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Crypsis versus intimidation—anti-predation defence in three closely related butterflies

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Abstract Butterflies that hibernate exhibit particularly efficient defence against predation. A first line of defence is crypsis, and most hibernating butterflies are leaf mimics. When discovered, some species have a second line of defence; the peacock, *I. io*, when attacked by a predator flicks its wings open exposing large eyespots and performs an intimidating threat display. Here we test the hypothesis that butterflies relying solely on leaf mimicking and butterflies with an intimidating wing pattern, when attacked, exhibit different behavioural suites—because leaf mimicking is best implemented by immobility, whereas intimidating coloration is best implemented by intimidating behaviour. In laboratory experiments blue tits, *Parus caeruleus*, were allowed 40 min to attack single individuals of three species of butterfly: one relying solely on crypsis, the comma, *Polygonia c-album*; one relying on intimidating wing pattern in addition to crypsis, the peacock; and one intermediate species, the small tortoiseshell *Aglais urticae*. The results are in accordance with expectations and demonstrate that: (1) birds take longer to discover the leaf mimicking species, the comma, than the tortoiseshell and the peacock; (2) the comma remained motionless throughout experimental trials but small tortoiseshells and peacocks flicked their wings when attacked; (3) the most intimidating butterfly, the peacock, started flicking its wings at a greater distance from the attacking bird than the small tortoiseshell; and (4) the intimidating pattern and behaviour of peacocks was effective—when discovered, all peacocks survived interactions with blue tits, whereas only 22% of commas and 8% of small tortoiseshells survived.

Introduction

Predation exerts strong selective pressures on preys to evolve morphological and behavioural adaptations to decrease the risk of being detected, attacked, captured and consumed (Edmunds 1974). Examples of morphological anti-predatory adaptations range from cryptic coloration, decreasing the risk of being detected, to sharp fangs and spines, structures used for retaliation and defence (Edmunds 1974; Ruxton et al. 2004). Behavioural adaptations in prey include, for example, choosing a matching background to escape detection (Endler 1984) and displaying impressive weapons or armour to discourage an attacking predator (Edmunds 1974).

Butterflies generally suffer from a substantial predation pressure which has selected for effective anti-predation defence, especially in long-lived species such as those that live for several months and hibernate as adult butterflies (e.g., Wiklund et al. 2003; Wiklund and Tullberg 2004). For an edible butterfly the best option for prolonged survival is to remain undetected. This is usually achieved when the coloration and the outline of the insect blend so well with the natural background that the attention of the predator is never alerted. Another way to escape predator attention involves specific resemblance (sensu Cott 1940) when a predator mistakes an insect for an inedible object such as a leaf. Specific resemblance is regarded as mimicry by some researchers (but not by others—Endler 1981; Rothschild 1981; Pasteur 1982), but can be distinguished from general resemblance (Wiklund and Tullberg 2004). The latter aims to decrease detection risk, whereas the former aims to decrease recognition risk, giving a prey the appearance of an inedible object, thereby making specific resemblance coloration functionally similar to mimicry in the strict sense. A second line of defence involves animal defensive action as soon as the predator has discovered the butterfly and is just about to seize it.

Peacock butterflies (*Inachis io*), small tortoiseshells (*Aglais urtica*) and comma butterflies (*Polygonia c-album*) all hibernate as adults and exhibit leaf mimicry when resting (Brakefield et al. 1992). Recently, Wiklund and

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Tullberg (2004) demonstrated that overwintering commas were extraordinarily efficient in avoiding discovery/attacks by great tits, *Parus major*; in 30-min experimental trials less than one third of the birds ever discovered either of two commas placed on the trunk of an old willow log.

The behavioural action taken by a leaf-mimicking hibernating butterfly after being discovered is likely to be influenced by the coloration on the dorsal wing surface, because efficiency in thwarting predator attacks may be enhanced by intimidating patterns such as large eyespots (Cott 1940). For example, Blest (1957), described the special wing-flicking behaviour of the peacock butterfly when attacked by yellowhammers (*Emberiza citrinella*) as follows: “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 specialisation 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)”. Recent experiments by Vallin et al. (2005) have shown that this behaviour is very effective in scaring small passerine predators; in experiments peacocks with their wings intact invariably survived attacks by blue tits because the birds ceased to approach the butterflies after one or more attacks.

In this article we address a number of predator defence issues in the three closely related nymphalids—the comma butterfly, the small tortoiseshell and the peacock butterfly. Recent phylogenetic analysis places *Inachis* and *Aglais* as sister genera, and the *Polygonia/Nymphalis* clade as the sister clade to the *Aglais/Inachis* clade (Nylin et al. 2001; Wahlberg and Nylin 2003). Hence, the assessment of variation in predator defence behaviour should give valuable insights into the evolutionary aspects of predator defence in this clade of adult overwintering butterflies. Previous studies suggested that all three nymphalids are palatable to birds, and peacocks and small tortoiseshells were also shown to be edible to bats (Blest 1957; Møhl and Miller 1976; Wiklund and Tullberg 2004).

Our first objective was to test whether the three butterfly species differed with respect to how easily they were discovered and attacked, how they behaved when attacked, and whether they survived close encounters with birds. Our second objective was to test if wing flicking occurs in all three species when a bird came close, and if so whether the wing-flicking behaviour differed between species with respect to frequency and at what distance from the bird wing flicking started. This was done to test our prediction that the most leaf-like butterfly, the comma, would be the least prone, whereas the peacock with its large eyespots would be most prone to flick its wings at a distance, with the small tortoiseshell, having contrasting colours but no eyespots on its wings, assuming an intermediary position. A third objective was to test whether wing-flicking behaviour in the peacock was released at a longer distance during the second encounter with a bird. We did this to test our prediction that this distance should be minimized during first encounters when the decision-making on the part of the butterfly

should incorporate the possibility that it has not been discovered by the bird, whereas this possibility can be ruled out during the second encounter (when following within minutes of the first encounter). This should result in wing-flicking behaviour being released at a longer distance during second encounters.

Materials and methods

Larvae of wild-caught females of the three butterfly species were raised on their host-plant, the stinging nettle, *Urtica dioica*. After eclosion the adult butterflies were transferred to flight cages maintained at a 8:16 light/dark cycle, and were allowed to feed on a 25% sucrose solution for 2 weeks, thus enabling them to fill their energy deposits before hibernation. Adult overwintering butterflies were then transferred to a cold room, maintained at 6°C, in which they were housed two or three together sitting on a gauze netting covering the top of small 350-ml plastic cups. In this way, the butterflies remained in hibernation until they were brought out for the predation experiments.

The study was carried out at Tovetorp Zoological Research Station, situated approximately 90 km south of Stockholm, Sweden. Blue tits, *Parus caeruleus*, were used as predators in these experiments. Blue tits are opportunistic omnivorous birds that raise their young exclusively on insects, which implies substantial insect catching skills. The birds were caught, outside of the breeding season (between mid-November 2001 and the end of February 2002), in the vicinity of the research station using trap cages and mist-nets (permit Swedish Bird Ringing Center 619:M03). Housing and experimental setup was reviewed and approved by the regional ethical committee (permit Linköpings djurförsöksetiska nämnd 49-01). Before the trials the birds were housed individually indoors in cages (80×60×40 cm), with the surrounding temperature varying between 15 and 17°C. In these cages they had free access to water, sunflower seeds, suet and mealworms, *Tenebrio molitor*. Every cage was also equipped with perches for the birds to rest upon. Birds and butterflies were only used once and after an accomplished trial, birds were ringed and then released at the site of their capture. All birds maintained their condition during captivity and were healthy upon release. No bird was kept in captivity for more than a week.

All trials were performed inside a room measuring 2.3×2.4×1.9 m, lit by three daylight fluorescent tubes, with one-way glasses (0.8×0.5 m) on two of the walls, which allowed us to observe the trials without disturbing the animals. On the floor, in the middle of the room, a longitudinally cut log of *Salix caprea* made up the stage for the experiment. At one end of the log, a transparent plastic feeding tray was placed 40 cm from the butterfly. Two 1.8-m-high wooden sticks, equipped with 10-cm-long perches at every decimeter of the stick's length provided resting and observation facilities for the birds. A bowl of fresh water was always present in the experimental room. The temperature was maintained at 12.4±0.1°C (mean±SE)

to reduce the risk of butterflies taking flight during trials. Before each trial two mealworms were placed in the feeding tray to help the birds associate the log with food and thereby encourage them to thoroughly examine this area for more food items. A butterfly was placed on a small mark at the centre of the log, facing the feeding tray, thus assuring a standardized presentation. The bird was then allowed into the room through a small hatch in the door. To ensure that the birds were actively foraging, timing of the trial started as soon as a bird seized a mealworm or attacked the butterfly. A trial lasted up to 40 min but was terminated earlier if the butterfly was consumed. Butterflies that were consumed or flicked their wings open during a trial were assumed to have been observed by the bird. When the maximum time of a trial had been reached, the remaining butterflies were considered to have successfully gone unnoticed by the bird. All trials were, in addition to being directly observed, recorded using a digital video camera (Sony DCR-VX1000E) mounted in the ceiling above the log, thus capturing an area measuring 90×68 cm of the floor on film. The recordings made it possible to analyze the interactions on a detailed level, for example, it was not always possible by direct observation to decide if a bird had made physical contact or merely approached the butterfly during a specific interaction.

Behavioural observations

At the beginning of a trial, the bird, after being allowed entry, initially flew around in the experimental room for a few minutes, keeping to the roof and the perches on the upper parts of the two wooden sticks. The bird would then approach the feeding tray on the log, seize one of the two mealworms, and carry it to a perch where it was consumed. The second mealworm was retrieved and eaten in a similar manner shortly after the first. Upon emptying the feeding tray the bird started to examine the log. All butterflies remained motionless until the blue tit was either very close or in actual physical contact with the butterfly. The butterfly's reaction after being disturbed differed between species. The peacocks suddenly flicked their wings open and engaged in the anti-predator behaviour described earlier. The bird's reaction was always to retreat from the wing-flicking butterfly by flying away from the log. The peacocks would now adopt one of three behaviours. Either they kept their wings open but motionless, thereby constantly exposing the eyespots, or they continued flicking their wings, thus adding continuous movement and sound to the display. The third behaviour was to close their wings, thereby resuming the same leaf-mimicking 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 peacock would always begin to flick its wings again regardless of its earlier behaviour. The small tortoiseshells' wing-flicking behaviour was not as distinct as that of the peacock butterfly, although variation between individuals was extensive. The birds did not seem to react as strongly to a wing-flicking

small tortoiseshell compared with a peacock butterfly but nevertheless they did retreat a small distance when the butterfly executed the first wing flick. Commas never flicked their wings open when disturbed by a bird. On the contrary, a comma would always remain perfectly still during the entire trial, no matter what the bird did. In two of the trials the bird actually stepped on the butterfly and then walked away, without attacking the prey item within its reach. All seized butterflies were decapitated, de-winged and subsequently consumed without hesitation by the blue tits. This implies that blue tits found these three butterfly species edible rather than distasteful.

Statistical procedures

Time to first interaction was log-transformed to achieve normal distribution. Data from the other measurements were more skewed and thus non-parametric methods were used. To compensate for multiple comparisons, sequential Bonferroni corrections (Dunn–Šidák method) were used to adjust α (Sokal and Rohlf 1995). All tests are two-tailed and all analyses were performed with Statistica 5.5 (StatSoft, Inc.).

Results

Median time in seconds to first interaction between blue tits and the three butterfly species was: peacocks, 414 s (first quartile=202; third quartile=688); small tortoiseshells, 492 s (first quartile=432; second quartile=868); commas, 976 s (first quartile=522; second quartile=2,024). Time to first interaction was not equal among butterfly species (ANOVA: $F_{2,29}=3.52$, $P=0.04$), with peacocks being discovered earlier than commas (Tukey's HSD: $P=0.04$). However, there was no difference in time to first interaction between small tortoiseshells and the two other species (Fig. 1).

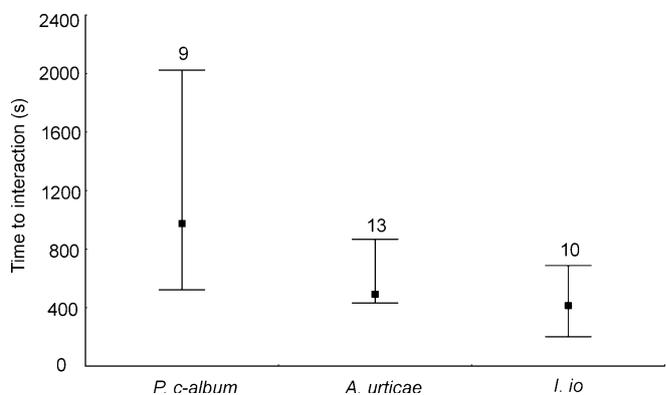
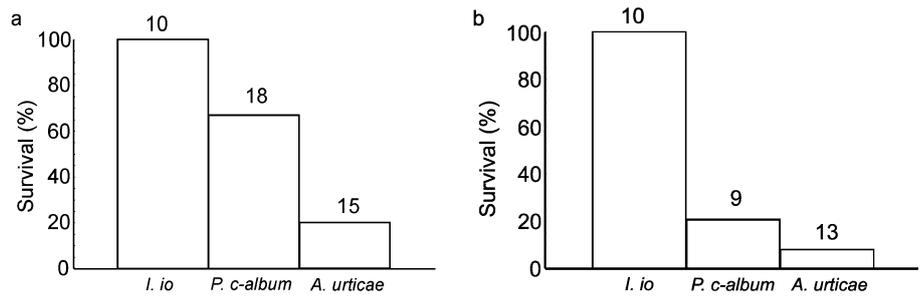


Fig. 1 Time (s) to the first interaction between bird and the tree butterfly species: commas (*Polygonia c-album*), small tortoiseshells (*Aglais urticae*) and peacocks (*Inachis io*); in trials where butterflies were discovered, as evidenced from bird and butterfly behaviour. Numbers above boxes are *n* values. Data are median and quartiles (whiskers)

Fig. 2 Difference in survival between peacocks (*Inachis io*), commas (*Polygonia c-album*) and small tortoiseshells (*Aglais urticae*) during 40-min trials with blue tits. **a** Total numbers of surviving butterflies, **b** survival of butterflies that were discovered by birds. Numbers above bars are *n* values



Survival varied between the three butterfly species—100% (10/10) of peacocks, 20% (3/15) of the small tortoiseshells and 67% (12/18) commas survived (Fig. 2a). Thus, peacocks survived to a higher degree compared to small tortoiseshells (Fisher's exact test: $P < 0.001$) and, also, more commas survived compared with small tortoiseshells (Fisher's exact test: $P = 0.01$). More peacocks survived, though not significantly, compared with commas (Fisher's exact test: $P = 0.06$).

When comparing butterfly survival following discovery by blue tits, the figures remain unchanged for the peacocks, because all ten peacocks were discovered by the birds and yet survived. In comparison, only one small tortoiseshell survived—out of 13 that were discovered, whereas two out of nine discovered commas survived (Fig. 2b). The two surviving commas were dropped by the birds during handling and ended up under the log where they were out of the birds' sight. Thus, peacocks survived in higher numbers after being discovered compared with small tortoiseshells (Fisher's exact test: $P < 0.001$) and commas (Fisher's exact test: $P = 0.002$). There was no difference in survival between discovered small tortoiseshells and commas (Fisher's exact test: $P = 0.53$).

In our experiments, none of the commas ($N = 18$), but all peacock butterflies ($N = 10$), and nine of the 15 small tortoiseshells tested started flicking their wings when disturbed by a bird. Thus, the peacocks were more prone to wing flick compared with commas (Fisher's exact test: $P < 0.001$) and they were more prone to wing flicking compared with small tortoiseshells (Fisher's exact test: $P = 0.05$). Also, more small tortoiseshells flicked their wings open compared with commas (Fisher's exact test: $P < 0.001$). When peacocks ($N = 10$) and small tortoiseshells ($N = 9$) flicked their wings open in the first interaction, peacocks did so at a greater distance from the approaching bird; peacock median value was 12 cm (first quartile = 0 cm; third quartile = 19 cm) compared to 0 cm for small tortoiseshells (first quartile = 0 cm; third quartile = 0 cm) (Mann–Whitney U test: $U = 2.0$, $P = 0.001$).

Discussion

In our experiments, both commas and peacocks survived experimental trials with blue tits better than small tortoiseshells, but for different reasons; the commas survived because they were seldom discovered whereas the peacocks survived because of their wing-flicking display after being

discovered and attacked (Fig. 2). The success of the peacock's intimidation behaviour is demonstrated by the fact that all peacocks survived after being discovered by the birds. Interestingly, two thirds of the small tortoiseshells flicked their wings open and thus they assume an intermediary position between the commas and the peacocks. However, the display of the small tortoiseshells was not very effective in scaring the blue tits away, and out of the tortoiseshells that flicked their wings open only one survived. This finding is in agreement with Blest's (1957) results, in which yellowhammers and reed buntings, *Emberiza schoeniclus*, were only slightly taken aback by the strong colours on the dorsal surface of small tortoiseshells. However, when discovered by a predator there is little to lose by engaging in some sort of a defensive behaviour, and although wing flicking was not very effective when they were attacked by blue tits, the same trend was observed during the trials, where small tortoiseshells flicked their wings and birds refrained from attacking when the butterfly executed the first wing flick and one out of nine butterflies managed to survive. Hence, the behaviour may be endowed with some success, and moreover it is conceivable that smaller predators than blue tits may be more intimidated by the wing-flicking behaviour of small tortoiseshells. For example, Exnerová et al. (2003) demonstrated that small species of insect-eating birds were more hesitant to attack the same aposematic and non-aposematic prey species compared with larger bird species. Moreover, we used wild-caught and consequently experienced blue tits as predators. Lyytinen et al. (2004) showed that naïve pied flycatchers (*Ficedula hypoleuca*) caught and ate fewer butterflies of the spotted wet-season morph of *Bicyclus anynana*, compared with experienced flycatchers. Thus, we can not eliminate the possibility that the small tortoiseshells' defence may be more effective against a naïve predator.

In some ways it is surprising that a butterfly such as the peacock, which is perfectly palatable to small passerine birds, is so efficient in surviving attacks from blue tits. Previous experiments have demonstrated that small tortoiseshells and peacocks are palatable to yellowhammers and reed buntings (*E. schoeniclus*) (Blest 1957) as well as to bats (Møhl and Miller 1976), and that commas are palatable to great tits (*P. major*) (Wiklund and Tullberg 2004). Recent experiments have shown that small tortoiseshells are less preferred compared to some other lepidopterans by domestic chicks (Rydell et al. 2001) and that they are "mildly distasteful" to great tits (Hagen et al. 2003). In our

experiments, 12 out of 13 small tortoiseshells that were discovered by the blue tits were attacked and subsequently consumed, which suggests that they are palatable. Other experiments have shown that peacocks are readily eaten by blue tits when captured (Vallin et al. 2005), and so it seems that peacocks, small tortoiseshells and commas are at least partly palatable to blue tits, and that they have no strong chemical defence that makes them unpalatable. Hence, these butterflies are dependent on leaf mimicry or intimidation for their protection against predators.

Our experiments demonstrate that commas and peacocks have taken different evolutionary routes and incorporate a suite of behaviours that go along with the road taken. Accordingly, commas rely completely on their leaf-mimicking properties and do not ever reveal themselves by flicking their wings open, no matter how closely the predator approaches. The other route is exemplified by the peacock, which is more easily discovered by the birds. In contrast to commas, which do not have a secondary line of defence once discovered, peacock survival after discovery relied on the wing-flicking behaviour which exposed the eyespots on the dorsal side of their wings. In agreement with our prediction, peacocks reacted by flicking their wings open when the bird was approaching the butterfly, and hence the peacock's decision-making is a difficult one incorporating the assessment of whether it has been discovered or not, and subsequently rapidly having to decide whether to flick the wings open at some distance from the approaching bird.

When tracing the phylogeny of the defensive behaviours exhibited by the comma, the small tortoiseshell and the peacock, it seems likely that crypsis and motionlessness was ancestral in the most basic clade consisting of the ancestors of *Polygonia/Nymphalis* plus *Inachis/Aglais* and their sister clade *Vanessa* (cf. Nylin et al. 2001; Wahlberg and Nylin 2003). This seems to have prevailed as an ancestral condition in *Polygonia*, whereas wing-flicking behaviour appears to have evolved after the split between of the *Inachis/Aglais* line from the *Polygonia/Nymphalis* line. Finally, extended wing flicking accompanied by the formation of eyespots on the wings appears to have occurred in *Inachis* after the split from *Aglais*. Thus, facing similar environmental conditions and sharing lifestyles are not absolute impediments for the evolution of fundamentally different anti-predation adaptations in closely related species.

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