Prey survival by predator intimidation: an experimental study of peacock butterfly defence against blue tits

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Long-lived butterflies that hibernate as adults are expected to have well-developed antipredation devices as a result of their long exposure to natural enemies. The peacock butterfly, *Inachis io*, for instance, is a cryptic leaf mimic when resting, but shifts to active defence when disturbed, performing a repeated sequence of movements exposing major eyespots on the wings accompanied by a hissing noise. We studied the effect of visual and auditory defence by staging experiments in which wild-caught blue tits, *Parus caeruleus*, were presented with one of six kinds of experimentally manipulated living peacock butterflies as follows: butterflies with eyespots painted over and their controls (painted on another part of the wing), butterflies with their sound production aborted (small part of wings removed) and their controls, and butterflies with eyespots painted over and sound production aborted and their controls. The results showed that eyespots alone, or in combination with sound, constituted an effective defence; only 1 out of 34 butterflies with intact eyespots was killed, whereas 13 out of 20 butterflies without eyespots were killed. The killed peacocks were eaten, indicating that they are not distasteful. Hence, intimidation by bluffing can be an efficient means of defence for an edible prey.

Keywords: eyespots; predator–prey interactions; deimatic behaviour

1. INTRODUCTION

Few organisms are above the threat of predation and the resulting selection pressure has, during the course of evolution, shaped both the morphology and behaviour of prey. Examples of morphological antipredatory adaptations range from cryptic coloration, decreasing the risk of being detected in the first place, to sharp fangs and spines, structures used for defence or retaliation (Edmunds 1974). In the same way, choosing a matching background can be crucial in escaping detection (Cott 1940), and a display of impressive weapons or armour can help to convince a predator to abort an initiated attack (Edmunds 1974).

The peacock butterfly, *Inachis io*, hibernates as an adult butterfly in northern temperate areas, and spends the seven to eight months of hibernation in hollow trees, barns and attics (Henriksen & Kreutzer 1982). Butterflies generally suffer from substantial predation pressure, which has selected for effective antipredator defence, especially in long-lived species such as the peacock butterfly (Wiklund *et al.* 2003; Wiklund & Tullberg 2004). When resting, only the dark-coloured ventral side of the wings is shown which, in combination with the irregularly shaped wing margins, make the butterfly mimic the appearance of a leaf (Brakefield *et al.* 1992). When the peacock is disturbed it will, however, dramatically change its appearance by suddenly opening the wings, thereby exposing bright colours and four major eyespots on the dorsal side of the wings in a manner described in detail by Blest:

A repeated sequence of movements whereby the wings are depressed exposing the forewing eyespots, and the forewings themselves strongly protracted, revealing those of the hind wings. The latter movement is accompanied by a hissing noise, produced by a specialization of the structure of the basal thirds of the anal veins of the forewings and the costal veins of the hind wings, which are rubbed together (Swinton 1876).

(Blest 1957, p. 228.)

In the same article, Blest gives an account of the intimidating effect of the eyespots on yellowhammers, *Emberiza citrinella*, and great tits, *Parus major*. Blest also describes how the peacock butterfly tilts its body to maximize the eyespot exposure towards the source of disturbance. The sound produced by the peacock during the eyespot display can be separated into three components: the hissing sound produced when the wing-veins are rubbed together and two components, outside the hearing range of humans, consisting of low- and high-intensity clicks (Möhl & Miller 1976).

The pioneering study by Blest (1957) was aimed at understanding what optical components (e.g. shape and pattern) make an eyespot intimidating to a passerine. In particular, Blest focused on the number of escape responses that peacock butterflies elicited from small passerines rather than butterfly survival *per se*. Blest suggested that butterflies with intact eyespots elicited higher numbers of escape responses from yellowhammers (*E. citrinella*) compared with butterflies with removed eyespots. However, although Blest's results are suggestive, his conclusions are hampered by his experimental protocol

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Figure 1. The six different morphs produced by a black marker pen and scissors. The upper row contains experimental morphs and the lower row contains their control morphs. Upper row: (a) no eyespots (eyesspots painted over), (b) no sound (wings cut to remove sound production), (c) no eyespots or sound (eyesspots painted over and sound production removed), (d) eyespots (eyesspots intact, instead painted at the base of the wings), (e) sound (wings cut but sound production still intact), (f) eyespots and sound (eyesspots still intact, instead painted at the base of the wings and wings cut but sound production still intact).

which is characterized by low sample sizes and pseudo-replication (cf. Ruxton *et al.* 2004). Thus, the extent to which the eyespot display and sound production promote butterfly survival during extended interactions with a predator has not previously been critically studied and therefore warrants further examination.

Signals that have more than one simultaneously emitted component in more than one sensory modality of the receiver are considered to be multimodal signals and have been suggested to be beneficial to prey (e.g. Rowe & Guilford 1999, 2001). Hence, with reference to the peacock butterfly it is reasonable to assume that an attacking bird will take into account not only the displayed eyespots, or the stridulatory sound, but will be simultaneously affected by the combined effects of the two defensive traits.

In this study we investigate the relative importance of the peacock's eyespots and sound, as well as the two traits in unison, for predator defence and survival when attacked by an avian predator. To test the prediction that the combination of eyespots and sound are more effective than either trait alone, we presented living peacock butterflies, manipulated to have either their eyespots or sound production or both traits simultaneously neutralized, to wild-caught blue tits, *Parus caeruleus*. This was carried out in a low temperature environment, simulating conditions during, for example, cold summer or spring; this low temperature prevented the butterflies from escaping by simply flying away. We also tested whether butterflies were more unwilling to flick their wings open in the first interaction with a bird compared with subsequent interactions. This is a reasonable prediction if the butterfly incorporates into its decision-making the possibility that it is not discovered by the bird. After the first interaction, however, when the butterfly has been forced to reveal itself, the possibility of being undetected can be ruled out and the butterfly may start to defend itself when the bird is further away.

2. METHODS

Larvae of wild-caught females were raised on their host-plant, the stinging nettle, *Urtica dioica*. After eclosion they were transferred to flight cages (0.8 × 0.8 × 0.5 m) where they were allowed to feed on a 25% sucrose solution *ad libitum* for two weeks to fill their energy deposits. The butterflies were then transferred to a cold storage room, maintained at 6 °C, where they were housed on a piece of gauze netting stretched over small plastic cups (350 ml)—two butterflies in each cup. The butterflies remained undisturbed in this way until the time of the experiment.

We manipulated butterflies, using a black permanent marker pen or scissors or both in order to create three different forms testing the importance of eyespots, stridulation and the combination of both, respectively. Each treatment morph had its own control morph yielding a total of six different types of manipulated butterflies. Butterflies were randomly assigned to the different treatments. To test the significance of eyespots, butterflies in the first group (no eyespots) had their eyespots painted over with the marker pen (figure 1a). The corresponding control specimens (eyesspots; figure 1d) were also painted on the dorsal side of their wings but closer to the body, thus leaving the four eyespots intact. To prevent the butterflies from producing a stridulatory sound, we removed a small part of the forewings in one group of butterflies (no sound; figure 1b) and cut off an equally large part of the hind wings in the corresponding control group (sound; figure 1e). Finally, to investigate the combined effect of eyespots and sound, a group of butterflies (no eyespots or sound; figure 1c) had their eyespots covered as well as the sound-producing parts of the forewings removed. Consequently, the butterflies in this control group (eyesspots and sound; figure 1f) were painted and cut in a manner as to leave their eyespots and sound-producing structures intact.

The experiments were carried out at Tovetorp Zoological Research Station, located in the southeast of Sweden, between February and April 2002. Blue tits, caught at the research station, were chosen as the butterfly predator because they are opportunistic birds that raise their young on a pure insect diet,

and accordingly, possess substantial insect-catching skills. They were housed individually in cages (80 × 60 × 40 cm) in a room indoors with the same light regime as that of the prevailing season. In their cages the birds had access to water, sun flower seeds and suet *ad libitum*. They were also offered mealworms, *Tenebrio molitor*, as complementary food. A total of 54 birds were used in this study. Birds were captured with permission from the Swedish Bird Ringing Center (permission 619:M03). Housing of animals and the experimental procedures were reviewed and approved by the regional ethical committee (permit Linköpings djurförsökstiska nämnd 49-01).

All trials were conducted in a room measuring 2.3 × 2.4 × 1.9 m. The room was lit by three daylight fluorescent tubes and had one-way mirrors on two of the walls to allow us to observe the course of events inside without disturbing the animals. On the floor, in the middle of the room, a log of willow, *Salix caprea*, made up the stage for the experiment. A 1.8 m high wooden stick, equipped with 10 cm long perches placed at every 10 cm of the stick's length, was placed in the room as a combined look out and resting place for the bird. At all times, a bowl of fresh water was accessible to the bird. Mounted in the roof above the log was a digital video camera capturing an area of the log and floor corresponding to 9 × 7 dm. The recordings were used as a complement to the direct observations and allowed us to analyse the interactions between bird and butterfly on a more detailed level.

Before a trial began, two mealworms were placed in a small plastic feeding tray at the far end of the log. This was done to help the birds to associate the log with food and encourage them to conduct a thorough search for food in this area of the room. To ensure a standardized presentation, the butterfly was always placed on a small mark at the middle of the log facing the food tray.

Birds were released into the experimental room through a hatch in the door and a trial would begin when either the bird seized one of the mealworms or attacked the butterfly. A trial would last 30 min but was terminated earlier if a butterfly was captured and consumed. Survival of the butterfly, distance to the bird when the butterfly initiated wing-flicking, number of interactions, as well as time between interactions, were measured. An interaction was considered to start when a butterfly reacted in any visible way to a bird present on the log, and ended with either the butterfly being consumed or the bird flying away from the log.

After a completed trial, birds were ringed and released at the site of their capture. No bird was kept in captivity for more than one week and no individual bird or butterfly was used in more than one trial.

3. RESULTS

All butterflies remained motionless until the blue tit was either very close or in actual physical contact with the butterfly. The peacocks would then suddenly flick their wings open and engage in the antipredation behaviour described earlier. The bird's reaction was invariably to retreat from the wing-flicking butterfly by flying away from the log. The butterflies would now adopt one of three behaviours. Either they kept their wings open but motionless, thereby constantly exposing the eyespots and strong colours, or they continued flicking their wings. Additionally, during wing-flicking, 48 of the total 54

Table 1. Frequencies of surviving and consumed butterflies after 30 min. Letters refer to the corresponding images in figure 1.

(Differences in survival were tested using Fisher's exact test (two-tailed). Treatments were as follows: no eyespots (eyespot painted over); eyespots (instead painted at the bases of the wings); no sound (wings cut to remove sound production); sound (wings cut but sound production still intact); no eyespots or sound (eyespots painted over and sound production removed); eyespots and sound (eyespots intact, instead painted at the bases of the wings and wings cut but sound production still intact.).)

treatment	total	survived	died	<i>p</i>
no eyespots (<i>a</i>)	10	5	5	0.03
eyespots (<i>d</i>)	9	9	0	
no sound (<i>b</i>)	8	8	0	1.00
sound (<i>e</i>)	8	7	1	
no eyespots or sound (<i>c</i>)	10	2	8	<0.001
eyespots and sound (<i>f</i>)	9	9	0	

butterflies tilted their bodies and rotated on the log to match the movement of the bird, possibly to increase the intimidating effect of the display. The third behaviour was to close the wings, thereby resuming the same cryptic posture as before the interaction with the bird. During a specific trial a butterfly would alternate between behaviours. However, when a bird returned for another attack, the butterfly would always begin to flick its wings again, increasing the rate of wing-flicking as the bird approached, regardless of its earlier behaviour.

During the 30 min trials, all butterflies were found and attacked by the birds. Forty butterflies survived and 14 were seized and consumed by the birds. All seized butterflies were consumed without hesitation, suggesting that the blue tits found the butterflies edible rather than distasteful.

A generalized linear model (binomially distributed response with logit link function, eyespots and sound as factors, each with three levels) revealed that more butterflies with eyespots survived compared with butterflies without eyespots ($\chi^2_2 = 24.34$, $p < 0.001$). However, there was no difference in survival between butterflies with or without sound ($\chi^2_2 = 3.48$, $p = 0.18$) and no interaction between eyespots and sound could be detected ($\chi^2_1 < 0.001$, $p = 0.99$). When comparing the frequencies of survival between the three treatments and their control groups respectively, more butterflies survived in the eyespots treatment compared with the no eyespots treatment (Fisher's exact test: $p = 0.03$) and more butterflies also survived in the eyespots and sound treatment compared with the no eyespots or sound treatment (Fisher's exact test: $p < 0.001$; see table 1 for frequencies). However, we found no difference in survival between the sound and the no sound treatments (Fisher's exact test: $p = 1.00$).

To test whether our manipulations had impaired the butterflies' abilities to perform the wing-flicking behaviour we compared the number of wing-flicks performed, relative to the duration of the experiments, for the three treatment groups with their control groups, respectively. There was a trend towards different frequencies of wing-flicking in the no eyespots/eyespots treatments (Mann-Whitney *U*-test: $U = 21.0$, $P_{\text{exact}} = 0.05$; figure 2*a*).

Figure 2. (a) The frequency of wing-flicks performed by each of the six experimentally manipulated groups of peacocks when they were attacked by blue tits; numbers are median, first and third quartile. (b) The time spent by blue tits within 10 cm of the peacocks in relation to the duration of the experiment; numbers are median, first and third quartile.

If this trend is to be believed, butterflies with covered eyespots showed a more intense wing-flicking behaviour compared with butterflies with intact eyespots. The comparison of the treatments no eyespots or sound and eyespots and sound showed that the butterflies with covered eyespots (and disabled sound production) flicked their wings more frequently than the control treatment (Mann–Whitney U -test: $U=1.0$, $P_{\text{exact}} < 0.001$). Finally, there was no difference between the no sound and the sound treatments (Mann–Whitney U -test: $U=31.0$, $p=0.96$).

When the time duration during which birds remained within 10 cm from the butterfly was analysed, a possible explanation for the vigorous wing-flicking in the groups of butterflies with covered eyespots emerged. Birds spent more time close to the butterflies in the no eyespots treatment compared with the eyespots treatment (Mann–Whitney U -test: $U=8.5$, $P_{\text{exact}}=0.001$; figure 2b). In the no eyespots or sound and eyespots and sound comparison the pattern appears to hint in the same direction, although no statistical difference could be found (Mann–Whitney U -test: $U=25.0$, $P_{\text{exact}}=0.11$), with birds spending more time close to butterflies with covered eyespots. No difference was found between the no sound

Figure 3. Distance between bird and butterfly at first and second interaction when the butterfly initiated wing-flicking. Median, first and third quartile (box), minimum and maximum values (whiskers).

and sound treatments (Mann–Whitney U -test: $U=26.0$, $P_{\text{exact}}=0.52$).

A total of 45 butterflies survived the first interaction. The distance to the bird when these butterflies initiated wing-flicking was compared, regardless of butterfly treatment, between first and second interaction, and this distance was found to be greater at the second interaction (Wilcoxon matched pairs test: $n=45$, $Z=5.49$, $p < 0.001$; figure 3).

4. DISCUSSION

In two treatments, no eyespots versus eyespots, and no eyespots or sound versus eyespots and sound, butterflies with eyespots survived in higher numbers compared with butterflies with covered eyespots. In fact, when considering only whether manipulated butterflies had their eyespots intact or not, the results show that only one of 34 butterflies with intact eyespots died during the 30 min trials, whereas 13 of 20 butterflies with covered eyespots were killed. Consequently, eyespots are very effective in protecting the peacocks against passerine predation. This result is noteworthy considering that peacocks appear to be perfectly edible to blue tits. In the classical literature there are some cases described in which harmless animals allegedly gain advantage by exhibiting startling, deimatic behaviours (cf. Cott 1940; Edmunds 1974; Sargent 1990), but we feel that convincing scientific evidence for this is lacking (cf. Ruxton *et al.* 2004). Hence, we believe that our demonstration of how peacocks survive predator attacks represents the first strong evidence for the idea that a harmless prey can increase its fitness by survival through the adoption of intimidation by bluffing.

In particular, the intimidating effect of the eyespots appears to be implemented by the butterfly's behaviour. When a blue tit approached a peacock for a second or third inspection the birds advanced only for as long as the butterfly remained still; when the butterfly resumed its wing-flicking behaviour the bird was visibly disturbed and usually cancelled its approach.

We did not find any indication that the combined effect of sound and eyespots is more effective compared with eyespots only, as there was no difference in frequency of

survival comparing soundless (no sound) with sound-producing butterflies (sound). Because the eyespots *per se* were so effective in protecting the peacocks in our experiment, this precludes the possibility of demonstrating any potentially synergistic effect of the peacocks' stridulation. None the less, our experiments do suggest that the hissing sound is not of crucial importance to the peacock butterfly when defending itself against the blue tits in this experiment.

The different components of the peacock butterfly's antipredator defence make the total signal multimodal. This is generally believed to increase the effect of a signal transmitted to a predator (Rowe & Guilford 1999, 2001). Möhl & Miller (1976) studied how three vespertilionid bats reacted to the sound produced during wing-flicking by peacock butterflies. The bats reacted to the sound even though the butterflies were out of sight, showing that the sound *per se* may have an intimidating effect on bats. Whether there are any synergistic effects to be found in the combination of eyespot and sound (i.e. that they work together to boost the total effect of the signal) remains to be investigated.

The manipulations did not compromise the butterflies' ability to perform wing-flicking. This is important as both eyespot display and stridulatory sound production is achieved through this behaviour. On the contrary, butterflies from both treatments with covered eyespots (no eyespots and no eyespots and sound) appeared to perform more wing-flicks compared with their control groups, respectively. The reason for the frequent wing-flicking behaviour in these two groups (figure 2a) is probably that birds spent more time close to these butterflies (figure 2b), which triggered the butterflies to increase their rate of wing-flicking.

Butterflies allowed the birds to come closer before initiating wing-flicking at the first interaction compared with the second interaction. This suggests that the butterfly incorporates into its decision-making the possibility that it has not yet been discovered by the bird. Peacock butterflies are leaf mimics when resting (Brakefield *et al.* 1992) and appear to rely on their crypsis until they are certain that they have been discovered, when they shift to a more active defence strategy. The risk of remaining cryptic, should the camouflage fail, is that the predator will be closer when the butterfly starts flicking its wings and its safety margins have, accordingly, been reduced. A butterfly that is already discovered, on the other hand, can prevent the bird from coming within pouncing distance by initiating wing-flicking earlier. In agreement with this interpretation, Vallin *et al.* (unpublished data) demonstrated that the distance at which a butterfly starts to flick its wings when approached by a bird is associated with the butterfly's type of antipredator device. A species that completely relies on crypsis, such as the comma, *Polygonia c-album*, never shifts its behaviour to more active defence no matter how closely the predator approaches, whereas a butterfly with an intimidating wing pattern, such as the peacock, often shifts

behaviour to active defence when the predator is very close.

In conclusion, eyespots appear to be very effective in protecting the peacock butterfly from passerine predation. Remarkably, all but one butterfly with intact eyespots survived the extreme situation of being attacked repeatedly by a hungry blue tit in a closed room. We were unable to elucidate the potentially synergistic effect of the peacock's sound production because the intimidating effect of eyespots was so effective in protecting the butterflies from predation by blue tits. However, it is possible that there are other butterfly predators and situations, yet to be investigated, where the combined effect is needed in order to protect the butterfly. Moreover, the peacock butterfly appears to adjust its wing-flicking behaviour depending on its posture. The adaptive value of this seems obvious, but whether or not these differences confer any advantage to the butterfly in terms of increased survival remains to be investigated.

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