CONSTRAINED CAMOUFLAGE FACILITATES THE EVOLUTION OF CONSPICUOUS WARNING COLORATION

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Abstract.—The initial evolution of aposematic and mimetic antipredator signals is thought to be paradoxical because such coloration is expected to increase the risk of predation before reaching a stage when predators associate it effectively with a defense. We propose, however, that constraints associated with the alternative strategy, cryptic coloration, may facilitate the evolution of antipredator signals and thus provide a solution for the apparent paradox. We tested this hypothesis first using an evolutionary simulation to study the effect of a constraint due to habitat heterogeneity, and second using a phylogenetic comparison of the Lepidoptera to investigate the effect of a constraint due to prey motility. In the evolutionary simulation, antipredator warning coloration had an increased probability to invade the prey population when the evolution of camouflage was constrained by visual difference between microhabitats. The comparative study was done between day-active lepidopteran taxa, in which camouflage is constrained by motility, and night-active taxa, which rest during the day and are thus able to rely on camouflage. We compared each of seven phylogenetically independent day-active groups with a closely related nocturnal group and found that antipredator signals have evolved at least once in all the diurnal groups but in none of their nocturnal matches. Both studies lend support to our idea that constraints on crypsis may favor the evolution of antipredator warning signals.

Key words.—Aposematism, artificial neural network, cryptic coloration, Lepidoptera, mimicry, predation.

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Many prey species use conspicuous warning coloration, either honestly or deceitfully, to signal that they are unprofitable to predators. In the case of aposematism, the prey has combined a conspicuous signaling coloration with a defense, such as distastefulness or toxicity (Cott 1940; Edmunds 1974). The protection gained from aposematism is based on the predator associating the warning coloration with the defense. Therefore, this protection should only be effective if the aposematic prey is abundant enough such that predators will effectively learn to avoid prey bearing the warning coloration. This appears to result in an evolutionary paradox, since a novel aposematic prey is expected initially to be both conspicuous, resulting in elevated predation risk, and rare. Therefore, it is not fully understood how an aposematic phenotype can increase above the threshold abundance for effective avoidance learning, and invade a population that originally consisted of nonaposematic individuals (Mallet and Singer 1987; Endler 1988; Guilford 1990; Lindström et al. 2001). Consequently, the study of aposematism has largely focused on the predator psychology of avoidance response. Such studies have increased our knowledge about how certain factors, such as the strength of the warning signal and prey gregariousness, are related to avoidance response and predation risk of aposematic prey (Gittleman and Harvey 1980; Leimar et al. 1986; Roper and Redston 1987; Alatalo and Mappes 1996; Gamberale and Tullberg 1998; Yachi and Higashi 1998; Lindström et al. 1999). However, factors other than predator psychology are also likely to be important in the evolution of aposematism (Brodie and Agrawal 2001; Merilaita and Kaitala 2002).

Aside from aposematism, mimicry is another antipredator strategy based upon conspicuous signaling coloration. In mimicry, the prey coloration imitates that of an aposematic model. The mimetic prey aims to gain a share of the aposematic prey's benefit from predator avoidance, but with weaker or no defense of its own (Cott 1940; Edmunds 1974). The initial evolution of mimicry is also considered problematic, primarily because the prey has to reach close enough resemblance to the aposematic model before the predator associates it with the distastefulness of the aposematic model. Only then would the strategy of mimicry become beneficial (Turner 1984; Endler 1991; Mappes and Alatalo 1997; Mallet and Joron 1999).

In this study, instead of focusing solely on signaling coloration, we attempt to widen the perspective by bringing it together with a somewhat deeper consideration of the alternative strategy to antipredator signaling, namely cryptic coloration. Cryptic coloration, or camouflage, functions to decrease prey detection risk (Cott 1940; Edmunds 1974). In many previous studies on aposematism, the underlying assumption has been that cryptic coloration is, in contrast to aposematism, unproblematic and always easily achievable. However, this critical assumption has never been closely scrutinized. In fact, the assumption appears ill grounded because there are at least two reasons why constraints on the evolution of cryptic coloration can be expected to be rather common. The first reason is visual differences between microhabitats. Habitat heterogeneity may impair crypsis achieved by background matching because it is difficult for a cryptic prey to match more than one visual background (Edmunds 1974; Merilaita et al. 1999, 2001). The second reason is that the motility of prey during the activity of visually hunting predators may give them away despite their cryptic coloration (Cott 1940; Edmunds 1974).

Here, we explore how constraints on the evolution of camouflage may affect the evolution of warning coloration. Our hypothesis is that constraints on camouflage might help to explain why some prey have evolved aposematism or mimicry in spite of the apparently high initial costs of such antipredator signals. We first constructed an evolutionary sim-

ulation model to study whether a constraint on the evolution of camouflage affects the probability of aposematic warning coloration invading a prey species. In this model the constraint was caused by visual heterogeneity between microhabitats, because such a constraint is feasible to model. To further test our hypothesis we then carried out a comparison, based on phylogeny, to test whether a constraint on crypsis caused by prey motility might affect the evolution of antipredator signals. Specifically, we compared the occurrence of antipredator signaling coloration between closely related diurnally and nocturnally active lepidopteran groups, as the former are more likely to encounter visual predators while motile than are the latter. We based this comparative analysis on the constraint caused by motility instead of the constraint based upon habitat heterogeneity, because the diurnal pattern of activity is known for many taxa, whereas visual heterogeneity of the habitat used is not.

<center>METHODS</center>

<center>*Evolutionary Simulation*</center>

General assumptions

In the first part of this research we studied the effect of constrained crypsis on the probability of aposematic warning coloration invading a prey species, using an evolutionary simulation model. This technique has several benefits. It allows us to model selection on true patterns instead of requiring the use of a single, simplistic variable such as conspicuousness. Furthermore, in contrast to most experiments, we are not limited only to the study of selection on a few predetermined prey coloration phenotypes; in our model the evolution of coloration is an open-ended process. Thus, mutation and recombination create new coloration phenotypes, and both evolutionary history (i.e., preceding phenotypes and selection on them) and current selection determine which phenotype will eventually become the optimal coloration at the end of a simulation.

In the present simulation the predator viewed its habitat by visually sampling it. These visual samples, and thus also the prey coloration, were described by vectors consisting of four cells, each cell occupied by a coloration element denoted by the numbers 1, 2, or 3. A coloration element can be considered a color or a figure such as a stripe or a spot (cf. Merilaita 2003). The model assumes that the predator is able to detect the prey simply by the visual deviation between prey coloration and the background. Furthermore, based on its previous encounters with prey, the predator has the ability to learn to avoid inedible prey by their coloration. The strength of such an avoidance response toward a coloration phenotype increased with the proportion of distasteful individuals with the coloration in question. There were two prey species, both of which had the potential for evolution of coloration. At the time of their respective introduction both the prey species had suboptimal coloration, because the habitat and the predator were new to them. In contrast to Prey 1, Prey 2 could produce an allele resulting in distastefulness. Thus, Prey 1 served as control for Prey 2. Prey 1 was introduced first and underwent evolution of coloration. After Prey 1 had reached an optimal coloration, Prey 2 was introduced

and underwent evolution until it too had reached an optimal coloration. We considered that a prey species had reached optimal coloration when the most protected 50% of the population had attained a coloration that perfectly matched the background (or a microhabitat) or remained unchanged (i.e., was not invaded by another coloration) for 30 successive generations. The optimal coloration was considered aposematic if it deviated from the background and the prey was distasteful. The adaptation of a prey would not necessarily come to an end at this point, but the protection might well continue evolving; for example, toward microhabitat choice that improves crypsis, or toward a stronger aposematic defense or signal. However, at this point the direction of evolution of antipredator coloration has been determined, either toward crypsis or aposematism.

There were two variants of the model. In the first model the evolution of camouflage was unconstrained, whereas in the second model it was constrained by difficulty in resembling the background in a variable environment consisting of two visually different microhabitats (Merilaita et al. 1999). We ran both simulation models 1000 times and compared the probability of aposematism invading Prey 2 between the models. Thus, the comparison reveals whether we should expect constrained camouflage to affect the probability of aposematism invading a population.

Visual background

In the first simulation the background was assumed to be uniform and consist of one visual element only. This element was randomly chosen to be either 1 or 3 at the beginning of each simulation run. Such visually homogeneous habitat allowed us to easily improve prey camouflage by increasing background matching. In the second simulation the background was, instead, heterogeneous so that it consisted of two visually different ''microhabitats.'' Thus, one microhabitat consisted of the visual element 1 while the other consisted of the visual element 3, with each of the microhabitats occupying 50% of the habitat. This visual difference between the microhabitats constrained the optimization of cryptic coloration, since the prey used both the microhabitats with equal probabilities, and it was impossible to evolve a coloration matching both microhabitats.

Prey species

We simulated the evolution of coloration of the two prey species in two 30-individual populations. Both prey species had four-cell body coloration, where each cell was occupied by one of the three possible coloration elements (1, 2, or 3). A simplified haploid genetic system coded for the antipredator adaptations (cf. Merilaita 2003). We used such a genetic system, first, because the simulation study focuses on selection on antipredator strategy (not on its genetics), and second, because we assumed for the sake of generality of the study that the genetic system has no complications, such as overdominance (Maynard Smith 1982; Hines 1987). Thus, both prey species had four loci, one for each cell of the coloration. For each locus there were three possible alleles, each corresponding to one of the three possible coloration elements. In the beginning of each run of the simulation, every Prey 1

individual was given the same randomly chosen combination of coloration alleles. When Prey 2 were introduced, they too were assigned the same randomly chosen initial coloration as Prey 1. Prey 2 differed from Prey 1 by having, in addition to the coloration genes, one locus with two alleles, coding for whether the individual was edible or distasteful. Initially all individuals of Prey 2 were edible.

During each prey generation the prey species that was currently undergoing evolution was exposed to predation. Thus, the individuals were ranked according to their predation risk, and the half of the population with the highest predation risks was removed before reproduction took place. Then, each individual of the half with the lowest predation risks produced two offspring to the next generation. Consequently, population size remained constant. Genetic variation was required for evolution of coloration to take place. Such variation was produced in each generation by two point mutations and one recombination event among the offspring, which were otherwise exact genetic copies of their parents. The two mutations each changed an allele in a random locus of a random individual. In addition, in Prey 2 there was in each generation a 25% probability for a point mutation that, in a randomly chosen individual, changed the allele that determined palatability, enabling the evolution of distastefulness. The recombination event took place between two randomly chosen individuals and resulted in the individuals exchanging a set of coloration alleles ranging from a randomly chosen locus to the last locus. The probability of mutation was high, to increase the probability of aposematism evolving. This enabled a powerful comparison between the two simulations with a feasible number of replications. Note that this does not present a problem for the interpretation of the results, because it does not affect the difference between the simulations in the probability of aposematism evolving, which is the crucial point here.

Predation

We used two different kinds of artificial neural networks to simulate predation. A radial basis network (Bishop 1995; Haykin 1999) with only one neuron simulated the ability of the predator to detect deviations from the visual background. Such a network compares an input vector with a template vector of equal size, and its output is determined by the difference between the two. More precisely, the output is given by the function $f(x) = e^{-x^2}$, where $x = |dist| \times [-\log(0.5)]^{1/2}$. Here $|dist|$ is the Euclidian distance between an input vector and the template vector, and it is multiplied by a constant that determines the width of the bell-shaped function. The template vector was a visual sample of the background, and the prey colorations of the current populations were presented as input vectors. Because $|dist| \geq 0$, it follows that $x \geq 0$ and $0 < f(x) \leq 1$. Thus, the output value for a given prey coloration could be used as the probability of escaping detection (i.e., the complement probability of the risk of detection) on a given visual background. Prey coloration equal to the background yielded the Euclidian distance of 0 and the maximal probability of escaping detection of 1. For increasing Euclidian distance between the prey coloration and habitat this probability decreased, asymptotically

approaching zero. Thus, for example, for the Euclidian distances of 1 and $2^{1/2}$ the respective outputs were 0.50 and 0.25. In the constrained evolution simulation, we calculated the probability of escaping detection for prey coloration in the whole habitat as the mean of the two microhabitat-specific probabilities.

The second neural network was used to simulate the decision of the predators about attacking a prey. However, this applied only after the introduction of Prey 2, because before that all prey were edible and thus equally willingly attacked. The second neural network consisted of four consecutive layers; that is, a layer of four input cells, the first intermediate layer of eight neurons, the second intermediate layer of four neurons, and the one-neuron output layer. It was a feed-forward network, and thus, the signals from the input cells traversed in only one direction through the intermediate layers to the output layer (Bishop 1995; Haykin 1999). The neural network was saturated such that each neuron was connected to every neuron or input cell of the adjacent layers. Each neuron consisted of specific weights for every incoming connection, a bias and a transfer function. The signals coming into a neuron were first multiplied with the connection-specific weights. The sum of the weighted signals and the bias formed the input to the transfer function. The output from the transfer function was then forwarded to the neurons of the next layer. All transfer functions were log-sigmoid (i.e., smooth threshold) functions except the transfer function of the output neuron, which was a linear function.

The response of a feed-forward neural network to a given input depends on its weight and bias values. When such a neural network is trained, these values are adjusted such that for a given input a desired output is received. Such a training procedure corresponds to the learning of natural neural systems. Training also affects the neural network's generalization ability, that is, the ability to correctly categorize data that it has not encountered previously (Bishop 1995; Enquist and Arak 1998; Haykin 1999). This is comparable with generalization in natural predators (e.g., Gamberale and Tullberg 1999). In the present model we included the coloration of each individual of Prey 1 and Prey 2 as inputs in the training dataset, and the proportion of inedible individuals within each coloration, ranging from 0 to 1, as the corresponding target outputs. The training was based on a back-propagation algorithm with an adaptive learning rate (Demuth and Beale 2000). The network was trained once during each generation of Prey 2. During a training pass, the weights and biases of the network were updated by the training algorithm after the entire training dataset had been presented. Thus, the higher the frequency of individuals of a given coloration phenotype, the more that phenotype influenced the outcome of the training. The presentation of the training set was continued until the mean square error of the output of the network was smaller than 10^{-3}, or until the training set had been presented 150 times.

As a consequence of the linear transfer function of the output neuron, the outputs of the second neural network ranged from 0 to 1 and corresponded to attack probability rather than being a binomial decision about attack. In other words, the training of the second neural network created, based on the proportion of inedible individuals within each

coloration phenotype, a relationship between coloration phenotypes and attack probabilities. This relationship determined the output of the neural network (i.e., the attack probability) both for coloration phenotypes that had been used in the training and for novel coloration phenotypes that were produced by mutation or recombination and that the network had not encountered before. The relationship between coloration phenotype and attack probability was affected by the average palatability of the coloration phenotypes that were available at the given generation, causing the relationship to change in time. Sometimes, minor changes could cause fluctuation and even inconsistency between generations in the ranked fitness of the colorations. To make the model less sensitive to such noise caused by minor fluctuations between generations, the outputs of the second neural network were rounded to a precision of 0.1. Also, because the outputs of the second neural network were sometimes a little larger than 1 or smaller than 0, they were truncated to range from 0 to 1, such that they could be used as probabilities.

During the evolution of a prey species, half of each prey generation was killed by predation. For Prey 1, the probability of detection (the output of the first neural network) alone determined the predation risk, because at this stage there was no distasteful prey and all prey were attacked equally willingly. However, both the neural networks were used to determine the predation risk of Prey 2. Thus, the first neural network gave the risk of detection based on the coloration of an individual, and the second one gave the probability of attack, also based on the coloration of the individual. The product of these probabilities determined the risk of predation for Prey 2. During the evolution of each prey species, the individuals were ranked according to their predation risk and the 50% of the population with the highest predation risk was removed. If some distasteful coloration phenotypes (i.e., phenotypes with some or all individuals distasteful) had equal predation risk, then these phenotypes were also ranked according to their frequencies, such that the most common phenotype had the lowest predation risk. This assumption corresponds to the fact that an increase in the frequency of an aposematic phenotype strengthens predator's avoidance response toward it (Lindström et al. 2001). In case there were unpalatable and palatable individuals of the same coloration phenotype, resulting in equal predation risk, the unpalatable individuals were assigned a lower rank than the palatable ones within the coloration phenotype in question. This assumption corresponds to the fact that defended prey often survive attacks by predators (Järvi et al. 1981; Wiklund and Järvi 1982), and it enabled the fixation of the allele for unpalatability. Otherwise, the locus would have been subject to random drift, which consequently would have substantially increased random variation in the outcome of the simulation.

Comparative Phylogenetic Study

Visual predators impose a risk for both nocturnal and diurnal species of Lepidoptera, but diurnal taxa are more likely to encounter visually hunting predators while motile than are nocturnally active, day-resting taxa. Accordingly, we should expect a much stronger motility constraint imposed on camouflage in diurnal than in nocturnal prey. Consequently, we

expected there to be an association between activity pattern and defense strategy, because for diurnal prey a good camouflage may be harder to attain. To test this idea we compared taxa in the order Lepidoptera, most of which are nocturnal, but where diurnal activity has evolved a number of times. Specifically, we carried out a matched-pairs comparison (Wickman 1992; Tullberg and Hunter 1996) of closely related diurnal and nocturnal groups in ditrysian Lepidoptera. The two groups in each matched pair were compared with respect to the occurrence of species with antipredator signaling coloration.

We based our comparison on Kristensen (1999), an edited volume with chapters written by specialists on various lepidopteran groups. The classification system and nomenclature in this volume are based on phylogenetic systematics, which means that the taxa that are included represent likely monophyletic groups. The volume provides a rich source of information on phylogenetic relationships, morphology, and ecology of various groups and we specifically used it to gather all basic information on phylogenetic relationships as well as on activity patterns and coloration for ditrysian taxa. The use of this source also ensured that the categorization of coloration was independent of our hypothesis. Furthermore, by keeping to one comprehensive source instead of several we ensured that our sampling of taxa was unbiased by our hypothesis. In other words, our knowledge or expectations did not affect the selection of the taxa for the analysis.

First, we perused the text and recorded all species or more inclusive taxa regarded as monophyletic groups that were described as diurnal or day flying. Then, we matched each diurnal taxon with its nocturnal sister-group or, alternatively, with the closest related group we could find described as nocturnal. Diurnal taxa for which no close nocturnal match could be found were excluded from the analysis. Thus, availability of information on activity pattern in our source was the first criterion for inclusion in the analysis. In addition, we included the monophyletic group Papilionoidea-Hesperioidea, known to be diurnal (e.g., Nordström et al. 1941), although this was not explicitly mentioned in our source (Kristensen 1999). Similarly, we regarded the taxa belonging to the family Noctuoidea as nocturnal (e.g., Nordström et al. 1941) unless otherwise mentioned in Kristensen (1999). We found 13 pairs of taxa, phylogenetically contrasted with regard to their lifestyle (diurnal or nocturnal).

The second criterion for inclusion in the analysis was the availability of description of coloration for both the diurnal and the nocturnal taxon of a matched pair. This was given for seven of the 13 matched pairs (Table 1). We regarded all colorations denoted (by a researcher who specialized in the taxon in question; Kristensen 1999) as aposematic or mimetic, as alternatives to camouflage, taking such occurrence as evidence that conspicuous antipredator coloration has evolved in that taxon at least once. Thus, in some of the taxa included in our analysis, all species seem to be signaling, whereas others (e.g., Papilionoidea-Hesperioidea) contain both signaling and nonsignaling species. However, the analysis is based on observations on whether antipredator signaling coloration has evolved at all in the included taxa.

The taxa included in the matched-pairs analysis differ to a great extent with regard to inclusiveness, from a few species

TABLE 1. The coloration in seven phylogenetically matched pairs of diurnal (d.) and nocturnal (n.) ditrysian lepidopteran taxa. The superscripts refer to the chapters in Kristensen (1999), from which information regarding phylogenetic relationships, activity pattern, and coloration has been obtained. Examples of aposematic or mimetic taxa are given within parentheses for larger diurnal groups. One comparison (*Hibrildes* sp.) is based on males and females of the same species.

Matched pair	Description of coloration
1. d. Zygaenidae[1]	aposematic (*Zygaena*)
n. Somabrachyidae[1]	cryptic
2. d. Sesioidea[2]	Batesian mimicry (Sesiidae)
n. Cossoidea[2]	cryptic
3. d. Thyridoidea[3]	aposematic (Charideinae)
n. Copromorphoidea[3]	cryptic
4. d. Papilionoidea-Hesperioidea[4]	mimicry, chemical defense (*Heliconius*)
n. Hedyloidea[4]	cryptic
5. d. *Hibrildes* sp. female[5]	mimicry
n. *Hibrildes* sp. male[5]	cryptic
6. d. Dioptinae[6]	mimicry, chemical defense
n. Nystaleinae[6]	cryptic
7. d. Cocytiinae[6]	mimicry
n. Bryophilinae[6]	cryptic

[1] Epstein et al. 1999.
[2] Edwards et al. 1999.
[3] Dugdale et al. 1999.
[4] Ackery et al. 1999.
[5] Lemaire and Minet 1999.
[6] Kitching and Rawlins 1999.

in the genus *Hibrildes* where the comparison has been made between the sexes within a species, to thousands of species as in the case of Papilionoidea-Hesperioidea, which is matched with its small sister-group Hedyloidea. Moreover, there is generally a lack of detailed information and low phylogenetic resolution within the more inclusive diurnal groups. Therefore, to ensure that the size of the various matched pairs did not bias the analysis, we chose to consider whether antipredator signals have evolved at all or not in a specific group, and one example of an aposematic or a mimetic taxon is given for each of the more inclusive diurnal groups (Table 1). Thus, the analysis is based on the observation of whether anti-predator signals have evolved at least once or not.

One important question is whether the matched pairs are phylogenetically independent from each other. Lepidopteran phylogeny is not well resolved when it comes to basal branches, such as certain relationships among the recognized 46 superfamilies (Kristensen and Skalski 1999). However, resolution is much better at a less basal level, and the families and superfamilies, respectively, are as a rule regarded as monophyletic (Kristensen 1999). Because the matched pairs in the present study were derived from different families and usually different superfamilies, phylogenetic dependence in our sample is unlikely.

RESULTS

Evolutionary Simulation

In the simulation where evolution of camouflage was unconstrained, Prey 1 always evolved coloration that visually matched the background. It normally took (median [min.–max.]) 17 (2–43) generations to reach the optimal coloration. Prey 1 also evolved to match the background in the simulation for constrained evolution. The coloration matched one of the two microhabitats in all of the 1000 simulation runs, except

in 22 runs where the population was polymorphic and consisted of individuals having either of the two background-matching colorations. Reaching the optimal coloration normally took 14 (2–37) generations. This slightly smaller number of generations for constrained than for unconstrained evolution is due to two optimal colorations instead of one and thus a smaller difference between the initial and optimal coloration in the constrained evolution simulation.

In many runs of the simulation, with both unconstrained and constrained evolution of camouflage, Prey 2 evolved aposematic coloration. The median number of generations for Prey 2 to reach the optimal coloration under unconstrained evolution was 14 (min.–max. = 2–613) for aposematic prey and 15 (2–44) for cryptic prey. Under constrained evolution, the median number of generations was 13 (2–40) for the aposematic prey and 15 (2–334) for the cryptic prey.

The most interesting result is that under constrained evolution of camouflage the probability of aposematism invading Prey 2 was 68.5% higher than under unconstrained evolution of camouflage (Fig. 1). This shows that constrained camouflage favors the evolution of aposematism. The average Euclidian distance of the aposematic coloration from perfect background matching was 1.99 ± 0.028 ($N = 543$) when camouflage was unconstrained. When camouflage was constrained, the average Euclidian distance to the closer of the two microhabitats was 1.48 ± 0.017 ($N = 915$).

The direction the evolution of antipredator coloration took was also dependent on the spread of distastefulness. Thus, when Prey 2 evolved to aposematism, on average ($\bar{X} \pm$ SE) $90.1 \pm 0.0083\%$ of the population in the unconstrained evolution simulation and $89.2 \pm 0.0067\%$ in the constrained evolution simulation was inedible when the optimal coloration was reached. The respective figures were significantly lower for prey that evolved camouflage, that is, in the unconstrained evolution simulation $57.9 \pm 0.017\%$ (Mann-Whitney U-test: $U = 13104.5$, $N_1 = 915$, $N_2 = 85$, $P <$
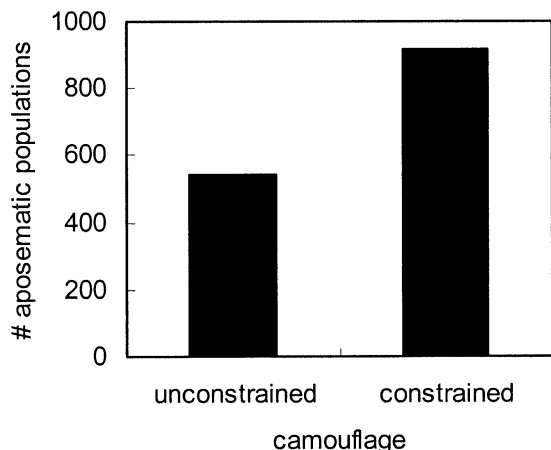
FIG. 1. The number of simulations, of 1000, in which the outcome of the evolution of Prey 2 was aposematism. The probability of aposematism invading was significantly higher when the evolution of camouflage was constrained than when it was unconstrained (χ^2 = 350.23, df = 1, $P < 0.001$).

0.001) and in the constrained evolution simulation 45.3 ± 0.041% ($U = 51662.5$, $N_1 = 543$, $N_2 = 457$, $P < 0.001$).

Comparative Phylogenetic Study

The phylogenetic comparison in ditrysian Lepidoptera showed that the evolution of antipredator coloration is dependent on activity period. In each of the seven phylogenetically matched pairs, aposematic or mimetic coloration can be inferred to have evolved at least once in every diurnal clade but not in their nocturnal matches, in which all species rely on camouflage (Table 1). This difference between the groups is statistically significant (sign test: $Z = 2.27$, $N = 7$, $P = 0.023$). In conclusion, our results clearly show that antipredator signaling coloration was much more likely to evolve in combination with a diurnal activity. Because motility is obviously more likely to disclose diurnal than nocturnal, day-resting Lepidoptera to visual predators, this result lends additional strong support to our general hypothesis that the evolution of signaling coloration is facilitated by constraints on crypsis.

DISCUSSION

Both the evolutionary simulation model and the comparative study strongly support our hypothesis that constrained camouflage can help to explain the evolution of antipredator signals. In the evolutionary simulation, habitat heterogeneity, which constrained camouflage, substantially increased the probability that prey would evolve aposematism. The general pattern of the evolution of antipredator coloration in the model can be summarized as follows. Without distastefulness the prey always evolved toward crypsis, as indicated by Prey 1. However, if the prey had not reached high degree of crypsis (because of the constraint or random events) and it gained distastefulness, then it was possible that the course of evolution was, instead, changed toward aposematic coloration. Accordingly, distastefulness had spread wider in those replicates in which Prey 2 evolved aposematism than in those

in which it evolved cryptic coloration. Consequently, in Prey 2, for which aposematism was possible, the probability of aposematism was considerably higher under the constraint. This suggests that we should expect warning coloration to be more common among species that have not been able to produce effective camouflage. Generally, the overall probability of aposematism invading Prey 2 was relatively high because conditions were favorable (high mutation rate of distastefulness, no costs for producing the defense). However, the same conditions applied in the simulations with and without the constraint. Therefore, this does not mar the main result that the probability of aposematism invading is increased when camouflage is constrained.

The comparative phylogenetic study showed that diurnally active lepidopteran taxa, which are more likely to encounter visual predators while motile, are also more likely to rely on antipredator signals than are nocturnally active lepidopteran taxa. An association between signaling coloration and diurnal activity was previously suggested by some lepidopterist researchers (Rothschild 1972; Turner 1984). However, our comparative study is, to our knowledge, the first phylogenetically controlled test for such an association, and, more importantly, the reason for this association has not previously been explored in a general context. The result of the comparative study accords with the result of the simulation study about the significance of constrained crypsis.

It should be noted that although we found a strong correlation between diurnal activity and signaling coloration, the present analysis does not tell us in which order these two traits have evolved. More detailed phylogenetic information would be needed for such a study. In the skippers (Hesperioidea) and true butterflies (Papilionoidea), which are likely sister groups (Ackery et al. 1999) with a shared origin of diurnal activity, aposematism and mimicry have evolved a number of times in various lineages whereas several lineages remain nonsignaling (Turner 1984). Thus, in this clade, the evolution of diurnal activity has obviously preceded signaling coloration. This also seems to be the case for some of the other, more inclusive diurnal groups, such as Zygaenidae and Sessioidea (Epstein et al. 1999; Edwards et al. 1999). That some diurnal taxa have remained cryptic indicates that diurnal activity is not a sufficient condition for the evolution of signaling coloration. This agrees with our simulation model, in which crypsis sometimes evolved even in the heterogeneous habitat, and the availability and spread of a defense was required, in addition to constrained crypsis, for the evolution of aposematism to take place. We also found two taxa denoted as both nocturnal and brightly colored, Lacturidae (Epstein et al. 1999) and Aganinae (''Often said to be diurnal, most fly at night . . . the bright colors of most adults are probably aposematic''; Kitching and Rawlins 1999, p. 375), implying that antipredator signaling coloration may evolve in nocturnal taxa, too. Thus, we have no reason to believe that signaling coloration should always be too costly relative to camouflage for nocturnal taxa. This also agrees with our model, in which constrained camouflage was not a necessary condition for aposematism, but aposematism sometimes evolved even without the constraint. In conclusion, we can expect this relative cost of aposematism and mimicry to be much reduced in taxa that cannot effectively avoid detection by visually

hunting predators. A related idea is that in highly exposed species defense strategies that work after detection, such as toxicity or structural defense mechanisms, should in general be more common than in less exposed species. This has been shown in coral reef invertebrates (Bakus 1981).

While focusing on the costs of aposematic or mimetic coloration, previous studies on the initial evolution of antipredator signals have not considered the possible costs of the alternative antipredator adaptation, that is, camouflage. However, for example, prey motility, heterogeneity of the visual background, or other functions of body coloration conflicting with camouflage, such as sexual signals or thermoregulation, may constrain camouflage (Edmunds 1974; Endler 1978; Merilaita et al. 1999, 2001). Under such circumstances, the additional increase in the risk of detection due to aposematism or mimicry may be low or nonexistent, and the initial evolution of antipredator signals is much easier to understand. In other words, it is not the cost of a strategy per se that determines whether it invades, but rather its cost in relation to other strategies available. Our results strongly support these conclusions and suggest that the evolutionary paradox of antipredator signaling coloration is at least partly a consequence of ignoring the cost of camouflage.

Literature Cited

Ackery, P. R., R. de Jong, and R. I. Vane-Wright. 1999. The butterflies: Hedyloidea, Hesperioidea and Papilionoidea. Pp. 263–300 in N. P. Kristensen, ed. Lepidoptera, moths and butterflies. Vol. 1. Evolution, systematics and biogeography. Walter de Gruyter, Berlin.

Alatalo, R. V., and J. Mappes. 1996. Tracking the evolution of warning signals. Nature 382:708–710.

Bakus, G. J. 1981. Chemical defense mechanisms on the Great Barrier Reef, Australia. Science 211:497–499.

Bishop, C. M. 1995. Neural networks in pattern recognition. Oxford Univ. Press, Oxford, U.K.

Brodie, E. D., III, and A. F. Agrawal. 2001. Maternal effects and the evolution of aposematic signals. Proc. Natl. Acad. Sci. USA 98:7884–7887.

Cott, H. B. 1940. Adaptive coloration in animals. Methuen, London.

Demuth, H., and M. Beale. 2000. Neural network toolbox for use with Matlab. Ver. 4. The MathWorks Inc., Natick, MA.

Dugdale, J. S., N. P. Kristensen, G. S. Robinson, and M. J. Scoble. 1999. The smaller microlepidopteran-grade superfamilies. Pp. 217–232 in N. P. Kristensen, ed. Lepidoptera, moths and butterflies. Vol. 1. Evolution, systematics and biogeography. Walter de Gruyter, Berlin.

Edmunds, M. 1974. Defence in animals. Longman, Harlow, U.K.

Edwards, E. D., P. Gentili, M. Horak, N. P. Kristensen, and E. S. Nielsen. 1999. The Cossoid/Sesioid assemblage. Pp. 181–198 in N. P. Kristensen, ed. Lepidoptera, moths and butterflies. Vol. 1. Evolution, systematics and biogeography. Walter de Gruyter, Berlin.

Endler, J. A. 1978. A predator's view of animal color patterns. Evol. Biol. 11:319–364.

———. 1988. Frequency-dependent predation, crypsis and aposematic coloration. Philos. Trans. R. Soc. Lond. B 319:505–523.

———. 1991. Interactions between predators and prey. Pp. 169–196 in J. R. Krebs and N. B. Davies, eds. Behavioural ecology: an evolutionary approach. 3rd ed. Blackwell, Oxford, U.K.

Enquist, M., and A. Arak. 1998. Neural representation and the evolution of signal form. Pp. 21–87 in R. Dukas, ed. Cognitive ecology. Univ. of Chicago Press, Chicago.

Epstein, M. E., H. Geertsema, C. M. Naumann, and G. M. Tarmann. 1999. The Zygaeonidea. Pp. 159–180 in N. P. Kristensen, ed. Lepidoptera, moths and butterflies. Vol. 1. Evolution, systematics and biogeography. Walter de Gruyter, Berlin.

Gamberale, G., and B. S. Tullberg. 1998. Aposematism and gregariousness: the combined effect of group size and coloration on signal repellence. Proc. R. Soc. Lond. B 265:889–894.

———. 1999. Experienced chicks show biased avoidance of stronger signals: an experiment with natural colour variation in live aposematic prey. Evol. Ecol. 13:579–589.

Gittleman, J. L., and P. H. Harvey. 1980. Why are distasteful prey not cryptic? Nature 286:149–150.

Guilford, T. 1990. Evolution of aposematism. Pp. 23–61 In D. L. Evans and J. O. Schmidt, eds. Insect defences. State University of New York Press, Albany, NY.

Haykin, S. 1999. Neural networks: a comprehensive foundation. 2d ed. Prentice Hall, Upper Saddle River, NJ.

Hines, W. G. S. 1987. Evolutionary stable strategies: a review of basic theory. Theor. Popul. Biol. 31:195–272.

Järvi, T., B. Sillén-Tullberg, and C. Wiklund. 1981. The cost of being aposematic: an experimental study of predation on larvae of *Papillio machaon* by the great tit *Parus major*. Oikos 36: 267–272.

Kitching, I. A., and J. E. Rawlins. 1999. The Noctuoidea. Pp. 355–402 in N. P. Kristensen, ed. Lepidoptera, moths and butterflies. Vol. 1. Evolution, systematics and biogeography. Walter de Gruyter, Berlin.

Kristensen, N. P. 1999. Lepidoptera, moths and butterflies. Vol. 1. Evolution, systematics and biogeography. Walter de Gruyter, Berlin.

Kristensen, N. P., and A. W. Skalski. 1999. Phylogeny and palaeontology. Pp. 7–26 in N. P. Kristensen, ed. Lepidoptera, moths and butterflies. Vol. 1: Evolution, systematics and biogeography. Walter de Gruyter, Berlin.

Leimar, O., M. Enquist, and B. Sillén-Tullberg. 1986. Evolutionary stability of aposematic coloration and prey unprofitability: a theoretical analysis. Am. Nat. 128:469–490.

Lemaire, C., and J. Minet. 1999. The Bombycoidea and their relatives. Pp. 321–354 in N. P. Kristensen, ed. Lepidoptera, moths and butterflies. Vol. 1. Evolution, systematics and biogeography. Walter de Gruyter, Berlin.

Lindström, L., R. V. Alatalo, A. Lyytinen, and J. Mappes. 2001. Strong antiapostatic selection against novel rare aposematic prey. Proc. Natl. Acad. Sci. USA 98:9181–9184.

Lindström, L., R. V. Alatalo, J. Mappes, M. Riipi, and L. Vertainen. 1999. Can aposematic signals evolve by gradual change? Nature 397:249–251.

Mallet, J., and M. Joron. 1999. Evolution of diversity in warning color and mimicry: polymorphism, shifting balance, and speciation. Annu. Rev. Ecol. Syst. 30:201–233.

Mallet, J., and M. C. Singer. 1987. Individual selection, kin selection, and the shifting balance in the evolution of warning colours: the evidence from butterflies. Biol. J. Linn. Soc. 32:337–350.

Mappes, J., and R. V. Alatalo. 1997. Batesian mimicry and signal accuracy. Evolution 51:2050–2053.

Maynard Smith, J. 1982. Evolution and the theory of games. Cambridge Univ. Press, Cambridge, U.K.

Merilaita, S. 2003. Visual background complexity facilitates the evolution of camouflage. Evolution 57:1248–1254.

Merilaita, S. and V. Kaitala. 2002. Community structure and the evolution of aposematic coloration. Ecol. Lett. 5:495–501.

Merilaita, S., J. Tuomi, and V. Jormalainen. 1999. Optimisation of cryptic coloration in heterogeneous habitats. Biol. J. Linn. Soc. 67:151–161.

Merilaita, S., A. Lyytinen, and J. Mappes. 2001. Selection for cryptic coloration in a visually heterogeneous habitat. Proc. R. Soc. Lond. B 268:1925–1929.

Nordström, F., E. Wahlgren, and A. Tullgren. 1941. Svenska Fjär-

ilar. Systematisk bearbetning av Sveriges storfjärilar Macrolepidoptera. Nordisk Familjeboks Förlag AB, Stockholm.

Roper, T. J., and S. Redston. 1987. Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance learning. Anim. Behav. 35:739–747.

Rothschild, M. 1972. Colour and poisons in insect protection. New Sci. 54:318–320.

Tullberg, B. S., and A. F. Hunter. 1996. Evolution of larval gregariousness in relation to repellent defences and warning coloration in tree-feeding Macrolepidoptera: a phylogenetic analysis based on independent contrasts. Biol. J. Linn. Soc. 57: 253–276.

Turner, J. R. G. 1984. Mimicry: the palatability spectrum and its consequences. Pp. 141–165 *in* R. I. Vane-Wright and P. R. Ackery, eds. The biology of butterflies. Academic Press, London.

Wickman, P-O. 1992. Sexual selection and butterfly design: a comparative study. Evolution 46:1525–1536.

Wiklund, C., and T. Järvi. 1982: Survival of distasteful insects after being attacked by naïve birds: a reappraisal of the theory of aposematic coloration evolving through individual selection. Evolution 36:998–1002.

Yachi, S., and M. Higashi. 1998. The evolution of warning signals. Nature 394:882–884.

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