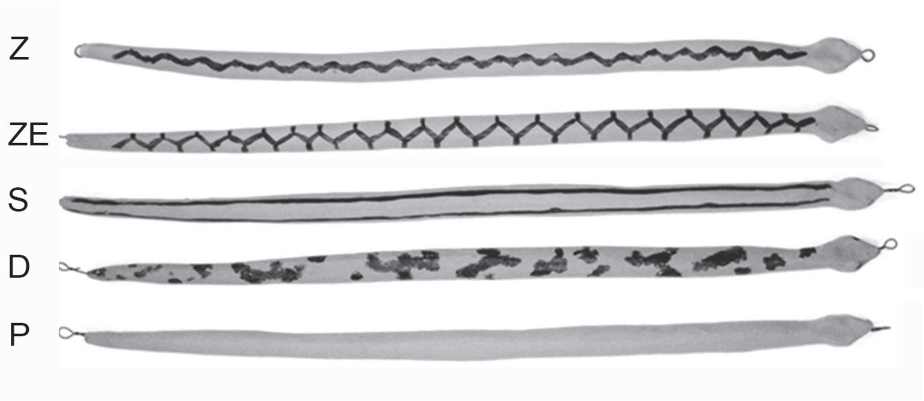

FIGURE 4 The five different pattern types of artificial snakes used in experiment (I). Pattern (Z) represented a typical dorsal zigzag pattern of European vipers, which has been suggested to function as a warning signal. Pattern (ZE) represents an aposematic zigzag pattern with a disruptive effect; the zigzag pattern was painted to break down body outlines. Pattern type (D) was produced to represent classic disruptive coloration without any other functions. Striped pattern (S) controlled the effect of the black paint and the contrast between the paint and the snake replicas. It also represented coloration without any known protective function that could decrease predation on it providing reference for comparing the protective effects of other colour patterns. Plain grey (P) snake replicas were constructed for controlling possible interactive effects of paint per se and different backgrounds (natural and white), which was used to control background matching of snake replicas. (The figure is modified from I).

To test if vipers' triangular head shape acts as a warning signal (III), four different kinds of artificial snakes were used: 1) zigzag-patterned snakes with triangular (viper type) head; 2) zigzag-patterned snakes narrow (colubrid type) head; 3) plain (grey) snakes with triangular head; and 4) plain snakes with narrow head. The use of plain-coloured snakes was crucial to tell apart the effect of the head shape from the overall appearance of vipers.

To study if variation in predator species affects the predation pressure on warningly and non-warningly coloured snakes (II), raptors flying over the experimental areas were surveyed during the predation experiments (I, III). The most commonly sighted raptor species were black kite (*Milvus migrans*), red kite (*Milvus milvus*), booted eagle (*Hieraaetus pennatus*), common buzzard (*Buteo buteo*) and short-toed eagle (*Circaetus gallicus*) (II, Table 1). These five species are likely to be the most important avian predators of snakes in the area and herein considered as key predators. These species are different in their foraging behaviour and food choice. The short-toed eagle is highly specialized in preying upon snakes and its diet consists mainly of snakes and lizards (Forsman 2007). The diet of the common buzzard consists mainly of small mammals and birds, but they are also known to commonly consume snakes (Selas 2001, Forsman 2007). The diet of the booted eagle consists mainly of medium-sized birds, big lizards, small mammals (Forsman 2007) and snakes (personal observation). Black and red kites are more generalist, feeding on carrion, small mammals, birds, insects, reptiles, and fish (Forsman 2007). Altogether, bird observations were conducted at a total of 40 transect lines. Predation experiments were conducted in the Coto de Doñana National Park in southern Spain (37°00 N, 06°38 W) during springs 2008–2010.

TABLE 1 Number of observed raptors of each species during the predation experiments. Numbers are given as observed individuals within one hour.

Species	Max	Mean	S.E.
Black kite (*Milvus migrans*)	68	22.20	3.33
Red kite (*Milvus milvus*)	27	5.16	1.06
Booted eagle (*Hieraaetus pennatus*)	14	4.13	0.54
Common buzzard (*Buteo buteo*)	7	0.95	0.24
Short-toed eagle (*Circaetus gallicus*)	2	0.60	0.11
Common kestrel (*Falco tinnunculus*)	2	0.16	0.07
Western marsh-harrier (*Circus aeruginosus*)	1	0.06	0.03
Imperial eagle (*Aquila adalberti*)	1	0.06	0.04
Peregrine falcon (*Falco peregrines*)	1	0.03	0.02
Lesser kestrel (*Falco naumanni*)	1	0.01	0.01

2.2 Origin and population structure of the smooth snake in Åland (IV)

To assess the phylogeography of the smooth snake and its population structure in Åland, I collected DNA- samples from the field and preserved museum samples. Samples were collected in the summer of 2010 and consisted of 41 buccal swabs and 21 tissue samples (shed skins and road-killed individuals) from 9 different localities throughout Åland. Swabs were taken by gently rubbing mucosa with cotton following the method in Beebee (2008). Swabs were then air dried for one minute and stored in 1.5 ml Eppendorf tubes at room temperature. Shed skins were stored dry, and road-killed individuals were kept in 70% Ethanol. For the remaining 82 samples, tissue was collected from different European museums, and shed skins were provided by collaborators. However, it was not possible to obtain enough DNA for some samples older than 10 years or excessively fixed with formalin. Hence, a final set of 58 samples originating from 15 different countries throughout Europe in addition to 62 samples from Åland was used.

I used maternally inherited 651 bp long COI-gene sequence to study the phylogeography of the smooth snake (methods described in (IV)). To evaluate the genetic structure of the smooth snake population in Åland, I genotyped all samples at 11 microsatellite loci (Bond *et al*. 2005, IV). The demographic history of the Åland Island was examined through a mismatch distribution test of the COI haplotypes by comparing the observed distribution of nucleotide differences between the haplotypes, against the expected nucleotide distribution for populations that have been stable (at equilibrium for no recombination). I also obtained an estimate of overall effective population size in Åland. As maternal inheritance of the mtDNA, the female effective population size (*Nef*) can be derived from the population mutation rate parameter θ, which is the product of 2*Nefv*, where ν is the sequence length multiplied by the mutation rate per generation. The parameter θ was calculated according to Watterson, (1975). Generation time was set to 3.5 years (Spellerberg 1977, Luisielli *et al*. 1996) and a nucleotide substitution rate to $5.2*10^{-9}$ substitutions/site/year, recently estimated for the Coloubridae family (Eo and DeWoody 2010). For more detailed description of methods see (IV).

2.3 Applying mimicry theory to conservation (V)

In order to encourage conservation biologists to consider, and apply, theoretical knowledge about frequency-dependent predation on mimicry systems, I present an example case from the Åland archipelago where the protected, endangered, non-venomous smooth snake (*Coronella austriaca*) mimics the non-protected venomous adder (*Vipera berus*). To illustrate the added predation risk on smooth snakes caused by rarity of vipers, I obtained risk estimates from experimental data published by Lindström *et al*. (1997). In their experiment Lindström *et al*. (1997) tested how the abundance of models in relation to their Batesian mimics affects predation pressure of mimics. They used three artificial prey species (model, mimic and alternative prey) and wild caught great tits (*Parus major*) as the predator. They manipulated the frequencies of the unpalatable model and the palatable mimic by keeping both the total size of prey community and the abundance of alternative palatable prey constant. Lindström *et al*. (1997) repeated their experiment with two unpalatability levels of the model species (for detailed description see Lindström *et al*. 1997). To obtain an estimated predation risk for each species (model, mimic and alternative) I fit generalized linear mixed models with Laplace approximation to their data. I then used the resulting estimates to illustrate the extent of the added predation risk on the mimic in relation to the decreasing abundance of the model.

3 RESULTS AND DISCUSSION

3.1 Warning signalling of European vipers (I, II)

Although intuitively distant strategies, aposematism and camouflage, via the disruptive coloration, are not necessarily mutually exclusive. In my first study (I), I manipulated the zigzag pattern in the way that the pattern can be classified as disruptive or non-disruptive and found that despite its seemingly inconspicuous nature, the dorsal zigzag pattern of European vipers is indeed a warning signal. Despite the fact that warning-signalling function of the zigzag pattern of European vipers is well supported (Wüster *et al*. 2004, Niskanen and Mappes 2005, I, II, III) the pattern may be cryptic from the distance but conspicuous and easy to distinct from a harmless prey after detection (Marshall 2000, Sherrat and Beatty 2003, Tullberg *et al*. 2005, Bohlin *et al*. 2008).

In my study I did not find any evidence supporting the idea that the zigzag pattern of vipers would also function as a disruptive colouration (I). However, that does not mean their colouration could not be multi-functional. In addition to its warning signalling function however, the dorsal zigzag pattern of European vipers may also have a motion dazzle function, which makes estimation of the speed and trajectory difficult for predators (Shine and Madsen 1994, Lindell and Forsman 1996, Stevens *et al*. 2008b, Scott-Samuel *et al*. 2011). Even though there is no evidence that the zigzag pattern would be more efficient at causing motion dazzle effect compared to other colour patterns, it has been shown to be more effective than the lack of any patterning (Stevens *et al*. 2008b, Scott-Samuel *et al*. 2011). The motion dazzle effect of the zigzag pattern could provide additional benefits against a predator that has already detected and made the decision to attack the viper. Because most prey animals suffer predation by more than one type of predator species, several different combined anti-predatory strategies would be highly beneficial. As I showed (II), some raptor species have no problems to attack and kill an adder despite its venomousness. Thus, more research is needed to examine my suggestion that protective colouration of prey can work in a multifunction manner by hindering detection and capturing, and also serving also as a warning signal.

Distance-dependent warning colouration can be particularly beneficial if the predator community consist of a high proportion of predators that do not avoid the aposematic prey. The results of my second study (II) revealed that the predation risk was higher among aposematic (zigzag patterned) snakes compared to non-aposematic snakes when common buzzards were abundant (Fig. 5). My results also show that conspicuous warning signals are more advantageous in locations where the majority of predators avoid the local warning signal. These results suggest that the abundant presence of specialised predators can cause local selection pressure favouring a less conspicuous warning signal within a prey population, as suggested by theory (Endler and Mappes 2004). Therefore, avoiding detection by specialist predators from the distance, but being distinctive if detected by a generalist, may be particularly beneficial for vipers in cooler climates for instance, where snakes are often exposed to visually hunting predators while basking. Snake species originating from warmer climate may be better able to hide from predators because they are not forced to spend as much time basking as snakes from northern or higher altitudes. Furthermore, the specialist common buzzard is a relatively more abundant raptor species in northern Europe compared to the south, where generalist raptors (e.g. black kites and red kites) can form a majority of the raptor community (Forsman 2007, II). That might explain the less conspicuous appearance of Northern European species of adders (e.g. *V. berus*) compared to the Southern European species (e.g. *V. latastei*) (De Smedt 2001, Kreiner 2007). This hypothesis, however, would require further experiments in both environments.

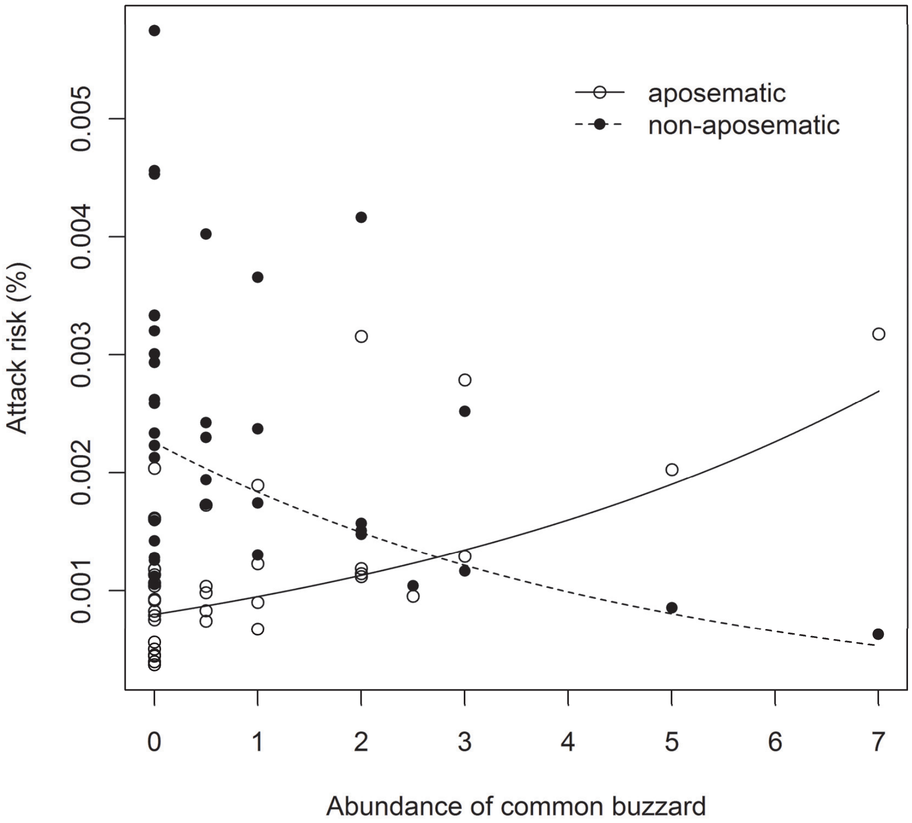

FIGURE 5 Attack risk of aposematic (open dots) and non-aposematic (closed dots) snakes related to abundance of common buzzard. Lines represent estimated attack risks (solid line, aposematic; dashed, non-aposematic snakes) (II).

My study serves as the first experimental evidence to support the hypothesis that varying predation can explain the existence of variable and inconspicuous warning signals. The significance of spatially and temporally varying predator community structure for selection on warning signalling is also supported recently by Nokelainen *et al*. (2014) and Valkonen *et al*. (2014). Nokelainen *et al*. (2014) found that spatially varying predator communities cause an opposing predation pressure on warning signalling in the wood tiger moth (*Parasemia plantaginis*). Male moths exhibit white and yellow colour morphs and, generally, the more conspicuous yellow morph is better protected against predators than the white morph due to its more efficient warning signal (Nokelainen *et al*. 2012, 2014). Nokelainen *et al*. (2014) however, showed that the success of each morph depends on the predator communities. They found that the white male morph of the wood tiger moth suffered higher predation compared to the yellow morph when Paridae were abundant whereas the opposite was true when Prunellidae were abundant. Their experiment shows that predator communities can generate mosaics of selection and partly explain diversity in anti-predatory strategies. My experiments along with Nokelainen *et al*. (2014) highlight that predator-prey interactions are continuously changing creating variable selection on anti-predatory strategies.

3.2 Viper head mimicry and conservation of the smooth snake in Åland (III–V)

> "*The most extraordinary instance of imitation I ever met with was that of a very large Caterpillar, which stretched from amidst the foliage of a tree which I was one day examining, and startled me by its resemblance to a small Snake. … I carried off the Caterpillar, and alarmed every one in the village where I was then living, to whom I showed it*" (Bates 1862).

Following the words of Henry Walter Bates (1862) several species have been suggested to gain protection from predation by mimicking the triangular head shape of venomous vipers (e.g. Greene 1977, Arnold and Burton 1978, Hailey and Davies 1986, Berenbaum 1995, Mattison 1995, Young *et al*. 2001). However, evidence that the head shape of vipers can be recognised and avoided by predators is missing. Results of my study (III) show that the triangular head shape of vipers alone can be recognized and avoided by raptors. This result means that the characteristics triangular head shape can act as a warning signal and allow the evolution of mimicry (IV).

Triangular head shape, however, did not provide additional anti-predator benefit when presented together with a zigzag-patterned snake body. Thereby suggesting that the zigzag pattern is a stronger signal of defence than the head shape (Fig. 6). Furthermore, the lack of additional effect of triangular head shape when presented with the zigzag-pattern indicated that predators might categorize prey dangerous based on only one, and the most obvious existing feature, of the prey's signals; rather than additively using all the available signal components (see also Rowe 1999, Hossie and Sherrat 2013).

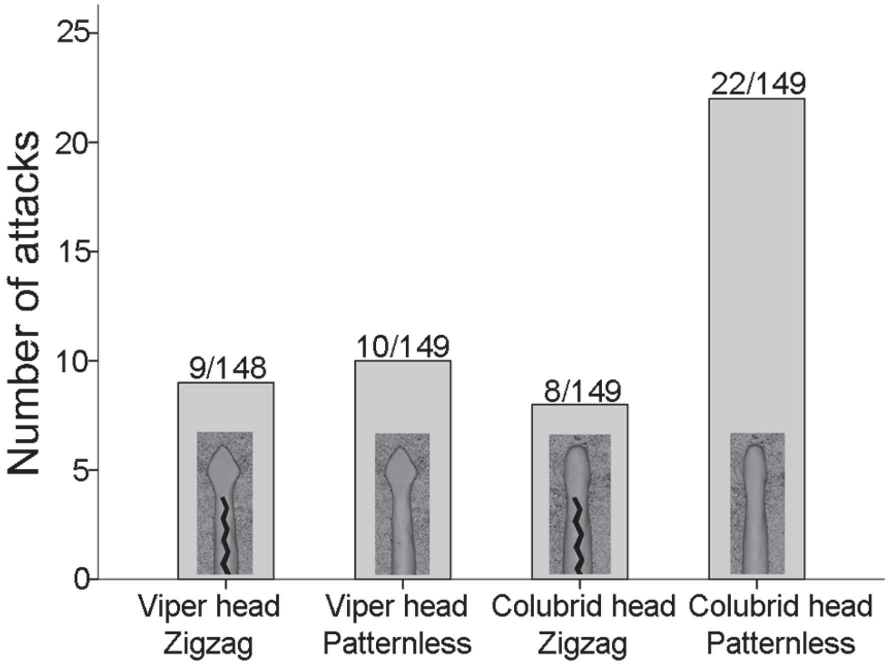

FIGURE 6 The observed number of raptor attacks on different types of snake replicas. Numbers at the top of each bar represent the total number of observed raptor attacks on each type of snake replica in relation to the total number of replicas of that type used in the experiment (III).

My results also suggest that behavioural mimicry (head triangulation) can significantly increase the survival of several non-venomous snake species that display head triangulation but do not notably mimic the dorsal zigzag pattern of vipers. The smooth snake (*Coronella austriaca*) is one of these non-venomous colubrid snake species that mimics vipers by head triangulation. As effectiveness of Batesian mimicry is dependent on abundance of the model species (Huheey 1964, Lindström *et al.* 1997, Joron and Mallet 1998, Mallet 1999, Honma *et al*. 2008) the abundance of viper population can be a crucial factor influencing survival of the smooth snakes in terms of ensuring the efficacy of their natural anti-predator strategy. To understand the efficiency of the mimicry in nature, we need to understand the geographic distribution and relative and absolute abundances of models and their mimics in the field, which was a goal in the fourth chapter of my thesis.

My study revealed the existence of three distinct maternal lineages of smooth snakes across its species range in Europe (IV). These clades expanded towards the north from Iberia, Balkan and Caucasus. Scandinavian individuals belong to the central clade originating from Balkan and colonized Åland islands from west via Sweden. Åland's smooth snake population is thus the northernmost smooth snake population in Europe. Edge populations are often more vulnerable for extinction than populations in the middle of a species's distribution range (Lawton 1993, Vucetich and Waite 2003) In addition, Åland is the island which obviously limits gene flow into the population. Indeed, I found that smooth snakes in Åland appeared to be fragmented into two sub-populations (IV) having alarmingly small population sizes. The sub-division of the population may be caused by several factors. First, in addition to being naturally fragmented by island mosaics the habitat is also further fragmented by anthropogenic influences (e.g. urbanization, road network, and agriculture). The smooth snake is shown to be highly susceptible to habitat fragmentation (Santos *et al*. 2009). Habitat-suitability models and field observations by Santos *et al.* (2009) showed that in the Iberian peninsula smooth snakes occur mainly in small and fragmented populations existing only on high grounds. Second, intrinsic life-history traits can have a major influence on the population structure of the smooth snakes. For example, in cool climates snakes often use the same hibernacula for overwintering year after year, which may limit their home range (Viitanen 1967, Larsen 1987, Weatherhead and Housak 1998), and also limit gene flow between different locations. This behaviour may also make them very vulnerable to the any natural or antropogenic disturbances because in the worst case scenario the whole population can vanish if the overwintering site is destroyed. Third, low movability of smooth snakes is also suggested to be a one of the major factors determining its population subdivisions in southern UK (Pernetta *et al*. 2011). Finally, in islands such as Åland, selection and drift can outweigh gene flow through immigration, thus enabling evolution of local adaptations and genetic differentiation between populations. Thereby, habitat fragmentation, phylopatric behaviour, and isolation, can all contribute to the fragmentation of smooth snake populations in Åland, making them vulnerable to local extinctions.

Indeed, the effective population size (number of breeding females) of the smooth snake in Åland is alarmingly low, only 41.35 individuals. This suggests the need to reconsider its national red list status and re-classification as endangered instead of its current vulnerable status (IV). Surprisingly, the effective population size of the adder, the model species that smooth snakes are mimicking, appeared to be even lower (28.93 breeding females) (J.A. Galarza unpublished data). According to the model I fit on the data of Lindström *et al.* (1997), mimicry is not anymore beneficial when the model mimic ratio is lower that 0.48 (V). Based on estimates of my model, predation risk of the mimics exceed the average predation risk of the prey community after model-mimic ratio reach that threshold value, thereby making mimicry disadvantageous. Thus the relative abundance of adders seems to be alarmingly low (0.41) compared to the mimetic smooth snakes. This result suggests that the natural anti-predator strategy (viper mimicry) of the smooth snakes might not be effective anymore until the populations of the adders are first secured. Thus, these results highlight the importance of considering the status of the model species as well when the mimic is in need of conservation actions; and in my specific system suggests that the adders as well as their smooth snake mimic, should be protected in Åland.

4 CONCLUSIONS

Predation is one of the most powerful selective forces in nature and various anti-predator strategies have evolved among prey animals. Aposematism is one of the most curious anti-predator strategies among prey animals because of its apparent contradiction of conspicuous self-advertisement for avoiding predation. Visual warning signals of aposematic preys are conventionally connected with conspicuous colours. However, also seemingly inconspicuous features of prey animals can serve as warning signals. In my thesis I show that the characteristic dorsal zigzag pattern of European vipers and their typical triangular head shape has a warning signal function. Plasticine snake models with these characters suffered less predation attempts by free ranging raptors than snake model without them (I, III).

When an effective warning signal that predators learn to avoid evolves it opens the possibility for mimicry to also evolve. Mimicry seems to be common among snakes, raising an interesting aspect on conservation of endangered snake populations. Mimicry as an anti-predator strategy is always dependent on the abundance of the other species that share the warning signals. Therefore during the conservation-management of endangered species, mimicry dynamics, if applicable, should be considered among other ecological and evolutionary factors. Sometimes protecting the model as well as the mimic can be crucial for ensuring the natural anti-predator strategy (mimicry) of the target species remains effective. This is especially useful in cases where humans also hunt the models. Such a case can be found in the Åland islands where endangered smooth snakes mimic the appearance of venomous adders. Adders are persecuted and often intentionally killed by humans, making viper mimicry of the smooth snake disadvantageous during encounters with humans. Protecting the adder in Åland could also prevent humans directly, though partly unintentionally, from killing smooth snakes as well. Furthermore, small effective population size of fragmented populations suggests the need for re-evaluation of the national IUCN red list status of the smooth snakes in Åland (IV, V). In future, it would be interesting and important to study other potential examples of model-mimic systems where one of the players (model or mimic) is declining or endangered. According to my knowledge those kind of systems are not yet described apart from the vipers and smooth snakes presented here. However, I believe that paying more attention to possible mimicry rings while studying endangered species will reveal more cases where considering mimicry among the other evolutionary interactions can lead more adequate conservation actions.

Variation in predator community structure can influence the strength and direction of selection on prey community. Plasticine snake models with the zigzag pattern suffered lower predation in comparison to the non-warning signalling ones when abundance of the specialist avian predators, common buzzards, was low but in high buzzard densities zigzag patterned snakes suffered higher predation than non-warning signalling ones (II). Thus, if specialist predators that are able to cope with the preys secondary defence are abundant in the predator community then selection on the signal conspicuousness can be relaxed favouring less conspicuous warning signalling. Thereby it can be expected that in the areas where specialist predators are rare, selection towards conspicuousness of warning signals is stronger than in the areas where proportion of the specialist in the predator community is higher. High abundance of specialist predators may also relax selection towards monomorphic warning signals and accurate mimicry. This is because variation in the prey appearance requires use of wider search image from the specialist predators leading to decreased probability of detection of the prey. This hypothesis, however, requires further studies to reveal if predation by itself as a selective force can explain the maintenance or origin of variation in warning signals and imperfect mimicry.

My results also show that predators are capable of recognising and avoiding inconspicuous features of prey that do not necessarily need to be initially evolved for signalling purposes (e.g. head shape of vipers). If predators can distinguish defended prey species based on some feature and readily avoid them, in some extent, that may allow gradual evolution of more conspicuous signalling which can further facilitate predators avoidance.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Kyykäärmeiden varoitussignalointi ja niiden matkijat.

Eläinten väritys on kiehtonut evoluutiobiologeja jo Darwinin ajoista lähtien, koska se antaa mahdollisuuden tutkia luonnonvalinnan toimintaa ja sen seurauksia hyvin tehokkaasti. Yksi tärkeimmistä värityksen tehtävistä on tarjota suojaa saalistusta vastaan. Osa eläimistä välttää havaituksi tulemista sulautuen taustaansa kryptisen värityksensä avulla. Aposemaattiset lajit puolestaan viestittävät saalistajille toissijaisesta puolustuksestaan, kuten myrkyllisyydestä, varoitussignaalein. Väitöskirjassani tutkin varoitussignaaleiden evoluutiota, malli–matkija-systeemeiden dynamiikkaa eurooppalaisilla kyykäärmeillä ja niiden matkijoilla sekä mallien ja matkijoiden vaikutusta Ahvenanmaan kangaskäärmeiden suojelun kannalta tarpeellisiin toimenpiteisiin.

Saalistuksen on perinteisesti ajateltu ajavan varoitusvärejä kohti yhä suurempaa näkyvyyttä, koska pedot tunnistavat ja oppivat yhdistämään silmiinpistävät signaalit helpommin saaliin haitallisuuteen verrattuna saaliseläimiin, jotka eivät signaloi tai signaloivat huomaamattomasti. Kaikki pedot eivät kuitenkaan vältä aposemaattisia saaliita vaan voivat tappaa ja jopa syödä niitä. Tällaiset pedot saattavat käyttää varoitussignaalia jopa vihjeenä; varoitusvärinen saaliseläinhän on helppo huomata. Tällaisessa tilanteessa näkyvän signaloinnin kustannukset kasvavat ja voivat jopa kääntyä saaliseläintä vastaan. Moni myrkyllinen laji ei olekaan erityisen huomiota herättävän värinen.

Yksi tällainen myrkyllinen lajiryhmä, jonka väritys näyttäisi olevan pikemminkin kryptinen kuin ylettömän silmiinpistävä, on eurooppalaiset kyykäärmeet. Kyiden tyypillisen sahalaitakuvioinnin on havaittu suojaavan niitä saalistukselta. Mihin mekanismiin suojaväritys perustuu, on ollut epäselvää. Sahalaitakuvioinnin on esitetty tekevän käärmeen värityksestä taustaan sulautuvan eli kryptisen. Sahalaitakuvion on esitetty rikkovan eläimen ääriviivat, joka vaikeuttaa sen havaittavuutta. Tätä puolustusstrategiaa kutsutaan hajottavaksi väritykseksi. Uudemmat tutkimukset ovat kuitenkin osoittaneet kyiden sahalaitakuvion toimivan varoitussignaalina niiden myrkyllisyydestä saalistajille. On myös mahdollista, että nämä eri strategiat eivät ole toisiaan poissulkevia; sahalaitakuvio voi haitata tietyssä tilanteessa käärmeen havaittavuutta toimien kuitenkin varoitussignaalina kun se on havaittu. Väitöskirjassani suunnittelin kokeen, jossa pystyin erottamaan Euroopan kyykäärmeille tyypillisen varoitussignaalin ja mahdollisen hajottavan värityksen aiheuttaman suojavaikutuksen toisistaan.

Tulokseni vahvistavat aiemmat havainnot siitä, että eurooppalaisille kyykäärmeille tyypillinen sahalaitakuviointi toimii petolinnuille varoitussignaalina kantajansa myrkyllisyydestä. En kuitenkaan havainnut sahalaitakuvion tarjoavan lisäsuojaa saalistusta vastaan sen havaittavuutta vähentävien vaikutusten kautta. Sahalaitakuvion tarjoama hyöty kuitenkin vaihteli alueittain riippuen petolintulajien keskinäisestä runsaudesta. Alueilla joilla hiirihaukka oli runsaslukuinen, saalistuspaine sahalaitakuvioisia keinokäärmeitä kohtaan oli suurempi verrattuna käärmeisiin, joilla ei ollut sahalaitakuviota. Tutkimusalueilla, joilla käärmeitä saalistavia spesialisteja ei ollut tai niitä oli vähän, sahalaitakuvio antoi kantajalleen suojan. Myrkyllisten käärmeiden käsittelyn osaava hiirihaukka ja muut käärmeitä saalistavat spesialistit kumoavat valintapaineen kohti suurempaa näkyvyyttä. Vähemmän huomiota herättävä varoitussignaali näyttäisi olevan hyödyllinen silloin, kun saalistajayhteisössä on petoja, jotka kykenevät murtamaan saaliin toissijaisen puolustuksen. Ajallinen ja paikallinen vaihtelu saalistajayhteisön rakenteessa saattaa selittää, miksi kaikki aposemaattiset lajit eivät vastoin oletusta näytä olevan väritykseltään ylettömän silmiinpistäviä.

Useat myrkyttömät lajit matkivat kyykäärmeiden ilmiasua, saaden näin suojaa saalistajilta, jotka erehtyvät välttämään niitä myrkyllisinä kyinä. Kyykäärmeiden matkiminen ei ole rajoittunut ainoastaan niiden väritykseen. Jotkut lajit, kuten myrkytön kyyrantakäärme, levittävät uhattaessa päänsä, jolloin se muistuttaa kyyn tyypillistä kolmiomaista pään muotoa. Myös kangaskäärme, jonka ainoat ja puutteellisesti tunnetut populaatiot Suomessa sijaitsevat Ahvenanmaalla, matkii uhattuna kyytä litistämällä päänsä kolmiomaiseksi. Mallien ja matkijoiden väliset suhteet saattavat muodostua erityisen tärkeiksi, mikäli malli on harvinainen tai uhanalainen, koska myrkyllisen lajin matkiminen on tehokas saalistuksenvälttämisstrategia vain mikäli mallilaji on riittävän yleinen. Tämä johtuu siitä, että saalistajien välttämisoppiminen ja opitun muistaminen perustuu epämiellyttäviin kohtaamisiin mallilajin kanssa, jota palkitsevat kohtaamiset, samankaltaisen ilmiasun omaavan matkijan kanssa heikentää. Keinokäärmeet, joilla oli kyille tyypillinen kolmiomainen päänmuoto joutuivat petolintujen hyökkäyksen kohteeksi harvemmin kuin käärmemallit, joilla oli kapea myrkyttömille tarhakäärmeille ominainen päänmuoto. Yllättävää kyllä, pelkkä kolmiomainen pään muoto oli yhtä tehokas kuin sahalaitakuvio tai sahalaitakuvio yhdistettynä pään muotoon. Usean signaalin läsnäolo ei siis välttämättä vahvista pedon välttämiskäyttäytymistä.

Kangaskäärme on Suomessa uhanalainen, sen ainoa populaatio esiintyy Ahvenanmaalla. Siitä huolimatta sen alkuperästä ja populaation koosta ei ole saatavilla lainkaan tutkimustietoa. Koska tutkimukseni osoittivat kangaskäärmeen matkivan kyitä, on erittäin tärkeää tutkia sekä mallin että matkijan populaatiokoot, koska niiden menestys on riippuvainen toistensa lukusuhteista. Geneettiset tutkimukseni osoittivat, että kangaskäärmeet ovat levittäytyneet pohjoiseen edellisen jääkauden jälkeen Iberian niemimaalta, Balkanilta sekä Kaukasukselta. Näistä kolmesta maternaalisesta linjasta Balkanin linja on ainoa, joka on levittäytynyt Fennoskandiaan. Ahvenanmaalle Ruotsin kautta levittäytyneen kangaskäärmepopulaation efektiivinen koko osoittautui hälyttävän pieneksi, ollen vain 41 lisääntyvää naarasta. Tämän lisäksi Ahvenanmaan kangaskäärmepopulaatio on jakautunut kahteen osapopulaatioon, joka ennestään lisää niiden riskiä kuolla sukupuuttoon. Yllättäen Ahvenamaan kyykäärmepopulaatio osoittautui jopa kangaskäärmepopulaatiota pienemmäksi, mikä viittaa vahvasti myös kyykäärmeen suojelun tarpeeseen Ahvenanmaalla. Kangaskäärmeiden luontainen saalistuksenvälttämisstrategia on tehokas vain, mikäli myrkyllinen mallilaji on riittävän runsaslukuinen. Jos Ahvenanmaan kyypopulaatio pienenee entisestään se todennäköisesti vaarantaa kangaskäärmeen luontaisen saalistuksenvälttämisstrategian toimivuuden, sekä kangaskäärmeeseen kohdentuvat suojelutoimet.

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ORIGINAL PAPERS

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DISRUPTION OR APOSEMATISM? SIGNIFICANCE OF DORSAL ZIGZAG PATTERN OF EUROPEAN VIPERS

by

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Disruption or aposematism? Significance of dorsal zigzag pattern of European vipers

Janne Valkonen · Martti Niskanen · Mats Björklund · Johanna Mappes

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Abstract European vipers (genus *Vipera*) are venomous and often have a distinctive dorsal zigzag pattern. The zigzag pattern of vipers has been suggested to be an example of disruptive colouration which reduces the detectability of a snake. However, recent studies suggest that the patterns have an aposematic function, although those experiments did not exclude the possibility of disruptive colouration. We used plasticine replicas of snakes to examine whether the zigzag pattern of European vipers provides protection from avian predator attacks via disruptive or aposematic function, or if the zigzag pattern might simultaneously serve both antipredatory functions. Experiments were conducted in the Coto Doñana National Park southern Spain. In the experiment, predation pressure caused by birds was compared between zigzag pattern (patterns were painted with and without disruptive effect i.e. breaking body outline or not), classical disruptive colouration (non-randomly placed patterns that breaks body outline) and control markings (replicas with length wise stripes and models without painted pattern) on natural and controlled backgrounds. We found that zigzag patterned snake replicas suffered less predation than striped ones regardless of the background, providing further evidence that the zigzag pattern of European vipers functions as a warning signal against predators. However, we did not find evidence that the zigzag pattern involves a disruptive effect.

Keywords Aposematism · Avian predation · Disruptive colouration · Protective colouration · Snakes · Vipera

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Introduction

Protection from predators is one of the most vital functions of animal colouration (Cott 1940; Ruxton et al. 2004). For example, animals can have highly detectable warning colour patterns that inform predators that the individual is toxic, unpalatable, or otherwise unprofitable (i.e. aposematism) (Poulton 1890; Ruxton et al. 2004). A more common strategy, however, is to hide from the predators by matching to the coloration of the background, or by making the detection of edges and boundaries of their body parts and/or outline more difficult (disruptive coloration) (Stevens and Merilaita 2009a, b). Disruptive colouration can decrease detectability even in backgrounds that do not perfectly match the colouration of an animal (Thayer 1909; Cott 1940; Stevens et al. 2006; Stevens and Merilaita 2009a). Disruptive colouration often contains both highly conspicuous and cryptic elements so that the signal noise ratio can be higher than in background matching, even though disruptive camouflage might work best when it shares a contrast range with the background (Stevens and Merilaita 2009b). The theory of disruptive colouration predicts that elements of colouration used to break up body outlines should be located more peripherally than elements of background matching colouration. The edge breaking elements are expected to make the body shape of an animal more difficult to detect than randomly placed elements (Cott 1940). Recently, experiments by Cuthill et al. (2005), Merilaita and Lind (2005), Stevens and Cuthill (2006) and Stevens et al. (2006) found support for this expectation. They designed artificial prey models with artificial pattern elements and found evidence for survival benefits of disruptive colouration. Schaefer and Stobbe (2006) used peach blossom (*Thyatira batis*) (L.) as a model for designing artificially disruptively-coloured moths and found that colour morphs with disruptively coloured edges were protected even with reduced background matching.

Unlike cryptic or disruptive colouration, warning colouration is generally assumed to be highly conspicuous as it ensures that the message is effectively delivered to predators (Poulton 1890; Cott 1940; Edmunds 1974; Evans and Schmidt 1990; Endler 1991). Predators learn to avoid unpalatable prey more quickly when they are conspicuous than when their colouration is cryptic (Gittleman et al. 1980; Gittleman and Harvey 1980; Roper and Wistow 1986; Linström et al. 1999). Increasing signal size (Linström et al. 1999; Lindstedt et al. 2008) and symmetry has also been shown to increase signal efficiency by increasing predator’s avoidance (Forsman and Merilaita 1999; but see Stevens et al. 2008). However, conspicuousness also increases detectability and therefore increases the risk of being attacked by naïve or specialist predators. In addition, if predators do not recognize the signal or they are immune to prey defence, conspicuousness may cause high costs for its carrier (Endler and Mappes 2004). According to the model by Endler and Mappes (2004), if there are significant within- and/or among-species variation in a predator’s tendency to attack aposematic prey, it is possible that selection will favour weak signalling in aposematic species, relaxing the selection for increased conspicuousness.

Sherrat and Beatty (2003) suggested that to be effective, the warning signal does not necessarily need to be conspicuous as long as it is distinctive from profitable alternatives. Also, if an increase in either toxicity or conspicuousness offers equally good protection against predators (Darst et al. 2006), it could enable the existence of weak visual signals in defended species. Furthermore, all aposematic species do not seem to maximise the conspicuousness of their signals and many species express only a moderate or weak signal. For example, adders (*Vipera berus*) (L.) are rather inconspicuously coloured, and can appear even cryptic against their natural background although they are considerably venomous. Thus, it seems that classifying prey protective colourations into two extremes, “cryptic” and “aposematic”, could be artificial since those different antipredatory strategies are not mutually exclusive and represent a continuum from crypsis to aposematism rather than two separate strategies. Similarly, prey palatability is likely to be a continuum rather than two extremes of prey profitabilities (Brower et al. 1968). Furthermore, the aposematic signal can be cryptic from a distance and easily recognizable from close by (Marshal 2000; Sherrat and Beatty 2003; Tulberg et al. 2005). For example, the colouration of *Parnassius apollo* (L.) larvae is suggested to have a distance-dependent switch from conspicuousness to camouflage with increasing distance (see Tulberg et al. 2005; Bohlin et al. 2008).

Many species of European vipers (genus *Vipera*) (Laurenti) exhibit a typical dorsal zigzag pattern. Several previous experiments have shown that the zigzag pattern provides some protection against predation (Andrén and Nilson 1981; Forsman 1995b; Lindell and Forsman 1996; Wüster et al. 2004; Niskanen and Mappes 2005). The protective effect of the dorsal zigzag pattern was first described by Andrén and Nilson (1981) when they compared predation pressures against melanistic and zigzag-patterned adders (*Vipera berus*). They found that melanistic individuals were attacked more often than those with a zigzag pattern and suggested that the zigzag pattern makes the snakes more cryptic. However, they did not control for the effect of the background and could therefore not rule out the possibility of an aposematic function of that pattern. In later experiments Wüster et al. (2004) confirmed that zigzag-patterned snakes were attacked less by avian predators also in the situation where conspicuousness (or background matching) was controlled. They placed snake replicas both on the natural background and on white paper sheets. Zigzag-patterned snake replicas on both backgrounds were attacked less than snake replicas without the pattern, suggesting that the pattern had a warning function. Later, Niskanen and Mappes (2005) repeated the experiment in southern Spain and confirmed the results of Wüster et al. (2004). However, the zigzag pattern of vipers is also used as an example of disruptive colouration (Cott 1940; Edmunds 1974; Shine and Madsen 1994) and the experiments by Wüster et al. (2004) and Niskanen and Mappes (2005) did not control the possibility of disruptiveness in the pattern and used only one type of zigzag pattern. Thus, it is possible that the higher survival of zigzag patterned snakes in those experiments was influenced/caused by disruptive colouration and not by aposematism. Furthermore, those experiments used paintless snake replicas as controls for the zigzag-patterned snakes and thus, there may have been a potential effect of black colouration (paint) per se, which could have potentially caused aversion in the predators. Aposematism and disruptive colouration may also interact and may therefore not necessarily be mutually exclusive. Even though some experiments have tested the protective effect of disruptive colouration (see Cuthill et al. 2005; Stevens and Cuthill 2006; Schaefer and Stobbe 2006; Stevens et al. 2006), they are focused on flat-bodied prey (e.g., moths). Unlike 2D patterns where an outline of the body is unequivocal, locations of boundaries in three dimensional objects depend on the viewing angle (see Stevens and Merilaita 2009a). Moreover, most previous experiments do not use patterns of real animals but are based on samples of natural background.

In this field study we used plasticine snake replicas to test if the zigzag pattern of European vipers has a disruptive or an aposematic function, or if the zigzag pattern has both antipredatory functions. To test this, we compared attack frequencies by natural predators towards artificial snakes on natural and white control backgrounds. In the first experiment, zigzag-patterned snake replicas with and without disruptive effect were used to see if the zigzag pattern has an aposematic or disruptive function. Disruptive-coloured snake replicas were used to compare the efficacy of disruptive and warning colourations. We used striped snake replicas as a reference to compare the protective values of disruptive and warning colourations, and also to control the possible effects of a black pattern per se. Effects of the white control background and interactions between pattern types and different backgrounds were controlled using patternless snake replicas. Due to an increased signal size in the snake replicas with the disruptive zigzag pattern in the first experiment, we conducted a second experiment to determine if the higher survival was caused by an enhanced signal size or by disruptive colouration. We compared predation pressure caused by avian predators between edge-breaking zigzag-patterned snake replicas, regular zigzag-patterned snake replicas with equal signal size and patternless snake replicas.

Materials and methods

Plasticine snake models

Plasticine models are an effective method of estimating attack rates by predators on snakes because a large number of replicas can be used. Predators such as raptors and mammals can be distinguished from the bite, beak and claw marks left on the soft clay model surface (see Andrén and Nilson 1981; Brodie and Janzen 1995; Wüster et al. 2004; Niskanen and Mappes 2005; Fig. 1). Differentiating between raptor and mammal attacks is crucial when plasticine prey items are used as the odour of plasticine may even attract mammals towards replicas (Rangen et al. 2000). Thus, we recorded bite marks made by mammals and claw marks made by raptors separately.

Snake replicas with five different pattern types were used in the experiment (Fig. 2). Pattern (Z) represented a typical dorsal zigzag pattern of European vipers, which has been suggested to function as a warning signal (Wüster et al. 2004; Niskanen and Mappes 2005). The second pattern (ZE) was aimed to represent an aposematic zigzag pattern with a disruptive effect; the zigzag pattern was painted to break down body outlines. Edge enhancing zigzag pattern (ZE) is artificial but it is a hybrid of common zigzag patterns of European vipers (e.g. *Vipera berus* and *V. latastei*) and cross-banded pattern typical for *Vipera aspis* (see for example De Smedt 2001).The third pattern type (D), which was produced to represent classic disruptive colouration without any other functions, was crucial because it allowed for a comparison between the protective effects of disruptive and warning colourations. The fourth pattern was striped (S) to control the effect of the black

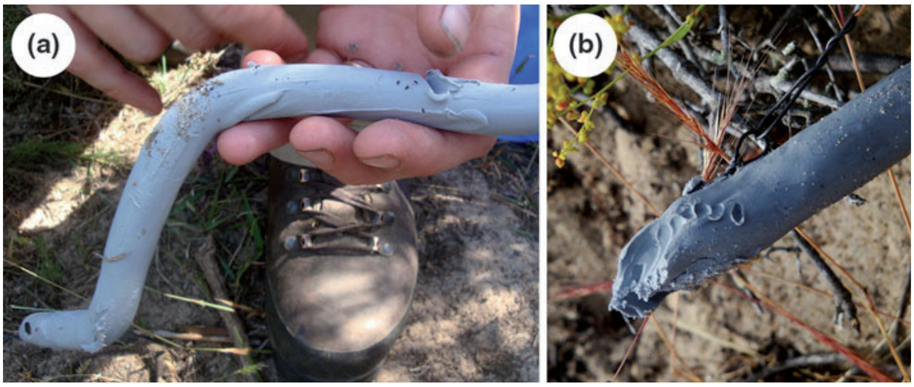

Fig. 1 Raptor (**a**) and mammalian (**b**) attacks can be distinguished from imprints left during attack

Fig. 2 The five different pattern types of plasticine snake replicas used in this experiment. Codes of model types left to right *Z* normal zigzag pattern, *ZE* zigzag pattern with edge violating, *S* striped, *D* classic disruptive and *P* plain

paint and the contrast between the paint and the snake replicas. Stripes were painted along body outlines. It also represented colouration without any known protective function that could decrease predation. It also acted as a reference in comparing the protective effects of other colour patterns. Plain grey (P) snake replicas were constructed for controlling possible interactive effects of paint per se and different backgrounds (natural and white) if some differences in the amount of attacks between the backgrounds had appeared. If the zigzag pattern is an aposematic signal as Wüster et al. (2004) and Niskanen and Mappes (2005) have suggested, both Z and ZE patterns should invite fewer attacks than S and P (see below and Fig. 2). If, in addition to its aposematic function, the wider ZE-pattern also has a disruptive function, we may expect ZE to invite fewer attacks than Z-patterned snake replicas.

Snake replicas were made from pre-coloured grey non toxic plasticine (Caran D’Ache, Modela Noir, grey 0259.005) and different patterns were painted on them with black paint (Bebeo acryl colour 374611 & Perinnemaali *art. nr.* 5511-05). A fifty percent mixture of both paints were used to ensure patterns were satin black. Snake replicas were sprayed with an insect repellent (Autan® by Johnson) to make them unpalatable and to reduce the amount of snake replicas eaten by mammals and insects (mainly beetles). The length of the snake models were 36.0—45.5 cm and approximately 100 g plasticine were used for each model. Ten of each model type were randomly chosen and photographed (Canon 350D with Canon EF-S 18—55 mm objective lens) to estimate the amount of black colouration in each type of snake replicas. Photographs were taken on a tripod, from a standard distance by using the same camera settings and focal length so that images were in a standard scale. Those images were printed afterwards with a black and white colour profile and the black pattern of each image was cut out and weighed to the nearest 0.1 mg (Mettler toledo XS204 by Delta Range®). The weights of the paper were used to estimate the total amount of black colouration in each snake replica.

Experiment areas

Experiments were conducted in Coto Doñana national park in southern Spain (37°00 N, 06°38 W) between 1st and 26th of April in 2008 and between 5th and 20th of May in 2009. Habitat types of the experiment locations were Monte Negro, Monte Blanco and sand dunes. Monte Negro and Monte Blanco habitat types were fairly open loosen sandy soil areas, consisting of low vegetation and a variable amount of pine trees *Pinus pinea* (L.), *Juniperus sp.* (L.) and *Erica sp.* (L.) bushes. Sand dune habitats were open and vegetation consisted mainly of wide-spaced bushes. Visibility of the snake replicas to avian predators was different between areas but we placed them as conspicuously as possible, not hidden by bushes or trees. Special care was also taken to make each snake replica equally visibly within each area. For a more specific description of the habitat types see (Niskanen and Mappes 2005).

Snake species in the experiment area

Eight species of snakes occur in Coto Doñana national park, including three venomous ones. The only front-fanged species in the area is *Vipera latastei gaditana* Saint-Girons and the other two venomous species are rear-fanged *Malpolon monspessulanus* (Hermann) and *Macroprotodon cucculatus* (Geoffroy). Non-venomous snake species in the park area are *Natrix natrix* (L.), *Natrix maura* (L.), *Rhinechis scalaris* (Schinz), *Hemorrhois hippocrepis* (L.) and *Coronella girondica* (Daudin).

V. l. gaditana exhibits the typical dorsal zigzag pattern of European vipers (Fig. 3) and it is one of the most defensive species in genus *Vipera* (De Smedt 2001). Another species with a dorsal zigzag pattern in the area is *N. maura* which potentially mimics the Lataste's viper (*V. l. gaditana*) colouration. When disturbed it makes the mimicry even more perfect by flattening its head to mimic the typical triangular head shape of vipers (Arnold and Burton 1978). Colouration of *R. scalaris* varies according to the age of individuals. Juveniles usually have a ladder-shaped dorsal pattern, which changes to two lengthwise stripes with age. Adults of the species are typically length-wise striped (Fig. 4). The rest of the snake species in the area have a speckled or uniform colour pattern.

Predators

There is a large number and variety of avian predators in Coto Doñana national park. The most commonly sighted species are Black Kites (*Milvus migrans*) (Boddaert), Red Kites

Fig. 3 Yearling captive bred *Vipera latastei gaditana* male. This species exhibits one of the most highly conspicuous colourations of the genus *Vipera*. This secretive species is highly defensive when disturbed. Photograph by J.V.

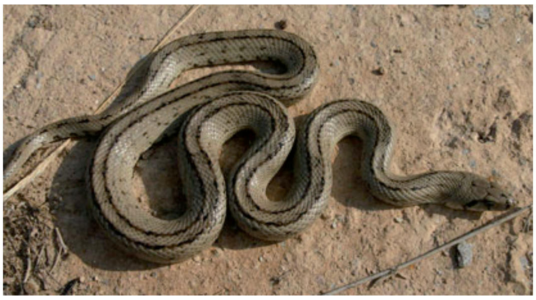

Fig. 4 This individual exhibits typical length-wise striped colouration of adult ladder (*Rhinechis scalaris*) snakes. Photo was taken in Murcia southern Spain by Matt Wilson

(*Milvus milvus*) (Lacepede), Common Buzzard (*Buteo buteo*) (L.) and Booted Eagle (*Aquila pennata*) (Gmelin). Some Short-Toed Eagle (*Circaetus gallicus*) (Gmelin) were also occasionally sighted and are expected to be responsible for the attacks on the snakes. The Short-Toed Eagle is a snake specialist and its diet consists mainly (95%) of snakes (Cramp 1985). The Common Buzzard is more of a generalist in its diet, feeding mostly on small mammals, but it has also been reported to consume amphibians and reptiles, including snakes (Selas 2001). Black Kites and Red Kites are both food generalist species (Cramp 1985), but there is also an observation of snake remains within Black Kite nests (Fabrizio Sergio personal communication April 2008). One Common Buzzard, one Short-Toed Eagle in 2008, and one Booted Eagle in 2009 were observed carrying or attacking a snake (personal observation).

Observations of hunting raptors were made by observing raptors flying above the experiment area with binoculars and a telescope. Hour-long observations were made at the start of each trial day between 10 am and 3 pm. During the first experiment (see next chapter), raptor observations were collected from the five study areas. For most species, except the black kite, the observations were few and restricted to one, or two, individual(s) seen once during the observation period (see Results) and hence the possibility of counting the same individual twice basically non-existing. The black kite often appeared in pairs or in larger numbers and were seen circling over a larger area for most of the observation time. This does not exclude the possibility that disappearing black kites were counted twice when new observations were made within the time frame and hence the number of black kites might be slightly overestimated. However, this does not affect the relative numbers more than marginally (black kites being the by far most abundant raptor present) and hence not the correlations presented.

Experiment I: effect of distruptiveness of zigzag patterns

In each trial, fifty snake replicas were used: ten of each pattern type. Half of the replicas of each pattern type were set on the natural background and other half on white A4-size paper sheets (Eclipse Quality paper 80 g/m^2 A-4). The white background controlled for possible background matching of models by making them conspicuous against the white control background. All plasticine snake replicas were set in the field in an s-shaped position, so that they looked as natural as possible (and also to fit the models on the background paper). Pattern types were placed in transect lines in random order, at approximately 15 m (15 steps) intervals. Lengths of the transects were approximately 0.75 km and they followed the shape of the terrain, thus varying from straight lines to U-shaped. Every other snake replica was placed on the white paper while the others were placed on the natural background. Snake replicas were tied to bushes or to dead branches with iron wire to prevent predators from taking or moving them during or after an attack. Trials were started in the evening between 5 and 8 pm. Snake replicas were then checked for the first time the next morning (after 12–16 h) and again in the evening (after 24–27 h). Snake replicas were checked a final time and collected from the field during the third day (after 44–47 h). In three trials when the weather was rainy, an exception to this schedule was made as one checking was skipped and the trial was left in the field for another 24-h period. This was done because raptors are observed to be inactive in rainy weather. The experiment was repeated 18 times in 15 different locations; two trials were going on simultaneously. The mean distance between experiment locations was 4.3 km (max. 9.7 km and min. 0.4 km) and the mean distance between simultaneously ongoing trials was 4.8 km (max. 9.4 km and min. 3.0 km). During the morning and evening checks, all attacked or damaged snake replicas were restored (claw marks etc. were hidden) or replaced for the following trial. If the same restored or replaced snake replica was attacked more than once by a raptor or mammalian predator during the trial, only the first occasion was included to reduce bias caused by multiple attacks from an individual predator.

Attacks by mammals that are potential predators of snakes (e.g., foxes, genets, lynx and wild boars) were recorded separately from raptor attacks. Footprints of animals were easily detectable in the soft sandy soil of the experiment areas, thus enabling the distinction. Differentiating between raptor and mammal attacks was quite easy if snake replicas were not removed after an attack, as claw marks were easily separated from tooth marks (Fig. 1). When a snake replica had been taken, the surroundings where it had been placed were investigated, and footprints of mammals or wing-marks of birds were usually found. If the snake replica had been taken away without any visible trace of a predator, the attack was considered as having been caused by a raptor. If there were multiple raptor attack marks in a snake replica, it was recorded as one attack because we could not say whether they had been caused by one or several predation events.

Experiment II: effect of signal size per se

In experiment I, the wide zigzag patterned replicates were found to have significantly more black paint compared to the other patterns (see above). Thus, it is possible that the better survival of wide zigzag patterned snakes was due to signal strength per se rather than disruptive colouration. We therefore carried out another experiment to separate between those effects. We compared attack rates on both zigzag patterned snake replicas (Z and ZE) with equal amount of black paint on the patterns and plain (P) type snake replicas (Fig. 2). The amount of black colouration in snake replicas Z and ZE was measured from five randomly chosen models of both colour types using the previously mentioned technique.

In each trial, thirty snake replicas were used: ten of each pattern type. The experiment was conducted in a similar way as the first experiment, except that models were placed only on a natural background because background has never been found to have a significant effect on attack rates (Brodie 1993; Wüster et al. 2004; Niskanen and Mappes 2005; experiment I). The trials started in the afternoon between 3 and 6 pm. Snake replicas were then checked for the first time the next morning (after 15–19 h) and again in the afternoon (after 20–27 h). Snake replicas were checked the final time and collected from the field during third day (after 42–49 h). Four exceptions from this schedule were made. In three trials one morning or afternoon checking was skipped and in one trial only the final checking was conducted. The experiment was repeated 12 times in 11 different locations with two trials going on simultaneously. Mean distance between experimental locations was 8.2 km (max. 28 km and min. 2.3 km) and mean distance between simultaneously ongoing trials was 7.1 km (max. 19.2 km and min. 2.3 km).

Statistical methods

When protective effects of the different pattern types were compared, the data was treated as one independent sample. When comparing predator community structures and the amount of attacks, different trials were treated as independent samples. Because of the dichotomy of the attack data and the reasonable sample size ($n = 900$), Chi-square tests and general log-linear models were used in the experiment 1. G-test of goodness of fit was used during model fitting because of additive properties of test values, which can be used to compare several models (see Sokal and Rohlf 1995). In the experiment 2, Fisher's exact test of independence was used to compare significance of number of attacks on the different pattern types (Sokal and Rohlf 1995), because sample size ($n = 360$) was smaller. We used the equation $OR = \frac{q1/p1}{q2/p2}$ (Sokal and Rohlf 1995) while odds ratios (*OR*) between pattern types were calculated from a two by two contingency table. All the statistical analyses were conducted with PASW statistic 18 and R 2.8.1.

Results

Experiment I: effect of disruptiveness on zigzag pattern

There were significant differences in the amounts of raptor attacks on the different pattern types (Chi-square test: $\chi^2 = 23.8$, $df = 4$, $P = < 0.001$), but no differences existed between natural and control backgrounds (Chi-square test: $\chi^2 = 0.014$, $df = 1$, $P = 0.91$). To make a more detailed conclusion about the interactions between different pattern types and backgrounds, a general log-linear model was fitted into the data (Table 1). In the log-linear model, attacks were independent of any interactions between background and pattern types. Snake replicas with pattern type ZE suffered fewer attacks than others. Pattern types Z and D performed equally well, and better than snake replicas with the striped (S) pattern. Odds ratios between different pattern types attacked by avian predators are shown in Table 2 (see also Fig. 5). Attacks by mammalian predators did not differ between pattern types (Chi-square test: $\chi^2 = 1.143$, $df = 4$, $P = 0.89$) but mammals attacked the snake

Table 1 Log-linear model fitting, relationships between attack rates, pattern types and different backgrounds

Model	Attacks are dependent on	G^2	*df*	Difference between models	*df*
1. A × P × B	Interaction between P and B	0.000	0		
2. A × P + A × B + P × B	Both P and B	3.026	4	1 and 2, $G^2 = 3.026$	4
3. A + P + B + A × P + A × B	Both P and B	3.026	8	2 and 3, $G^2 = 0.000$	4
4. A + P + A × P	Only P	3.040	10	3 and 4, $G^2 = 0.014$	2
5. A + B + A × B	Only B	27.386*	16		
6. A + P	Independent of P	27.399*	14		

P, is pattern type of snake replica (Fig. 2.), B, is background (natural or control) and A, is attack (attacked or not). The best fitting model is underlined. * Sig. < 0.05

Table 2 Odds ratios and G^2-test values between different pattern types of snake replicas attacked by avian predator

Pattern pair	Odds ratio	CI 95%	G^2 *df* 1	Sig. two tailed
Z and ZE	2.72	0.95–7.81	3.870	**0.049**
Z and D	1.00	0.45–2.22	0.000	1.000
Z and S	2.57	1.29–5.11	7.820	**0.005**
Z and P	1.61	0.77–3.34	1.646	0.199
D and ZE	2.73	0.95–7.81	3.870	**0.049**
S and D	2.57	1.29–5.11	7.820	**0.005**
P and D	1.61	0.78–3.34	1.646	0.199
S and ZE	7.00	2.65–18.49	21.737	**>0.001**
P and ZE	4.36	1.60–11.93	10.309	**0.001**
S and P	1.60	0.87–2.94	2.336	0.126

The likelihood of the pattern type mentioned first in each pattern pair being attacked is indicated by the odds ratio value. Codes of pattern types are *Z* normal zigzag pattern, *ZE* Zigzag pattern with edge violating, *S* striped, *D* classic disruptive and *P* = plain (see also Figs. 2, 5). Values of significance level under 0.05 are bolded

replicas more often on the natural background (Chi-square test: $\chi^2 = 5.612$, $df = 1$, $P = 0.018$). 9% of total 900 snake replicas were attacked by raptors and 6.9% attacked by mammalian predators.

The length of the models (ANOVA, normally distributed data assumed): $F_{4.45} = 0.88$, $P = 0.48$) and variation in length (Levene test: $F_{4.45} = 0.47$, $P = 0.75$) between different types of snake replica did not differ significantly. The total amount of black colouration on the snake replicas differed significantly (ANOVA, equal variances and normally distributed data assumed: $F_{3.36} = 27.72$, $P < 0.001$). Pattern type Z had a significantly lower amount of black colouration than other patterns. There were also no significant differences between other pattern types (Table 3).

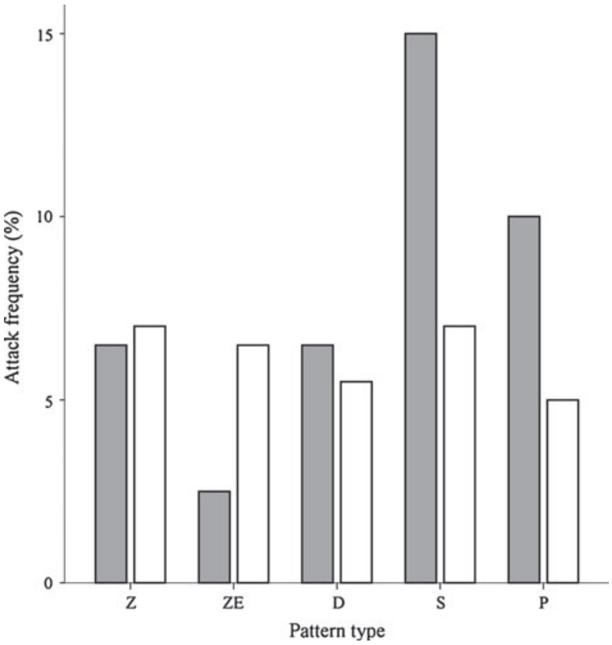

Fig. 5 Attack frequencies (raptor attacks shaded and mammalian attacks white bars) on plasticine snake replicas with different pattern types. Codes of pattern types are *Z* normal zigzag pattern, *ZE* zigzag pattern with edge violating, *D* classic disruptive, *S* striped, and *P* plain (see also Fig. 2)

Table 3 Differences in the amount of black colouration between different pattern types on snake replicas

(I) pattern	(J) pattern	Mean difference mg. (I-J)	CI 95%	*P*
Z	ZE	−6.460	−8.980 to −3.940	**<0.001**
	S	−6.110	−8.630 to −3.590	**<0.001**
	D	−7.840	10.360 to −5.320	**<0.001**
ZE	S	0.350	−2.170 to 2.870	0.982
	S	−1.380	−3.900 to 1.140	0.463
S	D	−1.730	−4.250 to 0.790	0.268

Codes of pattern types are *Z* normal zigzag pattern, *ZE* zigzag pattern with edge violating, *S* striped, *D* classic disruptive and *P* plain (see also Fig. 2). Tukey HSD test. Values of significance level under 0.05 are bolded

Effect of predator community on attack rate

There was a significant positive correlation between the overall raptor attacks towards snake replicas within the observation period and the number of Black Kites (Pearson correlation: $r = 0.911$, $N = 5$, $P = 0.031$). The number of Black Kites was also positively correlated with the number of Red Kites (Pearson correlation $r = 0.90$, $N = 5$, $P = 0.038$) and the total number of observed avian predators (Pearson correlation: $r = 0.98$, $N = 5$, $P = 0.002$), but a total number of raptors did not correlate significantly with overall attack rate (Pearson correlation: $r = 0.83$, $N = 5$, $P = 0.082$). This is important because it lends corrobative evidence for attacks categorized as raptor attacks. There were no significant

Table 4 Total number (over all observation periods) of raptors that were seen hunting in the experiment areas during the observations

Species	Count
Black Kite	25
Red Kite	7
Common Buzzard	5
Booted Eagle	2
Short-Toed-Eagle	1

Observations were conducted during first five trials

Table 5 Odds ratios between different pattern types of snake replicas attacked by avian predator

Pattern pair	Odds ratio	CI 95%	Fisher's exact sig. two tailed
ZE and Z	3.29	1.15–9.35	**0.033**
P and Z	3.29	1.15–9.35	**0.033**
P and ZE	1.00	0.47–2.15	1.000

The likelihood of the pattern type mentioned first in each pattern pair being attacked is indicated by the odds ratio value. Codes of pattern types are: *Z* normal zigzag pattern, *ZE* zigzag pattern with edge violating, *P* plain (see also Figs. 2, 6). Values of significance level under 0.05 are bolded

correlations between other predatory bird species or the number of individuals of raptor species and attacks (Pearson correlations, all $P \leq 0.152$, $N = 5$) see also Table 4.

Experiment II: to control effect of signal size per se

There were significant differences in the attack rates by raptors on different pattern types (Fisher's exact test: 6.92, $P = 0.035$). Pattern type Z was attacked less frequently than pattern types ZE and P. There was also no difference between attack rates on pattern types ZE and P (Table 5, Fig. 6). The amount of mammalian attacks did not differ between treatments (Fisher's exact test: 0.21, $P = 1.000$). Overall, 9.7% of 360 models were attacked by raptor and 4.4% by mammalian predators.

Length (ANOVA, normally distributed data assumed): $F_{2.27} = 0.32$, $P = 0.73$) and variation in length between the different types of snake replica did not differ significantly (Levene test: $F_{2.27} = 2.36$, $P = 0.11$). The total amount of black colouration (t-Test: $t = 0.55$, $df = 8$, $P = 0.60$) and variation of black colouration on the snake replicas with pattern type Z and ZE did not differ significantly (Levene test: $F = 0.28$, $df = 8$, $P = 0.61$).

Discussion

Results of this study confirm previous findings by Wüster et al. (2004) and Niskanen and Mappes (2005) that the zigzag pattern of European vipers is a warning signal. In previous experiments, the black paint was not controlled and there was therefore a possibility that better survival of zigzag-patterned snakes was due to the aversive effect of paint rather than the pattern. However, the results of this experiment clearly show that zigzag-patterned snakes survived better than striped snakes, which controlled for the effect of paint per se. When the signal size of the zigzag pattern was equal, the survival of the snakes with an

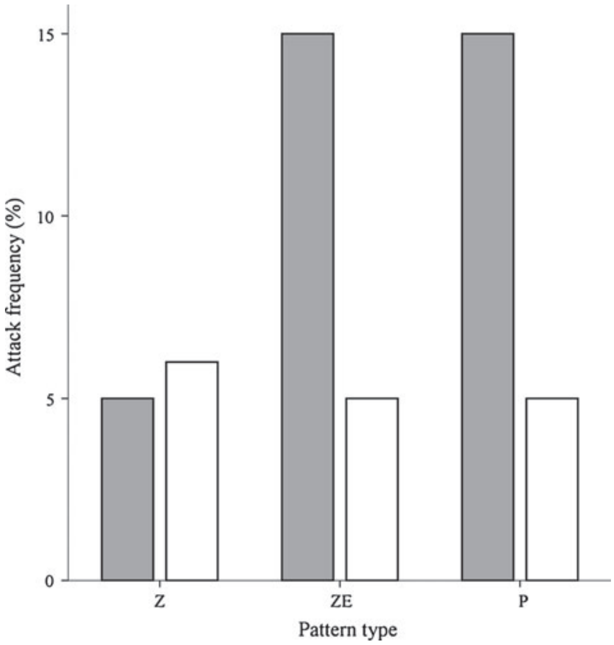

Fig. 6 Attack frequencies (raptor attacks shaded and mammalian attacks white bars) on plasticine snake replicas with different pattern types. Codes of pattern types are *Z* normal zigzag pattern, *ZE* zigzag pattern with edge violating, and *P* plain (see also Fig. 2)

edge-breaking pattern was significantly lower than the survival of snakes with a normal zigzag pattern (Experiment 2, Fig. 6). In addition, the survival of zigzag-patterned snakes (Z and ZE) was higher than the survival of striped ones. Thus, our results suggest that selection by avian predators should favour zigzag patterns in European vipers as it increases their survival. Snake replicas with zigzag (Z & ZE) patterns suffered fewer raptor attacks than striped (S) ones. Furthermore, in terms of signal efficacy, increased signal size (zigzag pattern) offered the best protection against avian predation (Experiment 1, Fig. 5).

In the first experiment, snake replicas with a wide, outline-breaking zigzag pattern (ZE) suffered fewer attacks than those with a typical, non-outline-breaking zigzag pattern (Z) and according to the results of the second experiment, that was caused by the increased signal size rather than a disruptive effect of the signal. In the first experiment the amount of black paint, and therefore the signal size, on Z patterned snake replicas was significantly lower than on the ZE patterned ones. In the second experiment when signal sizes of snake replicas did not differ between pattern types, the opposite was the case. It has previously been shown that increasing the size of a warning signal enhances its efficacy against predation (Gamberale and Tullberg 1996; Forsman and Merilaita 1999; Linström et al. 1999; Lindstedt et al. 2008) and that increased signal size explains the lower attack rate towards ZE compared to Z patterned snake replicas.

Attack rates on all pattern types did not differ significantly between natural and control backgrounds, which indicates that the snake replicas were equally visible to predators on both backgrounds, and therefore none of the pattern types can be considered to be cryptic in terms of background matching. Even if we cannot rule out other possible functions of European viper colouration (e.g. movement-related functions), these findings indicate that zigzag-patterned snakes benefit from aposematism, as described by Wüster et al. (2004) and Niskanen and Mappes (2005), rather than its disruptive or background-matching function. Moreover, the zigzag pattern of European vipers rarely meets the body outlines (see De Smedt 2001), which would be the first precondition of disruptiveness (Cott 1940; Stevens and Merilaita 2009a, b). The second precondition would be that the pattern elements should be distributed in a non-regular manner (Stevens and Merilaita 2009a, b). Neither is true in most European vipers (see De Smedt 2001).

Disruptively-coloured snake replicas (D) suffered fewer attacks by avian predators than striped (S) replicas and equal amount to the replicas with the smaller zigzag pattern. Both the striped and the smaller zigzag pattern types suffered more attacks than snake replicas with the edge-breaking zigzag pattern (ZE). Better survival of the ZE pattern replicas may indicate that the pattern is a more effective warning signal or that there is an additive effect of aposematism and disruptiveness. The wider pattern of ZE snake replicas may exhibit a reduced detectability due to disruptiveness, and the pattern may also act as an aposematic signal when noticed by predators (Cott 1940).

The lower attack rate towards pattern D compared to the striped pattern (S) indicates that disruptive colouration is providing protection against avian predators, even with relatively weak background matching. Previously, experimental research has focused more on flat-bodied animals (see Cuthill et al. 2005; Stevens and Cuthill 2006; Schaefer and Stobbe 2006; Stevens et al. 2006). Our results provide further support for the theory of disruptive colouration and suggest that disruptiveness is also effective with a three-dimensional body shape.

Since the white (control) background was rather unnatural and bright, it could have induced neophobic reactions in predators, or alternatively, the conspicuous background could have attracted them towards the replicas. However, there was no significant difference in the avian predator attack rate on snake replicas between the natural and control backgrounds. Neither were there interactions between pattern types and backgrounds. It is therefore reasonable to assume that the white background only controlled the background matching of different snake replicas.

Overall raptor attacks towards snake replicas correlated positively with the total amount of hunting Black Kites which were the most numerous raptors in our experiment areas. However, the correlation between the total amount of hunting raptors and the attack rate was not significant. These results indicate that raptors, and in particularly Black Kites, were likely to be the main cause of attacks on the snake replicas in our experiment area. Black and Red Kites are both generalist predator species (Cramp 1985) and there are some observations of snake remains in Black Kite nests (Fabrizio Sergio personal communication April 2008). Snake specialists like Short-Toed Eagles (Cramp 1985) are likely to handle venomous snakes without getting injured and therefore may ignore the zigzag pattern of snakes. Thus, we suggest that generalist raptors like Red and Black Kites are more important in selecting warning colouration of snakes than specialist species. However, the present data is only suggestive and this hypothesis would need further testing.

We did not find any evidence that mammalian predators avoided any of the pattern types of model snakes. Using plasticine prey items might not be a suitable study method for mammalian predation, as mammals largely use olfactory cues rather than visual cues during hunting and the odour of plasticine may attract them (Rangen et al. 2000). We also believe that mammals may bite artificial snakes out of curiosity. During the experiments, we observed foxes following our tracks along transect lines and biting every snake replica in their path.

Several species of European vipers are also known to have melanistic (at least almost uniform black) forms (De Smedt 2001). Black colouration is shown to offer some thermoregulation benefits (Forsman 1995a), but the zigzag-patterned forms are still more common. Only in one species of European viper *Vipera nikolskii* (Vedmederja, Grubant & Rudayeva) is the melanistic form more common than the zigzag form (De Smedt 2001). Melanistic individuals are capable of reaching their preferred body temperature faster than zigzag patterned ones and therefore benefit during digestion, growth and reproduction (Forsman 1995a; Herczeg et al. 2007). On the other hand, the melanism of adders is most likely to be a continuum between extremes rather than a dichotomous feature (JV personal observation). However, the aposematic feature of the zigzag pattern (supported by data) may offer an evolutionary explanation to the question of why zigzag-patterned individuals are usually more common than melanistic ones, despite the thermoregulation benefits of melanism being obvious.

Even if the aposematic function of the zigzag pattern of European vipers is now well supported, other simultaneous adaptive benefits of that pattern cannot be excluded. The zigzag pattern cannot be regarded as an overtly conspicuous signal and the pattern may have a distance-dependent function of crypsis, meaning that an animal may be cryptic from the distance but easily recognisable when noticed (Marshal 2000; Sherrat and Beatty 2003; Tulberg et al. 2005). Distance-dependent qualities of colouration could be particularly important in colder climates were ectothermic animals are forced to expose themselves to visually hunting predators during basking.

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II

VARIATION IN PREDATOR SPECIES ABUNDANCE CAN CAUSE VARIABLE SELECTION PRESSURE ON WARNING SIGNALING PREY

by

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Variation in predator species abundance can cause variable selection pressure on warning signaling prey

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Keywords
Aposematism, predation, selection, snake, viper, warning signal.

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Abstract

Predation pressure is expected to drive visual warning signals to evolve toward conspicuousness. However, coloration of defended species varies tremendously and can at certain instances be considered as more camouflaged rather than conspicuous. Recent theoretical studies suggest that the variation in signal conspicuousness can be caused by variation (within or between species) in predators' willingness to attack defended prey or by the broadness of the predators' signal generalization. If some of the predator species are capable of coping with the secondary defenses of their prey, selection can favor reduced prey signal conspicuousness via reduced detectability or recognition. In this study, we combine data collected during three large-scale field experiments to assess whether variation in avian predator species (red kite, black kite, common buzzard, short-toed eagle, and booted eagle) affects the predation pressure on warningly and non-warningly colored artificial snakes. Predation pressure varied among locations and interestingly, if common buzzards were abundant, there were disadvantages to snakes possessing warning signaling. Our results indicate that predator community can have important consequences on the evolution of warning signals. Predators that ignore the warning signal and defense can be the key for the maintenance of variation in warning signal architecture and maintenance of inconspicuous signaling.

Introduction

Various conspicuously colored animals advertise their defense to potential predators with bright colors. For example, many toxic poison frog and butterfly species exhibit bright warning coloration (Poulton 1890; Cott 1940). The conspicuousness of warning signals enhances predator avoidance as improved detection and recognition facilitate predator learning. Warning signals can thereby be expected to evolve toward conspicuousness (reviewed in Ruxton et al. 2004). However, not all defended prey species advertise themselves to predators by having overtly conspicuous coloration (Endler and Mappes 2004). In their model, Endler and Mappes (2004) showed that "weak" warning signals can evolve and be maintained if predators vary in their willingness to attack defended prey. In other words, if some predators are able to cope with the secondary defenses of conspicuous prey, predation pressure should increase due to detectability of the prey and lead to selection for reduced conspicuousness. An example of this is suggested in the seemingly inconspicuous pine sawflies (*Neodiprion sertifer* and *Diprion pini*) that are preyed upon by both ants and great tits (*Parus major*), although they are chemically defended and seemingly not palatable food for birds (Lindstedt et al. 2011).

Indeed, most prey species are preyed upon by more than one predator species and predators may vary in their willingness to attack defended prey. Thus, in theory, fitness of warning signals may depend on the given predator community structure (Endler and Mappes 2004; Mappes et al. 2005; Nooan and Comeault 2009; Mochida 2011), however, studies quantifying this in natural predator communities are lacking.

Several species of European vipers (genus *Vipera*) seem to exhibit rather inconspicuous coloration despite being venomous (De Smedt 2001; Fig. 1a). They share a characteristic dorsal zigzag pattern (see De Smedt 2001), which has been suggested to offer protection through camouflage by hindering detection by predators (Andrén and Nilson 1981). However, more recent studies have shown that the zigzag pattern of vipers acts as a warning signal despite its seemingly inconspicuous nature. Studies by Wüster et al. (2004), Niskanen and Mappes (2005), and Valkonen et al. (2011a,b) have demonstrated that zigzag patterned snakes are preyed upon less by avian predators than snakes without a zigzag pattern, indicating that the zigzag pattern is in fact aposematic. However, these studies do not take into account regional variation in predation pressure or its relationship with local predator community structure. Niskanen and Mappes (2005) found large variation in predator pressure among locations which may indicate that local predator community structure can have a significant effect on strength and direction of predation. Here, we assess if variation in composition of predatory community structure affects the predation on warningly and non-warningly colored snakes. We combined data collected during three field experiments by Valkonen et al. (2011a,b) to test whether the abundance of natural predator species is related to the benefits of warning signaling.

Materials and methods

We observed birds of prey during three different experiments conducted in Coto Doñana National Park, Southern Spain during springs 2008–2010 (Valkonen et al. 2011a,b). Experiments spanned approximately 25% of the 6794 hectare reserve. Following previously employed methods, (Andrén and Nilson 1981; Brodie 1993; Pfennig et al. 2001; Wüster et al. 2004; Niskanen and Mappes 2005) artificial snake replicas with zigzag (wide and narrow) and other patterns (plain, striped, or blotched) were placed in the field in transect lines and imprints on the replicas caused by avian predator attacks were observed. Two to five different pattern types of snakes were used in each transect lines and length of the transect lines varied from 300 to 750 m (20–50 snake replicas). Snake replicas were placed in 15 m intervals and the number of differently colored replicas were balanced within transect. Artificial snakes were made of gray pre-colored plasticine (CaranD'Ache, Modela Noir) and had patterns painted on them with black paint. As we did not find any effect of the background matching of the snake replicas in the previous experiment (Valkonen et al. 2011a; but see also Wüster et al. 2004; Niskanen and Mappes 2005) background effect is not included in data presented here. For more detailed descriptions of the methods and coloration of snake replicas, see Valkonen et al. (2011a,b). Only attacks caused by birds were included in the analyses because mammals might recognize artificial prey items from olfactory cues and be attracted by the odor of plasticine (Rangen et al. 2000; Valkonen et al. 2011a,b; Valkonen and Mappes 2012). During our experiments, we often observed mammalian predators (e.g., red fox, *Vulpes vulpes*) following our tracks along the transect lines and non-selectively biting almost all snake replicas in the area. Furthermore, we were not able to estimate the number of preying mammals in the study areas.

During the experiments, raptors flying over the experimental areas were surveyed using a telescope and

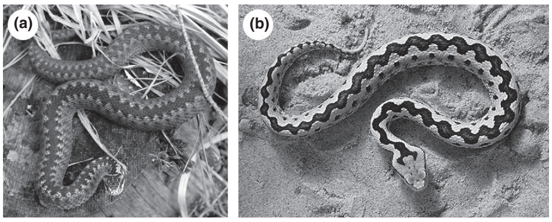

Figure 1. European vipers (*Vipera* sp.) exhibit characteristic dorsal zigzag pattern which is shown to act as a warning signal for avian predators. However, despite the signaling function of the zigzag pattern some species like *Vipera berus* (a) are seemingly inconspicuous whereas others like *Vipera latastei* (b) exhibit more conspicuous coloration.

binoculars. The duration of observations varied from 55 to 75 min and the observations were repeated one to three times at each transect line in different days between 10 am and 3 pm. Thereby our data does not consider possible owl attacks. However, the only relevant owl in the area is the barn owl (*Tyto alba*), which is almost exclusively a rodent predator with only few observations on other prey of which few, except small lizards, are reptiles (Herrera 1974). The most commonly sighted raptor species were black kite (*Milvus migrans*), red kite (*Milvus milvus*), booted eagle (*Hieraaetus pennatus*), common buzzard (*Buteo buteo*), and short-toed eagle (*Circaetus gallicus*) (Table 1). These five species are likely to be the most important avian predators of snakes in the area and herein we consider them as key predators. These species are different in their foraging behavior and food choice. The short-toed eagle is highly specialized in preying upon snakes and its diet consists mainly of snakes and lizards (Forsman 2007). The diet of the common buzzard consists mainly of small mammals and birds, but they are also known to commonly consume snakes (Selas 2001; Forsman 2007). The diet of the booted eagle consist mainly of medium-sized birds, big lizards, small mammals (Forsman 2007), and snakes (Valkonen et al. 2011a). Black and red kites are more generalist, feeding on carrion, small mammals, birds, insects, reptiles, and fish (Forsman 2007).

In all three experiments, bird observations were conducted at a total of 40 transect lines and 1443 snake replicas. Based on findings of previous experiments (Wüster et al. 2004; Niskanen and Mappes 2005; Valkonen et al. 2011a), snake replicas were divided into two categories: (1) Aposematically colored snake replicas, which included all zigzag patterned replicas (n = 722); and (2) non-aposematic, which included plain, striped, and blotched patterns (n = 721). The abundance of each raptor species in each location was calculated by dividing the total number of observed individuals of each species by total observation time. A generalized linear mixed model (GLMM) with binomial distribution was used to analyze the data. As a response variable we used fate of each snake replica (attacked or not). As time that transect lines were in the field varied from 41.92 to 73.67 h (mean = 52.25 h), we corrected our response variable by the catching effort (binomial trial with 1 or 0 attack out of hours that transect line was in the field). Coloration of the snake replica and the abundance of each key raptor species were used as explanatory variables. To account for the sampling structure of our data (six of 40 transects were conducted in the same location in consecutive years) we included "year" and "location" as random effects in our model. The model selection procedure started from the model including all possible two-way interactions of raptor species and snake replica coloration then simplified. Model selection was based on significance of the terms in the model (Table 2). Statistical analyses were conducted using R 2.11.1 and lme4 package.

Table 1. Number of observed raptors of each species/observation time (h).

Species	Max observations/h	Mean observations/h	SE
Black kite *(Milvus migrans)*	68	22.20	3.33
Red kite *(Milvus milvus)*	27	5.16	1.06
Booted eagle *(Hieraaetus pennatus)*	14	4.13	0.54
Common buzzard *(Buteo buteo)*	7	0.95	0.24
Short-toed eagle *(Circaetus gallicus)*	2	0.60	0.11
Common kestrel *(Falco tinnunculus)*	2	0.16	0.07
Western marsh-harrier *(Circus aeruginosus)*	1	0.06	0.03
Imperial eagle *(Aquila adalberti)*	1	0.06	0.04
Peregrine falcon *(Falco peregrines)*	1	0.03	0.02
Lesser kestrel *(Falco naumanni)*	1	0.01	0.01

Results

The overall predation pressure on aposematically colored snake replicas was lower than the predation pressure on non-aposematic snake replicas ($Z = -4.56$, $P < 0.001$) (Fig. 2). The only raptor species that caused significant deviation from the general trend of lowered predation on aposematic snakes was the common buzzard (Table 3). The predation on snake replicas increased by function of the interaction of aposematic coloration of snake replicas and abundance of common buzzard ($Z = 2.47$, $P = 0.013$). In other words, if common buzzards were abundant there was a higher probability of attack on aposematic snakes possessing warning signals compared with non-aposematic snakes (Fig. 3). We did not find significant interactions between snake replica coloration and abundance of black kites, red kites, or booted eagles, which indicates that these species generally do avoid warningly signaling vipers (Table 2).

Discussion

Overall, aposematically colored snakes suffered less avian predation than non-aposematic snakes. However, sup-

Table 2. Generalized mixed model selection. Response variable is the fate of the individual snake replica balanced by times that transect line was in field (catching effort).

Model	df	AIC	χ^2	sig. χ^2	Z	sig. Z
1 ~A*B+A*ML+A*MI+A*H+A*C+1|Y+1|L	14	645.88				
2~MI+A*B+ A*ML+A*H+A*C+1|Y+1|L	13	643.95	0.078	0.78	−0.271	0.78
3~MI+ML+A*B+A*H+A*C+1|Y+1|L	12	642.12	0.166	0.68	0.405	0.69
4~MI+ML+C+A*B+A*H+1|Y+1|L	11	641.41	1.293	0.26	−1.118	0.26
5~MI+ML+C+H+A*B+1|Y+1|L	10	641.33	1.925	0.17	1.398	0.16
6 ~MI+ML+C+A*B+1|Y+1|L	9	639.34	0.003	0.96	0.057	0.95
7~MI+C+A*B+1|Y+1|L	8	637.35	0.009	0.92	−0.151	0.88
8~C+A*B+1|Y+1|L	7	636.95	1.606	0.21	−1.519	0.13
9~A*B+1|Y+1|L	6	637.60	2.651	0.10	1.64	0.10

Abbreviations of the explanatory variables are: A, coloration of snake replica (aposematic or not); B, abundance of common buzzard; ML, abundance of red kite; MI, black kite; H, booted eagle; C, short-toed eagle; Y, year; L, location. Asterisk indicates interaction term of the variables and + indicates main effects. If interaction term is indicated also main effect is included. χ^2 value and significance level of χ^2 indicates change from higher model. Z value and its significance are for significance of the removed term in the higher model. Model selection was based on significance of the terms in the model.

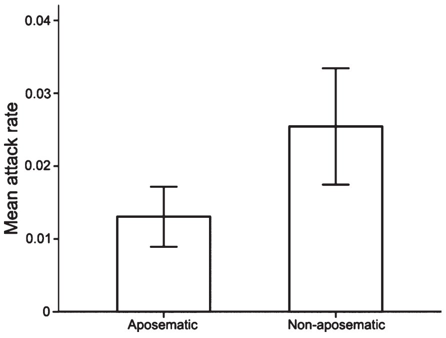

Figure 2. Avian attack rates (attacks in 10 h/snake replica) on aposematic (zigzag) and non-aposematic (plain, stripe, or disruptive pattern) snakes. Bars represent 95% confidence interval.

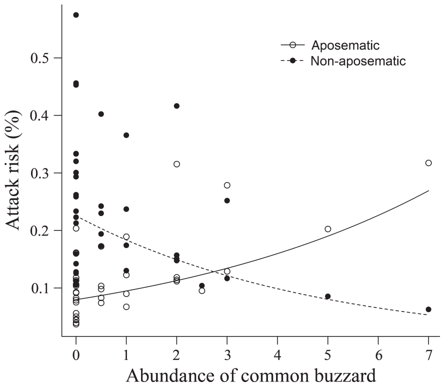

Figure 3. Attack risk of aposematic (open dots) and non-aposematic (closed dots) snakes related to abundance of common buzzard. Lines represent model estimates (solid line, aposematic; dashed, non-aposematic snakes).

porting the hypothesis that predator species do vary in their tendency to attack warningly colored prey, we found significant interaction of prey coloration and abundance of only the common buzzard. More importantly, if common buzzards were abundant enough, the probability to get attacked by raptor was higher among aposematic snakes compared with non-aposematic ones. This suggests that the abundant occurrence of specialized predators may cause local selection pressure to favor a less conspicuous warning signal within a prey population. Although predator community structure has been suggested to affect the benefits of conspicuous warning signaling of a prey population (Endler and Mappes 2004; Nooan and Comeault 2009; Mochida 2011), these suggestions are so far based on theoretical models or observations on predation pressures in different locations without detailed data about predatory community structure. Empirical results presented here with more detailed natural predator community data provide rare support that abundant occurrence of specialist predators may select reduced conspicuousness of warning signal.

Avian predators that are specialized snake predators are expected to not hesitate to attack vipers. Although the common buzzard is not considered to be a snake specialist, this species is known to commonly attack snakes, including vipers (Selas 2001; Forsman 2007). Common buzzard is also the only raptor species that significantly decreased sur-

Table 3. Generalized linear mixed model (model 9 in Table 2) fitted in predation data. Dependent variable, number of attacks on snake replicas is balanced by time. Year and location is included in the model as random effects.

Terms in the model	Estimate	SE	Z	P
Intercept	−6.11	0.23	−25.72	<0.001
Aposematic	−1.04	0.22	− 4.56	<0.001
Common buzzard	−0.21	0.13	−1.54	0.125
Aposematic × Common buzzard	0.38	0.15	2.47	0.013

vival advantage of warningly colored snake replicas in our data. Surprisingly, the abundance of short-toed eagles did not significantly affect the survival advantage of warningly colored snake replicas because this species is known to be a highly specialized snake predator (Forsman 2007). It is possible, however, that the effect of the short-toed eagle cannot be observed in our data due to their low abundance (Table 1). Alternatively, short-toed eagles are shown to prefer larger prey than we used in these experiments (Gil and Pleguezuelos 2001) and they might have ignored our snake replicas. Abundance of more generalist predators; black kite, red kite, and booted eagle did not cause significant deviation on the general trend of the attack probabilities. The fact that these species did not have significant interaction with prey coloration indicates that they generally do avoid preying on warningly colored snakes. By avoiding attacking conspicuously signaling vipers, generalist raptors may favor protective coloration of the local viper species (*Vipera latastei gaditana*). As being very abundant in Southern Spain, these generalists may cause seemingly conspicuous warning coloration of *V. l. gaditana* compared with most European vipers (see De Smedt 2001; Fig. 1b).

Besides the fact that several predators can learn to recognize and avoid aposematic species, many predators can also learn to kill and handle defended prey (Skelhorn and Rowe 2006). If a predator is capable of handling defended prey without extra costs, the direction of selection toward conspicuous warning signal can disappear or reverse. In addition, there is experimental evidence that several characteristics, not only the conspicuous colors of prey animals can be recognized and avoided by predators. For example, natural predators are shown to avoid triangular head shape of vipers (Valkonen et al. 2011b), dragonflies (*Aeshna grandis*) can recognize and avoid body shape of wasps (Kauppinen and Mappes 2003); body shape and size of the prey has been observed to affect foraging behavior of praying mantis (*Sphodromantis lineola*) (Prete 1990). Such features can thereby serve a signaling function to predators without increased cost of conspicuousness.

Prey animals are subjected to predation by several predators which can wary their behavior and cognitive capabilities. Data presented here provide further support for the idea that some players of the natural predatory community can cause selection pressure that leads to reduced conspicuousness of aposematic signal. According to our results, conspicuous warning signals can be expected to evolve in the locations where majority of predators avoid local warning signal. Whereas in locations where increasing abundance of predators that do not hesitate attacking on signaling prey, costs of conspicuousness may lead the evolution of moderate or less conspicuous warning signaling.

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Conflict of Interest

None declared.

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III

ANTIPREDATORY FUNCTION OF HEAD SHAPE FOR VIPERS AND THEIR MIMICS

by

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Antipredatory Function of Head Shape for Vipers and Their Mimics

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Abstract

Most research into the adaptive significance of warning signals has focused on the colouration and patterns of prey animals. However, behaviour, odour and body shape can also have signal functions and thereby reduce predators' willingness to attack defended prey. European vipers all have a distinctive triangular head shape; and they are all venomous. Several non-venomous snakes, including the subfamily Natricinae, commonly flatten their heads (also known as head triangulation) when disturbed. The adaptive significance of this potential behavioural mimicry has never been investigated. We experimentally tested if the triangular head shape typical of vipers offers protection against predation. We compared the predation pressure of free-ranging predators on artificial snakes with triangular-shaped heads against the pressure on replicas with narrow heads. Snakes of both head types had either zigzag patterned bodies, typical of European vipers, or plain (patternless) bodies. Plain snakes with narrower Colubrid-like heads suffered significantly higher predation by raptors than snakes with triangular-shaped heads. Head shape did not, however, have an additive effect on survival in zigzag-patterned snakes, suggesting that species which differ from vipers in colouration and pattern would benefit most from behavioural mimicry. Our results demonstrate that the triangular head shape typical of vipers can act as a warning signal to predators. We suggest that head-shape mimicry may be a more common phenomenon among more diverse taxa than is currently recognised.

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Introduction

Research into warning signals and defensive mimicry has largely concentrated on colouration and colour patterns in prey animals. Body shape, odour, behaviour or a combination of these features may also act as warning signals and can therefore be mimicked by other species [1–5]. Myrmecomorphy, among the group of spiders that mimic ants, is a famous example of behavioural mimicry. Myrmecomorphs often reinforce their morphological resemblance to ants through mimicry of ant leg movements and zigzag walking [6–7]. Behavioural mimicry combined with colouration has also been recorded among cephalopods: long-armed octopus species inhabiting Indonesian waters mimic venomous sea snakes [8]. When disturbed, these animals alter their colouration to present black and white bands, and then adopt a specific posture. Norman et al. [8] have reported cases where six of the octopus arms were hidden in the burrow while the remaining two arms were held straight, away from the burrow, imitating a local sea snake. Perhaps the most famous example of behavioural mimicry was described by Henry Bates in 1862 who observed that some butterfly larvae seem to mimic snakes. When the late instar larvae of swallowtail butterflies (*Papilo sp.*) and hawk moths (Sphingidae) are threatened by a predator, they mimic small tree vipers by hiding their heads and inflating the thorax or abdomen [1,9–10].

A triangular head shape and dorsal zigzag pattern are common features among venomous European vipers (genus *Vipera*) [11] (Fig. 1c). However, non-venomous snakes in the family Colubridae [12] often have a narrower head shape. Some, such as viperine snakes (*Natrix maura*) [13–14] (Fig. 1a), grass snakes (*Natrix natrix*) and smooth snakes (*Coronella austriaca*) (personal observation) flatten their heads (head triangulation) when disturbed (Fig. 1b). Viperine snakes exhibit a dorsal zigzag pattern that resembles vipers' zigzag pattern whereas grass snakes and smooth snakes commonly do not [15]. Viperine snakes have been observed excreting a strong-smelling liquid from their cloacal glands when disturbed, which may be unpleasant to predators [15] and, thus, this species could be considered a Müllerian [2] or quasi-Batesian viper mimic [5,16]. Head triangulation alone or combined with a viper-like dorsal zigzag pattern may improve non-venomous snakes' resemblance to vipers [15]. If several groups of predators avoid vipers based on their triangular head shape, a range of species could benefit from mimicry of this head shape.

The significance of head triangulation and its advantages for mimics have not been empirically tested. To experimentally test if a triangular head shape, and the head triangulation in particularly, acts as a warning signal, we compared predation caused by free-ranging predators on snake replicas with triangular (viper-like) and narrow (colubrid-like) heads. To differentiate the effect of the triangular head shape from the overall appearance of European vipers we used snake replicas with and without the characteristic zigzag pattern typical of European vipers (Fig. 1e,g).

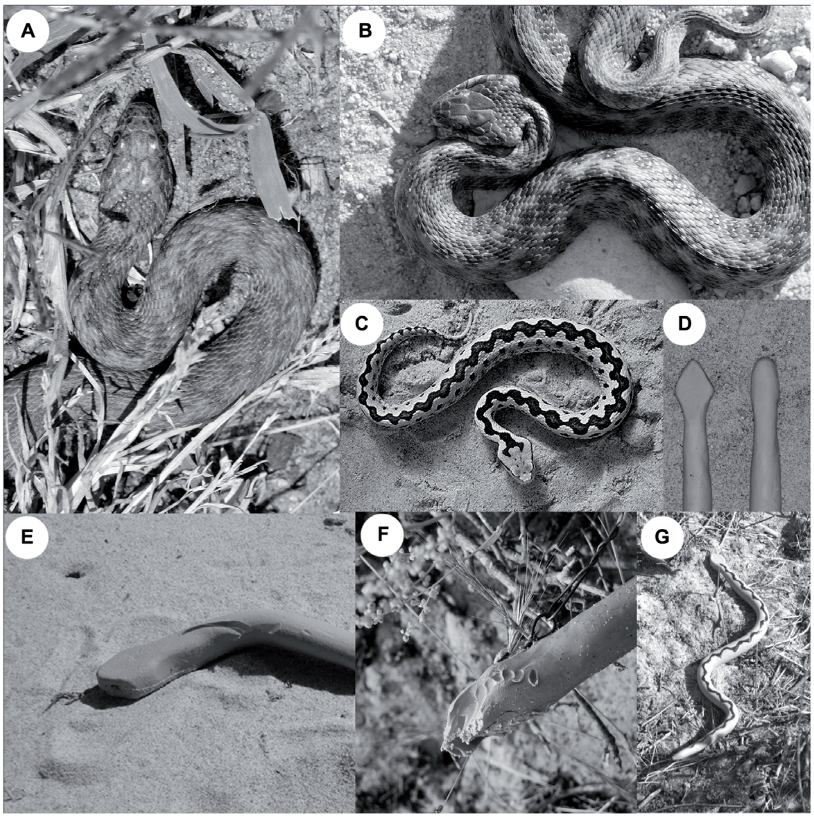

Figure 1. Head shapes of viper and viperine snakes. *Natrix maura* has a narrow colubrid-like head shape (a). When disturbed, they flatten their heads making the head more triangular in shape (b). *Vipera latastei gaditana* exhibit the typical triangular head shape of European vipers (c). Triangular, viper-like and narrow, colubrid-like head shapes of plasticine models (d). Attacks of raptors (e) and mammals (f) can be separated from imprints left during predation events. A dorsal zigzag pattern was painted on half of the snake replicas (g).
doi:10.1371/journal.pone.0022272.g001

Materials and Methods

Following previously employed methods [17–23], we used artificial snakes made of non-toxic grey plasticine (Caran D'Ache, Modela Noir, 0259.005). We painted the characteristic zigzag pattern on half of them using black paint (Bebeo acryl colour 374611 & Perinnemaali 5511-05) (Fig. 1. g). A 50:50 mixture of two types of paint were used to achieve a matte-black finish. To test if vipers' triangular head shape acts as a warning signal, four different kinds of artificial snakes were used: 1) zigzag-patterned snakes with triangular (viper type) heads and 2) with narrow (colubrid type) heads and 3) plain (grey) snakes with triangular heads and 4) with narrow heads (Fig. 1d,e,g). The use of plain snakes was crucial to separate the effect of the head shape from the overall appearance of vipers. The length and diameter of artificial models were identical in all treatments and in correspondence to the size of a sub adult/adult viper (*Vipera latastei gaditana*) [11,15]. This was confirmed by measuring 82 randomly-chosen clay models (range = 36–45 cm, mean = 41.8 cm, s.e. = 0.2 cm). The models did not differ in length between treatments ($F_{3,78} = 1.10$, $p = 0.355$). The average width of the triangular heads was 27.8 mm (s.e. = 0.5 mm, $n = 12$) and of narrow heads 18.3 mm (s.e. = 0.3 mm, $n = 13$) corresponding to the natural variation of both viper and viperine snake head width (Fig. 1d).

The experiment was conducted in Coto Doñana National Park, southern Spain, between 5 and 17 May 2009 and 28 April and 10 May 2010. Six trials (transects) were conducted in 2009 and thirteen in 2010. Trials were conducted in 17 different locations 0.5–38.9 km apart (mean 9.7 km). We used five to ten replicas of each type (4 types) in each transect (595 snake replicas in total). The replicas were placed on the natural background in random order at approximately 15 metre (15 paces) intervals following features of the terrain. All model types were equally represented within transects. However, one zigzag patterned model with a triangular head was accidentally omitted from one transect (nine replicas instead of ten). Between one and four trials were conducted simultaneously and with a minimum distance of 2.5 km between them. Snake replicas were tied to bushes with iron wire to prevent predators taking them during an attack. The replicas were sprayed with insect repellent (Autan® by Johnson) making them distasteful to deter mammals (mainly foxes and boars) from eating them. In a previous experiment [23], we observed foxes following our tracks along transect lines and systematically biting every snake replica, destroying all traceable evidence of attacks by other predators. The use of insect repellent significantly reduced this indiscriminate predation.

In 2009, snake replicas were first checked after approximately 12 hours and then again after approximately 24 hours. After approximately 48 hours in the field, they were checked a final time and then removed. In 2010, snake replicas were left in the field for a longer period to accumulate more attacks. The prey items were checked every 24, 48 and 72 hours and the number of attacks on each item recorded. All the attacked snake replicas were repaired after each inspection so that the probability of models being attacked remained constant. Attacks by raptors and mammals, e.g. foxes, were recorded separately. Raptor attacks (Fig. 1e) can be separated from those caused by corvids, gulls or mammals (Fig. 1f) by the imprints that result from the attacks. While raptors use their talons during an attack, gulls and corvids tend to approach the prey from the ground and use their beaks. The footprints of the diverse predators were easily detectable in the soft sandy soil of the experimental areas. No corvid or gull attacks were observed during the experiment.

Raptors can fly long distances in a short period of time making it likely that an individual raptor saw snake replicas in several transects, violating the independence of transect lines. Therefore data from all trials was treated as one independent sample. The Pearson Chi-squared test of independence was used to test differences in the number of attacks on each of the four snake types. There was no significant main effect or interaction between years and attack rates (neither of mammals nor of raptors) among treatments (Logit model, all Z values $\leq \pm 1.618$, and p values ≥ 0.106) and thus we pooled the data from both years.

Results

There was a significant difference in the number of raptor attacks among treatments ($\chi^2 = 11.393$, $df = 3$, $N = 595$, $p = 0.010$). Plain snakes with narrow heads were attacked significantly more often by raptors than were plain snakes with triangular heads ($\chi^2 = 5.04$, $df = 1$, $p = 0.025$) (Fig. 2). There was no difference in the number of raptor attacks on patterned, triangular-headed snake replicas and on patterned replicas with narrow heads ($\chi^2 = 0.07$, $df = 1$, $p = 0.792$) (Fig. 2). When we pooled attack data on snake replicas based on their patterns, plain snakes were attacked by raptors significantly more frequently than were zigzag patterned replicas ($\chi^2 = 6.49$, $df = 1$, $p = 0.011$). In total, 8.2% of the 595 snake replicas were attacked by raptors and 18.5% by mammalian predators. Attacks by mammalian predators did not differ between treatments ($\chi^2 = 5.10$, $df = 3$, $p = 0.165$).

Discussion

We have shown that the triangular head shape of vipers is recognized and avoided by raptors and does, therefore, act as a warning signal. Plain, triangular-headed snake replicas suffered significantly fewer raptor attacks than suffered by plain replicas with narrow heads. Moreover, plain snake replicas were attacked more often overall than were patterned replicas. However, a triangular head shape did not have any antipredator benefit when presented together with a zigzag-patterned body, suggesting that pattern and colouration together are a sufficient warning signal. Many colubrid species that display head triangulation notably do not mimic the body pattern of vipers. Our results also suggest that behavioural mimicry (head triangulation) can, in particular, significantly increase the survival of species that do not mimic the body pattern of vipers.

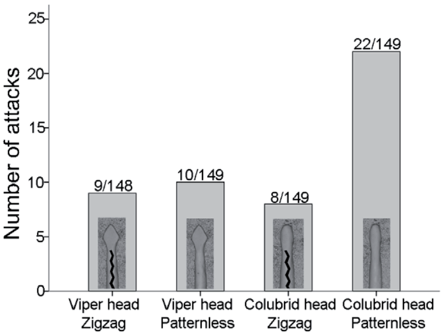

Figure 2. The number of attacks on snake replicas. The observed number of raptor attacks on different types of snake replicas. Numbers at the top of each bar represent the total number of observed raptor attacks on each type of snake replica in relation to the total number of replicas of that type used in the experiment.
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Although raptors recognized head shape as a signal, we did not find any evidence that mammalian predators avoided the triangular head shape or zigzag pattern of the snake replicas. Using plasticine prey items might not be a suitable method to study mammalian predation, as mammals use olfactory cues rather than visual cues during hunting. The odour of plasticine is distinctive and that can influence predators' behaviour [24]. Previously, we observed foxes following our tracks along transect lines and systematically biting and eating every snake replica in their path [23]. These observations suggest that mammalian predators (e.g. foxes) do not consider plasticine prey items as real living (or dead) snakes. It would be interesting to study the efficacy of head-flattening behaviour against mammalian predators using replicas with various olfactory cues.

Vipers (family Viperidae) are venomous, widely distributed and tend to be avoided by predators [21–23], making them good models for several groups of animals. We suggest that the triangular head shape of venomous snakes is mimicked by prey animals more broadly than was previously thought. Indeed, potential head shape mimicry has independently evolved several times among snakes. Horizontal head display is known to exist among 13 Old World and 9 New World genera of the families Colubridae, Elapidae, and Viperidae [25]. Snakes commonly flatten or inflate their bodies as defensive behaviour to make themselves appear bigger than they are [26]. Enlargement may occur over the whole body or it may be concentrated in a particular area, such as the neck or head [26]. While head triangulation in snakes is often combined with body flattening/inflating behaviour, it also occurs among species that do not exhibit body-flattening behaviour. Smooth snakes (*Coronella austriaca*), for example, flatten (triangulate) their heads when disturbed but do not flatten their bodies (personal observation). Given the taxonomic and geographic diversity of these genera, much of the similarity in defensive behaviour must be due to convergence [25], which we suggest has been driven by defensive mimicry. Further support for these behavioural observations comes from Young et al. [14] who describe the mechanical basis of head triangulation in distantly-related colubrid snakes. They found that the morphological mechanisms required to triangulate the head differ greatly between the genus *Hetrodon* and *Dasypeltis*.

In addition to snakes, the larvae of several moth and butterfly species enhance their resemblance to tree vipers by concealing their heads and inflating their thorax or abdomen to express a false triangular-shaped head [1,9–10]. Hawkmoth (*Leucorampha sp.*) caterpillars also "strike" the objects that threaten them making the behavioural mimicry even more accurate [26]. Although the adaptive significance of this behaviour against predators has never been tested experimentally, it is reasonable to assume that the triangular head shape is effective against predators as it is known to be against humans. Henry Bates described the power of this mimicry in 1862; "The most extraordinary instance of imitation I ever met with was that of a very large Caterpillar, which stretched from amidst the foliage of a tree which I was one day examining, and startled me by its resemblance to a small Snake. … I carried off the Caterpillar, and alarmed every one in the village where I was then living, to whom I showed it."[1]. The fascinating anecdotal evidence combined with intriguing research data argue the need for a survey other animals to discover if head-shape mimicry can be found elsewhere in the animal kingdom.

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Author Contributions

Conceived and designed the experiments: JV ON JM. Performed the experiments: JV ON JM. Analyzed the data: JV. Contributed reagents/materials/analysis tools: JM. Wrote the paper: JV ON JM.

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IV

BIOGEOGRAPHY OF THE SMOOTH SNAKE (*CORONELLA AUSTRIACA*): ORIGIN AND CONSERVATION OF THE NORTHERNMOST POPULATION

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Juan Galarza, Johanna Mappes & Janne K. Valkonen 2014

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V

RESEMBLING A VIPER: IMPLICATIONS TO THE CONSERVATION OF THE ENDANGERED MIMIC, CORONELA AUSTRIACA

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