Significance of the dorsal zigzag pattern of *Vipera latastei gaditana* against avian predators

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Summary

1. Aposematism is an antipredatory strategy in which prey animals conspicuously signal their noxiousness to potential predators. This successful strategy is based on avoidance learning of predators.

2. Most species in the viperid genus *Vipera* have a characteristic dorsal zigzag pattern. It has previously been suggested that the dorsal zigzag pattern of *Vipera berus* (the adder) has a cryptic function and thus makes the snake less conspicuous to avian predators. However, a recent experiment suggested that the pattern may function as an aposematic signal rather than being cryptic, and therefore induces active avoidance in avian predators.

3. We conducted a field experiment in Doñana national park in Spain, testing whether the zigzag pattern gives survival value to *Vipera latastei gaditana* against avian predators. We used two sizes of plasticine models with a zigzag pattern vs. without a zigzag pattern to record attacks by avian predators. The background was controlled (white vs. natural) to exclude the possibility that one morph would be more cryptic to predators than the other one. We also tested size-dependent mortality against the signalling and nonsignalling snakes as aposematic signal is expected to enhance the survival of the aposematic species in a size-dependent manner.

4. Predation pressure against snakes was severe, and on average 39% of models were attacked. Coloration did not enhance the survival of juvenile-sized zigzag-patterned snakes, but significantly increased the survival of adult-sized zigzag-patterned snakes on both backgrounds.

5. Our results provide further support for the aposematism hypothesis of zigzag-patterned noxious snakes, although the advantage due to disruptive coloration is not mutually exclusive.

Key-words: aposematism, avian predation, snakes, size-dependent selection, *Vipera latastei*.

Introduction

Unpalatable or otherwise unprofitable prey are often conspicuously coloured to potentially induce avoidance in predators. This conspicuous coloration is termed aposematic coloration (Poulton 1890). Several benefits of conspicuous coloration for the survival of aposematic prey have been identified: (1) a greater reluctance to attack (Sillén-Tullberg 1985; Marples, Brakefield & Cowie 1989); (2) enhanced learning to avoid aposematic prey (e.g. Gittleman & Harvey 1980; Mappes &

Alatalo 1997; Lindström *et al*. 1999); and (3) a lower probability of killing aposematic prey in an attack (Sillén-Tullberg 1985). The protective effect of an aposematic signal increases with increased conspicuousness. It has been shown that the conspicuousness of an aposematic signal can be increased by increasing signal size and symmetry (Forsman & Merilaita 1999). Increasing body size is a good way of increasing signal conspicuousness and decreasing predation risk due to predators negatively selecting large aposematic prey (Gamberale & Tullberg 1996). It has been suggested that selection imposed by visual predators may promote an increase in body size in conspicuously coloured species (Hagman & Forsman 2003). An increase in conspicuousness may be caused by a large individual's

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inability to be cryptic due to its size, which would restrict crypsis to small individuals. Owing to this, predation can be expected to affect large and small individuals differently. King (1992, 1993) has shown that small and large water snakes are preyed on differently regarding their colour pattern. Although the different predation on large and small water snakes is related to crypsis and not aposematism, a nonconspicuous warning signal may be affected by predation in a similar way.

Aposematism in snakes is probably best known in coral snakes. Coral snakes of the genera *Micrurus* and *Micruroides* are small to medium size terrestrial elapids with strong, neurotoxic venom. They are conspicuously coloured with usually red, yellow and black bands (the 'tricolor monad' of Savage & Slowinski 1992). The tricolour banded pattern functions as an aposematic signal to avian predators and the snakes are thus avoided by them (Smith 1975; Greene & McDiarmid 1981; Pough 1988a,b; Brodie 1993; Brodie & Janzen 1995; Pfennig, Harcombe & Pfennig 2001). Other snake species, in which aposematism has been found to occur, include the venomous Pacific sea snake *Pelamis platurus* (Rubinoff & Kropach 1970; Caldwell & Rubinoff 1983) and *Natrix natrix* (Madsen 1987).

Aposematic colours are expected to be most effective when they create a strong contrast between the coloration of the animal and its background (Endler 1978, 1980, 1988, 1991a,b, 1992; Endler & Théry 1996). Snake species in the venomous genera *Micrurus* and *Micruroides* are conspicuously coloured and the colorations function as aposematic signals to predators. Many considerably venomous species have defensive dispositions and strong venom, but are still not conspicuously coloured, and the same applies for European vipers, which usually have a dorsal zigzag pattern and are fairly inconspicuously, even cryptically coloured. However, these species are considerably venomous and may use weak signals coupled with strong defences to deter predators. Although most of the examples of aposematism include conspicuously coloured species, the use of weak signals with strong defences can be a common phenomenon (Endler & Mappes 2004). Snakes are largely preyed by birds, some of which are ophiophage specialists and feed almost exclusively on snakes (Gil & Pleguezuelos 2001) and have behavioural adaptations for restraining venomous species during an attack (Smith 1973, 1976). If some predators avoid venomous snakes due to their coloration but some do not, it might not be a good strategy to be overtly conspicuous, but rather cryptic, to gain protection from specialist predators (Endler & Mappes 2004). The coloration could therefore have a cryptic function and prevent the individual from being seen by predators, but also have an aposematic function once the individual has been seen. The zigzag pattern of European vipers is not very conspicuous and could therefore also have both, a cryptic and an aposematic function. An aposematic colour pattern can be rather cryptic as long as it's distinct (Sherratt & Beatty 2003), and the zigzag pattern of

European vipers could provide protection from avian predators by making them less visible, but simultaneously functioning as an aposematic signal to the less efficient predator species. The usually cryptic zigzag pattern may change into a highly conspicuous and characteristic one, once the snake has been seen.

The function of the characteristic, dorsal zigzag pattern of *Vipera berus* has been suggested to induce a 'flicker-fusion' illusion while searching for mates or while escaping from predators (Jackson, Ingram & Campbell 1976; Lindell & Forsman 1996). This illusion makes it more difficult for the predator to focus on the escaping snake and therefore increases the snake's chance to escape. In addition to providing a snake with protection by inducing a 'flicker-fusion' illusion, the dorsal zigzag pattern of *Vipera berus* has been suggested to function as an aposematic signal to avian predators (Wüster *et al.* 2004). The protective function of the pattern was first discovered by Andrén & Nilson (1981) who experimented on the avian predator attacks on normal and melanistic *Vipera berus*. They found that there were less attacks on zigzag-patterned *Vipera berus* than on melanistic ones and suggested that it is due to the zigzag pattern making the snake more cryptic. However, they did not control the background of the models in their experiment, and it therefore remained unclear whether the zigzag pattern functions as crypsis or an aposematic signal. In a previous experiment, it was suggested that the dorsal zigzag pattern of *Vipera berus* functions as an aposematic signal to avian predators (Wüster *et al.* 2004). The experiment was conducted by using painted plasticine models to record attacks by avian predators (see also Brodie 1993). Unlike in Andrén & Nilson's (1981) experiment, crypsis was controlled for by placing pieces of white card underneath half of the models. The number of attacks on zigzag-patterned models on white and natural backgrounds did not differ, suggesting that the difference in attack rate was not due to crypsis, but due to the zigzag pattern functioning as an aposematic signal. However, predation pressure is low in Britain and Finland compared with southern Europe and unlike in southern Europe, avian predators that prey almost exclusively on snakes are very rare.

The aims of the experiment were to test (1) whether the dorsal zigzag pattern functions as an aposematic signal against avian predators in southern Europe, where there are a lot of avian predator species that prey on snakes, and (2) Does the potential aposematic signal of the dorsal zigzag pattern function in a size-dependent manner, i.e. are adult snakes better protected compared with smaller juveniles?

Materials and methods

VIPERA LATASTEI

Vipera latastei gaditana Saint Girons is the 'prey species' in the experiment. It grows to about 60 cm long

but usually remains smaller, approximately 40–50 cm. Despite its small size, *Vipera latastei gaditana* is considered to be one of the most defensive, even aggressive species in the genus *Vipera* (De Smedt 2001). If threatened, it will strike repeatedly while producing a loud hissing sound. Although the venom toxicity of *Vipera latastei gaditana* is considered to be relatively low, three to seven people are killed annually (Gonzalez 1982a,b). The venom is haemotoxic and causes mostly local symptoms. The effects of a bite include swelling, discoloration and pain.

Vipera latastei gaditana is the only solenoglyphous (front fanged) venomous snake species occurring in Coto Doñana national park. Other snake species include the nonvenomous *Rhinechis scalaris* (Schinz), *Natrix maura* (L.), *Natrix natrix* (L.), *Hemorrhois hippocrepis* (L.), *Coronella girondica* (Daudin) and the opisthoglyphous (rear fanged) *Malpolon monspessulanus* (Hermann) and *Macroprotodon cucullatus* (Geoffroy).

PLASTICINE MODELS

Our methods follow those of Madsen (1987), Andrén & Nilson (1981), Brodie (1993), Brodie & Janzen (1995), Pfennig *et al*. (2001) and Wüster *et al*. (2004). Plasticine replicas of *Vipera latastei gaditana* were manufactured from pre-coloured grey, nontoxic plasticine (Caran D'Ache, Modela Noir 1000 g). The use of plasticine is an effective method in estimating the number of attacks on the models because large numbers can be used and the attacks by predators leave

imprints on the surface of the models. Two sizes of models were constructed: small 18 ± 0 cm models representing juvenile, yearling *Vipera latastei gaditana*, and larger; 41 ± 4 cm models representing adult *Vipera latastei gaditana*. Twenty models of both sizes were constructed. A black, dorsal zigzag pattern was painted on to half of the models by using acrylic paint [Pébéo acryl colour (3746 11) 500 mL]. The heads of the models were distinguished by flattening them to resemble the wide head shape of *Vipera latastei gaditana*. The tails were also made narrower than the heads.

The reflectance of the background paper, natural background, plasticine and the paint was measured and compared with the reflectance of two captive bred, yearling *Vipera latastei gaditana* (Fig. 1). In the snakes, the reflectance was measured from the middle of the dorsal zigzag pattern and the grey background coloration. We did not have chance to use a spectroradiometer in the field and we conducted measurements of 'natural background' afterwards. For that, we tried to find typical background (mixture of sand and tried vegetation with patches of green plants) that dominated our field site. It is important to keep in mind that presented reflectances show only relative rather than absolute contrasts between snake models and different backgrounds. All reflectances were measured at 320–700 nm at 0.33 nm intervals using a spectroradiometer (Ocean Optics USB2000 spectrometer, Ocean Optics PX-2 pulsed xenon light source, Ocean Optics OOI-Base 32 software, Ocean Optics Inc., Dunedin, FL, USA). Reflectance was measured as the proportion of
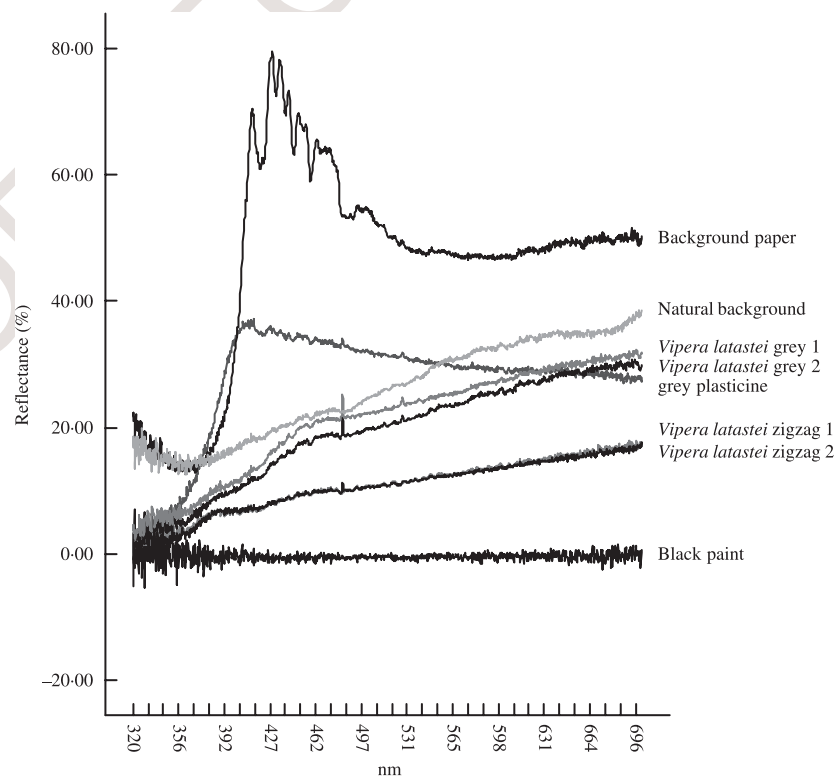
Fig. 1. Reflectances of the white background paper, natural background, grey plasticine, black paint and the grey coloration and zigzag pattern coloration of two different individuals of *Vipera latastei gaditana*.

light reflected from a calibrated 98% white standard (LabSphere). This was done to compare the natural colours of the snakes to the colours of the paint and plasticine. As measured snakes were captive bred and not originated from Doñana (geographical variation in snake colour pattern is common) the direct comparison of the plasticine and snake coloration is not meaningful. Still it shows the scale of the contrast between black and grey colour. Contrast between background paper and snake models show that both snake types (plain and zigzag) are highly visible on the white paper, whereas on the natural background the contrast is not as obvious.

In every treatment, eight combinations of grey plasticine models resembling *Vipera latastei gaditana* were used: small grey on natural background, small grey on white background, small zigzag-patterned on natural background, small zigzag-patterned on white background, large zigzag-patterned on natural background, large zigzag-patterned on white background, large grey on natural background and large grey on white background. Five models of each combination were used in each treatment. An A4-sized white card was used as the white background to determine whether avian predators actively avoid a type of model or if they are simply not noticed (i.e. cryptic). The plasticine models were placed conspicuously on to the ground or on to the sheet of paper into an S-shaped posture at approximately 17 m intervals (20 steps) and anchored down into the ground or into bushes with iron wire from both ends to prevent avian predators from taking the models as a result of an attack. The models were set into an S-shaped posture to make them as natural as possible and also to enable the large models to fit on to the A4 sheet of paper. The location of each model was marked by attaching a piece of white plastic tape on to a plant nearby.

The plasticine models were set in the evening between 17:00 and 19:00 h. They were checked the next morning between 08:30 and 09:30 h and again during the evening between 18:00 and 19:00 h. The models were checked the final time and collected during the second morning between 08:30 and 09:30 h. When checked, it was recorded whether the plasticine models were attacked, where they were attacked (head, middle or tail) and the intensity of the attack. If a model had disappeared from where it had been set, it was classified as attacked due to the strength of the large avian predators and the tendency of the attacks of being very intense. The classification of disappeared models as attacked was based on observations of attacked models and their surrounding area. In several cases, wing marks (depression marks made by the wings of taking off or hovering avian predators) could be seen in the sand in the location where a model had been set, and where it had disappeared from, indicating that the model had indeed been attacked by an avian predator instead of, for example, a mammal. If a model had more than one attack mark on it, it was recorded as one attack because

of the possibility that they were multiple marks from one attack. Only certain attacks that were considered to have been caused by avian predators, and had clear marks from bird claws or beaks on them, were used in the analyses.

During every treatment, the number of avian predators sighted was recorded to gain information about the predator frequencies in each area. Predators were looked out for during the treatments by simply looking around during the setting of the models. This simple way of estimating predator frequencies is not very accurate, and it is probable that the same predator was recorded more than once. However, this method does give a general conception of the numbers of predators and helps assessing their activity.

The survivals of the plasticine models were classified into four categories according to the time of attack. The first category included all attacks that occurred between the setting of the models and the first check. The second category included the attacks that occurred between the first and the second check. The third category included all attacks that occurred between the second and third check. Models that had not been attacked at all during the treatment, were classified in the fourth category.

EXPERIMENT AREAS

The experiment was conducted in Coto Doñana national park (37°00N, 06°38W) in southern Spain between 23 April 2003 and 19 May 2003. The 11 experiments were conducted in nine locations, at 0–40 m above sea-level, where *Vipera latastei gaditana* is known to occur (Adolfo Marco and José Luis Arroyo personal communication, April 2003). The areas were characteristic habitat for *Vipera latastei gaditana*, consisting of low vegetation and variable amounts of *Pinus pinea* L. More detailed information about different habitat types are given in Supplementary Material, Table 1, available on the *Journal of Animal Ecology* Web site. Predator learning was minimized by using areas that were a minimum of 1·5 km distance from each other. An exception to this were two replications conducted in two areas that were less than 1·5 km apart. However, the latter experiment was conducted 13 days later than the first one to avoid predator learning. The replications were conducted in different habitats to use different types of background to prevent a certain type of model being more cryptic or conspicuous than the other models, and to conduct the experiment on a wider range of avian predators.

The nine experiment areas were all different in terms of the models' visibility to avian predators. The amounts of *P. pinea* were variable between the experiment areas. The height of bushes also varied between low *Lavandula* sp. and *Halimium* sp. bushes and tall *Juniperus* sp. and *Erica* sp. bushes. Although the models were set conspicuously instead of being hidden, these factors potentially affect the model's visibility to predators.

Table 1.

Experiment area	Habitat type and assessed model visibility to predators	Dominant plant species
Casa de Santa Olalla	Monte Blanco. Very dry, open and sandy. Visibility of models very good	*Halimium halimifolium* (L.), *Lavandula stoechas* L., *Cistus libanotis* L. and *Halimium calycinum* (L.)
Corral del Toro	Monte Blanco. Visibility of models to predators restricted by *Pinus pinea*	*Pinus pinea* L., *Halimium halimifolium*, *Rosmarinus officinalis* L., *Lavandula stoechas*, *Erica scoparia* L., *Cistus libanotis* L., *Halimium calycinum*, *Cytisus grandiflorus* (Brot.) DC., *Ulex australis* Clemente, *Thymus albicans* Hoffmanns & Link and *Stauracanthus genistoides* (Brot.)
El Raposo	Wet Monte Blanco with *Pinus pinea*. Visibility of models slightly restricted by *Pinus pinea*	*Pinus pinea*, *Helichrysum picardii* Boiss. & Reut., *Stauracanthus genistoides*, *Thymus albicans*, *Cytisus grandiflorus*, *Rosmarinus officinalis*, *Cistus libanotis*, *Lavandula stoechas*, *Halimium halimifolium*, *Osyris quadripartita* Salzm. ex Decne, *Corema album* (L.) and *Armeria pungens* Hoffmanns & Link
La Retuerta	Monte Blanco. Located close to a *Pinus pinea* forest. Often covered with water, especially during the winter and spring. Visibility of models to predators good	*Rosmarinus officinalis* (dominant), *Lavandula stoechas*, *Stauracanthus genistoides*, *Halimium calycinum*, *Asparagus acutifolius* L., *Phyllirea angustifolia* L., *Osyris alba* L. and to a lesser extent, *Erica scoparia*
Laguna del Taraje	Mostly wet Monte Negro habitat surrounded by ponds as well as dry sand dunes. Visibility of models to predators good but slightly restricted by tall *Erica* bushes	*Calluna vulgaris* L., *Ulex minor* Roth, *Erica scoparia*, *Osyrisalba* and *Cytisus scoparius* L.
Las Baquetas	Wet Monte Blanco with *Pinus pinea*. Visibility of models to predators very slightly restricted by *Pinus pinea*	*Pinus pinea*, *Helichrysum picardii*, *Stauracanthus genistoides*, *Thymus albicans*, *Cytisus grandiflorus*, *Rosmarinus officinalis*, *Cistus libanotis*, *Lavandula stoechas*, *Halimium halimifolium*, *Osyris quadripartita*, *Corema album* and *Armeria pungens*
Nave del Panteón	Monte Blanco. Visibility of models to predators good	*Helichrysum picardii*, *Stauracanthus genistoides*, *Thymus albicans*, *Cytisus grandiflorus*, *Rosmarinus officinalis*, *Cistus libanotis*, *Lavandula stoechas*, *Halimium halimifolium*, Osyris, *quadripartita* and *Corema album*
Sabinar del Ojillo	Monte Blanco or Sabinar (Monte Blanco with *Juniperus*). Visibility of models to predators slightly restricted by tall *Juniperus* bushes	*Juniperus phoenicea* L., *Helichrysum picardii*, *Stauracanthus genistoides*, *Thymus albicans*, Cytisus grandiflorus, Rosmarinus *officinalis*, *Cistus libanotis*, *Lavandula stoechas*, *Halimium halimifolium*, *Osyris quadripartite*, *Corema album* and *Pinus pinea*
Nave de Pedro Pérez	Monte Blanco or Sabinar (Monte Blanco with *Juniperus*). Near a Monte Blanco / Monte Negro border where the habitat changes from a dry, low-vegetation environment to a wetter environment with more *Juniperus* and *Pinus*. Visibility of models to predators very slightly restricted by *Juniperus* and *Pinus*	*Juniperus phoenicea*, *Helichrysum picardii*, *Stauracanthus genistoides*, *Thymus albicans*, *Cytisus grandiflorus*, *Rosmarinus*, *officinalis*, *Cistus libanotis*, *Lavandula stoechas*, *Halimium*, *halimifolium*, *Osyris quadripartita*, *Corema album* and *Pinus pinea*

Special care was taken, however, that different models were placed equally visibly within the location.

ATTACKS

Attacks by avian predators were recognized as U- or V-shaped marks on opposite sides of the models or as closely spaced deep scratches on the dorsal surface of the models (Supplementary Material, Fig. 1a,b). In some instances, wing marks could be seen on the sand around the attacked model. Some models had been attacked with such force that the iron wire had been torn off the bushes or the ground, or from inside the model itself, and some models had been moved up to 2 m away from their original location by strong predators. They were occasionally broken into pieces as a result of very strong attacks (Supplementary Material, Fig. 1c) or had been taken and moved away completely. The shapes and nature of the marks recorded as attacks were assessed as being the result of an avian predator

attack, not from random events not specifically directed at the replicas (e.g. falling branches, thorns of plants). The shapes or nature of the attack marks cannot be used reliably to differentiate attacks by hawks and owls.

PREDATORS

A large amount and a wide range of avian predators occur in Doñana National Park. The short-toed eagle *Circaetus gallicus*, common buzzard *Buteo buteo*, marsh harrier *Circus aeroginosus*, Montagu's harrier *Circus pygargus*, lesser kestrel *Falco naumanni*, common kestrel *Falco tinnunculus*, northern hobby *Falco subbuteo*, red kite *Milvus milvus*, black kite *Milvus migrans*, black-shouldered kite *Elanus caeruleus*, honey buzzard *Pernis apivorus*, tawny owl *Strix aluco*, Eurasian scops owl *Otus scops* and barn owl *Tyto alba* have all been reported to feed on snakes (Cramp & Simmons 1980; Cramp 1985). The short-toed eagle *Circaetus gallicus* is an ophiophage specialist with 95% of its prey consisting of snakes (Cramp 1985). High numbers of avian predators were sighted during the treatments. Especially *Milvus migrans* were seen in high numbers and in some cases up to 20 individuals could be seen flying simultaneously. *Buteo buteo* were also sighted daily as were *Milvus milvus* and *Falco naumanni*. However, *Milvus migrans* were by far the most commonly sighted raptor species and their numbers clearly exceeded those of other species.

The numbers of avian predators varied considerably between the experiment areas. Predators seemed to be most common at Palacio de Doñana and the surrounding *Pinus pinea* forest, and seemed to decrease westward towards the entrance to the reserve. Experiment areas closest to Palacio de Doñana seemed to have higher numbers of *Milvus migrans* than the other areas. The number of sighted predators was lowest in the experiment area outside the reserve, where no raptors were sighted. To estimate the effect of predator density on predation pressure on snake models we used nesting data of raptors, which was provided by the Doñana Biological Station Monitoring Group of Natural Processes in Doñana. Unfortunately they did not have data from two of our study locations and no data on nesting owls. The breeding data are from 1999 but the variation in predator numbers between years is low (Begoña Arrizabalaga personal communication, December 2003).

Results

ATTACK RATE ON DIFFERENT MORPHS

The statistical analyses were conducted by using SPSS 11·0 for Windows. Owing to the categorical nature of the data, it was analysed by using nonparametric tests, such as Spearman's Rank Correlation, Chi-square, Kruskal–Wallis and Wilcoxon Signed Rank tests. All *P*-values are for two-tailed tests.
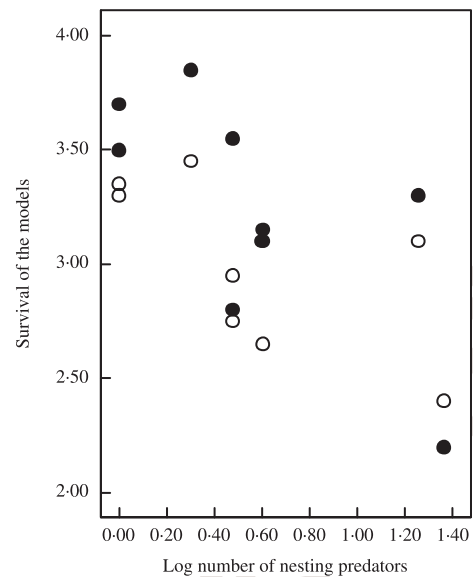
Fig. 2. Survival of grey (open dots) and zigzag-patterned (black dots) plasticine models decreased with increasing density of nesting raptors in the experiment area. Each dot represents mean survival of each snake type. Survival is indicated by numbers from 1 to 4, referring to how long a model survived. 1 meaning that the model was attacked before the first check and 4 meaning that it was not attacked at all.

In the analyses, survival was determined as the check, before which the model had been attacked; 1 meaning that the model had been attacked before the first check, and 4 that it had not been attacked at all. The predation pressure differed between areas because there were significant differences between the total survivals of plasticine models (Kruskal–Wallis $\chi^2 = 40·942$, d.f. = 10, $P < 0·001$). The survival of the plasticine models was lower in the area, where the highest number of avian predators were nesting (for zigzag models $r_s = -0·70$, $n = 9$, $P = 0·037$ and for the grey models $r_s = -0·72$, $n = 9$, $P = 0·030$) (Fig. 2).

The overall frequencies of attacks on the models during subsequent treatments (= areas) did not change with time (Spearman's rank correlation $r_s = -0·102$, $n = 11$, $P = 0·766$), which means that even though the attack frequencies differed between the experiment areas, they did not change with time. This is important because even though it can be assumed that attacks in separate treatments were probably made, to some extent, by the same predators; it suggests that the avian predators did not learn that the plasticine models were uneatable. The frequencies of attacks on the models did not differ between the first and last checking of the models (Wilcoxon $Z = -0·889$, $n = 11$, $P = 0·374$) which suggests that local avian predators did not learn that the models were unpalatable in the different areas during individual treatments. However, the frequency of attacks was significantly lower on the second check compared with the first (Wilcoxon $Z = -2·847$, $n = 11$, $P = 0·004$) and the third check (Wilcoxon $Z = -2·666$, $n = 11$, $P = 0·008$). This is expected to be due to the activity of predators being highest during the morning
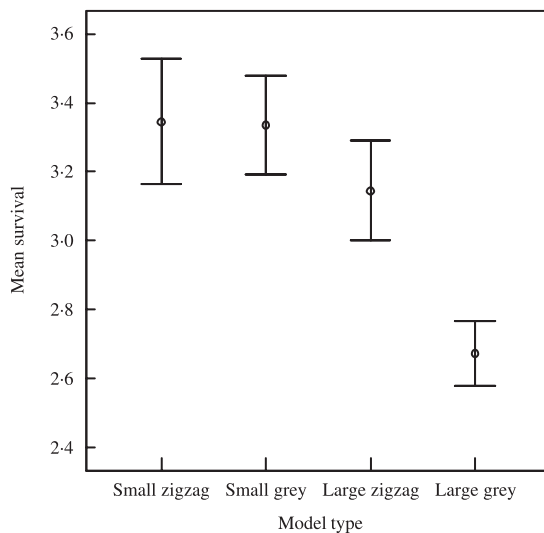
Fig. 3. The fate of different sized and patterned models. For 'survival', the numbers from 1 to 4 indicate the time how long a model survived, 1 meaning that it was attacked before the first check and 4 meaning that it was not attacked at all. Bars show standard errors.

Fig. 4. The numbers of attacks on different parts of the small and large models. The attacks were directed significantly towards the head in both model sizes.

and evening, therefore resulting in lower predation pressure during midday. Another possible explanation is that owls were responsible for the attacks during nights. Unfortunately we could not separate attacks of hawks and kites from attacks of owls.

The survivals of different model types were tested by treating the different experiment areas ($n = 11$) as independent cases by calculating the average survival rate of each model type. The survivals of the two model sizes differed significantly with the survival of the smaller models being higher (Wilcoxon Signed Ranks Test $Z = -2.499$, $n = 11$, $P = 0.012$), suggesting that large snakes are more attractive prey to avian predators. The survivals of the model types did not differ significantly between the two different backgrounds (Wilcoxon Signed Ranks Test, all $P < 0.151$, $n = 11$), and therefore the data from the two backgrounds were pooled to increase the power of the test. The survivals of small grey and zigzag-patterned models did not differ significantly (Wilcoxon Signed Ranks Test $Z = -0.211$, $n = 11$, $P = 0.833$) but there was a significant difference between the survivals of large grey and zigzag-patterned models, the survival of zigzag-patterned models being significantly higher than that of grey models (Wilcoxon Signed Ranks Test $Z = -2.539$, $n = 11$, $P = 0.011$, Fig. 3).

Looking more closely at the data on the attacks on different parts of the models, it can be seen that the models were significantly attacked towards the flattened head ($\chi^2 = 38.254$, d.f. = 2, $P < 0.001$) both in small ($\chi^2 = 19.733$, d.f. = 2, $P \le 0.001$) and large ($\chi^2 = 19.425$, d.f. = 2, $P \le 0.001$) models (Fig. 4). The frequencies of attacks towards the heads were at least twice as high as the ones towards the middle parts or the tails of the models.
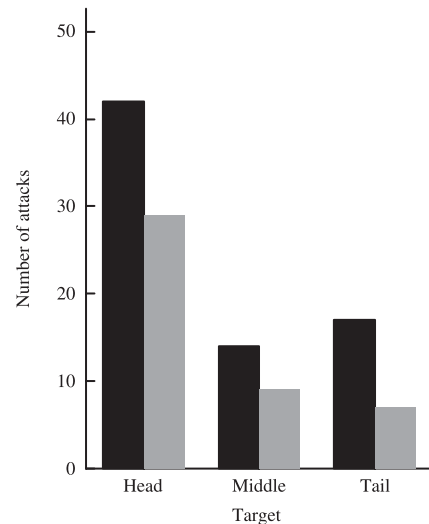
Discussion

SURVIVAL OF DIFFERENT MORPHS

Most *Vipera* species have a characteristic dorsal zigzag pattern. A small number of species also have an additional melanistic morph that occurs mainly in the higher latitude/altitude of the species' distribution and has a thermoregulatory advantage over the zigzag-patterned morph (Gibson & Falls 1979). Andrén & Nilson (1981) were the first to show that the zigzag-patterned snakes are predated on less than the melanistic ones. This difference in predation has been thought to be due to the zigzag pattern's cryptic function making the snake less visible to predators (Andrén & Nilson 1981). In the current study, we controlled crypsis by using a white background to make the plasticine models equally visible to predators. Thus, we suggest that crypsis does not explain the lower attack rate against zigzag-patterned snakes. Recently, Wüster *et al.* (2004) found similar results in northern Europe and suggested that the zigzag pattern of European vipers is more likely to have an aposematic rather than cryptic function.

Zigzag pattern of snakes has been used as an example of disruptive coloration (Cott 1940; Edmunds 1974; Forsman 1995) as the zigzag pattern effectively brakes up the body outline of the snake. Thus one may argue that lower attack rates on zigzag models might be due to that. Although we cannot exclusively rule out that possibility, we believe that disruptive coloration alone cannot explain the higher survival of large zigzag-patterned models, as both model types (plain and zigzag) were highly visible on the white background. It is good to keep in mind that an animal can use both aposematic and disruptive coloration simultaneously (Cott 1940) and thus the hypotheses for disruptive coloration and aposematism are not mutually exclusive. However,

future experiments with critical controls for disruptive coloration are needed to determine the ultimate reason for predator avoidance.

Predation in nature is often difficult to study. Partly because attack frequencies in natural conditions are often very low, and partly because laboratory experiments don't necessarily produce results applicable to natural conditions. For these reasons the use of plasticine models is a very effective method of determining attack rate on snakes (Andrén & Nilson 1981; Madsen 1987; Brodie 1993; Brodie & Janzen 1995; Pfennig *et al*. 2001; Wüster *et al*. 2004). Another advantage of using plasticine models is that the possible behavioural differences between morphs are controlled for by the nonmoving plasticine model and the attack rate can be assessed as the function of the colour pattern itself. On the other hand, a possible disadvantage of using motionless plasticine models is a different attack stimulus created by the different sized plasticine models. Some medium sized birds rely on movement to spot their prey and the difference in attack frequency on the small and large models may reflect a difference in attack stimulus between small and large motionless prey items.

Predation pressure against snake models during our experiments was very high. On average 39% of the models were attacked during the 36 h experiment period and thus it was possible to effectively calculate the differences between attack rates on different morphs. It seems that predation pressure against snakes in southern Spain is higher compared with northern Europe or Great Britain (Wüster *et al*. 2004). On the other hand, in the study of Wüster *et al*. (2004), only juvenile size snake models were used, which were found to be less attractive prey items compared with adult size models in the current experiment. There are also more avian predators present that specialize in preying on snakes in Spain than in northern Europe, which can be expected to increase the predation of snakes.

Interestingly, predation pressure against snakes differed widely among experiment locations and was strongly correlated with the local raptor density (Fig. 2). Differences in local predator community can lead to variable selection against different antipredator strategies (Mappes, Marples & Endler 2005). In areas with high density of snake specialist predators, selection may favour cryptic rather than aposematic strategy. In our data, both snake types were preyed on more in areas with high predator density. Even though our experiment was not designed to compare differences between localities, this method provides interesting prospects for studying variable selection on different colorations.

There was no significant difference between the survivals of the colour morphs in the small models. However, survival of the large models was lower than the small models, and furthermore, the survival of the large grey models was lower than that of the large zigzag-patterned models. The models were also attacked sig-nificantly towards the head. Attack rate did not differ between the natural background and the white background, which suggests that the observed differences in survival are not due to the zigzag pattern's cryptic effect, but due to predator avoidance.

A white background was used under half the plasticine models to make the models equally visible to avian predators and to exclude the possibility of the zigzag pattern making the models more cryptic. The white background cannot be considered to be natural and may possibly have some other effect than making the plasticine models more visible to avian predators. One possibility is that the white background might induce neophobia in the avian predators or attract them towards the models and affect the results in this way. However, the function of the white background was not to be natural, but to make the model types equally visible to predators. No statistically significant differences were found between the numbers of attacks on models on natural and white backgrounds. This similarity in attack frequency may be interpreted either as the effect of the white background being small, or as the high visibility of the models on a white background being counteracted by the effect of the white background as a cause of neophobia. However, the former seems more plausible than the latter due to the generally high rate of attack and insignificant difference between attacks on models on the two different backgrounds.

The results on predation pressure and survival from using motionless plasticine models cannot be considered to be completely natural due to the models lacking the behaviour of real snakes. Despite this, plasticine models have been used successfully several times to determine attack rates by avian predators (Andrén & Nilson 1981; Madsen 1987; Brodie 1993; Brodie & Janzen 1995; Pfennig *et al*. 2001; Wüster *et al*. 2004). With real snakes, the aposematic function of the zigzag pattern may cause an avian predator to hesitate before an attack, thus allowing the snake to escape and giving it a selective advantage over snakes without a zigzag pattern. This hesitation of predators before an attack, however, short, can be a great advantage to a snake trying to escape.

Moreover, *Vipera latastei gaditana* tends to be very defensive and antipredator behaviour against avian predators should, at least in some cases, result in unsuccessful attacks. On the other hand, avian predators may use movement to detect their prey, resulting in no attacks on the plasticine models. However, these factors should not affect the results of the experiment due to all the models having the same behavioural potential to repel or attract avian predators.

The survival of the smaller models of both 'morphs' was higher than that of the larger models. This may be due to prey-size selection by avian predators, large prey being more attractive. It has been shown by Gil & Pleguezuelos (2001) that *Circaetus gallicus* positively selects snakes with a snout-vent length (SVL) of 700–1000 mm and negatively selects snakes with a SVL of

less than 600 mm. However, this may not apply to other raptor species, due to the short-toed eagle being an ophiophage specialist and its diet consisting almost exclusively on snakes (Cramp & Simmons 1980). An alternative explanation to the higher survival of the small models is that they are less apparent and therefore less often noticed by predators (see also King 1992, 1993). Thus, selection pressure on coloration is expected to affect adult and juvenile snakes differently.

There was no significant difference between the survivals of small grey and zigzag-patterned plasticine models, but there was a significant difference between the large models. This may be due to the smaller amount of attacks on small models than large models and thus we were not able to find any statistical significicance between two different morphs. Alternatively, signal efficiency in the small models may be lower than in the larger models, thus leading to similar levels of predation on the morphs. Gamberale & Tullberg (1996) showed that the larger an aposematic prey is, the more effectively it induces avoidance in predators. Even though juvenile *Vipera latastei gaditana* are considerably venomous, they may not be able to inflict serious bites to large avian predators due to their short striking distance or comparative weakness. Because of this, juvenile *Vipera latastei gaditana* may not be dangerous enough to the predators to be able to induce avoidance in them, thus restricting the aposematic function of the zigzag pattern to larger, more dangerous individuals. One has to keep in mind that overall predation pressure was rather low against small snake models making comparison between zigzag and grey juvenile-sized snakes difficult.

Survival was significantly lower in large, grey models than in large zigzag-patterned models, suggesting that zigzag-patterned snakes are subjected to less predation than those without the pattern (Fig. 3). However, while this suggests a selective advantage to a viper's zigzag pattern, it does not clarify the function of the pattern. The difference in attack frequency may have occurred either due to the pattern's cryptic effect, whereby the predators do not notice the snake because of the pattern's disruptive effect, or due to an aposematic effect, whereby the avian predators actively avoid the zigzag-patterned plasticine models because they associate the pattern with toxicity. However, the number of attacks did not differ significantly between natural and white backgrounds, which suggests that the difference in attack rate is not due to crypsis, but due to pattern *per se*. Differences in attack rates on uniform grey and zigzag-patterned grey can be due to either active avoidance or differential preference of these colorations by avian predators. While the results suggest that the zigzag pattern functions as an aposematic signal to avian predators, it does not mean that vipers advertise themselves to predators. Some forms of behaviour may function to make zigzag-patterned live snakes more difficult to detect. These may include flicker fusion effect and evasive flight (Brattström 1955; Jackson *et al.* 1976;

Brodie 1989). As the zigzag pattern does not seem to be very conspicuous, it could be considered to be a compromised strategy (Endler & Mappes 2004) in a multi-predator community, as many of the avian predator species prey on snakes regardless of their toxicity. The zigzag pattern could therefore function as an aposematic signal to the species that are not ophiophage specialists. The high-contrast zigzag pattern may also function as an aposematic signal to mammals, such as genets and mongooses as a visual signal or as a combination of colour pattern and behaviour (Brodie 1992; Forsman & Appelqvist 1998) so that the pattern does not attract mammalian predators but functions as an aposematic signal when an encounter occurs. Most mammalian predators have dichromatic vision (Jacobs 1993) and the black and white zigzag pattern may potentially be a very effective signal to them.

AVIAN PREDATOR ATTACKS

The position of the attack marks on the models suggests that avian predators treat the models as prey, rather than nonprey items. The attacks were inflicted significantly towards the heads of the models and the claw marks were often very deep, indicating the strength of the strike. Smith (1973, 1976) has shown that avian predators of snakes have a tendency to attack snakes to the head, which is considered to be an adaptation to handling dangerous prey that can inflict bites on the predator with their fangs if the head is not restrained during the strike (Smith 1973, 1976). Although the treatments were conducted in different areas, it is still very likely that some of the same predators attacked the plasticine models during separate treatments. However, the frequencies of attacks did not decrease with time from the first to the last treatment, suggesting that the predators did not learn that the plasticine models were not real prey but attacked them as real snakes.

Some models had small incisor marks from rodents. These were not recorded as attacks because rodents do not predate on snakes and are more likely to be drawn by the apparent sweet taste of plasticine. Mammalian predators of snakes in Doñana include *Herpestes ichneumon* (L.) and *Genetta genetta* (L.). No marks from mammalian predators were recorded during the experiment and mammalian predators were not expected to attack the plasticine models due to the models not looking that much like real snakes close up and not having a behavioural repertoire. Mammals are also expected to be able to distinguish plasticine models from real snakes by odour.

Our results indicate that snakes are very attractive prey to avian predators and that the predation pressure is very heavy. Also, large snakes are predated on significantly more than small ones. Even though the zigzag pattern did not seem to have a protective function in the small snakes, their predation pressure may be too low for the advantage to be detectable. The zigzag pattern provided large snakes with protection from avian

predators. A snake has to grow large to be able to breed and it is advantageous for it to be zigzag-patterned compared with being uniform grey. So even though the zigzag pattern is not conspicuously aposematic, it is still more advantageous for a snake to be zigzag-patterned than not.

Acknowledgements

References

Andrén, C. & Nilson, G. (1981) Reproductive success and risk of predation in normal and melanistic colour morphs of the adder, *Vipera berus*. *Biological Journal of the Linnean Society*, **15**, 235–246.

Brattström, B.H. (1955) The coral snake 'mimic' problem and protective coloration. *Evolution*, **9**, 217–219.

Brodie, E.D. (1989) Genetic correlations between morphology and antipredator behaviour in natural populations of the garter snake *Thamnophis ordinoides*. *Nature*, **342**, 542–543.

Brodie, E.D. (1992) Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. *Evolution*, **46**(5), 1284–1298.

Brodie, E.D. (1993) Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. *Evolution*, **47**, 227–235.

Brodie, E.D. & Janzen, F.D. (1995) Experimental studies of Coral Snake mimicry: generalized avoidance of ringed patterns by free-ranging avian predators. *Functional Ecology*, **9**, 186–190.

Caldwell, G.S. & Rubinoff, R.W. (1983) Avoidance of venomous sea snakes by naive herons and egrets. *Auk*, **100**, 195–198.

Cott, H.B. (1940) *Adaptive Coloration 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.

Cramp, S. & Simmons, K.E.L. (1980) *Handbook of the Birds of Europe, the Middle East and North Africa*, Vol. III. Oxford University Press, Oxford.

De Smedt, J. (2001) *Die europäischen Vipern. Artbestimmung, Systematik, Haltung und Zucht*. Johan De Smedt, Füssen.

Edmunds, M. (1974) *Defence in Animals*. Harlow: Longman.

Endler, J.A. (1978) A predator's view of animal color patterns. *Evolutionary Biology*, **11**, 319–364.

Endler, J.A. (1980) Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, **34**, 76–91.

Endler, J.A. (1988) Frequency-dependent predation, crypsis and aposematic coloration. *Philosophical Transactions of The Royal Society (Series B)*, **319**, 505–523.

Endler, J.A. (1991a) Interactions between predators and prey. *Behavioral Ecology*, 3rd edn (eds J. Krebs & N. Davies), pp. 169–196. Blackwell Scientific Publications, Oxford.

Endler, J.A. (1991b) Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Research*, **31**, 587–608.

Endler, J.A. (1992) Signals, signal conditions, and the direction of evolution. *American Naturalist*, **139** (Suppl.), s125–s153.

Endler, J.A. & Mappes, J. (2004) Predator mixes and the conspicuousness of aposematic signals. *American Naturalist*, **163**(4), 532–547.

Endler, J.A. & Théry, M. (1996) Interacting effects of lek placement, display behavior, ambient light and color patterns in three neotropical forest-dwelling birds. *American Naturalist*, **148**, 421–452.

Forsman, A. (1995) Opposing fitness consequences of color pattern in male and female snakes. *Journal of Evolutionary Biology*, **8**, 53–70.

Forsman, A. & Appelqvist, S. (1998) Visual predators impose correlational selection on prey color pattern and behavior. *Behavioral Ecology*, **9**(4), 409–413.

Forsman, A. & Merilaita, S. (1999) Fearful symmetry: pattern size and asymmetry affects aposematic signal efficacy. *Evolutionary Ecology*, **13**, 131–140.

Gamberale, G. & Tullberg, B.S. (1996) Evidence for peak-shift in predator generalization among aposematic prey. *Proceedings of the Royal Society of London (Series-B)*, **263**, 1329–1334.

Gibson, A.R. & Falls, J.B. (1979) Thermal biology of the common garter snake *Thamnophis sirtalis* (L.). II. The effects of melanism. *Oecologia (Berlin)*, **43**, 99–109.

Gil, J.M. & Pleguezuelos, J.M. (2001) Prey and prey-size selection by the short-toed eagle (*Circaetus gallicus*) during the breeding season in Granada (south-eastern Spain). *Journal of Zoology London*, **255**, 131–137.

Gittleman, J.L. & Harvey, P.H. (1980) Why are distasteful prey not cryptic? *Nature*, **286**, 149–150.

Gonzalez, D. (1982a) Epidemiological and clinical aspects of certain venomous animals of Spain. *Toxicon*, **20**(5), 925–928.

Gonzalez, D. (1982b) Clinical aspects of bites by viper in Spain. *Toxicon*, **20**, 349–353.

Greene, H.W. & McDiarmid, R.W. (1981) Coral snake mimicry: Does it occur? *Science*, **213**, 1207–1212.

Hagman, M. & Forsman, A. (2003) Correlated evolution of conspicuous coloration and body size in poison frogs (Dendrobatidae). *Evolution*, **57**(12), 2904–2910.

Jackson, J.F., Ingram, W. & Campbell, H.W. (1976) The dorsal pigmentation pattern of snakes as an antipredator strategy: a multivariate approach. *American Naturalist*, **110**, 1029–1053.

Jacobs, G.H. (1993) The distribution and nature of colour vision among mammals. *Biology Reviews*, **68**, 413–471.

King, R.B. (1992) Lake Erie water snakes revisited: morph- and age-specific variation in relative crypsis. *Evolutionary Ecology*, **6**, 115–124.

King, R.B. (1993) Color-pattern variation in Lake Erie water snakes: prediction and measurement of natural selection. *Evolution*, **47**(6), 1819–1833.

Lindell, L.E. & Forsman, A. (1996) Sexual dichromatism in snakes: support for the flicker-fusion hypothesis. *Canadian Journal of Zoology*, **74**, 2254–2256.

Lindström, L., Alatalo, R.V., Mappes, J., Riipi, M. & Vertainen, L. (1999) Can aposematic signals evolve by gradual change? *Nature*, **397**, 249–251.

Madsen, T. (1987) Are juvenile grass snakes, *Natrix natrix*, aposematically coloured? *OIKOS*, **48**, 265–267.

2

Mappes, J. & Alatalo, R.V. (1997) Effects of novelty and gregariousness in survival of aposematic prey. *Behavioral Ecology*, **8**, 174–177.

Mappes, J., Marples, N. & Endler, J.A. (2005) Unexplored factors retard our understanding of aposematism. *Trends in Ecology and Evolution*, in press.

Marples, N.M., Brakefield, P.M. & Cowie, R.J. (1989) Differences between the 7-spot and 2-spot ladybird beetles (Coccinellidae) in their toxic effects on a bird predator. *Ecological Entomology*, **14**, 79–84.

Pfennig, D.W., Harcombe, W.R. & Pfennig, K.S. (2001) Frequency-dependent Batesian mimicry. *Nature*, **401**, 323–323.

Pough, F.H. (1988a) *Mimicry and Related Phenomena, Biology of the Reptilia*, Vol. 16: *Defense and Life History* (eds Gans, C. & Huey, R.B.), pp. 153–234. Alan R. Liss, Inc., New York.

Pough, F.H. (1988b) Mimicry of vertebrates: Are the rules different? *American Naturalist*, **131**, S67–S102.

Poulton, E.B. (1890) *The Colours of Animals*. Kegan Paul, Trench, Trübner & Co Ltd, London.

Rubinoff, I. & Kropach, C. (1970) Differential reactions of Atlantic and Pacific predators to sea snakes. *Nature*, **228**, 1288–1290.

Savage, J.M. & Slowinski, J.B. (1992) The coloration of the venomous coral snakes (family Elapidae) and their mimics (families Aniliidae and Colubridae). *Biological Journal of the Linnean Society*, **45**, 235–245.

Sherratt, T.N. & Beatty, C.D. (2003) The evolution of warning signals as reliable indicators of prey defense. *American Naturalist*, **162**(4), 377–389.

Sillén-Tullberg, B. (1985) Higher survival of an aposematic than cryptic form of distasteful bug. *Oecologia*, **67**, 411–415.

Smith, S.M. (1973) A study of prey-attack behaviour in young loggerhead shrikes, *Lanius ludovicianus* L. *Behaviour*, **44**, 113–141.

Smith, S.M. (1975) Innate recognition of coral snake pattern by a possible avian predator. *Science*, **187**, 759–760.

Smith, S.M. (1976) Predatory behaviour in young turquoise-browed motmots, *Eumomota superciliosa*. *Behaviour*, **56**, 309–320.

Wüster, W., Allum, C.S.E., Bjargardóttir, I.B., Bailey, K.L., Dawson, K.J., Guenioui, J., Lewis, J., McGurk, J., Moore, A.G., Niskanen, M. & Pollard, C.P. (2004) Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. *Proceedings of the Royal Society of London, Biology Sciences*, **271**(1556), 2495–2499.

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