

Aposematic Coloration of Gregarious Insects Can Delay Predation by an Ambush Predator

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ABSTRACT An important hypothesis concerning the role of aggregation in the antipredator defense of aposematic insects is that a group projects a larger aposematic signal to a predator. The nearly exclusive use of birds as model predators may be leading us to over-emphasize the importance of signal amplification as an explanation for the gregarious behavior of aposematic insects. Ambush (sit-and-wait) predators such as amphibians and praying mantids make predatory decisions relying primarily on prey movement, and secondarily on prey size, with prey color being less important. Therefore, we tested the interaction of aposematic coloration and gregarious behavior in insect defense from frogs. We offered frogs four types of mealworm prey: (1) cryptic and solitary, (2) cryptic and gregarious, (3) aposematic and solitary, and (4) aposematic and gregarious. The frogs ate aposematic and gregarious prey significantly later than they ate cryptic and gregarious prey and cryptic and solitary prey. Our results support the hypothesis that aposematic coloration in gregarious prey, but not in solitary prey, can function to produce a sufficient aposematic signal to delay attack by an ambush predator. This result was not due to predator learning. Hence, the antipredator benefits of aposematic coloration in aggregated prey may function in encounters with a wide range of predators, including frogs.

KEY WORDS warning coloration, aggregation, chemical defense, frogs, ambush predator

THE PUTATIVE INTERACTION of aposematic coloration and gregarious behavior in the defense of insects has been the subject of much theoretical work (reviewed in Vulinec 1990) but less experimental work. An important hypothesis concerning the role of aggregation in the antipredator defense of aposematic insects is that a group projects a larger aposematic signal to a predator. Hence, individuals within groups are attacked later or less often than singletons, even if they are located more rapidly (Sillén-Tullberg 1990; Gamberale and Tullberg 1996, 1998; Mappes and Alatalo 1997). In addition, gregarious behavior seems to have antipredator benefits to aposematic prey when in concert with chemical defense (Alatalo and Mappes 1996; but see Tullberg et al. 2000), which often exists with conspicuous coloration. Together, these studies have suggested that aggregations play a special role in the antipredator ensemble that includes aposematic coloration. However, all of the experiments cited above used birds as predators.

Although birds exert strong predation pressure on insects (Schuler 1990), so do a host of other predators. One review suggested that reptiles, bats, and fishes are more important vertebrate predators of insects than birds (Schoenly 1990). Several studies on insect defenses have employed non-avian predators (e.g., Brower and Brower 1962, Johki and Hidaka 1979, Bowers and Stamp 1997, Hatle and Faragher 1998, Strohmeyer et al. 1998), but these studies have not addressed the interaction of aposematic coloration and gregarious behavior.

The nearly exclusive use of birds as model predators may be leading us to over-emphasize the importance of signal amplification as an explanation for the gregarious behavior of aposematic insects. Ambush (sit-and-wait) predators such as amphibians (Roth 1986, Ewert 1987) and praying mantids (Rilling et al. 1959) make predatory decisions relying primarily on prey movement, and secondarily on prey size, with prey color being less important. For example, leopard frogs attack only moving prey (Anderson 1993), and frogs will starve rather than feed in a cage filled with dead flies (Roth 1986). Although frogs are primarily motion-oriented predators, they have retinal cones for color vision (Duellman and Trueb 1986) and can presumably see color. In contrast to frogs, birds are searching (seek-and-find) predators that use prey size, quality, and frequency in predatory decisions (Schuler 1990). Several studies have shown that birds use color in predatory decisions by demonstrating that birds learn to avoid unpalatable, aposematic prey (e.g., Gittleman and Harvey 1980, Roper and Wistow 1986).

Birds have been shown to attack aggregations of aposematic prey less often than either aggregations of cryptic prey or solitary, aposematic prey (Gamberale and Tullberg 1998). Because frogs rely on different prey characteristics to release their attack response, one could predict that frogs would respond differently to aposematic and gregarious prey. Specifically, we predicted that frogs would not be significantly deterred by aposematic coloration, gregarious behavior, or the interaction of aposematic coloration and gregarious behavior. Therefore, we tested the interaction

of aposematic coloration and gregarious behavior in insect defense from frogs. We predicted *a priori* that the warning signal of aggregations of aposematic prey would not be sufficient to deter frog predatory behaviors in comparison to both aggregations of cryptic prey and solitary, aposematic prey.

Materials and Methods

We used the American bullfrog, *Rana catesbeiana* Shaw, as a model predator. We collected 22 postlarval bullfrogs from a small pond near Route 8 and north of Lake Evergreen in McLean County, IL. Initially, they were kept communally in a large plastic container (75 by 50 by 50 cm) at $22 \pm 2^\circ\text{C}$. The frogs were offered two crickets each twice weekly for 2 wk.

Approximately 10 d before the start of the experiment, we transferred 22 frogs to individual plastic containers (30 by 30 by 10 cm) with 150 ml of pea gravel and 250 ml of aged tap water and held them at $22 \pm 2^\circ\text{C}$ and a natural photoperiod. Each frog was offered a larval *Tenebrio molitor* (L.) 3 d before the beginning of the experiment. We offered these prey to the frogs in the same way that the experimental trials would be performed, to assess whether the frogs would feed. This initial feeding was to ensure that all frogs began the experiment at similar hunger levels and that the prey would be recognized as potential prey. One frog failed to attack the prey in this design and was therefore dropped from the study (final $n = 21$).

We sought to test whether the increased aposematic signal arising from gregarious, aposematic insects would confer an increased deterrence of predatory attacks. To accomplish this, we established five prey treatments for this experiment: (1) cryptic and solitary = singleton painted dark brown, (2) cryptic and gregarious = group of 10 all painted dark brown, (3) aposematic and solitary = singleton painted bright red, (4) aposematic and gregarious = group of 10 all painted bright red, (5) cryptic and solitary and motionless = singleton painted dark brown, and frozen. Mealworms were painted with a Uni Paint oil-based paint marker (Mitsubishi Pencil Co., Bellwood, IL) the night before the experiment to ensure proper drying and reduce any potential odors caused by the paint. The experiments were conducted on white posterboard, so that both brown and red mealworms were equally conspicuous. We defined brown prey as *cryptic*, despite the fact that they were conspicuous on a white background, because brown prey are typically cryptic in their natural environment. The cryptic and solitary and motionless treatment was used as a negative control to demonstrate that the frogs were discriminating among prey. These nonmoving prey were frozen for ≈ 2 min (and then allowed to warm) before the start of the trial. Only large third- and small fourth-instar mealworms were used so that all prey were of similar size.

When presented to the frogs, mealworms were arranged in two petri dishes (5 cm in diameter), one placed on top of the other. When groups were pre-

sented, one mealworm was placed in the top dish (which was open) and the remaining nine were enclosed in the bottom dish. When a solitary mealworm was presented, it was placed in the top dish and the bottom dish remained empty (after Gamberale and Tullberg 1998). This was done to control any potential odors (i.e., so that there would be no difference in odor between the solitary or groups of mealworms). Hence, we specifically tested the visual affects of aposematic coloration and gregariousness. Our design also ensured that only one mealworm could be eaten per trial; therefore, subsequent trials were not affected by uncontrolled hunger levels. In addition, a thin layer of petroleum jelly was applied to the rim of the top dish to prevent the test mealworm from escaping.

This experiment was conducted in a randomized design, so that by the end of the experiment, each frog had received one of each of the five prey types in a random order. All the trials were conducted on a single day, and trials for an individual frog were not consecutive. We conducted a trial by transferring an individual frog to an 11-liter glass aquarium that was covered on the side facing the experimenter with a cardboard screen with a window in the center. The frog was placed under an opaque container until the trial began. One of the five mealworm treatments was placed directly in front of the opaque container and the trial begun immediately by removing the opaque container and starting the timer. We measured three predatory frog behaviors: (1) the time the frog first turned toward the prey (orientation), (2) the time the frog first projected its tongue at or moved toward the prey (snapping), and (3) the time when the prey was completely inside the frog's mouth (eating). In the remainder of the article, we refer to orientation, snapping, and eating collectively as *attack*. Each trial ended when the prey was eaten or at the arbitrary time limit of 180 s, whichever occurred first. Frogs that did not attack by the end of the trial were assigned 180 s as the time of attack.

The data did not meet the assumptions of normality and homogeneous variance. Using data transformations, we were able to meet the assumption of normality, but not the more important assumption of equal variance. Hence, we used Friedman's rank test (the nonparametric equivalent of a repeated-measures analysis of variance [ANOVA]) with Dunn's multiple comparisons post-test to analyze the data (Siegel and Castellan 1988). The test was performed with InStat version 3.01 (GraphPad Software 1998).

Results

The frogs ate none of the 21 frozen mealworms and all but one of the 84 live mealworms. Hence, we conclude that our negative control was effective, and we infer that the frogs were selectively attacking moving prey. Hereafter, we disregard the frozen mealworms in the analysis and discussion of our results.

Time to eating was significantly different among the four prey types (Fig. 1; overall $P = 0.0014$, $F_r = 15.59$). Frogs ate aposematic and gregarious prey significantly

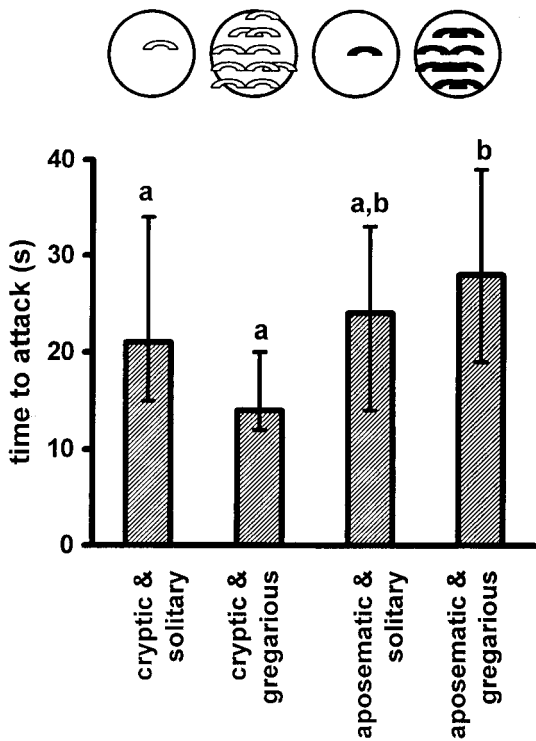


Fig. 1. Median \pm inter-quartile ranges to attack mealworm prey items by bullfrogs. Prey were in enclosed petri dishes so that all mealworms were visible but only one could be attacked. Letters above error bars indicate significant differences by a Friedman's test with Dunn's multiple comparisons posttest (Siegel and Castellan 1988) (the nonparametric equivalent of a repeated-measures ANOVA).

later than they ate cryptic and gregarious prey ($P < 0.01$) and cryptic and solitary prey ($P < 0.05$). No other pairwise comparisons of frog eating times were significantly different. The statistical analyses of the snapping and orientation responses showed qualitatively identical significant differences as eating responses, and hence the snapping and orientation data are not shown.

Discussion

Our results, contrary to our prediction, support the hypothesis that aposematic coloration in gregarious prey can function to produce a sufficient aposematic signal (Gamberale and Tullberg 1996, 1998) to delay attack by an ambush predator. Aposematic and gregarious prey were attacked significantly later than the cryptic and gregarious prey and cryptic and solitary prey (Fig. 1). In contrast, aposematic and solitary prey were not attacked significantly later than either type of cryptic prey. We used an ambush predator that we predicted would not be significantly deterred by aposematic coloration. Hence, the antipredator benefits of aposematic coloration in aggregated prey may function in encounters with a wide range of predators, including frogs.

In our experiments, the times at which frogs attacked aposematic and gregarious prey in laboratory trials were significantly delayed. In nature, this delay in predatory responses could increase prey survivorship by increasing the amount of time in which prey could escape. We hypothesize that prey survivorship in nature would be increased by the synergy of aposematic coloration and gregarious behavior during encounters with frogs.

The results of our experiment suggest that attack abatement of aposematic and gregarious prey does not necessarily rely on learning (e.g., Gittleman and Harvey 1980, Lindström et al. 1999), because each frog encountered each prey type only once. Whether the delay to attack aposematic and gregarious prey would increase, decrease, or remain the same with further trials is unknown. Because we did not train these frogs with the painted prey used in the experiment, our results may be in part attributable to prey novelty (sensu Coppinger 1969). We used artificial prey, so our results are not likely caused by novelty specifically to the prey we used. It may be, though, that the frogs were accustomed to brown prey relative to red prey, producing a delayed attack of red prey due to novelty. Regardless of the role of novelty, the aposematic and gregarious prey had an initial benefit in comparison to cryptic prey.

Phylogenetic studies have suggested that gregarious behavior evolved after aposematic coloration in larval lepidopterans (Sillén-Tullberg 1988). Hence, it seems more likely that aposematic coloration evolved before gregarious behavior in other aposematic insects, rather than vice-versa. In this light, one could predict that aposematic and gregarious prey in our study would be attacked significantly later than aposematic and solitary prey (from which they presumably evolved). The times at which frogs attacked these two prey groups were not significantly different in our study (rank sum difference = 19.5, $P > 0.05$). The nonsignificant trend was for frogs to attack aposematic and gregarious prey later than aposematic and solitary prey (specifically, 8.4 ± 4.6 s later), which is the predicted direction. We hypothesize that aposematic coloration is most effective in deterring ambush predators when prey are aggregated and producing a larger warning signal.

Our data suggest that frog predation, like bird predation, is delayed by the combination of aposematic coloration and gregarious behavior. Despite this similarity, we believe that experiments with nonavian predators will be important in developing our understanding of the aposematic defensive ensemble.

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