The white ‘comma’ as a distractive mark on the wings of comma butterflies

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Distractive marks have been suggested to prevent predator detection or recognition of a prey, by drawing the attention away from recognizable traits of the bearer. The white ‘comma’ on the wings of comma butterflies, Polygonia c-album, has been suggested to represent such a distractive mark. In a laboratory experiment using blue tits, Cyanistes caeruleus, as predators, we show that the comma increased survival, since the blue tits attacked butterflies with overpainted commas more often than sham-painted butterflies with intact commas. In a field experiment we placed hibernating, similarly manipulated, comma butterflies on tree trunks of two different species and noted their survival. Although survival was higher on birch trees than on oak trees, there was no effect of treatment, probably because the butterflies were preyed on by both diurnal and nocturnal predators and the latter are unlikely to attend to small conspicuous markings.

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Under the incessant selection pressure from a variety of vertebrate and invertebrate predators, butterflies and other insects have evolved a multitude of defences. Insect defence against predation can usefully be divided into (1) primary defences, which operate before a predator launches an attack, and (2) secondary defences, which operate during an encounter with a predator, typically after the predator has launched its prey capture attempt (Edmunds 1974).

Although prey animals have evolved a variety of secondary defences such as flight behaviours and startle displays, it is obvious that they benefit more by not being attacked at all; hence, if possible, primary defence is to be preferred (Edmunds 1974). Avoiding discovery by cryptis is arguably the most common device used by prey (Cott 1940; Edmunds 1974; Ruxton et al. 2004), but, contrary to old belief, cryptis or the ability for an organism to ‘initially preventing detection’, is not always so easy to achieve (Stevens & Merilaita 2009a, page 424). Moreover, an animal's outline may in many situations reveal its presence, thereby making camouflage by means of disruptive coloration, ‘where patterns break up the animal’s appearance and body outline’, more effective (Stevens & Merilaita 2009b, page 481). Yet another means of avoiding being identified as prey by a predator includes masquerade, by which an insect mimics an inedible object, such as a leaf or a twig, so effectively that it is mistaken for the inedible object itself (Skelhorn et al. 2010a, b). Finally, some prey have small conspicuous marks (‘distractive marks’) that purportedly attract the predator’s attention towards them to such a degree that it prevents detection or recognition of the prey itself (Thayer 1909; Stevens & Merilaita 2009a).

Previous laboratory experiments have demonstrated that high-contrast marks, printed on otherwise camouflaged artificial prey, can indeed improve concealment from blue tit, Cyanistes caeruleus, predators (Dimitrova et al. 2009). On the other hand, Stevens et al. (2008, 2013) performed field experiments by deploying artificial prey on tree trunks and found that conspicuous marks impaired cryptis of the targets. Moreover, humans have been shown to detect background-matching targets on a computer screen more readily if the targets are endowed with small contrast marks, compared to when the targets are only fabricated to match the background (Stevens et al. 2013). Nevertheless, to the best of our knowledge, no study has investigated whether a small contrast...
marking can aid survival of a natural prey. Here we present such a study. We tested whether the small, conspicuous mark on the under-side of the hindwing of the comma butterfly, *Polygonia c-album*, reduce their risk of attack from wild-caught blue tits. The wings of the comma butterfly have an elaborate jagged outline, the under-sides of which are dark brown, resembling a withered leaf, and centred on each hindwing there is a highly conspicuous white ‘comma’. The undersides of the wings, which are exposed when the butterfly is at rest, are highly sexually dimorphic: males’ wings have a variegated appearance with lighter and darker brown fields that provide strong contrasts, whereas females’ wings have a much more uniformly brownish appearance (Emmet & Heath 1989). Using controlled two-choice experiments, we tested (1) whether the conspicuous ‘comma’ influenced a predator’s choice between one butterfly with the comma intact and another butterfly with the comma painted over, and (2) whether the predator’s choice was influenced by the degree of contrast (males versus females) between the comma and the surrounding wing appearance.

In butterflies, a short adult life span is the rule (Scott 1973), but the comma butterfly is one of the few exceptions. Along with some other close relatives in the Nymphalini tribe, the comma butterfly hibernates as an adult and may thus live for almost an entire year (Ellisson et al. 2005). Two closely related species, the small tortoiseshell butterfly, *Aglais urticae*, and the peacock butterfly, *Inachis io*, hibernate in dark sheltered places (e.g. outhouses, earth-cells and abandoned mines, which suggest caves, crevices and hollow trees as natural hibernacula; (Dvořák et al. 2002; Wiklund et al. 2008; Olofsson et al. 2011)), whereas the comma butterfly is never found in such places. This suggests that the comma butterfly may be exposed to visually oriented predators during hibernation more often than its relatives the tortoiseshell and the peacock (Wiklund & Tullberg 2004). To test the protective function of the comma under field conditions, we placed live hibernating comma butterflies, with the comma intact or painted over, on tree trunks (one per tree), and assessed their survival via repeated censuses during 2–3 months in winter. Our main objectives for the field experiment were (1) to determine whether the butterflies could survive at all when completely exposed to inclement weather and predators, (2) to assess whether the presence of the comma improved such long-term winter survival, (3) to compare male and female vulnerability to predation, and (4) by means of searching for wing remains, to assess which predators were responsible for the disappearance of hibernating comma butterflies.

**METHODS**

**Laboratory Experiment**

**The prey**

Larval descendants from wild-caught comma butterflies were reared communally on cuttings of stinging nettle, *Urtica dioica*, at 25 °C and a 12 h daylength, which produced adults of the dark overwintering morph. Eclosing butterflies were put in glassine envelopes and euthanized by freezing (−20 °C). After being euthanized the butterflies were removed from the freezer and stored at room temperature until the experiment started. Butterflies were sexed, and males and females were visually inspected and matched into pairs (male/male and female/female) with respect to size and general coloration. Coloration within males showed little variation, whereas females varied from being almost uniformly brown to having a more ‘male-like’ coloration. Of the females, we used only uniformly brown individuals for the laboratory experiment. In each pair, we painted over the comma of one butterfly (randomly chosen) using a black permanent marking pen (Faber-Castell Multimark) and sham-painted between the comma and the body of the other butterfly so that its comma was still intact. To get a uniform presentation of the butterflies, we detached the legs, antennae and either the left or the right wing pair (depending on their location during the presentation). The matched butterfly pairs were glued onto a circular piece of uniformly coloured light brown cardboard (which fitted exactly into a petri dish, 9 cm diameter) with their bodies placed approximately 1 cm from each other and oriented mirror reversely, exposing the ventral side of the butterflies (i.e. the left butterfly had its right ventral surface exposed and the right butterfly had its left ventral surface exposed; hence the butterflies were highly visible against the cardboard background; Fig. 1).

**The predators**

Wild-caught blue tits, *Cyanistes caeruleus*, were used as predators. The experiment was carried out in 2010 between 17 February and 1 April at Tovetorp Research Station (Stockholm University), situated approximately 100 km south of Stockholm (58°56’N, 17°08’E). The birds were caught in mist nets at the research station and held individually in cages (80 × 60 × 40 cm) on a lighting regime that was correspondingly adjusted to mimic that of the prevailing season. The temperature in the room where the birds were kept was 16–18 °C. Each cage was supplied with litter on the floor and was furnished with three perches for the birds to rest upon. Water, sunflower seeds and suet were supplied ad libitum. After the experiment, birds were ringed and released at the site of capture. The birds spent no more than 2 weeks in captivity. All birds maintained their weight during captivity and were healthy upon release. Observational evidence suggests that the birds were not negatively affected by the capture process or by their time in captivity; we base this inference on the fact that we often recapture ringed birds, both within and between seasons. Moreover, the research station has two large feeding stations outside where recently released birds have ad libitum access to suet, peanuts and sunflower seeds.

![Figure 1. Presentation of the butterflies in the experiment. The left butterfly is sham-painted (‘comma intact’) and the right butterfly has had its comma painted over (‘comma obliterated’). The butterflies were glued onto a circular piece of brown cardboard against which they were fully visible to the blue tits.](image-url)
The experiment described herein was reviewed and approved by the regional ethical committee (Linköpings djurförsöksetiska nämnd, permit 62-08). Permit to keep birds in captivity was approved by the Swedish Board of Agriculture (Dnr 31-11980/10). The birds were captured and ringed with permission from the Swedish Museum of Natural History (Dnr 52-00060/2010).

Training and experimental procedures

Experiments were conducted in a plywood box (55 × 70 and 90 cm high) illuminated from the ceiling with two daylight-mimicking, high-frequency fluorescent lamps (15 W, BIoLight, Narva, Knoxfield, Victoria, Australia). A small opening on the bottom of the box, on the right long side, was used to present the butterflies to the birds. We made observations through an opening (10 × 12 cm, on one of the short sides, close to the top of the box) that was covered with one-way plastic. In the middle of the left long side, a perch was mounted 20 cm beneath the ceiling. The experimental box was placed in a dark room with a constant temperature (16 °C).

We used 62 birds in the present experiment that had been previously trained to search for artificial prey on artificial backgrounds for another study (S. Merilaita & M. Dimitrova, unpublished data). Of these birds, only 13 were subsequently tested in that study. In the present study, we deprived these 13 birds of food for about 5 min prior to testing; the remaining birds (N = 49) were deprived of food for a mean ± SD of 34 ± 8 min prior to testing. All birds were provided with a small bowl of water in the experimental box. The experimental set-up in the previous study differed from that in the current experiment and thus should have had no influence on the birds’ choice of which butterfly to attack; however, to be sure of this we tested whether these two groups of birds differed with respect to their choice of prey, and we detected no such effect (Fisher’s exact test: P = 0.516).

Training was initiated when we presented a decapitated mealworm, *Tenebrio molitor*, on a circular piece of cardboard fitted in a petri dish. An experimental trial was initiated as soon as the bird attacked and consumed the mealworm. We then replaced the empty petri dish with a petri dish containing a butterfly pair (see above; Fig. 1). The petri dish was always placed in the same spot on the floor in the middle of the experimental box, but it was haphazardly rotated when inserted into the experimental box. Together with altering the right/left positioning of the butterflies (comma intact/comma obliterated), this ruled out potential right/ left biases in the birds.

We noted which butterfly each bird attacked first (comma intact or comma obliterated). We also documented where the birds aimed their first attack (head, body or wings of the butterfly). Butterflies that were attacked on the head or body would have been killed instantaneously (had they been alive) and were scored as ‘died’. Butterflies that were attacked on the wings would have survived the initial attack, and could have had the opportunity to escape, and were therefore scored as having ‘survived’. We did this to compare whether blue tits would attack the purported leaf-mimicking comma butterfly in a different manner than what has been reported for attacks on satyrine butterflies, which are more ‘butterfly-like’ and are typically attacked in the head region by blue tits (Olofsson et al. 2010, 2013). When a bird attacked one of the butterflies in the pair, we terminated the experiment and rewarded the bird with peanuts before returning it to its home cage. Each butterfly pair was used only once, and each of the 62 birds was used in only one experimental trial and was presented with only one butterfly pair (i.e. either a pair of males or a pair of females); thus, the data consisted of 62 independent trials and were analysed accordingly.

Statistical analysis

We used Fisher’s exact test (two-tailed) to examine whether the comma affected the attack decision (i.e. which of the two butterfly forms was attacked) of blue tits differently when confronting a pair of males compared to a pair of females. A binomial test was used to investigate the attack decision with respect to the comma treatment (comma intact/comma obliterated). Furthermore, we used a Fisher’s exact test to investigate whether attacks were targeted differently (head/body versus wings) when the butterfly had an intact comma or an obliterated comma.

Spectrophotometry and Size Measurements

To document the degree of sexual colour dimorphism, we obtained reflectance spectra measurements on 10 females and 10 males from our laboratory culture of *P. c-album* (rearing conditions as described above). We used a spectrometer (Ocean Optics USB 2000 equipped with a PX-2 pulsed xenon light source) to measure spectral reflectance of the butterflies’ wings; we took two measurements of the ventral forewing (one dark patch and one light patch) from each specimen (Fig. 2). Note that we actively chose 10 females whose wings were uniformly brown, whereas we chose the 10 males at random. Thus, reflectance measures of the females were not representative of the natural range of variation in females and were performed only to assess the visual appearance of the specimens used in our laboratory predation experiment. Additionally, we measured the reflectance of the comma on three males and three females. We also photographed 20 males and 18 females (of the brown form) with a Canon D60 digital camera equipped with a macro lens (Sigma 150 mm 1:2.8 APO MACRO DG HS). All pictures were taken from the same distance using a tripod, and a scaled sheet of paper served as a size reference. We used the Analysis Tool in Photoshop (CS3) to analyse the pictures and measure the area of the comma. We assessed the size of the butterflies by measuring the length of the hindwing (from the basal part where the wing attaches to the thorax to the most distal outgrowth in the wing margin; Fig. 2).

Statistical analysis

The size of the comma was used as the response variable in an ANCOVA. Wing length was used a continuous variable and butterfly sex was included as a covariate. We also included the interaction between wing size and butterfly sex.

Field Experiments

All butterflies that partook in the winter censuses had been reared communally on stinging nettle as larvae (rearing conditions as described above) and, upon eclosion, were sexed and exposed to a lighting regime that induces diapause (8:16 h light:dark cycle) with ad libitum access to food (sugar solution) for 2 weeks. Thereafter, the butterflies were individually kept in small plastic cups in a cold room (ca. 10 °C) until the experiment started. We assessed winter survival of comma butterflies by means of two census studies. In the censuses, all females, including those with a more variegated coloration, were used. These censuses were carried out in the Royal National City Park located adjacent to Stockholm University (between 14 January and 15 April 2009) and in the surroundings of Tovetorp Research Station (between 9–11 December 2008 and 9 April 2009). In Stockholm, we placed 72 butterflies on old, mature oak trees, *Quercus robur*, 1–2 m above ground. The butterflies were painted with a black permanent marker following the same procedure as described above for the laboratory experiment: 35 butterflies (15 females and 20 males) were sham-painted (comma intact) and 37 butterflies (16 females...
At Tovetorp, we placed 32 butterfiles on oak trees, 33 butterfiles on birch trees (Betula sp.) and 15 butterfiles on other tree species (willow, Salix sp.; aspen, Populus tremula); spruce, Picea abies; alder, Alnus glutinosa). Half of the butterfiles were distributed on 9 December and the other half on 11 December. All butterfiles were placed 1–2 m above ground. Of these 80 butterfiles, 41 had their comma intact (19 females and 22 males) and 39 (18 females and 21 males) had their comma obliterated. At both localities, the butterfiles were checked about 24 h after they had been placed on the trees and were thereafter revisited at 4–5-day intervals in Stockholm and at 4–14-day intervals (typically every 10th day) at Tovetorp. At each census, we noted whether the butterfly was present or missing. If a butterfly was missing, we searched carefully for wing remains, both on the tree trunk and on the ground in the immediate vicinity of the tree.

Unfortunately, the area at Tovetorp was partly felled approximatively 10 days after the experiment was initiated and six of the butterfiles ‘disappeared’ since the trees on which they were placed were cut down. Additionally, three butterfiles in this census were found dead (but fully intact) on the ground. These nine butterfiles were treated as censored in the survival analyses (i.e. included until they disappeared for known, nonpredation causes). In the Stockholm census, all butterfiles that disappeared were assumed to have been taken by predators. After 14 March in Stockholm and after 20 March at Tovetorp, the censuses were no longer reliable because of increasing temperatures (i.e. butterflies that disappeared after these dates could have terminated their diapause and simply flown away); this means that butterflies that survived until these dates had been fully exposed to predators and inclement weather (including snow and temperatures below 0 °C) for 99 days in the Stockholm census and for 99 days and 101 days in the Tovetorp census.

**Statistical analysis**

We performed survival analyses with the Survival package in R (R Foundation for Statistical Computing, Vienna, Austria) using Cox proportional hazard regression (coxph). To compare the two localities, we performed survival analyses on the first 59 days of exposure since this was the maximum duration of the Stockholm census when temperatures were still low (see above). The survival analysis within the Tovetorp census was performed on the first 99 days of exposure for the same reason. We performed two analyses. First, we tested whether survival on oaks was dependent on comma treatment, butterfly sex or locality. Second, we tested whether butterfly survival differed between tree species; we did this by comparing survival of butterflies that were placed on oaks and birches within the Tovetorp census, using comma treatment, butterfly sex and tree species as factors. We limited our analysis to oaks and birches because sample sizes for the other tree species were inadequate for reasonable interpretations; however, we descriptively present the fate of all butterflies in the Results.

**RESULTS**

**Laboratory Experiment**

The blue tits’ choice of which butterfly to attack (comma intact or comma obliterated) was not significantly affected by butterfly sex; 23 of 31 birds attacked female butterflies with the comma obliterated and 17 of 31 birds attacked male butterflies with the comma obliterated (Fisher’s exact test: two-tailed \( p = 0.184 \)). Thus, we pooled the sexes and analysed the overall effect of comma treatment using a binomial test. More birds directed their initial attack towards the butterfly with an obliterated comma (40 of 62 birds; binomial test: \( p = 0.03 \); Fig. 3).

Approximately one-third of the birds (19 of 59) launched their initial strike towards the wings of the butterfly, whereas the remaining birds attacked the butterfly’s head or thorax (in three trials the bird’s position obscured the observer’s view of the point of attack; thus, the deviance from \( N = 62 \)). However, the comma mark treatment did not affect the attack distribution of the birds: in eight of 20 trials (eight of 22 trials, including trials when the bird obscured the observer’s view), butterflies with intact commas ‘survived’ (the bird attacked the butterfly’s wings instead of its head or thorax), and in 11 of 39 trials (11 of 40 trials, including trials when the bird obscured the observer’s view), butterflies with obliterated commas ‘survived’ (Fisher’s exact test: two-tailed \( p = 0.39 \)). Note that we found no evidence that the comma marks attracted the birds’ attacks per se; strikes towards the wings were typically aimed at the wing margin and not towards the centrally located comma.

**Spectrophotometry and Size Measurements**

Our spectrophotometric measurements confirmed that male and female comma butterflies differ in ventral wing coloration (cf. Emmet & Heath 1989). On the ventral forewings of males, the dark patch was darker, and the bright patch was brighter, compared to females whose patches were more similar in coloration (Fig. 4, and...
see Fig. 2 for differences in coloration). Furthermore, the commas in both males and females had a broad range of reflectance, including UV (Fig. 5), but as they are small and difficult to measure the probe also measures the considerably darker area that surrounds the comma and thus the reflectance spectra presented in Fig. 5 are underestimates. The size of the comma was positively correlated with the size of the butterfly and, moreover, was bigger in males than in females (ANCOVA: size: $F_{1,34} = 11.69, P = 0.0017$; butterfly sex: $F_{1,34} = 95.9, P < 0.0001$; size*butterfly sex: $F_{1,34} = 0.361, P = 0.55$; Fig. 6).

**Field Experiment**

**Winter survival**

At the first census (ca. 24 h after the butterflies were placed on the trees) all butterflies were recovered alive ($N_{\text{Stockholm}} = 72$; $N_{\text{Stockholm}} = 72$).
Our study suggests that, in addition to enhancing camouflage of background-matching prey (Dimitrova et al. 2009), a conspicuous marking can also be effective when the prey per se is perfectly visible against a mismatching background (Schaefer & Stobbe 2006). Indeed, evidence favours the idea that comma butterflies use masquerade (leaf mimicry) as a first line of defence (Wiklund & Tullberg 2004; Vallin et al. 2006). Moreover, previous experiments have shown that blue tits tend to direct their attacks towards the head of butterflies (Olofsson et al. 2010, 2013) and therefore it is noteworthy that 19 of 59 (32%) blue tits did not launch their attack towards the head of the comma butterflies, which adds further support for the idea that the birds are not fully ‘convinced’ that the comma butterfly is actually a prey. Nevertheless, we found no evidence that the comma marking itself was responsible for the misdirected attacks of the blue tits, as butterflies with their comma obliterated were equally likely to be attacked at the wing margin as were butterflies with their comma intact.

Why should a leaf-mimicking butterfly incorporate a conspicuous marking on its wings? Two alternatives come to mind: (1) either the marking functions as a distractive marking in the sense of drawing the predator’s attention towards it so that the outline of the prey body escapes attention (Dimitrova et al. 2005), or (2) the marking is part and parcel of the prey’s leaf-mimicking design in which the predator’s misidentification is enhanced (Emmet & Heath 1989). Since both of these alternatives are contingent on the predator’s cognitive system, it is difficult to decide which alternative is likely to apply, because essentially, the decisive issue

DISCUSSION

The results show that the presence of a conspicuous white comma on the otherwise brown underside of the wings of the comma butterfly has a preventive effect against attacks from blue tits. The birds attacked the butterfly with the obliterated comma significantly more often than they did the alternative butterfly with the comma intact. We found no evidence that the protective effect of the comma differed between males and females, despite the prominent sexual dimorphism, with males having larger commas and, moreover, showing a more variegated ventral coloration than females. If any, the effect of the comma was seemingly stronger in the female sex, but further experiments are needed to investigate whether, and how, small conspicuous markings interplay with the general coloration of the prey.

Our field experiments demonstrated that a substantial proportion of comma butterflies survived up to 3 months even when fully exposed on tree trunks during the winter; however, there was no effect of the comma on either sex, suggesting that being endowed with a comma had neither positive nor negative effects on survival during long-term exposure to a variety of predator species. We observed no actual predation events, but findings of gnawed-off wings suggest that rodent predators accounted for a non-negligible part of the missing butterflies and that their attacks were presumably confined to the dark hours when selection on visual traits becomes largely irrelevant (cf. Olofsson et al. 2011). This added diversity in potential predators may hence account for the different results relating to the adaptive benefit of the white comma between the laboratory and the field experiment. Moreover, we argue that the evidence of rodent predation in the present study should be taken into account in future studies attempting to address questions relating to adaptive coloration when prey items are left exposed on tree trunks to complex predator communities.

The raison d’être of the marked sex dimorphism on the underside of the wings of the comma butterflies is intriguing. Insofar as this results from selection for concealment from predators, one possibility would be that males and females use different microhabitats for roosting and/or hibernation, or that it results from disruptive selection (Bond 2007). Regardless of which of these explanations apply, it may be suggested that, although both sexes masquerade as leaves, males have a more disruptive wing coloration on which the comma does not stand out as much as it does on the wings of females, where the comma may add a more conspicuous element to the general leaf mimicry.

Dimitrova et al. (2009) demonstrated in a laboratory experiment that conspicuous markings on artificial prey increased the effective search time of blue tit predators. Here, the artificial prey items largely matched the background. A high level of background matching makes the actual discovery of prey a challenge for predators, and the increased effective search time is likely to be further influenced by the prey’s markings, which draw attention away from its outline (Dimitrova et al. 2009). In our laboratory experiment, however, the two butterflies in each pair were highly apparent when presented; thus, ‘discovery’ of the butterflies as contrasted objects against a background was not an issue. Thus, our study suggests that, in addition to enhancing camouflage of background-matching prey (Dimitrova et al. 2009), a conspicuous marking can also be effective when the prey per se is perfectly visible against the background. A parallel could be drawn to animals that use disruptive body coloration. On the one hand, this defence relates to partly blending with the background, whereas other conspicuously coloured parts help breaking up the outline, hindering detection of the prey (Cuthill et al. 2005). On the other hand, evidence suggests that disruptive coloration can also hinder recognition (which is contingent on the birds’ cognitive system) when disruptive prey are fully exposed against a mismatching background (Schaefer & Stobbe 2006). Indeed, evidence favours the idea that comma butterflies use masquerade (leaf mimicry) as a first line of defence (Wiklund & Tullberg 2004; Vallin et al. 2006). Moreover, previous experiments have shown that blue tits tend to direct their attacks towards the head of butterflies (Olofsson et al. 2010, 2013) and therefore it is noteworthy that 19 of 59 (32%) blue tits did not launch their attack towards the head of the comma butterflies, which adds further support for the idea that the birds are not fully ‘convinced’ that the comma butterfly is actually a prey. Nevertheless, we found no evidence that the comma marking itself was responsible for the misdirected attacks of the blue tits, as butterflies with their comma obliterated were equally likely to be attacked at the wing margin as were butterflies with their comma intact.

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$N_{\text{Tovetorp}} = 80$. We found no differences in survival rates of butterflies on oak trees ($N = 104$) over the first 59 days of exposure with respect to comma treatment ($\text{hazard ratio} = 1.08, z = 0.351, P = 0.73$), butterfly sex ($\text{hazard ratio} = 1.26, z = 1.049, P = 0.29$) or location ($\text{hazard ratio} = 0.631, z = -1.81, P = 0.071$).

A higher proportion of butterflies survived when placed on birch than when placed on oak in the Tovetorp census ($\text{hazard ratio} = 2.88, z = 3.013, P = 0.003$), but there was no effect of comma treatment ($\text{hazard ratio} = 0.893, z = -0.331, P = 0.74$) or butterfly sex ($\text{hazard ratio} = 1.21, z = 0.554, P = 0.58$). This may reflect an overall preference for a variety of predators to search for prey on oak trunks. Another, nonexclusive, explanation might be that rodents find it more feasible to climb on rough oak bark than on the typically smoother birch bark. We repeated the two survival analyses, but censored all events ($N = 21$) when butterflies were assumed to have been taken by small rodent predators (as was indicated by wing remains); the results (not presented here) from these analyses were similar and did not change our conclusions.

Three of 15 butterflies that were placed on trees other than oak or birch were either found dead (one butterfly) or disappeared because of the felling (two butterflies). Of the remaining 12 butterflies, four survived 59 days or longer and eight had a median survival of 38 days.

Including all tree species, seven of 72 butterflies survived for at least 59 days (until 14 March) in the Stockholm census and 27 of 80 butterflies survived for at least 99 days (until 20 March) in the Tovetorp census. Given that the comma butterfly’s natural hibernation sites are unknown, this result is interesting because the number that survived even when fully exposed to predators and inclement weather was not negligible, demonstrating that they can survive the winter without seeking dark and sheltered hibernacula. We found wing remains from 14 of 72 (19.4%) butterflies in Stockholm and seven of 80 (8.8%) butterflies at Tovetorp. The wings we found were almost invariably gnawed off and intact, which suggests that birds were not the predators on these butterflies; this is rather the typical attribute of what small rodents such as mice leave when they have devoured a butterfly (Wiklund et al. 2008; Olofsson et al. 2011).

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is how birds perceive their world (see Birkhead’s (2012) question: ‘What is it like to be a bird?’, page xi). Without resorting to anthropomorphism, it is obvious to us that a bird in a situation as that presented in our laboratory experiment makes at least two ‘deliberate’ decisions: (1) whether to attack the object in front of it or not, and (2) if the object is identified as a prey, where to target its attack.

In a suite of field experiments, Stevens et al. (2008, 2013) found that mealworms attached to artificial prey targets that were endowed with small, high-contrast markings were more susceptible to predation than were targets with low-contrast or no markings. Based on this result, the authors rejected the idea that small contrast markings can have a distinctive function and aid survival of a prey (sensu Thayer 1909). These results may, however, also reflect an elevated detection ability of marked pieces of papers from a distance, whereas, when close by, the predators’ attention may have shifted to the mealworm that partly projected out from the comma mark primarily operates when the predator is close by (cf. Dimitrova et al. 2009). Note, however, that our two-choice experiment in the laboratory did not allow us to assess the absolute strength of the protective effect of the comma, because the bird’s choice of prey did not reflect its probability of choosing to attack a solitary butterfly with and without a small conspicuous marking. Nevertheless, it is a challenge for future research to investigate how common putative distinctive marks are in the natural world, but in the Lepidoptera alone there are a multitude of marks that appear to be likely candidates, both letter-like marks on adult butterflies, moths and geometrids and metallic colours on the pupae of many butterfly taxa such as the Nymphalidae, Danaeinae and Ithomiinae. Future experiments are required, preferably in the wild, to ascertain the general importance of such markings on prey.

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