Ultraviolet reflection and predation risk in diurnal and nocturnal Lepidoptera

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According to our extensive data on Lepidoptera (883 species), UV wing patterns are almost three times more common in nocturnal than in diurnal Lepidoptera. This might be due to predation, because the primary diurnal predators, birds, utilize UV light in foraging and even prefer UV-reflecting prey. To test this hypothesis, we conducted a field experiment with tethered living moths whose wings were artificially manipulated to reflect (UV+, reflection at UV wavelength: 15%) or absorb (UV−) UV light, keeping longer wavelengths identical. Thus, any difference found in survival rates would be the result of the difference in wing patterns in UV spectrum. Significantly more UV+ moths than UV− ones were eaten in the daytime, but no difference in predation rates could be detected when moths were exposed to nocturnal predators. The different survival rates indicate that UV reflection increased predation risk by visually orienting diurnal predators. The lack of difference at night arises from the lack of UV-sensitive predators. UV wing patterns, even if they are important in intraspecies communication, seem to be costly to diurnal Lepidoptera by attracting predators. Key words: Lepidoptera, predation, prey detection, ultraviolet reflection. [Behav Ecol 15:982–987 (2004)]

 Ultraviolet reflecting wing patterns (UV, 320–400 nm) are a widespread characteristic in Lepidoptera (e.g., Eguchi and Meyer-Rochow, 1983; Meyer-Rochow, 1991; Rutowski, 1985; Silberglied, 1979) that may, researchers suggest, have multiple functions. Butterflies may use UV cues as signals in species recognition (Meyer-Rochow, 1991; Silberglied and Taylor, 1973, 1978) and sexual selection (Brunton and Majerus, 1995; Burghardt et al., 2000). However, it is not known whether the UV wing patterns have a function in prey-predation interactions. It is possible that UV reflection attracts predators (see Lyytinen et al., 2001; Viitala et al., 1995). However, it has also been suggested that UV cues serve as aposematic signals, increasing predators’ reluctance to attack (Church et al., 1998a; but see Lyytinen et al., 2001). Birds are the primary vertebrate predators of adult butterflies (Dempster, 1984) and at least 35 bird species have been shown to have UV vision (reviewed by Cuthill et al., 2000). Other potential predators, such as reptiles (Fleishman et al., 1993) and some mammals (Jacobs et al., 1991), have also been shown to extend their vision into the UV range.

Although it is reasonable to suppose that birds might use UV cues in prey detection (Bennett and Cuthill, 1994), only a few researchers have investigated UV colors in terms of their relationship to foraging (Church et al., 1998b, 2001; Koivula et al., 1997; Lyytinen et al., 2001; Siitari et al., 1999; Viitala et al., 1995). The work of Viitala et al. (1995) indicated that kestrels (Falco tinnunculus) utilize UV-visible vole scent-marks to locate areas rich in rodents. Church et al. (1998b) reported that cryptic prey search performance in blue tits (Parus caeruleus) is better under UV light conditions than under UV-absent illumination. In addition, the fact that many natural backgrounds, such as bark, leaves, and soil, do not greatly reflect UV light (Endler, 1993; Finger and Burkhardt, 1994) has led to reassessments of the level of crypsis of insects by taking into account the whole light spectrum (Church et al., 1998a; Majerus et al., 2000). Both Church et al. (1998a) and Majerus et al. (2000) reported that at least some insects are cryptic only in the human-visible range (~400–700 nm) but not necessarily in the UV spectrum. Remarkably, none of these papers investigating the relationship of UV colors to foraging directly compares the survival of two prey types that are dissimilar only in the UV range. There is, however, experimental evidence that the UV reflection of prey seems to attract rather than repel predators (Lyytinen et al., 2001).

If UV wing patterns increase the predation risk by visual predators, they would be selected against in diurnal butterflies. Thus, UV reflection should be a less common trait among day-active than among night-active Lepidoptera. Our first aim was to classify Lepidoptera species into two categories: UV wing patterns present or absent. This was done using photography to assess the degree of UV reflectance in Finnish butterflies. We then collected activity data from the literature to identify which were day-active and which were night-active. The relationship between degree of UV reflectance and activity times in Lepidoptera suggests that UV wing patterns are more often associated with a nocturnal than a diurnal life-style. Based on this relationship, we tested the hypothesis that UV reflection indeed increases predation risk. We manipulated the wings of moths to either reflect or absorb UV wavelengths and observed the survival of these two forms at different times of the day in nature. We expected that moths possessing UV wing patterns would experience higher predation only in the daytime and not at night. This is because the primary diurnal predators are birds, whereas the primary mortality agents at night are non-visual predators, for example, rodents. Diurnal birds possess UV-sensitive cones (Cuthill et al., 2000), and they could use UV cues in prey detection, resulting in a higher predation risk for butterflies with UV wing patterns than for those without.

MATERIALS AND METHODS
The occurrence of UV wing patterns
The study subjects were of 883 species of Lepidoptera representing 16 families. This included almost all Finnish species. All Lepidoptera used in the analysis of UV wing patterns were dried specimens from a private collection (K. Kulmala).
The presence or absence of UV patterns was determined photographically comparing the wing patterns against a grey scale that was included in all photographs. The amount of UV light reflected (i.e., reflection in %) is indicated by numbers next to the grey scale. This UV photograph shows the upper side of *Aporia crataegi* (four males on the left and four females on the right), whose males appear to absorb UV light (i.e., they appear darker than 12% on the grey scale) while the forewings, but not the hindwings, of females reflect UV light.

The presence or absence of UV patterns was determined photographically, which is a rough but practical, qualitative method for a large data set. Lepidoptera individuals were placed on a white background and a photograph was taken. Because individuals of a given species may vary in their UV coloration, at least six individuals per species were placed together for each photograph. Care was taken to ensure that specimens were always lit from the same direction (from the right) since the appearance of UV wing patterns might depend on the angle of the light (Ghiradella et al., 1972; Nekrutenko, 1965). The upper sides of the butterflies were photographed in daylight with a Nikon camera through a UV transmission filter (Nikon UV filter, UR-2; Kodak Tmax 400pro black-and-white film), which excludes all but UV light. A scale showing shades of grey from light to dark (LabSphere) was placed beside the specimens for each photo. In the photographs, wing areas that reflect UV light strongly appear white whereas UV-absorbing areas appear black. Wing areas with intermediate UV reflectance appear grey.

The differences in the appearance of the butterflies in these UV photographs (black-and-white photos) were used to assess UV wing patterns (Figure 1). One of the authors (A.L.) and two people who were unaware of the hypothesis judged the degree of UV reflection against the grey scale (LabSphere). Species were placed in one of two categories: (1) UV reflection present (corresponding to 12–98% on the grey scale) or (2) UV reflection absent (reflectance less than 12%). The cutoff point for a UV pattern being present or absent corresponds to the value used in our previous experiment, which showed that birds are able to perceive the difference between these two treatments (Lyytinen et al., 2001). We pooled the classifications made by three judges into one file, from which any single deviate observation was discarded (this happened in some cases). That is, at least two of the three persons had to agree with their classification.

Data on activity times were collected from the literature (Marttila et al., 1990, 1996; Miikola et al., 1985; Miikola and Jalas, 1977, 1979) and compared to the UV pattern data. Although Lepidoptera species are usually divided into diurnal, crepuscular, and nocturnal according to their flying activity patterns, we pooled the two latter classes into one class, nocturnal. Thus, we had only two categories (diurnal and nocturnal). This was done because Finnish summer nights are not dark but only dusky and, furthermore, extremely short. There is no strong distinction between twilight and night. Also, most birds stop foraging by sunset, further minimizing a distinction between the usual evening and night categories.

Phylogenies for moths have not been completed and, thus, it was impossible to statistically analyze the data on the occurrence of UV colors using an independent number of losses and gains of UV pattern. Therefore, we had to use taxonomic arrangements to analyze the data. We divided the data according to the activity time of the species (i.e., diurnal or nocturnal) and calculated the proportion of species with UV-reflecting wing patterns within each activity time. We used the Fisher’s Exact test to test whether UV-reflecting wing patterns are more likely than expected by chance to be associated with either of the activity times. The test was performed using species and family as independent variables (Harvey and Pagel, 1991; see also Hausmann et al., 2003). For the family level test, one species showing a typical family trait for activity time and wing patterns was selected from each family. Because in some species the sexes are dimorphic with respect to activity time and dorsal UV coloration, statistical tests were performed separately for the sexes.

UV reflection in Lepidoptera wings has been associated with human-visible colors like white, blue, and yellow whereas dark colors usually absorb UV light (Eguchi and Meyer-Rochow, 1983). Therefore, we used color photographs to record whether a species has or lacks a certain human-visible color (white, blue, yellow, grey). Then we calculated the frequencies of occurrence of these colors among diurnal and nocturnal Lepidoptera.

**The field study**

To test the predation risk of UV-reflecting versus UV-absorbing Lepidoptera, we chose the male gypsy moth (*Lymantria dispar*, *Lyantriiidae* (Linnaeus)) as a prey insect. Gypsy moths are known to be a very unpalatable food item for birds (Sargent, 1995) and are even toxic to great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*) (Lyytinen A, personal observations). Using gypsy moths decreases the possibility that only one or very few birds consumed all the disappeared moths in our large study area (see below). Additionally, we changed the appearance of the moths (see below) so that even if birds had confronted the moth before, they were unlikely to recognize the species. This manipulation severely diminished the possibility that the unpalatability of the moths affected birds’ reactions to them. Birds would confront the gypsy moths as new, potentially delicious, prey items.

Larvae from five clutches of five different females were fed on willow foliage (Salix). After emerging, the moths were stored at 4°C. Moths are incapable of moving at low temperatures and thus they remained in good condition until all larvae were hatched.

Adult male moths were manipulated to either reflect (UV+) or absorb (UV−) UV light. Gypsy moths are brownish with mottled pattern on their wings. We wanted to retain their original basic wing color, brown, because it is the color usually associated with palatability. Since the manipulation powders (UV-reflecting chalk and UV-absorbing TiO$_2$) are white, we added brown chalk to the UV+ and cocoa powder to the UV− mixture. As an end product, we had two brownish powders that were very similar in the longer wavelengths (400–700 nm) but dissimilar in the UV region of the spectrum (Figures 2 and 3).

The forewings of the live moths were sprayed with a thin layer of spray glue (producer: 3M) and then coated with...
UV-reflecting or UV-absorbing powder. A piece of paper was slipped between the front and hind wings to prevent the wings sticking together during gluing. This ensured that moths were able to move their wings after the manipulation. The head was protected with a sheet of paper during the manipulation. When the wings were dry the moths were glued ventrally to tiny oval-shaped balls made of cotton wool. Since the cocoa powder used in the UV+/C0 treatment is odorous, the cotton wool was also dyed with cocoa powder to ensure that predators could not distinguish the moth types from each other on the basis of odor.

We conducted the field experiment in the vicinity of Konnevesi Research Station, Central Finland, over two consecutive days at the beginning of July 1999. UV+ treated moths (n = 80) were paired with UV− treated moths (n = 80) of similar size, and they were placed in nature. Each field site contained one UV+ and one UV− moth, tethered to a twig with 95 cm of black thread. The distance between the moths in the pair was about 20 m and each pair was separated from the adjacent pair by at least 50 m. Moths were placed so that one could see two individuals of a given pair simultaneously but not the next pair. The experimental area of approximately 1.5 km2 was characterized by mixed forest, a power line, forest truck roads, and cultivated gardens.

To evaluate the importance of UV reflectance in predation at different times of the day, the experiment was conducted both by day (40 pairs) and by night (40 pairs). The daytime experiments were run from 0900 to 1700 h. Each site was checked for the presence or absence of the moth in total three times every 2–3 hours, for a total of three checks. Because summer nights are short, the night experiments were shorter and each site was checked only once. They were started at 2200 h and terminated at about 0300 h.

To test whether the manipulation was resistant to deterioration, the spectral reflectance of UV+ and UV− moths was recorded at 5 nm intervals relative to 98% white standard (LabSphere®) using a spectroradiometer (EG&G Gamma Scientific GS3100 Radiometer) before and after the experiment (n = 10 in both treatments). The coatings were resistant to wear, showing only a slight drop in the average brightness. The average change in brightness in the UV region, 320–400 nm, was −1.0 (± 0.12) percentage unit and 0.21 (± 0.04) for UV− (n = 10; Figure 4).

RESULTS

The occurrence of UV wing patterns

UV wing patterns were found in females of 41 out of 171 (24%) and in males of 46 out of 167 (28%) strictly diurnal species. Among strictly nocturnal Lepidoptera, UV wing patterns occurred in females of 557 out of 712 species (78%) and males of 557 out of 714 species (78%). Fisher's Exact tests on species revealed that UV wing pattern are associated with nocturnal lifestyles more often than expected by chance. In the wings of diurnal species, the trait is usually absent (for both sexes: p < .001). Family-level analyses performed separately for both sexes confirm the result (Table 1). Thus, the results point to the conclusion that UV reflection is more often associated with a nocturnal than with a diurnal lifestyle in Lepidoptera. When hind- and forewings differed with respect to UV reflection (23% of the species), UV colors were usually present in hindwings and absent in forewings (92% of such species).

We also tested whether UV wing patterns were associated with human-visible colors. UV color wing patterns were found in 41.5% of those diurnal species that had white wings, 9.1% in those with yellow, 82.4% in those with blue (present in only a few species), and 52.6% of those that had grey wings. The equivalent frequencies for nocturnal species were, respectively, 91.5, 68.9, 75.0, and 83.2%. When we compare the diurnal and nocturnal families with respect to these
The presence of UV wing patterns in Lepidoptera families

<table>
<thead>
<tr>
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<th>UV−</th>
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<td>1</td>
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<td></td>
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The Fisher’s Exact test was performed using family typical values as independent data points. Sexes were analyzed separately because of sex-linked differences in activity times and wing traits. UV+ = number of families with UV-reflecting wings. UV− = number of families without UV-reflecting wings.

The field study

A comparison of the UV+ and UV− treated moths along the entire spectrum showed major differences only in the UV region (see Figure 2). This result indicates that the manipulation produced two moth types differentiated only in their wing patterns in the UV range. The amount of UV light reflected from the wings of UV+ manipulated moths corresponds to the most common category found in the photographed species. Thus, the manipulation created UV-reflecting wing patterns that could be found also in nature.

The duration of the day and the night experiments was not the same and hence the number of individuals surviving and eaten during each period could not be used in survival rate calculations. Therefore, we calculated the Mayfield estimates (Mayfield, 1975) of the hourly survival rates (S) for each individual. The calculation proceeded as follows: \( S = 1 - (\text{number of deaths}) / \left( \frac{1}{L} \times \left( n_{LS} + 0.5 n_{LF} \right) \right) \), where \( L \) = interval length in hours, \( n_{LS} \) = number of survivors, and \( n_{LF} \) = number of deaths. In this method, each individual is followed until death (or end of the experiment) and the estimates assume that each loss occurred halfway between visits. The earlier the individual is eaten, the lower value (between 0 and 1) it receives. If the individual is still alive at the end of the experiment, it receives the value of one. The estimates were used as variables in statistical tests.

Because the structure of the data did not meet assumptions of parametric statistics, nonparametric statistics were applied. We applied nonparametric two-way ANOVA (factors: treatment, time) for ranked Mayfield estimates. The test values \( H = \left( SS_{source} / SS_{total} \right) \) follows asymptotically the \( \chi^2 \)-distribution with \( df_{source} \) (Zar, 1996). There was an interaction between treatment and time of day (\( H = 3.879, p = .049 \)) in survival rates, which indicates that moths survived differently at different times of the day. Predation pressure was higher in the daytime than at night (\( H = 12.627, p < .001 \)) (Figure 5). Interestingly, the survival rates for UV+ were lower than for UV− moths (\( H = 3.831, p = .050 \)). When moths were exposed to diurnal predation, the survival rates for UV+ moths were significantly lower than for UV− treated moths. No difference could be found at night. The results imply that UV reflection on wings attracts diurnal predators.

DISCUSSION

Nocturnal Lepidoptera species appear to have wing patterns visible in UV light more often than one could expect by chance. In contrast, the majority of the diurnal species and families have UV-absorbing wing patterns (see Table 1). Thus, UV reflection in Lepidoptera seems to be connected to a nocturnal lifestyle. A similar link between activity time and wing coloration was not observed with three human-visible colors (white, blue, yellow). Therefore, nocturnal-diurnal difference is restricted only to the UV part of the spectrum. A possible explanation is that UV reflection in diurnal butterflies was under a negative selection pressure. There is some evidence that UV reflection attracts the avian predators (Lyytinen et al., 2001; Viitala et al., 1995), and this might cause substantial selection pressure acting against UV wing patterns in diurnal Lepidoptera. Alternatively, we could assume that UV-reflecting wing patterns are a more ancestral trait since moths are an older group than true butterflies (Kristensen and Skalski, 1999). Because there are so few UV-reflecting butterflies, it is possible that UV patterns were already lost in early stages of evolution of butterflies. However, the question of precisely when UV patterns were lost in butterflies cannot be resolved until an extensive phylogeny has been constructed.

We tested the predation hypothesis by a field experiment. UV+ moths suffered higher predation than UV− moths during the day, but this pattern was not found during the night (see Figure 5). This suggests that UV reflection attracted diurnal predators, which in this case were most likely birds. The difference in survival rates can be reasonably attributed to UV-sensitive predators because the UV+ and UV− treated moths were markedly dissimilar only in the UV region of the spectrum. One can argue that the proportion of UV-reflective moths eaten was relatively low. However, the proportion roughly corresponds to the reported levels of avian predation on Lepidoptera (see Bowers et al., 1985; Bowers and Wiernasz, 1979). It is unlikely that differences in manipulation could confound the results, as both prey types had a similar faint odor. As gypsy moths are very unpalatable to birds (Sargent, 1985; Lyytinen A, personal observation), it is implausible that manipulation made them more unpalatable. If, however, the treatment had changed the taste, its effect could be excluded since a previous experiment (Lyytinen et al., 2001) has shown that birds learn to associate unpalatability with UV reflection only with great difficulty. Therefore, differences in UV reflectance of moth wings, rather than in unpalatability, causing avoidance learning of potential predators explains the observed difference in predation (see Figure 5).
At night, when visually hunting predators are virtually absent, predation was so low that we could not say whether or not there is disadvantage to nocturnal insects in exhibiting UV patterns. The main nocturnal predators were most likely rodents that use senses other than vision in predation. We can rule out bats as potential predators because the moths could not fly (although they were able to move their wings) and bats ignore nonmoving objects. It should be noted that night-active moths are also exposed to diurnal predators in nature. The effect of diurnal predation on these moths is probably negligible because they are inactive and hidden among the foliage that makes visually oriented predation more difficult. Furthermore, resting moths cover their hindwings with their forewings, which do not usually reflect UV light. Thus, they are able to merge into their background and stay concealed even from predators with UV vision. Butterflies at rest are especially vulnerable to predation (Dennis et al., 1986; Myneshonadt and Myneshondt, 1976; Shapiro, 1977; Young, 1980). Resting butterflies fold their wings together into an upright position, concealing the upper side of their wings, which is typically colorful. Thereby, they expose only the under side of their wings, which is often characterized by a cryptic coloration. Furthermore, the under-surface of butterflies’ wings does not usually reflect UV light (Ghiradella et al., 1972; Meyer-Rochow, 1991), so predators are incapable of using UV patterns on butterflies wings in prey detection.

The fact that moths have UV vision (Eguchi et al., 1982) and possess UV wing patterns raises the question of the function of UV colors in these species. Because UV light levels are relatively low at night compared to the daylight (Koivula et al., 1997), UV patterns might be of little use in visual communication. On the other hand, at least some nocturnal moths are able to discriminate colors at very low light intensities (Kelber et al., 2002) and therefore it might pay them to reflect UV light in order to be as detectable as possible by their conspecifics. Even if pheromones are more important than visual cues in mate detection (see, e.g., Eisner and Meinwald, 1995), UV cues might, after all, contribute to sexual selection in moths. At the very least, they do not significantly decrease the fitness of night-active insects.

Although UV reflectance is reported to be associated with certain human-visible colors in Lepidoptera wings (Eguchi and Meyer-Rochow, 1983), we did not find this trend when we recorded the presence of white, yellow, blue, and grey and linked this with UV wing patterns. The wings of Lepidoptera species seem to have UV reflectance regardless of what color they are in visible light. Furthermore, nocturnal species reflect UV light relatively more often than diurnal ones in all colors except blue, which is, however, only seldom present in wings (in Lycaenidae). This result is only tentative because to precisely link a certain color with UV reflection, we should measure each color patch with a spectrophotometer. Grey, for example, might be a part of mottled coloration and does not necessarily reflect UV light itself.

We cannot simply conclude that Lepidoptera with UV reflection are more conspicuous than those without UV wing patterns. We need to take into account the color perception of a predator as well as the entire spectral range of the insect and that of its background. Then we could assess the insect’s degree of conspicuousness. We should also remember that the UV waveband is not special compared to other regions of the avian-visible spectrum (Hunt et al., 2001; Keven et al., 2001). However, if the major part of the difference in visibility is found in the UV region, UV reflection might facilitate detectability by those animals sensitive to UV light. Since we created two prey types dissimilar only in the UV spectrum, the higher mortality of UV reflecting prey could be interpreted as evidence of the use of UV as a cue in prey detection, and thus UV reflection increases predation risk. This conclusion is indirectly supported by a previous experiment which tested whether prey could use UV signals to advertise their unpalatability to bird predators (Lyytinen et al., 2001). In that experiment, birds received a series of binary choices of palatable and unpalatable prey items that differed only in the UV range. Birds seemed to more readily associate UV reflection with palatability than unpalatability. Together these two separate experiments suggest that UV reflection invites attacks rather than discourages predators.

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