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Batesian Mimicry: Selective Advantage of Color Pattern

Abstract. *Field studies of releases and recaptures of diurnal moths painted with yellow to resemble the edible tiger swallowtail and of black moths that resemble a toxic species of swallowtail produced these results: (i) A greater proportion of the black moths were recaptured; (ii) daily trapping for a week after each release showed that the black moths survived longer than the yellow-painted moths; (iii) an analysis of wing injuries shows that most attacks can be attributed to birds and that the yellow-painted moths were attacked more often, more vigorously, or more persistently than the black moths. These results are interpreted as showing a greater predation pressure on the yellow-painted than on the black moths and, therefore, as confirming the Batesian theory of mimicry.*

The concept of Batesian mimicry has aroused interest for more than a century. Observational, theoretical, and laboratory studies with caged predators have demonstrated the apparent effectiveness of Batesian mimicry in protecting butterflies, flies, and beetles from birds, toads, and lizards (*1*). Other experiments with caged predators and artificially created models and mimics (*2*) also support the assumption that Batesian mimicry is effective in the wild. These studies constitute a strong but not conclusive case. The obvious next step is the experimental demonstration and measurement of the selective advantages of Batesian mimicry in nature, but, because of difficulties with experimental design, this has yet to be accomplished.

Jones (*3*) and Kettlewell (*4*) showed that the color patterns of insects can affect the predatory responses of insectivorous birds in the field. Jones offered dead insects of various species to wild birds, finding that insects with aposematic color patterns were rejected and that such insects were often distasteful. Kettlewell's studies on industrial melanism in moths demonstrated that birds act as selective agents on insect populations with color morphs that have different adaptive values as camouflage.

Brower and his co-workers did a series of field experiments on Trinidad in order to test experimentally the Batesian mimicry theory (*5, 6*). They released black diurnal males of the edible North American saturniid moth *Callosamia promethea* which had been painted with various patterns on the upper surface of the wings. Some had been painted to resemble unpalatable butterflies found on Trinidad; others had similar quantities of black paint applied to the black areas of the wings so as not to change their appearance. They made the reasonable assumption that differential recaptures indicated differential predation. They concluded that their experiments had not demonstrated that mimics have a selective advantage in the field.

However, Waldbauer and Sternburg (*7*) contended that the experiments by the Brower group can be interpreted differently and that similar techniques could demonstrate mimetic advantage. They argued that the presumed control moths, although edible and essentially unaltered in appearance, were protected from predation by their similarity to the toxic, aristolochia-feeding *Battus* spp. of Trinidad, especially the abundant *B. polydamas*. That is, the Brower group actually compared artificial mimics of one model with mimics of another model. According to this interpretation, the Brower group's results (*5*) would be expected if promethea males are actually mimics of *Battus* spp.

Table 1. Number of moths of each painted type recaptured on each day after their release. The difference between the number of yellow- and black-painted moths recaptured is significant ($\chi^2 = 14.7$, d.f. = 6, $P < .025$).

Category	Days after release							Total
	0	1	2	3	4	5	6	
Black-painted	54	26	12	4	2	2	2	102
Yellow-painted	54	7	3	5	3	3	0	75

We now report that, with the proper controls, the release and recapture of painted promethea can demonstrate the selective advantage of one color pattern over another under natural conditions. Furthermore, because our controls were painted to resemble a palatable butterfly found in the experimental area, our results strongly support the Batesian mimicry hypothesis.

Waldbauer and Sternburg (*7*) presented evidence that male promethea belong to a well-known Batesian mimicry complex for which *Battus philenor* is the model. This complex includes as mimics all females of *Papilio troilus*, *P. polyxenes*, and *Speyeria diana*; the black females of *P. glaucus*; and both sexes of *Limenitis arthemis astyanax* (*8*). *Battus philenor* is moderately abundant in central Illinois, and all of the mimetic butterflies in the complex are common here except for the more southern *S. diana*.

We released and recaptured painted male promethea (*9*). Pupae were stored during the winter in an outdoor insectary. The moths emerged between mid-May and late August. A limited supply necessitated a design with only two groups of males. Those of one group were painted to resemble the yellow morph (a color form) of the palatable tiger swallowtail, *P. glaucus*; the others, resembling the toxic blue swallowtail, *B. philenor*, were essentially unaltered in appearance although they bore a comparable amount of black paint (Fig. 1). The flight of male promethea closely resembles that of the swallowtails, but near-perfect realism is impossible in painting the moths to resemble yellow tiger swallowtails. However, birds generalize patterns and avoid potential prey that only vaguely resemble the unpalatable model (*10*). Therefore, we applied a suggestive pattern (Fig. 1), which deceives the human eye at a distance of 5 or 6 m. The standard release point was the center of a circle (1.6 km diameter) of seven evenly spaced traps. Since native promethea are rare (*11*) in this area, there was little interference from wild, pheromone-releasing females.

On 12 days between late June and early September we released a total of 436 moths in groups of between 14 and 50 consisting of equal numbers of the two painted types. The traps were checked daily for at least 7 days after each release. From the total number recaptured (177), we calculated recapture

Table 2. Total recapture of black-painted and yellow-painted promethea males.

	Black		Yellow		Total	
	No.	%	No.	%	No.	%
Recaptured	102	58	75	42	177	40
Not recaptured	116		143		259	
Total released	218		218		436	

frequencies and the length of time each moth was in the field before recapture. Of the recaptured moths, 122 were pinned and spread for analysis of any injury to their wings. Fifty-five were excluded from the latter analysis for various reasons: A few were damaged in handling, some had been partially eaten in the traps by predaceous tettigoniids, and 12 may have been injured by a bird trapped with them. The proportions of black- and yellow-painted moths excluded did not depart significantly from a random distribution (chi-square test).

We recaptured an excess of black-painted moths ($\chi^2 = 6.43$, d.f. = 1, $P < .025$). Thus, the black-painted moths, which resembled a toxic swallowtail, had a selective advantage over yellow-painted moths, which resembled a morph of the edible tiger swallowtail. We assume that the differential recapture indicates differential predation by visually orienting, diurnal predators, almost surely birds. Of the 177 recaptured males, 69 spent one or more nights in the field (Table 1) and thus could have been exposed to nocturnal predators. However, the effect of nocturnal predators was probably negligible because, at night, promethea males are inactive and hidden among foliage. The data for all days on which recaptures were made are lumped in Table 2, since a χ^2 test indicated homogeneity ($.30 > P > .20$).

In the field the black-painted moths were more likely to survive beyond the day of release than were the yellow-painted moths (Table 1). This finding further indicates the selective advantage of the moths that resembled *B. philenor*.

Most wing injuries to recaptured moths suggest bird damage; more than 40 percent (beak-shaped rips, for example) are identical to known bird damage on moth wings (*12*). None of the injuries are inconsistent with the bird damage hypothesis, and few can be attributed to accidents such as flying into obstacles. Male promethea allowed to fly freely in a large cage for several days suffered relatively little damage to their wings, and this damage does not resemble the damage to the recaptured males.

The yellow-painted moths were more likely to have wing injury and were more severely injured than the black-painted moths (Fig. 1). Fifty-nine percent of the blacks were uninjured or only slightly injured ($\leqq 0.5$ cm^2 missing), and only 8 percent were severely injured (> 2 cm^2 missing). On the other hand, none of the yellows were uninjured or only slightly injured, and 59 percent were severely injured.

Twenty-seven recaptured moths (11 black- and 16 yellow-painted) had matching notches on opposite wings or other bilaterally symmetrical injuries that could have occurred only if they were attacked while resting with the upper wing surfaces held together and hidden (*13*). Bilaterally symmetrical injuries, therefore, indicate attacks by predators that may not have seen the painted pattern. This possibility is consistent with our finding that the proportions of black- and yellow-painted moths with bilaterally symmetrical injuries do not differ significantly ($\chi^2 = 1.1$, d.f. = 1, $.30 > P > .20$). Eliminating moths with recognizable bilaterally symmetrical injuries from the data resulted in little change with respect to the black-painted moths, although it resulted in an increase in the proportion of severely injured yellow-painted moths because more than half of the eliminated yellow-painted moths were from the categories with the least injury (Fig. 2).

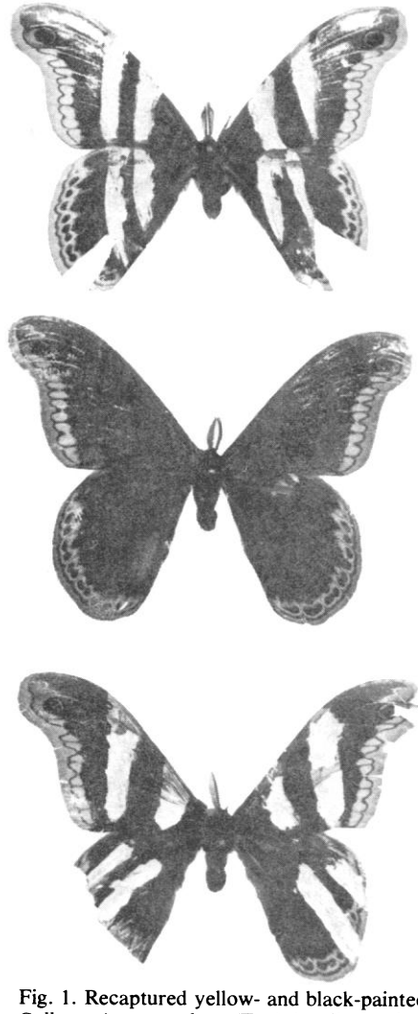

Fig. 1. Recaptured yellow- and black-painted *Callosamia promethea*. (Top) A yellow-painted moth with symmetrical injury to the hind wings; (center) an uninjured black-painted moth; (bottom) moth with asymmetrical injury to the hind wing.

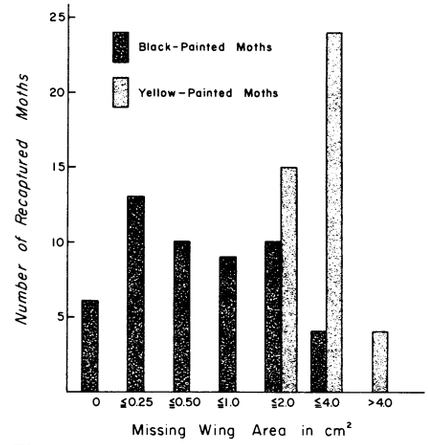

Fig. 2. The amount of wing injury to black- and yellow-painted *Callosamia promethea* released and recaptured in a central Illinois woodland. These data do not include moths with bilaterally symmetrical injury.

There are two possible interpretations of our results. Either our black-painted moths were protected by their resemblance to *B. philenor* or they were simply less conspicuous to predators than the yellow-painted moths (*14*). Brower and his co-workers (*5, 6*) believed that the latter interpretation was demonstrated by their experimental comparison of black-painted controls with conspicuously painted moths, which they said did not resemble any Trinidad butterfly. However, Waldbauer and Sternburg (*7*) argued that the data of the Brower group (*5*) support the Batesian mimicry theory, pointing out that their controls actually closely resembled unpalatable Trinidad species of *Battus*, while their conspicuously painted moths differed from the controls not only in conspicuousness but also in their resemblance to several palatable Trinidad butterflies.

Our experimental design and results differ from those of the Brower group in at least three important ways. (i) Our palatable control moths had been painted to resemble a palatable butterfly that occurs in the experimental area, whereas the Brower group's controls were male promethea essentially unaltered in appearance. Our control is essential because male promethea closely resemble toxic butterflies found on Trinidad or in

central Illinois. (ii) We recaptured a significantly smaller proportion of the yellow-painted control moths (Table 2). (iii) We found that the control moths were more often and more severely injured than the experimental moths (Fig. 2), and that much of this injury can be attributed to attacks by birds. We believe that the most plausible interpretation of our results is that the black-painted moths were protected by their resemblance to the toxic *B. philenor* and that the yellow-painted moths were more frequently attacked because of their resemblance to the palatable and nonmimetic yellow form of the tiger swallowtail.

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9. Each day before 2:00 p.m., the newly emerged moths were placed in envelopes and stored at 4°C until they were used (within 3 days). Undamaged males were painted on the dorsal surface of the wings with Flopaque paint (Flo-quil Products, Inc., Cobbleskill, N.Y.). An inconspicuous mark on the thorax distinguished each day's release. Painted moths were stored in a cage at 4°C until the next morning. After being allowed to warm up, they were released in the experimental area at the University of Illinois's Robert Allerton Park near Monticello, Illinois. Each trap consisted of a wire box (61 by 71 by 71 cm) with two staggered and inwardly directed funnels on opposite sides of the trap. Each trap contained a small cage baited with at least two pheromone-releasing promethea females. The area within the circle of traps was a mixture of upland and floodplain forest and a small area of prairie. A large and varied population of birds inhabits the area in summer (J. Bursewicz, thesis, University of Illinois, 1961). Unlike the Brower group, we did not release moths again after they had been recaptured.
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11. Only two unmarked wild male promethea were captured in the pheromone traps during the entire summer.
12. T. D. Sargent [*J. Lepid. Soc.* **27**, 175 (1972)] presents photogaphs of the damage to moth wings resulting from observed attacks by caged birds.
13. *Callosamia promethea* always rests with the wings held together vertically, and never holds them in the flexed position typical of many moths.
14. To our eyes, the black butterflies and moths are very conspicuous in flight, probably no less conspicuous than the yellow ones.
15. Supported in part by the Graduate Research Board of the University of Illinois at Urbana, and by NIH Biomedical Sciences Support grant RR 07030-09. We thank Dr. W. Keith and F. Turok for making available the natural areas of Robert Allerton Park. We also thank Dr. W. H. Luckmann and the Illinois Natural History Survey for facilities used to rear *Callosamia promethea*.

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Selective Display of Histamine Receptors on Lymphocytes

Abstract. *Histamine, acting on histamine type 2 receptors, increases intracellular cyclic adenosine monophosphate (AMP) and thus modulates the immunologic functions of lymphocytes. Lymphocyte cyclic AMP levels were used to follow the development of histamine receptors. The B lymphocytes have no functional histamine receptors. As T lymphocytes "mature" in immunologic function—from thymocytes to cortisone-resistant thymocytes to splenic T lymphocytes—their response to histamine increases. The response of these subpopulations of lymphocytes to isoproterenol is the inverse of the histamine response. It is suggested that the changing display of histamine receptors plays an important part in the control of immunologic responses.*

Histamine is a low-molecular-weight hormone, widely distributed in mammalian tissues, and is released from storage sites by immunologic and other stimuli (*1, 2*). The activities of histamine are mediated through two specific receptors which can be distinguished by the competitive effects of specific histamine type 1 or histamine type 2 antagonists. Stimulation of histamine type 1 receptors causes a variety of well-recognized "pro"-inflammatory events. At higher concentrations histamine mediates several "anti-inflammatory" effects via histamine type 2 receptors (*2*).

Lymphocytes and other inflammatory cells have specific receptors for a wide variety of hormones (*3*), including histamine. Histamine inhibits the in vitro cytolytic (tumor destroying) activity of alloimmunized effector T spleen cells, and this effect is paralleled by an increase in cyclic adenosine monophosphate (AMP) levels in lymphocytes. Both effects are blocked by the histamine type 2 antagonists burimamide and metiamide, suggesting that histamine acting through specific histamine type 2 receptors activates adenylate cyclase, and that the resulting increase in intracellular cyclic AMP leads to the inhibition of cytotoxic activity (*4*). Histamine can inhibit other lym-

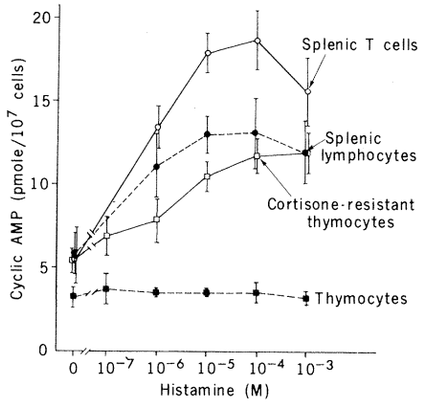

Fig. 1. Young adult age-matched C57B1/6 mice were divided into three groups. For preparation of cortisone-resistant thymocytes, one group of mice received an intraperitoneal injection of 10 mg of cortisone acetate suspension (Merck). Twenty-four hours later, these mice were killed. Thymocytes were also obtained from a second group (untreated mice). The cell yield in cortisone-resistant thymus glands was approximately 1×10^7 (that is, 5 percent of the cell yield in thymuses of untreated mice). A third group of mice was used as a source of spleen cells. The spleen cells were treated with ammonium chloride to remove red blood cells and were then either filtered through glass wool to deplete adherent cells (*14*) (splenic lymphocytes), or filtered through glass wool and then incubated for 45 minutes at 37°C in nylon wool columns (*14*), and the effluent, nonadherent cells were obtained (T cell–enriched splenic lymphocytes, designated "splenic T cells"). All cell preparations were then resuspended to 2×10^7 viable cells per milliliter and incubated for 10 minutes with or without histamine. Intracellular cyclic AMP was assayed as described in the text. The percentage of immunoglobulin (Ig) positive cells (that is, B cells) was estimated by direct immunofluorescence staining by rhodamine-conjugated goat antiserum to mouse Fab fragments. The preparation and properties of this antiserum have been described (*21*). Unfractionated spleen cells contained 55 percent of Ig-positive cells, while T cell–enriched spleen cells contained 19 percent Ig-positive cells. Similar results were obtained in a total of four experiments with cortisone-resistant thymocytes, and in a total of three experiments with spleen cells that were passed through nylon wool. (○) T cell-enriched splenic lymphocytes; (●) splenic lymphocytes; (□) cortisone-resistant thymocytes; (■) thymocytes of normal mice. Each point represents the mean ± standard deviation of quadruplicate determinations.