



Hairiness and warning colours as components of antipredator defence: additive or interactive benefits?

C. LINDSTEDT, L. LINDSTRÖM & J. MAPPES

Department of Biological and Environmental Science, University of Jyväskylä

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To deter predator attack, aposematic prey species advertise their unprofitability with one or more conspicuous warning signals that, in turn, enhance the avoidance learning of predators. We studied the costs and benefits of multicomponent signalling in *Parasemia plantaginis* moths. The hairy moth larvae have an orange patch on their otherwise black bodies. The patch varies phenotypically and genetically in size. We studied whether the detection risk associated with patch size varied against two backgrounds (green or brown) with two different predators: naïve chicks, *Gallus gallus domesticus*, and experienced great tits, *Parus major*. We also evaluated the signal value of different defence traits within a multicomponent signal by testing which combination of two traits, hairiness and the presence or size of the orange patch, most affected the avoidance learning rate of predators. Larvae with a large orange patch were at greater risk of detection by birds against both backgrounds. This higher detection risk was traded-off with enhanced avoidance learning rate. The orange patch had a higher signal value for the predators than did hairiness, which only slightly increased the survival of totally black or small-patched larvae but did not affect the defence of larvae with a large orange patch. Multicomponent defences are therefore not necessarily additive and variation in the warning coloration of aposematic animals may be partly explained by variation in the relative benefits of different components of a warning signal to different predators.

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Aposematism is an antipredator strategy where predators learn to associate the unprofitability of prey (e.g. chemical defences) with conspicuous and bright coloration, consequently avoiding similar prey in the future (Sillén-Tullberg 1985; Roper & Redston 1987; Alatalo & Mappes 1996; Gamberale & Tullberg 1996; Forsman & Merilaita 1999; Lindström et al. 1999; Ihalainen et al. 2007). Given the variation in predator susceptibility to aposematic prey defences, such as resistance to toxins (Calvert 1979; Fink & Brower 1981) or behavioural adaptation to overcome prey defence mechanisms (Yosef & Whitman 1992), many aposematic displays include many defence components simultaneously (Marples et al. 1994). Different defence components may be aimed at different predators with dissimilar search behaviour and perception (Pearson 1989) or against separate phases of predation (Endler

1991). Studies with real (Marples et al. 1994) and artificial (Rowe 2002) prey items have shown that the avoidance learning of a single predator becomes more effective if a prey species provides multiple defence cues (but see Vallin et al. 2005 for nonadditive benefits).

Empirical work on the function of multicomponent signals has concentrated on the interactions between warning coloration and odour (Rowe & Guilford 1999a; Lindström et al. 2001a; Kauppinen & Mappes 2003), sound (Rowe 2002; Hauglund et al. 2006) or grouping behaviour (Tullberg et al. 2000; Gamberale-Stille 2000). That many aposematic animals also use a physical trait, such as hair or spines, as a defence mechanism together with other antipredator repellents has been ignored until recently (Inbar & Lev-Yadun 2005; Speed & Ruxton 2005). Speed & Ruxton (2005) have shown mathematically that physical defences may act as visual cues to a prey's unprofitability and improve both the detectability of prey and the avoidance learning by predators. However, data that directly test the value of physical defences (e.g. spines or hairiness) as signals of unprofitability for visual

Correspondence: C. Lindstedt, Department of Biological & Environmental Science, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland (email: carlind@cc.jyu.fi).

predators and how they contribute to a prey individual's survival combined with other defence components are rare (but see Barnhisel 1991; Mikolajewski & Rolff 2004 for nonaposematic species).

We evaluated the signal value of two defence traits: hairiness and conspicuousness of the colour pattern. We asked whether physical defences such as spines or hair can (1) function as warning signals that enhance predators' avoidance learning rate, (2) function as secondary defence mechanisms that reinforce the effect of other defence components or (3) perform both of these functions simultaneously. Because conspicuousness incurs the cost of increased attention from predators (Riipi et al. 2001), we also tested possible differences in the conspicuousness of different prey morphs. We performed two separate experiments where we used wood tiger moth, *Parasemia plantaginis*, larvae as the prey species. *Parasemia plantaginis* is a useful model to test the interaction of colour and another physical trait in aposematic signalling because the larvae are hairy and have a moderately conspicuous orange patch, which varies in size phenotypically and genetically, on their otherwise black bodies (Ojala et al. 2007).

In the first experiment, we compared the relative detection risk of *P. plantaginis* larvae with small and large patches on two backgrounds: a dark brown background on which the larvae are rather cryptic and a green background on which the larvae are more visible. We used young, naïve domestic chicks, *Gallus gallus domesticus*, as inexperienced predators and wild-caught great tits, *Parus major*, as more experienced wild predators. We predicted that larvae with large orange patches would be more conspicuous, that is, more quickly detected, than larvae with small patches. We further predicted that detection time would be lower on the green background than on the brown background because of increased conspicuousness. Conspicuousness should be more costly when the predators are naïve domestic chicks than when predators are wild great tits that probably have already encountered warning-coloured unpalatable prey (see e.g. Lindström et al. 2001b).

The second experiment specifically examined the relative importance of the different defence components (hairiness and coloration) of the *P. plantaginis* larvae on avoidance learning rate by experienced predators, great tits. To test this, we first manipulated the hairiness (hairy or bald) and presence of the orange patch (patch or no patch) of the larvae (experiment 2a). We further (experiment 2b) investigated the effect of variation in warning coloration among prey on the avoidance learning rate of the predator. Hairiness was manipulated as in experiment 2a. We also manipulated the size of the orange patch (large or small).

We formulated three hypotheses that consider the relationship between hairiness and the size of the orange patch in experiments 2a and 2b (Fig. 1). Hypothesis 1 posits that both hairiness and patch size operate additively and improve prey defence, thus increasing the defence capacity of the prey (e.g. Rowe 1999). Therefore, if the orange colour patch is an important signal for predators, they should learn quicker to avoid prey with large patches than prey

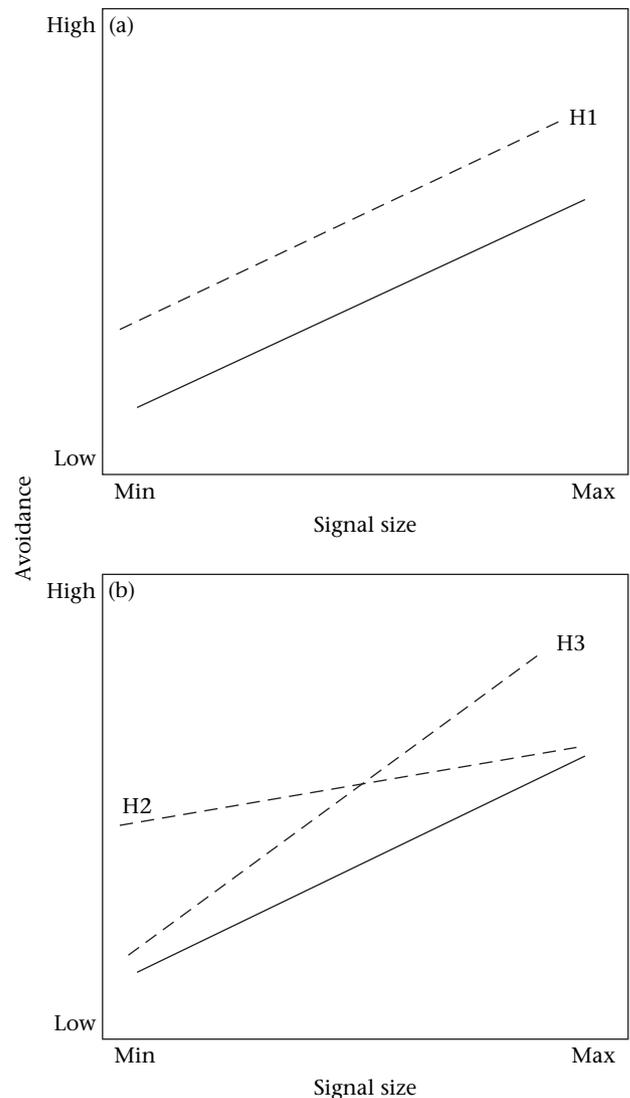


Figure 1. Hypothetical responses of predators towards prey with multicomponent warning displays. Three possible interactions between hairiness and signal size can be predicted: (a) hairiness and signal size additively increase defence capacity (H1) (Rowe 1999; Speed & Ruxton 2005), (b) hairiness contributes more to the defence capacity of prey with a weak signal (H2) or with a strong signal (H3). Dashed line shows the responses of predators towards the hairy prey and solid line shows the responses towards the hairless prey.

with smaller patches. Hairiness should make avoidance learning rate even more effective if it acts as a visual signal or deterrent (Inbar & Lev-Yadun 2005; Speed & Ruxton 2005). Both hypotheses 2 and 3 posit that hairiness and warning colour interact and, thus, hairiness could contribute to the defence capacity of prey with a large (hypothesis 2) or a small (hypothesis 3) colour signal (see Fig. 1b; e.g. Partan & Marler 2005).

GENERAL METHODS

Parasemia plantaginis larvae and adults are warningly coloured and unpalatable for several different types of

vertebrate and invertebrate predators (C. Lindstedt, unpublished data); that is, they are aposematic. For our experiments we selected large- and small-patched last-instar larval phenotypes from the laboratory stock. A large orange patch covers approximately 80% of the body of an individual, while a small orange patch covers 30% of an individual's body. Larvae have five to seven instars and the patch is expressed from the third instar onwards (Ojala et al. 2007). The last-instar larvae used in the experiments were on an average 2–2.3 cm long and weighed 180–250 mg. All larvae were reared on a similar diet (lettuce for the first 10 days after hatching and, after that, only *Taraxacum* sp.) to ensure similar levels of defence chemicals.

Twenty-two mixed-sex domestic chicks (our naïve predators) were obtained from a commercial hatchery (Mänttä, Finland) in August 2006 and transported to the Konnevesi Research Station on the day they hatched. Eleven chicks were housed together in two plywood cages (width 40 × depth 50 × height 50 cm). The bottom of each cage was made from metal netting and half of it was covered with paper, hay and sawdust that were changed daily. At the age of 7 days, the chicks were distributed between four cages, with five or six birds per cage. All of the birds were marked with nontoxic coloured marker pens, which did not appear to have any adverse effect on their behaviour. Water and chick's starter crumbs (Pikku-punahelttä; Suomen Rehu, Finland) were provided ad libitum. Chicks were kept at approximately 25°C using heat lamps and on a 16:8 h light:dark cycle with fluorescent tubes which do not emit UV light. After the experiment, the chicks were relocated to a small free-range farm.

We chose great tits as the wild and experienced predator species for the experiments (1, 2a and 2b; see below) because it is bold enough to attack aposematic prey, to the extent of causing mortality, which allows us to test the effectiveness of different defence components. Preliminary experiments excluded blue tits and pied flycatchers from this role as they caused zero mortality to *P. plantagin*is larvae (C. Lindstedt, unpublished data). Furthermore, great tits are common in Finland and well suited to the experiment because insect larvae compose the largest part of their diet during the breeding season (Royama 1970).

Wild great tits were trapped from feeding sites around Konnevesi Research Station (central Finland) and subsequently ringed for identification. We used a prebaited trap (a box 13 × 17 × 40 cm) containing peanuts to catch study birds. The trap had an entrance that could be closed with a trap door and was positioned so that we could see when a bird entered. The trap door was used only when capturing birds and the door was shut by pulling the string attached to the door. Each bird was removed from the trap immediately after capture. This method allowed us to selectively catch unringed great tits.

Each bird was kept individually in an illuminated plywood cage (65 × 65 × 80 cm) with a daily light period of 11.5 h and at 15°C. Each cage contained three perches. One side of the cage could be opened to transfer birds into or out of the cage. Each cage also had two hatches which were used when cleaning the cage, adding food or water or checking the condition of the birds without disturbing the

bird. Birds were unable to see out of these plywood cages and were therefore calm and retained their plumage better than birds kept in common metal bird cages. Sunflower seeds, tallow and fresh water were available ad libitum. The birds were released at their capture sites after the experiments. The maximum time in captivity for the birds was 2 weeks. All birds remained in good health throughout their captivity. The Central Finland Regional Environment Centre gave us permission to capture and keep the great tits (permission numbers: experiment 1: KSU-2006-L-249; 2a: KSU-2004-L-238/254; and 2b: KSU-2005-L-309/254) and all experiments (permission numbers: 1: 18/22.5.2006; 2a: 29/31.5.2004; and 2b: 39/30.5.2005) were run under licence from the Experimental Animal Committee of the University of Jyväskylä.

EXPERIMENT 1: DETECTION RISK

Methods

First we tested how the orange patch size (small or large) and background colour affected the detectability risk of *P. plantagin*is larvae when the predators were naïve chicks or experienced wild-caught great tits. Two study arenas (width 1 m, depth 1 m, height 30 cm) made of plywood were used in the predation experiments with chicks, one with a green and one with a brown plastic mat (Bristlex; Stesika, Lithuania) background. Either fresh green birch leaves (collected every day) or pine wood chips (Aurinko Kuorike; Loimaan Turve ja Humus Oy, Finland) and black soil (Biolan kukkamulta; Kekkila Oyj, Finland) were scattered over the entire green and brown backgrounds, respectively, covering at least 60% of it, to increase background complexity. The chicks (11/background) were randomly assigned to a test arena.

We had four training sessions before the experiment, in which birds were familiarized with the experimental arena and trained to search for food. Chicks were trained to eat dead mealworms to familiarize them with insect food. Dead mealworms were also used as a familiar and palatable control in the experiments. Before each training session and testing birds were deprived of food for 1 h. We performed training sessions on the second, third and fourth posthatching days. The number of chicks foraging on the background was decreased with each successive training session (11, five or six, two and one, respectively) so the chicks learned to forage alone on the background. Chicks usually consumed most of the mealworms they found during the training sessions. All but one of the chicks readily ate food from the arena from the first session. This bird, trained on the brown background, never accepted mealworms and was excluded from the experiments.

Five last-instar *P. plantagin*is larvae with a large patch and five larvae with a small patch (total 10 larvae) were placed in the arena for the experiment. The size of the large orange patch was seven segments (covering approximately 80% of the body) and the size of the small patch was four segments (covering approximately 30% of the body) (see [Electronic appendix](#) for the colour analysis of experimental preys). To increase motivation to forage, 12 mealworms were also placed in the arena. Mealworms

and moth larvae were placed alternately on the arena in the shape of a square with ca. 20 cm separating each from the next. Two mealworms were placed in the middle of the square. The experiment was then initiated by placing a chick in the middle of the 'prey square' near the two mealworms.

Due to the multidimensionality of the backgrounds, all prey items were placed in the grass, but none was covered with debris and thus all were equally visible. The order of differently signalling (small or large patch) *P. plantagin* larvae was randomized to ensure that birds had an equal likelihood of detecting both prey types. The order of the larvae was changed between the experiments. The experiment lasted 10 min, and we recorded the order in which the bird detected, attacked and/or killed the prey items. If the birds clearly noticed an item (e.g. the bird moved towards the item to make a closer examination) this was counted as 'apparent detection', if the bird pecked the prey this was counted as an attack and if the bird ate at least part of the prey the item was counted as killed. We tested 10 birds on the brown background and 11 birds on the green background.

The detection risk experiment with great tits was performed in two aviaries (2.4 m high with a floor area of 13.5 m²) between October and November 2006. The same backgrounds as in the chick experiments (1 m²) were used and placed in the middle of each aviary. The aviaries contained two perches, a filled water bowl and a video camera to record the behaviour of each bird during the experiment. Before the experiment, the great tits were trained to feed on the background in an aviary, making the setting familiar. During training, 12 large mealworms killed by freezing were scattered on the background. Training was stopped when a bird had found at least 10 mealworms. The learning period took ca. 2 h per bird. Birds were randomly distributed to either the green or the brown background treatments.

The birds were deprived for food 1 h before the experiment to increase their motivation to forage. In addition, two mealworms were offered to the birds at the beginning of the experiment in the middle of the background square, motivating them to further foraging. After the bird had eaten the mealworms, we switched off the lights in the aviary, entered the aviary and placed one *P. plantagin* larvae in the middle of the background square. The lights were switched off to decrease the stress of human disturbance to the birds. The lights were switched on again and the trial was started when the bird landed on the square. Each trial lasted 10 min or less if the bird ate the prey item offered. During the trial, we recorded the time the bird spent on the background square until it detected the prey, the latency to attack (the time between detection and attack) and the handling time of the prey. The behavioural patterns were recorded as defined above. If a bird did not attack the prey it was included in the data set as the maximum time of attack delay (10 min). To estimate the hunger level of the birds, we offered six mealworms after the experiment and counted the number eaten by each bird within 10 min. We tested 40 birds: 10 birds with small-patched larvae and 10 with large-patched larvae on both green and brown backgrounds.

Statistics

We used a sign test to analyse the order in which prey were detected (small or large patch) without regard to the background. The comparison of detection order and attack order on different backgrounds in the chick experiment and the differences in the attack and killing risks in the great tit experiment were analysed with Fisher's exact tests. A univariate ANOVA was used to test whether signal size and background affected (a) the total number of prey attacked in the chick experiment and (b) the time it took great tits to detect larvae and their latency to attack in experiment 1. The detection time or latency to attack(s) of the birds was used as a dependent variable in the model and background and patch size were fixed factors. Only detection time did not meet the assumptions of a parametric ANOVA (due to the heteroscedastic structure of the data) and therefore we used log transformed values of the detection time in the analysis.

Results

The naïve chicks found the larvae with the large orange patch first on both backgrounds (sign test: $N = 21$, $P = 0.001$; Fig. 2). The detection order did not differ significantly between the backgrounds (Fisher's exact test: $N_1 = 11$, $N_2 = 10$, $P = 0.09$). In addition, the background (Fisher's exact test: $N_1 = 11$, $N_2 = 10$, $P = 0.563$) or patch size (sign test: $N = 21$, $P = 0.227$) did not affect the attacking order. However, the total number of prey attacked was higher on the brown background than on the green background (ANOVA: $F_{1,38} = 4.163$, $P = 0.048$; Table 1). There was a marginally nonsignificant trend that the chicks attacked more larvae with large patches than larvae with small patches ($F_{1,38} = 3.339$, $P = 0.076$). There was no interaction between the background and the patch size in the number of larvae attacked ($F_{1,38} = 2.319$, $P = 0.136$). None of the *P. plantagin* larvae was killed during the experiment (Table 1).

Great tits found *P. plantagin* larvae more quickly on the green background than on the brown background

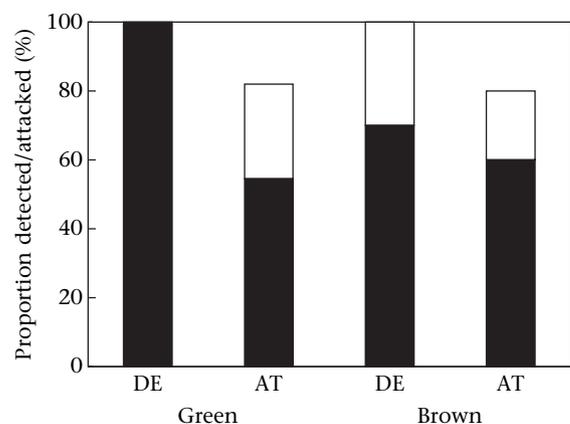


Figure 2. Proportion of larvae with a small orange patch (white) and with a large orange patch (black) first detected (DE) and attacked (AT) by naïve chicks on the green and brown backgrounds.

Table 1. Results of birds (domestic chicks and great tits) attacking and killing the *P. plantagin* larvae in relation to the patch size and the background that the prey were presented to birds in experiment 1

Predator	N	Patch size	Background	Total number of prey	Number of attacked	Attack risk	Number of killed	Mortality risk
Domestic chick	11	Large	Green	55	7	0.13	0	0
	11	Small	Green	55	4	0.07	0	0
	10	Large	Brown	55	17	0.31	0	0
	10	Small	Brown	55	7	0.13	0	0
Great tit	10	Large	Green	10	10	1	5	0.5
	10	Small	Green	10	8	0.8	3	0.3
	10	Large	Brown	10	10	1	6	0.6
	10	Small	Brown	10	8	0.8	1	0.1

(ANOVA: $F_{1,36} = 26.270$, $P < 0.001$) and they discovered larvae with a large patch faster than those with a small patch ($F_{1,36} = 4.492$, $P = 0.041$). There were no interactions ($F_{1,36} = 0.000$, $P = 0.989$): the detection time was always lower for the large-patched than for the small-patched larvae, irrespective of the background colour (Fig. 3).

After the great tits had detected the prey, the latency to attack (the time between detection and attack) was similar for both large- and small-patched larvae ($F_{1,36} = 1.733$, $P = 0.196$) and background colours ($F_{1,36} = 0.004$, $P = 0.947$). No interaction between patch size and background colour was found ($F_{1,36} = 0.030$, $P = 0.863$). Although birds attacked 80–100% of the larvae, both the small- and the large-patched morphs had similar attack risks (Fisher's exact test: $N_1 = N_2 = 20$, $P = 0.106$) which were not affected by background colour (Fisher's exact test:

$N_1 = N_2 = 20$, $P = 1.000$). However, the mortality risk was higher for the larvae with a large patch than for the larvae with a small patch (Fisher's exact test: $N_1 = N_2 = 20$, $P = 0.048$; Table 1). The background did not affect the probability of being killed (Fisher's exact test: $N_1 = N_2 = 20$, $P = 1.00$).

Discussion

Our results suggest that a large orange patch decreased the detection time of the prey, indicating that large-patched morphs are more conspicuous to both predator species. However, the detection risk varied between the predator species. The detection risk for the prey by the naïve chicks was small because chicks attacked only 16% of larvae offered (Table 1) and they did not eat any of the larvae after an attack, whereas great tits attacked nearly all offered prey. After similar delays, the attack and killing risk by the great tits was slightly higher for the larvae with large patch, suggesting that increased conspicuousness was costly (Table 1). Surprisingly, larvae with a small patch were abandoned by great tits more often after few pecks, suggesting that they were more unattractive for them. The larvae with small patches had a larger amount of black on their body, thereby increasing the contrast between the orange and the black in their colour pattern. Thus, this increased contrast could have made the prey appear more unprofitable (Roper & Redston 1987; Gamberale-Stille 2001).

The detection risk also varied between the backgrounds. The background did not significantly affect the order in which chicks detected the larvae, but great tits detected both prey types faster on the green background than on the brown background, suggesting that contrast with the background increased the conspicuousness of both small- and large-patched larvae (see Fig. 2). Whereas chicks attacked larvae more often on the brown background than on the green background, great tits found larvae more readily on the green background. This suggests that increased conspicuousness on the green background decreased chicks' willingness to attack larvae but did not affect that of great tits.

Contrary to our expectations, naïve chicks were more wary of the *P. plantagin* larvae than great tits. It appears that chicks showed unlearned wariness against the *P. plantagin* larvae possibly because of its novelty as a prey

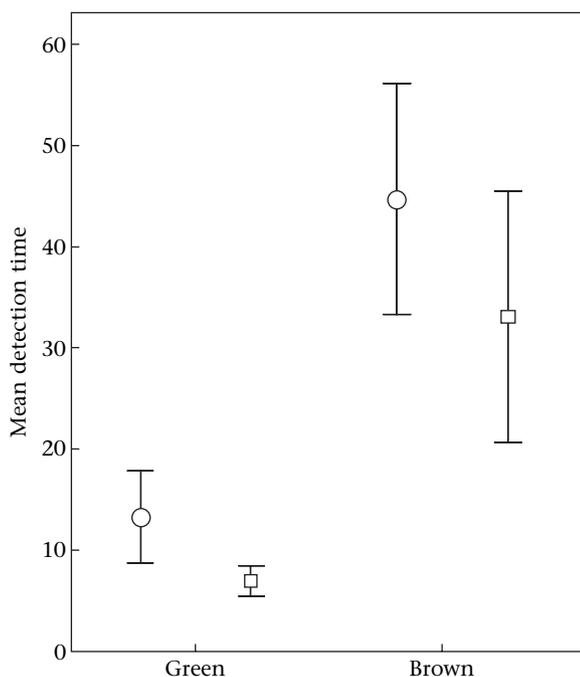


Figure 3. Time(s) taken for great tits to detect prey on different backgrounds (mean detection time) (○: larvae with a small orange patch; □: larvae with a large orange patch). Bars show \pm SE (experiment 1).

(Marples et al. 2005) or because naïve avian predators have biases against typically warningly coloured prey in their food preferences leading to initial avoidance of warningly coloured prey (Rowe & Guilford 1996; Rowe & Skelhorn 2005). Furthermore, the hairiness of the prey could have increased the chicks' wariness (Inbar & Lev-Yadun 2005; Speed & Ruxton 2005). The result that experienced great tits were attacking and killing *P. plantaginis* prey is not very surprising in the light of a previous study with several passerine species (Exnerová et al. 2003) which showed that experienced birds also readily attack aposematic prey and their reactions to the prey vary both within and between species. Thus, in addition to inexperienced and naïve predators, experienced unwary predators can also cause selection on warningly coloured defended prey (Exnerová et al. 2003, 2006; Endler & Mappes 2004).

EXPERIMENTS 2A AND 2B: EFFECTS OF WARNING COLOUR AND HAIRINESS ON AVOIDANCE LEARNING

Methods

In the second experiment, we tested whether the presence of an orange patch, the hairiness of the larvae or both together were most effective in the defence against predators. Birds were first familiarized to forage sunflower seeds from a white dish in the experimental cage (plywood, 50 × 50 × 70 cm) for 2 h. The cages were lit with energy-saving bulbs (Osram Dulux el longlife 7 W) which emitted a minimum of UV light. Thus, any possible differences in the UV reflectance of the prey colour patterns were not visible to the birds. The behaviour of the birds was observed through a small mesh-covered window in one side of the box and experiments took place in a dark room so that the birds were less aware of the presence of the observer. The cages contained a perch and a water bowl. Prey items were offered through a hatch behind a visual barrier during both training and experiments. The visual barrier enabled us to measure the exact time of prey detection because the bird had to come from behind the barrier or fly to the top of it to observe the prey.

We used dead last-instar *P. plantaginis* larvae in the experiments. For animal welfare reasons, we killed larvae by freezing before removing their hair. Plucking the hair after freezing is also easier and does not damage the skin of the larvae. Since the orange signal of the larvae consists of orange hairs, hair removal also removed the orange patch. Therefore, we had to paint a patch on bald larvae and cover the patch of hairy patchless larvae with a mixture of nontoxic, water-soluble orange (Folk Art, glazed carrots; Plaid Enterprises, Inc., Norcross, GA, U.S.A.) and black (deep black; Pebeo Deco, BP 106-13881 Gemenos Cedex) acrylic colours (see [Electronic appendix](#) for colour analysis of the prey items).

We ran a two by two factorial design (hair/no hair, signal/no signal) experiment with four treatments: (1) *P. plantaginis* larvae with hair but no orange patch (patch was covered with black paint), (2) larvae with

hair and an orange patch (size five to seven segments) (control larvae), (3) hairless and patchless larvae (completely black larvae) and (4) bald larvae with an orange patch (the patch was created with orange paint). In manipulations 2 and 4, we added the same amount of black paint on the ventral side of the larvae to control for any possible taste effects of the paint. Birds ($N = 42$) were randomly assigned to one of the treatments.

Prey was offered to the predators on a white dish, making all *P. plantaginis* larvae equally conspicuous irrespective of their hairiness or patch sizes ([Tables A1 and A2 in Electronic appendix](#)). Before the experiment birds' motivation to feed was tested by offering them a mealworm. After the bird ate the mealworm the test was started. In the experiment birds were offered one *P. plantaginis* larvae per trial in three consecutive trials. We measured the bird's latency to attack as the time from observation to the time it made contact with the prey using its beak (attack) in each trial. Latency to attack has been used previously as a variable testing the effectiveness of avoidance learning (Shettleworth 1972; Terrick et al. 1995; Marples & Roper 1997; Roper & Marples 1997; Gamberale-Stille 2000, 2001). After attacks larvae were recorded as killed if the bird ate at least part of the larvae. If the bird did not take the prey after it observed it, the trial was terminated after 5 min. The birds that did not attack were included in the data set with maximum attack latency (5 min). Since hunger level can affect a predator's readiness to attack defended prey (Sherratt et al. 2004; Barnett et al. 2007), we also measured the hunger level of birds quantitatively by giving them 5 min time to eat as many larvae as they wanted from 12 mealworms on a petri dish after the experiment. We weighed the mealworms before and after offering them to the birds and used the difference as a covariate to test whether hunger level affected the attack latency. The experiment was conducted between November and December 2004.

Experiment 2b was conducted as was experiment 2a, except that we manipulated the size of the orange patch along with the hairiness of the larvae. We had four treatments: (1) larvae with hair and a small orange patch (three or four segments), (2) larvae with hair and a large orange patch (six or seven segments), (3) bald larvae with a small orange patch and (4) bald larvae with a large orange patch. The sizes of the patches in treatments 3 and 4, added with orange paint, matched the sizes of those in treatments 1 and 2, respectively. As in experiment 2a, the same amount of paint as used for treatments 3 and 4 was painted on the ventral side of the larvae of treatments 1 and 2. Birds ($N = 64$) were randomly assigned to one of the treatments. The experiment was conducted between October and December 2005.

Statistics

To test for differences in avoidance learning in experiments 2a and 2b, we analysed the change in attack latencies (s) between the three consecutive trials of each bird with repeated-measures ANOVA, which takes in account the dependent structure of the data. We used

the 'change in attack latency' (described as avoidance learning rate in Results and Discussion) between the trials as a dependent variable. The between-subject factors were the presence of an orange patch (experiment 2a), the size of the patch (experiment 2b) and the hairiness. The hunger level of the great tits was used as a covariate in analyses, but because it did not significantly affect the avoidance learning rate (main effect in experiment 2a: $F_{1,51} = 3.894$, $P = 0.056$ and in experiment 2b: $F_{1,51} = 0.128$, $P = 0.722$), it was omitted from the final analyses. Due to the heteroscedastic structure of the learning data, we used log transformed values of hesitation delay in experiment 2a. In experiment 2b only ranking removed the heteroscedasticity problem from the data (which was due to the treatment group 3), but it violated the biological structure of the data remarkably by increasing the means and variances of attack delays in the first trial in treatments 1, 2 and 4 (Fig. 4c, d). Therefore we chose to test the learning rates in two different ways to elucidate the effects of hairiness and signal on the learning rates. First, we ran repeated-measure ANOVA analyses on non-transformed data with two factors, hairiness and signal size. Second, we ran analyses for each treatment group

separately to test whether the hesitation times changed over the course of experiment within each treatment. The latter test was conducted because the investigation of the data (see Fig. 4c, d) revealed that the avoidance learning rate appeared clear in all other treatment groups but not in treatment group 3 (bald larvae with small patch). A Mann–Whitney U test was used to analyse the amount of *P. plantaginis* larvae killed (i.e. eaten) during the experiment. The data on the number of larvae killed were pooled from the first, second and third trials. All statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, U.S.A.) and all P values are two tailed.

Results

Experiment 2a

In general, the avoidance learning rate increased significantly during the experiment, irrespective of the presence of orange patch or hairiness (learning rate: $F_{2,37} = 3.486$, $P = 0.041$). Great tits learned to avoid larvae with an orange patch more quickly than those without one (learning rate* presence of patch: $F_{2,37} = 3.875$, $P = 0.030$). However, the hairiness of the larvae did not affect the learning rate

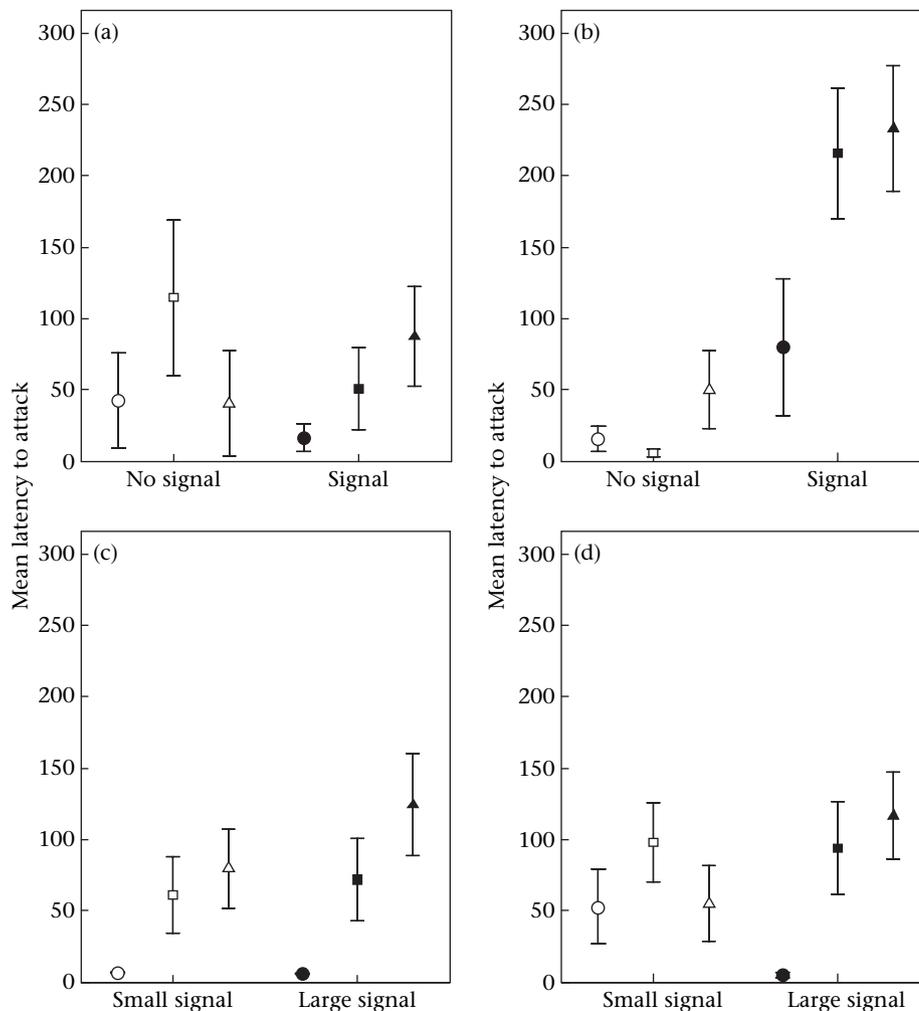


Figure 4. Attack latencies of great tits to (a) hairy prey and (b) nonhairy prey with or without an orange patch and (c) hairy prey and (d) nonhairy prey with small or large orange patch (○, ●: first trial; □, ■: second trial; △, ▲: third trial) (experiments 2a and 2b). Bars show \pm SE.

(learning rate * hairiness: $F_{2,37} = 0.839$, $P = 0.440$; Fig. 4a, b). There was a significant interaction between patch presence and hairiness on learning rate in the main effects (presence of patch * hairiness: $F_{1,38} = 10.230$, $P = 0.003$; Fig. 5a). No significant three-way interaction was found (repeated-measure ANOVA: $F_{2,37} = 2.759$, $P = 0.076$).

After an attack, birds killed significantly more bald patchless larvae than bald larvae with a patch (Mann–Whitney U test: $U = -2.567$, $N_1 = 15$, $N_2 = 8$, $P = 0.010$).

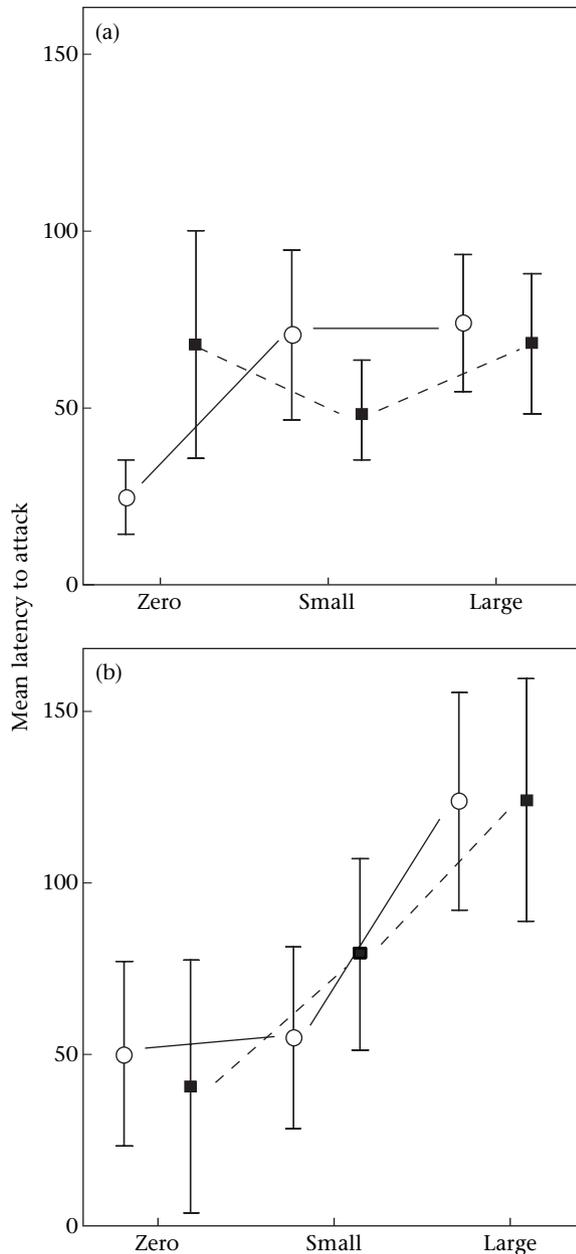


Figure 5. Functional response of great tit predators towards the hairy and nonhairy prey with different signalling levels combined from two data sets (experiments 2a and 2b). Error bars show (a) the mean latency to attack (\pm SE) pooled from the three trials showing the overall avoidance of prey and (b) the mean latency to attack (\pm SE) in the third trial showing the outcome of avoidance learning after two encounters of prey (○: hairless prey; ■: hairy prey).

The presence of a patch did not affect the rate at which hairy larvae were killed ($U = -1.342$, $N_1 = 8$, $N_2 = 11$, $P = 0.180$; Table 2). Birds also killed significantly more hairy, patched larvae than bald, patched larvae ($U = -2.711$, $N_1 = 8$, $N_2 = 11$, $P = 0.007$). Hairiness did not affect the total amount of larvae without a patch that were killed ($U = -0.899$, $N_1 = 8$, $N_2 = 15$, $P = 0.369$). Even though the birds did attack and kill the *P. plantaginis* larvae, it is important to note that the mean attack delay (68.2 s) was much higher than that for mealworms for which the mean attack delay was only 15.8 s.

Experiment 2b

When we manipulated the size of the orange patch and the hairiness of the larvae, there were no significant three-way interactions between hairiness, patch size and avoidance learning rate ($F_{2,59} = 0.745$, $P = 0.369$). However, the great tits learned to avoid prey with a large patch more quickly than prey with a small patch (patch size * learning rate: $F_{2,59} = 3.741$, $P = 0.030$). The hairiness of the larvae did not interact with the learning rate ($F_{2,59} = 1.215$, $P = 0.304$). In addition, there was no main effect of patch size ($F_{1,60} = 0.645$, $P = 0.425$) or hairiness ($F_{1,60} = 0.499$, $P = 0.483$) on the learning rate. Also, there was no significant interaction between hairiness and patch size in the main effects ($F_{1,60} = 0.030$, $P = 0.863$). Irrespective of the patch size or hairiness of the larvae, the avoidance learning rate increased significantly during the experiment (learning rate: $F_{2,59} = 17.226$, $P < 0.001$; Fig. 4c, d). However, when we analysed the avoidance learning rates in each treatment group separately, we found that in all other treatments the avoidance learning rate increased significantly (all $P < 0.023$) except in group 3 (bald larvae with small signal: $F_{2,14} = 2.223$, $P = 0.145$), suggesting that hairiness is beneficial especially for the larvae with small patch size.

The birds killed larvae with small and large patches similarly among the hairy (Mann–Whitney U test: $U = -1.051$, $N_1 = N_2 = 16$, $P = 0.293$) and bald ($U = -0.901$, $N_1 = N_2 = 16$, $P = 0.367$) larvae. In addition, hairiness did not affect the number of large-patched larvae killed ($U = -1.110$, $N_1 = N_2 = 16$, $P = 0.267$). However, the birds killed almost significantly more small-patched hairy larvae than small-patched bald larvae ($U = -1.839$, $N_1 = N_2 = 16$, $P = 0.066$; Table 2). Again, *P. plantaginis* larvae were clearly unpalatable to great tits compared to mealworms because birds were more hesitant to attack *P. plantaginis* larvae (mean attack delay 63.7 s) than mealworms (mean attack delay 12.7 s).

Discussion

We found that the overall contribution of hairiness to the defence capacity of *P. plantaginis* larvae against great tits was low compared to the black-orange warning coloration. In experiment 2a hairiness was beneficial only for the completely black larvae, increasing the overall latency to attack compared to that for the black and bald larvae shown in interaction in main effects, but did not significantly contribute to the survival of the prey with an orange patch (Fig. 5a). Separate analyses for the learning rates in

Table 2. Attack and mortality risks imposed by great tits on *P. plantagin* larvae in respect to their hairiness (hairs versus no hairs) and the size of the orange patch (no patch versus patch in experiment 2a and small patch versus large patch in experiment 2b) over three trials

Experiment	Predator	N	Treatment	Attack risk			Mortality risk		
				1st Trial	2nd Trial	3rd Trial	1st Trial	2nd Trial	3rd Trial
2a	Great tit	15	No patch+no hairs	1	1	0.87	0.73	0.91	0.73
		8	Patch+no hairs	0.5	0.5	0.25	0.25	0.25	0.25
		8	No patch+hairs	1	0.63	0.88	0.63	0.5	0.5
		11	Patch+hairs	1	0.9	0.82	0.82	0.82	0.73
2b	Great tit	16	Small patch+no hairs	0.94	0.88	0.88	0.5	0.44	0.44
		16	Large patch+no hairs	1	0.75	0.75	0.38	0.31	0.38
		16	Small patch+hairs	1	0.88	0.88	0.75	0.69	0.63
		16	Large patch+hairs	1	0.88	0.69	0.5	0.63	0.5

experiment 2b revealed the same pattern, suggesting that hairiness is beneficial for the inconspicuous larvae but not for the conspicuous larvae. In contrast to hairiness, the orange patch against a black body had a high signal value for predators because the avoidance learning rate was higher when larvae had an orange patch than when larvae were without one (see Fig. 4a, b). This is in accordance with previous studies which have shown that warning coloration is the most important trait in a multiple defence strategy (Sillén-Tullberg 1985; Marples et al. 1994). The size of the orange signal also mattered. A large patch enhanced the avoidance learning rate of avian predators, as shown by a longer latency to attack (Fig. 4a, b). After the predators had encountered two prey individuals, the survival of a larva with a large orange signal was two times higher than that of a larva with an inconspicuous signal (i.e. all black; see Fig. 5b). Furthermore, even though the attack rates were considerably high and did not differ between the larval morphs, the *P. plantagin* larvae were clearly more aversive prey than palatable mealworms.

GENERAL DISCUSSION

All predators imposed a higher detection risk on larvae with a large warning signal on both green and brown backgrounds. Large-patched larvae were found more quickly and attacked more eagerly than small-patched larvae. However, this high detection risk of a large patch trades-off with a learning benefit as shown in experiment 2b because a large orange patch enhances the avoidance learning rate of predators. It could be that conspicuousness can be sustained only by well-defended prey (Sherratt 2002). The brazen advertisement of a warningly coloured animal can be very risky if predators vary in their susceptibility to its defences (Exnerová et al. 2003). Thus, intermediate or weak warning signals may be selected for instead of overtly conspicuous warning signals because of their too high detection risk (Endler & Mappes 2004). Although larger orange patches enhanced the avoidance learning rate of great tits, causing selection for increasing conspicuousness (see Gamberale-Stille & Tullberg 1999; Lindström et al. 1999; ; Riipi et al. 2001), the cost of educating predators to avoid a large orange signal appears to be very high due to the high detection risk. Therefore, the overall benefit of a large orange signal appears to be

small and could result in only a weak selection pressure towards larger signal sizes. This trades-off between detection risk and learning benefit can partly explain the variation seen in the colour patterns of *P. plantagin* (but see Ojala et al. 2007) and possibly other aposematic prey species.

When comparing the signal value of hairiness and coloration in experiments 2a and 2b, we were surprised to find that, contrary to coloration, hairiness did not increase the learning rate of the predators. In contrast to our hypotheses H1 and H3 (Fig. 1) and earlier studies (Marples et al. 1994; Rowe & Guilford 1999b; Lindström et al. 2001a), all of the defence components do not necessarily additively increase the survival of the prey. Instead, our results (see Fig. 5) support hypothesis H2 (Fig. 1b) that hairiness benefits only prey with inconspicuous coloration (see also Vallin et al. 2005). One explanation for this unexpected finding is that the bald larvae with the painted orange patch may have appeared more unprofitable to the birds because the patch was more clearly pronounced and was brighter than that of the hairy larvae. Moreover, because the great tits were experienced wild predators, they probably knew how to handle hairy larvae. When handling the prey, most birds (67%) plucked the hairs out before eating the edible parts of the prey, suggesting that handling hairy prey items was a familiar procedure.

There are three potential explanations for the observed benefit of hairiness to the inconspicuous larvae: (1) the hair is a visual cue for the bird predator to the defence capability of the prey, (2) the hair functions as a physical deterrent that increases the secondary defence of the hairy prey compared to bald prey and (3) the hair performs both these functions. In our study, we cannot explicitly exclude any of these explanations. However, if the hair acts solely as a secondary defence mechanism, the black and hairy larvae should have been more repellent and less consumed than the black and bald larvae. The mean latency to attack on black hairy larvae was higher than that on the bald morphs (Fig. 5a), but predators ate both prey types similarly. Moreover, in experiment 2b birds killed hairy small-patched larvae almost significantly more than bald inconspicuous morphs, but at the same time hairiness enhanced the avoidance learning rate of hairy morphs compared to bald morphs. This indicates that hairs have at least a low signal value for great tits. By increasing the defence capacity of inconspicuously coloured prey, hairiness also allows

variation in warning colour pattern. The variable coloration does not have great consequences for an individual's fitness when combined with physical defences.

Instead of additive effects, components of multicomponent warning displays can also work independently with each part having its own target audience (Pearson 1989; Endler 1991; Partan & Marler 2005). Thus, hairiness may be a more effective defence mechanism against more wary bird predators (e.g. naïve birds such as chicks) as well as insect predators (Dyer 1995) whose predation behaviour and sensory capabilities differ remarkably from these birds. There is also a lot of variation in the form of the physical defences, which could influence their effectiveness against predators. Thus, whereas sharp spines are known to increase survival against predators (Barnhisel 1991; Mikolajewski & Rolff 2004), hairiness is not necessarily as effective. Hairs can have other important functions such as thermoregulation and protection against physical injuries or parasites, which makes hairiness a beneficial trait to maintain. Alternatively, hairiness can increase the survival of the prey through increased handling costs (see also Rowell-Rahier et al. 1995): the amount of energy obtained per unit time can be lower compared to prey with a lower handling time. According to the optimal diet model, when having the possibility to choose, predators should prefer prey with lower handling costs (Krebs & Davies 1993).

In conclusion, our results show that the benefits of multicomponent defences are not necessarily additive: if the value of the visual signal is high, there is only a small additional effect from physical defence mechanisms. More study on the interactions between warning coloration and physical defences is needed to further elucidate the functioning of multicomponent warning displays. Our study further underlines the need to study different predator species to generate a more comprehensive picture of the selection pressures acting on populations of aposematic animals because predators' responses to aposematic prey can vary remarkably and cause differential selection pressures on different defence components.

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Supplementary Material

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.anbehav.2007.10.024](https://doi.org/10.1016/j.anbehav.2007.10.024)

References

- Alatalo, R. V. & Mappes, J. 1996. Tracking the evolution of warning signals. *Nature*, **382**, 708–710.
- Barnett, C. A., Bateson, M. & Rowe, C. 2007. State-dependent decision making: educated predators strategically trade-off the costs and benefits of consuming aposematic prey. *Behavioral Ecology*, **18**, 645–651.
- Barnhisel, D. R. 1991. Zooplankton spine induces aversion in small fish predators. *Oecologia*, **88**, 444–450.
- Calvert, W. H. 1979. Mortality of the monarch butterfly (*Danaus plexippus* L.): avian predation at five overwintering sites in Mexico. *Science*, **204**, 847–851.
- Dyer, L. A. 1995. Tasty generalists and nasty specialists? Antipredator mechanisms in tropical Lepidopteran larvae. *Ecology*, **76**, 1483–1496.
- Endler, J. A. 1991. Interactions between predators and prey. In: *Behavioural Ecology* (Ed. by J. R. Krebs & N. B. Davies), pp. 169–196. Oxford: Blackwell Scientific.
- Endler, J. A. & Mappes, J. 2004. Predator mixes and the conspicuousness of aposematic signals. *American Naturalist*, **163**, 532–547.
- Exnerová, A., Landová, E., Štys, P., Fuchs, R., Prokopová, M. & Cehláriková, P. 2003. Reactions of passerine birds to aposematic and non-aposematic firebugs (*Pyrrhocoris apterus*; Heteroptera). *Biological Journal of the Linnean Society*, **78**, 517–525.
- Exnerová, A., Svadová, K., Štys, P., Barcalová, S., Landová, E., Prokopová, M., Fuchs, R. & Socha, R. 2006. Importance of colour in the reaction of passerine predators to aposematic prey: experiments with mutants of *Pyrrhocoris apterus* (Heteroptera). *Biological Journal of the Linnean Society*, **88**, 143–153.
- Fink, L. S. & Brower, L. P. 1981. Birds can overcome the cardenolide defence of monarch butterflies in Mexico. *Nature*, **291**, 67–70.
- Forsman, A. & Merilaita, S. 1999. Fearful symmetry: pattern size and asymmetry affects aposematic signal efficacy. *Evolutionary Ecology*, **13**, 131–140.
- Gamberale-Stille, G. 2000. Decision time and prey gregariousness influence attack probability in naïve and experienced predators. *Animal Behaviour*, **60**, 95–99.
- Gamberale-Stille, G. 2001. Benefit by contrast: an experiment with live aposematic prey. *Behavioral Ecology*, **12**, 768–772.
- Gamberale, G. & Tullberg, B. S. 1996. Evidence for a more effective signal in aggregated aposematic prey. *Animal Behaviour*, **52**, 597–601.
- Gamberale-Stille, G. & Tullberg, B. S. 1999. Experienced chicks show biased avoidance of stronger signals: an experiment with natural colour variation in live aposematic prey. *Evolutionary Ecology*, **13**, 579–589.
- Hauglund, K., Hagen, S. P. & Lampe, H. M. 2006. Responses of domestic chicks (*Gallus gallus domesticus*) to multimodal aposematic signals. *Behavioral Ecology*, **17**, 392–398.
- Ihalainen, E., Lindström, L. & Mappes, J. 2007. Investigating Müllerian mimicry: predator learning and variation in prey defences. *Journal of Evolutionary Biology*, **20**, 780–791.
- Inbar, M. & Lev-Yadun, S. 2005. Conspicuous and aposematic spines in the animal kingdom. *Naturwissenschaften*, **92**, 170–172.
- Kauppinen, J. & Mappes, J. 2003. Why are wasps so intimidating: field experiments on hunting dragonflies (odonata: *Aeshna grandis*). *Animal Behaviour*, **66**, 505–511.
- Krebs, J. R. & Davies, N. B. 1993. Economic decisions and the individual. In: *An Introduction to Behavioural Ecology* (Ed. by J. R. Krebs & N. B. Davies), pp. 48–76. Oxford: Blackwell Scientific.
- Lindström, L., Alatalo, R. V., Mappes, J., Ripi, M. & Vertainen, L. 1999. Can aposematic signals evolve by gradual change? *Nature*, **397**, 249–251.
- Lindström, L., Rowe, C. & Guilford, T. 2001a. Pyrazine odour makes visually conspicuous prey aversive. *Proceedings of the Royal Society of London, Series B*, **268**, 159–162.

- Lindström, L., Alatalo, R., Lyytinen, A. & Mappes, J. 2001b. Predator experience on cryptic prey affects the survival of conspicuous aposematic prey. *Proceedings of the Royal Society of London, Series B*, **268**, 357–361.
- Marples, N. M. & Roper, T. J. 1997. Response of domestic chicks to methyl anthranilate odour. *Animal Behaviour*, **53**, 1263–1270.
- Marples, N. M., Van Veelen, W. & Brakefield, P. M. 1994. The relative importance of colour, taste and smell in the protection of an aposematic insect *Coccinella septempunctata*. *Animal Behaviour*, **48**, 967–974.
- Marples, N., Kelly, D. J. & Thomas, R. J. 2005. Perspective: the evolution of warning coloration is not paradoxical. *Evolution*, **59**, 933–940.
- Mikolajewski, D. J. & Rolff, J. 2004. Benefits of morphological defence demonstrated by direct manipulation in larval dragonflies. *Evolutionary Ecology Research*, **6**, 619–626.
- Ojala, K., Lindström, L. & Mappes, J. 2007. Life-history constraints and warning signal expression in an Archtiid moth. *Functional Ecology*, **21**, 1162–1167.
- Partan, S. R. & Marler, P. 2005. Issues in the classification of multimodal communication signals. *American Naturalist*, **166**, 231–245.
- Pearson, D. L. 1989. What is the adaptive significance of multicomponent defensive repertoires? *Oikos*, **54**, 251–253.
- Riipi, M., Alatalo, R. V., Lindström, L. & Mappes, J. 2001. Multiple benefits of gregariousness cover detectability costs in aposematic aggregations. *Nature*, **413**, 512–514.
- Roper, T. J. & Marples, N. M. 1997. Odour and colour as cues for taste-avoidance learning in domestic chicks. *Animal Behaviour*, **53**, 1241–1250.
- Roper, T. J. & Redston, S. 1987. Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance learning. *Animal Behaviour*, **39**, 466–473.
- Rowe, C. 1999. Receiver psychology and the evolution of multicomponent signals. *Animal Behaviour*, **58**, 921–931.
- Rowe, C. 2002. Sound improves visual discrimination learning in avian predators. *Proceedings of the Royal Society of London, Series B*, **269**, 1353–1357.
- Rowe, C. & Guilford, T. 1996. Hidden colour aversions in domestic chicks triggered by pyrazine odours of insect warning displays. *Nature*, **383**, 520–522.
- Rowe, C. & Guilford, T. 1999a. The evolution of multimodal warning displays. *Evolutionary Ecology*, **13**, 655–671.
- Rowe, C. & Guilford, T. 1999b. Novelty effects in a multimodal warning signal. *Animal Behaviour*, **52**, 341–346.
- Rowe, C. & Skelhorn, J. 2005. Colour biases are a question of taste. *Animal Behaviour*, **69**, 587–594.
- Rowell-Rahier, M., Pasteels, J. M., Alonso-Mejia, A. & Brower, L. P. 1995. Relative unpalatability of leaf beetles with either biosynthesized or sequestered chemical defence. *Animal Behaviour*, **49**, 709–714.
- Royama, T. 1970. Factors governing the hunting behaviour and selection of food by the great tit (*Parus major* L.). *Journal of Animal Ecology*, **39**, 619–668.
- Sherratt, T. N. 2002. The coevolution of warning signals. *Proceedings of the Royal Society of London, Series B*, **269**, 741–746.
- Sherratt, T. N., Speed, M. P. & Ruxton, G. D. 2004. Natural selection on unpalatable species imposed by state-dependent foraging behaviour. *Journal of Theoretical Biology*, **228**, 217–226.
- Shettleworth, S. J. 1972. The role of novelty in learned avoidance of unpalatable 'prey' by domestic chicks (*Gallus gallus*). *Animal Behaviour*, **20**, 29–35.
- Sillén-Tullberg, B. 1985. Higher survival of an aposematic than of a cryptic form of a distasteful bug. *Oecologia*, **67**, 411–415.
- Speed, M. P. & Ruxton, G. D. 2005. Warning displays in spiny animals: one (more) evolutionary route to aposematism. *Evolution*, **59**, 2499–2508.
- Terrick, T. D., Mumme, R. L. & Burghardt, G. D. 1995. Aposematic coloration enhances chemosensory recognition of noxious prey in the garter snake *Thamnophis radix*. *Animal Behaviour*, **49**, 857–866.
- Tullberg, B. S., Gamberale-Stille, G. & Solbreck, C. 2000. Effects of food plant and group size on predator defence: differences between two co-occurring aposematic Lygaeinae bugs. *Ecological Entomology*, **25**, 220–225.
- Vallin, A., Jakobssen, S., Lind, J. & Wiklund, C. 2005. Prey survival by predator intimidation: an experimental study of peacock butterfly defence against blue tits. *Proceedings of the Royal Society of London, Series B*, **272**, 1203–1207.
- Yosef, R. & Whitman, D. W. 1992. Predator exaptations and defensive adaptations in evolutionary balance: no defence is perfect. *Evolutionary Ecology*, **6**, 527–536.