

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

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Received: 9 June 2010 / Accepted: 22 January 2011 / Published online: 5 February 2011
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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 coloration 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 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 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 (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 coloration. 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 coloration, warning coloration 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 coloration 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 (Marshall 2000; Sherratt 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 (Andr n and Nilson 1981; Forsman 1995b; Lindell and Forsman 1996; W ster et al. 2004; Niskanen and Mappes 2005). The protective effect of the dorsal zigzag pattern was first described by Andr n 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 W ster 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 W ster 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 W ster 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 Andr n and Nilson 1981; Brodie and Janzen 1995; W ster 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 (W ster 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

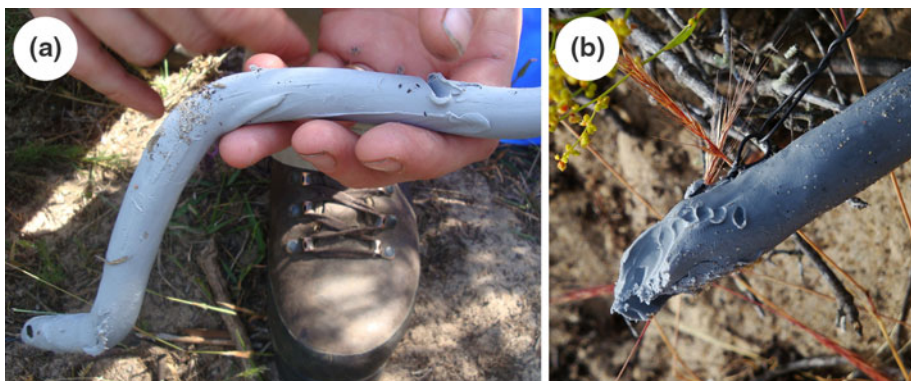
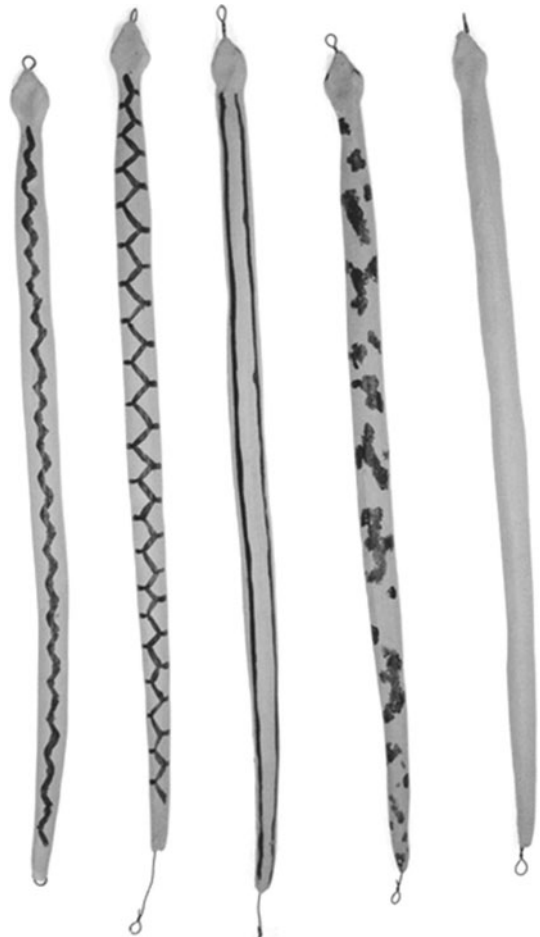


Fig. 1 Raptor (a) and mammalian (b) attacks can be distinguished from imprints left during attack

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 cucullatus* (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 disruptiveness 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/m² 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. <u>A × P × B</u>	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. <u>A + P + A × P</u>	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 G²-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).

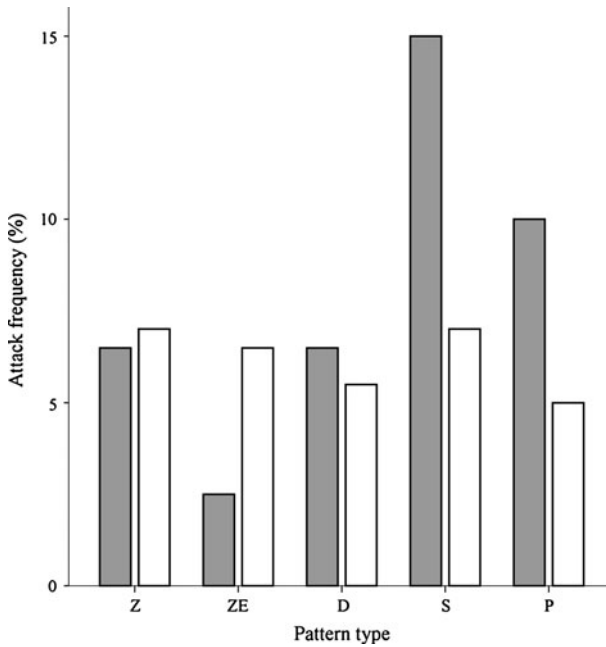


Fig. 5 Attack frequencies (raptor attacks shaded and mammalian attacks white bars) on plasticine snake replicas with different pattern types. Codes of pattern types are *Z* normal zigzag pattern, *ZE* zigzag pattern with edge violating, *D* classic disruptive, *S* striped, and *P* plain (see also Fig.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

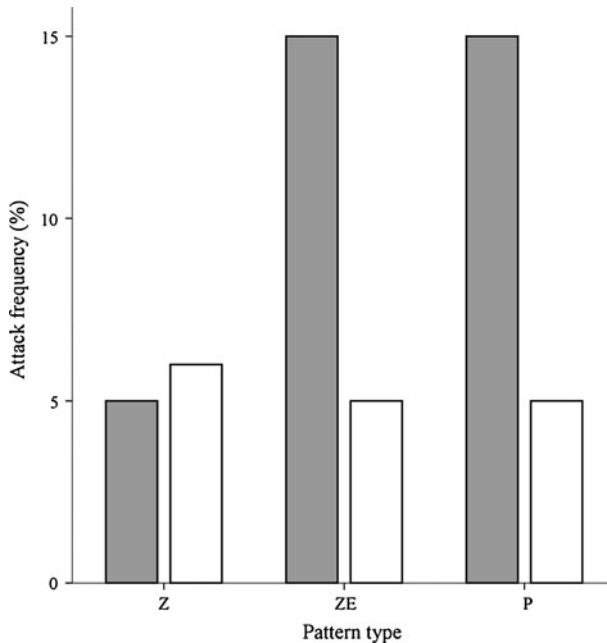


Fig. 6 Attack frequencies (raptor attacks shaded and mammalian attacks white bars) on plasticine snake replicas with different pattern types. Codes of pattern types are *Z* normal zigzag pattern, *ZE* zigzag pattern with edge violating, and *P* plain (see also Fig. 2)

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 (Marshall 2000; Sherratt and Beatty 2003; Tulberg et al. 2005). Distance-dependent qualities of colouration could be particularly important in colder climates where ectothermic animals are forced to expose themselves to visually hunting predators during basking.

Acknowledgments We would like to thank Consenjo Superior De Investigaciones Científicas (CSIC) for providing funding and the opportunity to work in Coto Doñana national park. Also, many thanks to Begoña Arrizabalga and Rosa Rodríguez for arrangements and help in Sevilla. Dr. Martin Stevens, Dr. Carita Lindstedt, Prof. Mikko Mönkkönen, Prof. Janne Kotiaho, MSc Robert Hegna and three anonymous reviewers gave many helpful comments on the earlier version of the manuscript. MSc Ossi Nokelainen assisted with field work and Matt Wilson provided photographs of the ladder snake. Part of the funding was provided by the Centre of Excellence in Evolutionary Research and the University of Jyväskylä.

References

- Andrén C, Nilson G (1981) Reproductive success and risk of predation in normal and melanistic colour morphs of the adder, *Vipera berus*. *Biol J Linn Soc* 15:235–246
- Arnold EN, Burton JA (1978) A field guide to the reptiles and amphibians of Britain and Europe [Finnish edition—Viitanen P, Koskela P, Lindholm E (1981)] Kustannusosakeyhtiö Tammi, Helsinki
- Bohlin T, Tullberg BS, Merilaita S (2008) The effect of signal appearance and distance on detection risk in an aposematic butterfly larva (*Parnassius apollo*). *Anim Behav* 76:577–584
- Brodie ED (1993) Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. *Evolution* 47:277–285
- Brodie ED, Janzen FD (1995) Experimental studies of Coral Snake mimicry: generalized avoidance of ringed patterns by free-ranging avian predators. *Funct Ecol* 9:186–190
- Brower LP, Ryerson WN, Coppinger LL, Glazier SC (1968) Ecological chemistry and the palatability spectrum. *Science* 161:1349–1351
- Cott HB (1940) Adaptive colouration in animals. Methuen, London
- Cramp S (1985) Handbook of the birds of Europe, the Middle East and North Africa, vol IV. Oxford University Press, Oxford
- Cuthill IC, Stevens M, Sheppard J, Maddocks T, Parraga CA, Troscianko TS (2005) Disruptive colouration and background pattern matching. *Nature* 434:72–74
- Darst CR, Cummings ME, Cannatella DC (2006) A mechanism for diversity in warning signals: conspicuousness versus toxicity in poison frogs. *Proc Natl Acad Sci USA* 103:5852–5857
- De Smedt J (2001) Die europäischen Vipern. Artbestimmung, Systematic, Haltung und Zucht. Johan De Smedt, Füssen

- Edmunds ME (1974) Defence in animals: a survey of anti-predator defences. Longman, Harlow, Essex
- Endler JA (1991) Interactions between predators and prey. In: Krebs JR, Davies NB (eds) Behavioural ecology, 3rd edn. Blackwell Scientific Publications, Oxford, pp 169–196
- Endler JA, Mappes J (2004) Predator mixes and the conspicuousness of aposematic signals. *Am Nat* 163: 532–547
- Evans DL, Schmidt JO (1990) Insect defenses: adaptive mechanisms and strategies of prey and predators. University of New York Press, New York
- Forsman A (1995a) Heating rates and body temperature variation in melanistic and zigzag *Vipera berus*: does colour make a difference? *Ann Zool Fenn* 32:365–374
- Forsman A (1995b) Opposing fitness consequences of color pattern in male and female snakes. *J Evol Biol* 8:53–70
- Forsman A, Merilaita J (1999) Fearful symmetry: pattern size and symmetry affects aposematic signal efficacy. *Evol Ecol* 13:131–140
- Gamberale G, Tullberg BS (1996) Evidence for a peak-shift in predator generalization among aposematic prey. *Proc R Soc Lond B* 263:1329–1334
- Gittleman JL, Harvey PH (1980) Why are distasteful prey not cryptic? *Nature* 286:149–150
- Gittleman JL, Harvey PH, Greenwood PJ (1980) The evolution of conspicuous colouration: some experiments in bad taste. *Anim Behav* 28:897–899
- Herczeg G, Saarikivi J, Gonda A, Perälä J, Tuomola A, Merilä J (2007) Suboptimal thermoregulation in male adders (*Vipera berus*) after hibernation imposed by spermiogenesis. *Biol J Linn Soc* 92:19–27
- Lindell LE, Forsman A (1996) Sexual dichromatism in snakes: support for the flicker-fusion hypothesis. *Can J Zool* 75:2254–2256
- Lindstedt C, Lindström L, Mappes J (2008) Hairiness and warning colours as components of antipredator defence: additive or interactive benefits? *Anim Behav* 75(5):1703–1713
- Linström L, Alatalo RV, Mappes J, Riipi M, Vertainen L (1999) Can aposematic signals evolve by gradual change? *Nature* 397:249–251
- Marshall NJ (2000) Communication and camouflage in reef fishes. *Phil Trans Soc Lond B* 355:1243–1248
- Merilaita S, Lind J (2005) Background-matching and disruptive colouration and the evolution of cryptic colouration. *Proc R Soc B* 272:665–670
- Niskanen M, Mappes J (2005) Significance of the dorsal zigzag pattern of *Vipera latastei gaditana* against avian predators. *J Anim Ecol* 74:1091–1101
- Poulton EB (1890) The colours of animals: their meaning and use especially considered in the case of insects. Kegan, Trennch, Trubnes & Co
- Rangen SA, Clarl RG, Hobson KA (2000) Visual and olfactory attributes of artificial nests. *Auk* 117: 136–146
- Roper TJ, Wistow R (1986) Aposematic colouration and avoidance-learning in chicks. *Q J Exp Psychol B* 38:141–149
- Ruxton GD, Sherrat TN, Speed MP (2004) Avoiding attack. Oxford University Press, Oxford
- Schaefer MH, Stobbe N (2006) Disruptive colouration provides camouflage independent of background matching. *Proc R Soc B* 273:2427–2432
- Selas V (2001) Predation on reptiles and birds by the common buzzard, *Buteo buteo*, in relation to changes in its main prey voles. *Can J Zool* 79:2086–2093
- Sherrat TN, Beatty CD (2003) The evolution of warning signals as reliable indicators of prey defence. *Am Nat* 162:377–389
- Shine R, Madsen T (1994) Sexual dichromatism in snakes of the genus *Viperia*: a review and a new evolutionary hypothesis. *J Herpet* 28:114–117
- Sokal RR, Rohlf JF (1995) Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Company, New York
- Stevens M, Cuthill IC (2006) Disruptive colouration, crypsis and edge detection in early visual processing. *Proc R Soc B* 273:2141–2147
- Stevens M, Merilaita S (2009a) Animal camouflage: current issues and new perspectives. *Phil Trans R Soc B* 364:423–427
- Stevens M, Merilaita S (2009b) Defining disruptive colouration and distinguishing its functions. *Phil Trans R Soc B* 364:481–488
- Stevens M, Cuthill IC, Windsor AMM, Walker HJ (2006) Disruptive contrast in animal camouflage. *Proc R Soc B* 273:2433–2438
- Stevens M, Castor-Perry SA, Price JRF (2008) The protective value of conspicuous signals is not impaired by shape, size, or position asymmetry. *Behav Ecol* 20:96–102

- Thayer GH (1909) *Concealing-colouration in the animal kingdom: an exposition of the laws of disguise through color and pattern: being a summary of Abbott H. Thayer's discoveries*. NY Macmillan, New York
- Tulberg BS, Merilaita S, Wiklund C (2005) Aposematism and crypsis combined as a result of distance dependence: functional versatility of the colour pattern in the swallowtail butterfly larva. *Proc R Soc B* 272:1315–1321
- Wüster W, Allum CSE, Bjargardóttir IB, Bailey KL, Dawson KJ, Guenoui J, Lewis J, Mc Gurk J, Moore AG, Niskanen M, Pollard CP (2004) Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. *Proc R Soc B* 271:2495–2499