4-2008

EFFECTS OF GREGARIOUSNESS, CONSPICUOUSNESS, AND NOVELTY ON BLUE JAY (*CYANOCITTA CRISTATA*) LEARNED AVOIDANCE AND STIMULUS GENERALIZATION OF UNPALATABLE PREY

Joyce M. Dykema
University of Nebraska-Lincoln

Dykema, Joyce M., "EFFECTS OF GREGARIOUSNESS, CONSPICUOUSNESS, AND NOVELTY ON BLUE JAY (*CYANOCITTA CRISTATA*) LEARNED AVOIDANCE AND STIMULUS GENERALIZATION OF UNPALATABLE PREY" (2008). *Avian Cognition Papers*. 9.
http://digitalcommons.unl.edu/bioscaviancog/9

EFFECTS OF GREGARIOUSNESS, CONSPICUOUSNESS, AND NOVELTY ON BLUE JAY (*CYANOCITTA CRISTATA*) LEARNED AVOIDANCE AND STIMULUS GENERALIZATION OF UNPALATABLE PREY

by

Joyce M. Dykema

A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Biological Sciences

Under the Supervision of Professor Alan C. Kamil

Lincoln, Nebraska

April, 2008

EFFECTS OF GREGARIOUSNESS, CONSPICUOUSNESS, AND NOVELTY ON BLUE JAY (*CYANOCITTA CRISTATA*) LEARNED AVOIDANCE AND STIMULUS GENERALIZATION OF UNPALATABLE PREY

Joyce Marie Dykema, M.S.

University of Nebraska, 2008

Adviser: Alan C. Kamil

I examined a variety of factors hypothesized to be important in the evolution and maintenance of aposematism. Aposematism occurs when prey individuals advertise their toxic or otherwise aversive nature to potential predators via evolved conspicuous signals. I conducted three experiments in which blue jays (*Cyanocitta cristata*) were allowed to search a printed grayscale pixilated background for grayscale pixilated moths in an open room. I manipulated moth appearance and food reward, and recorded jay predation on the varying moth stimuli. In my first experiment, I repeated Alatalo & Mappes' (1996) study examining the effects of prey gregariousness, or grouping, on predation rates of cryptic (difficult to detect) palatable, cryptic unpalatable, and conspicuous (easy to detect) unpalatable (aposematic) artificial prey. I found that gregariousness does not provide a benefit to prey, suggesting gregariousness did not facilitate the initial evolution of aposematism, in contrast to Alatalo & Mappes (1996). My second study investigated why predation on aposematic prey was continually low in experiment 1. I found that the moth stimuli used in experiment 1 were truly cryptic and conspicuous, so the low predation on conspicuous unpalatable (aposematic) moths in experiment 1 was likely due to very rapid learned avoidance of aposematic prey. Finally, in experiment 3, I asked whether jays from experiment 1 and 2 would attack novel cryptic and conspicuous moths differently based on their prior experience: experience with unpalatable food (experiment 1), or no experience with unpalatable food (experiment 2). Jays that had experienced unpalatable moths previously attacked significantly more novel cryptic moths than novel conspicuous moths, both overall and in the first attack of the first trial. In contrast, jays that had not experienced unpalatable moths previously attacked significantly more novel conspicuous moths than novel cryptic moths. This may suggest a conspicuousness-dependent generalization threshold for food aversions.

Acknowledgements

I would first like to thank my advisor, Al Kamil, for his continuous support throughout my graduate career. When I was struggling to continue experiments that just weren't working, Al encouraged me to keep going and then supported me when we realized they were never going to work and I had to start again from scratch. Al's critical thinking, attention to detail, and his astounding ability to identify the slightest nuances in scientific arguments are challenging and inspiring, and I feel privileged to have worked with him.

My committee members have been wonderful; encouraging, always willing to discuss a problem or issue with me, and I have greatly appreciated the time and energy they each spent advising my work. Alan Bond allowed me to be creative with my ideas and also taught me practically all I know about computer programming. Bill Wagner always had time for me, whether I needed to bounce ideas off of someone or help with a specific statistical problem. Svata Louda's "outsider's" perspective challenged me to reexamine and clarify my own ideas as well as the many conventions within my field, and her compassion helped make my time at UNL easier and more enjoyable. John Flowers and Larry Harshman both served on previous versions of my committee, and they both stimulated my scientific curiosity in amazingly diverse directions. In addition to these faculty members, I received much needed statistical instruction and clarification from Robert Gibson and Cal Garbin, and Eileen Hebets' behavioral ecology course was a breath of fresh air in a time when I needed it most. Thank you to these and all the other professors who have influenced me and my work.

A huge thank you to all of my lab mates, post-docs, graduate students, technicians, and undergraduates, both past and present. I am very lucky to have worked with such a huge group of people who not only do excellent research but also care deeply about the well-being of our animals. I want to especially thank our lab technicians, particularly Marianna Burks, for always keeping the health of our birds a priority and for being so knowledgeable and helpful at the same time. Special thanks to Jody Lewis, Lynnsey Morrison, Christine Muller, Kate Webbink and Cindy Wei, who have been great friends as well as great coworkers.

To the many friends and family members who have cared about me and encouraged me in pursuing my education, thank you, and I love you all.

Thank you to the blue jays, who made every day worthwhile and for teaching me how to whistle.

My husband, Justin Dykema, never ceases to encourage me. He has helped me throughout this process in ways I cannot count or describe. Thank you for being you. I love you so much, and I’m looking forward to the next chapter of our life together.

Finally, to God. I pray that my work has pleased you, and I thank you for all that you do for me. I couldn’t have done this without you.

Table of Contents

Abstract i

Acknowledgements iii

Chapter 1: Introduction 1

- Gregariousness 2
- Dietary Wariness 6

Chapter 2: Experiments 11

- General methodology for all experiments 12
- Experiment 1: Effects of unpalatability on predator food choices 18
- Experiment 2: Relative visibility differences of cryptic and conspicuous moths 22
- Experiment 3: Effects of prior experience on palatable novel cryptic and conspicuous prey acceptance 25
- General Discussion 29

Figures and Tables 33

References 49

Chapter 1: Introduction

Aposematism occurs when individuals advertise their aversive nature via evolved, conspicuous signals to potential predators. As a result, predators recognize and can avoid unprofitable or toxic prey while prey can avoid predation by informed predators (Darwin, 1881; Joron, 2002). Aposematism is widespread and has evolved independently many times in distantly related taxa, perhaps because of its value to predator and prey. Despite this ubiquity, however, the question of how aposematism evolved remains unresolved (Guilford, 1992; Joron, 2002; Lindstrom, Alatalo, Mappes, Riipi, & Vertainen, 1999; Sillen-Tullberg, 1988).

A generally accepted assumption is that cryptic and palatable is the ancestral state, and aposematism is derived (Guilford, 1990; Harlin & Harlin, 2003). This hypothesis is suggested by phylogenetic evidence. Phytophagy in insects appears derived from predatory, parasitic, or detritivorous ancestors (Farrell, Dussourd, & Mitter, 1991; Mitter, Farrell, & Wiegmann, 1988) and once phytophagy evolved in insects, plants began evolving anti-herbivory defenses, such as latex and resin canals (Farrell et al., 1991). As it is well known that many aposematic organisms sequester their protective toxins from consuming defended plant sources (for example: Brower, Brower, & Corvino, 1967), it seems likely that, at least in insects which sequester toxins, the ancestral state is palatable and presumably cryptic. Although the current consensus is that aposematism is derived, the specific selection regimes that favor evolution from cryptic and palatable to aposematic are still strongly debated (Alatalo & Mappes, 1996, 2000; Tullberg, Leimar, & Gamberale-Stille, 2000).

The theoretical problem concerning how aposematism first evolved arises because aposematism incorporates two traits, aversiveness and conspicuousness, neither of which appears adaptive by itself. A conspicuous mutant individual in a population of cryptic individuals would be at a selective disadvantage because of its increased probability of detection, regardless of whether or not aversive traits have already evolved. A mutant aversive but cryptic individual, indistinguishable visually from its palatable conspecifics, would not be avoided because predators have no opportunity to learn its aversive nature (Krebs & Davies, 1993). In addition, such an organism would bear the fitness or fecundity costs of aversiveness (Joron, 2002; Mallet, 1999). Therefore, while we understand how aposematic prey and cryptic and palatable prey exist, the initial evolution of aposematism remains problematic. Many hypotheses have been proposed to explain why aposematism is so widespread despite the inherent difficulties in imagining its origination. These hypotheses cover a variety of topics, including prey gregariousness and predator dietary wariness.

Gregariousness

Many aposematic organisms are found in large conspicuous aggregations (Reader & Hochuli, 2003; Ruxton & Sherratt, 2006). Aggregations of prey enhance the aposematic signal (Gagliardo & Guilford, 1993; Gamberale & Tullberg, 1998; Hatle & Salazar, 2001), result in reduced antiapostatic selection (Lindstrom, Alatalo, Lyytinen, &

Mappes, 2001), and although grouping increases the risk of being detected (Gamberale & Tullberg, 1996), it also results in a dilution effect (Riipi, Alatalo, Lindstrom, & Mappes, 2001). Gregariousness may also be important for the initial evolution of aposematism (Alatalo & Mappes, 1996, 2000; Mappes & Alatalo, 1997), or may not be (Skelhorn & Ruxton, 2006; Tullberg et al., 2000). In some instances, gregariousness and kin or green-beard (individuals gain indirect fitness from unrelated individuals that share a particular phenotype (Dawkins, 1976)) selection might be necessary to allow the evolution of conspicuousness in aversive yet cryptic prey (Alatalo & Mappes, 1996; Fisher, 1958; Joron, 2002). An aversive, cryptic individual in an aggregation that is attacked by a naïve predator may confer a survival benefit to other individuals in the group because the predator should subsequently avoid attacking others in the aggregation. However, there is also evidence that individual selection is sufficient to produce aposematism (Joron, 2002; Sillen-Tullberg, 1988; Tullberg et al., 2000), perhaps because aversive prey that are attacked often survive to produce offspring themselves (Wiklund & Jarvi, 1982).

The aposematism research group headed by Dr. Johanna Mappes at the University of Jyvaskyla in Finland has investigated whether group or individual selection is most likely to lead to the evolution of aposematism. Alatalo & Mappes (1996) investigated what prey conditions are necessary for the evolution of aposematic coloration under Great tit (*Parus major*) predation. In their key experiment, tits preyed upon three different prey types: cryptic and palatable, cryptic and unpalatable, and conspicuous and unpalatable, or aposematic. Tits encountered prey either arranged solitarily or aggregated in groups of four. Gregariousness led to a significantly lower relative predation rate for cryptic and unpalatable and aposematic prey, leading the authors to hypothesize that aggregation was necessary for the initial evolution of warning coloration via the following pathway: unpalatability, then gregariousness, then warning coloration. However, there are methodological issues that bring these results into question.

The prey stimuli used in Alatalo & Mappes (1996) were camouflaged mimetically, rather than cryptically. Mimetically camouflaged prey are prey that resemble a specific feature in the environment, like a walking stick that resembles a twig (Poulton, 1890). This type of camouflage is very different from crypsis/disruptive coloration, in which prey blend into their natural background, as noctuid moths seem to disappear into tree bark. Also, the prey stimuli used in this experiment were not truly camouflaged; prey consisted of a small piece of brown straw filled with animal fat with two white paper “wings,” with the cryptic or conspicuous symbol printed on them, attached to the ends of the straw. As the prey were presented to the tits atop a flat background of white paper printed with the cryptic symbol, the brown straw pieces were quite conspicuous, both in color and in space, regardless of their similarity or dissimilarity to the background symbol (Tullberg et al., 2000).

A reanalysis of Alatalo & Mappes’ (1996) data by Tullberg et al. (2000) suggested that the apparent benefit of gregariousness to cryptic and unpalatable prey was a result of tit foraging strategies, leading to higher use of aggregated palatable prey rather than a decreased use of unpalatable prey types. Once a bird encountered an unpalatable aggregation, it would not attack any more individuals from the group, but if it encountered a palatable aggregation, it would attack all items in the group. As a result, there was an increase in the relative proportion of cryptic and palatable prey that are attacked in gregarious conditions and an apparent, and not necessarily absolute, decrease in the relative proportion of cryptic and unpalatable prey that were attacked that disappeared once cryptic and palatable predation was removed from the analysis (Tullberg et al., 2000). Unfortunately, neither Alatalo & Mappes (1996) nor Tullberg et al. (2000) presented their data in a way that would allow us to distinguish between this and the possibility that grouping truly lends a benefit to cryptic and unpalatable prey. Tullberg et al. (2000) then conducted two new experiments using the same predator species, prey stimuli, and general methods to investigate whether there is a benefit of gregariousness to cryptic and unpalatable prey and whether tits differed in their predatory strategies when encountering solitary or aggregated cryptic and palatable and cryptic and unpalatable prey. They found no benefit of gregariousness to cryptic and unpalatable prey, suggesting the following order of evolutionary steps: unpalatability, then conspicuous coloration, which may or may not then lead to gregariousness.

In a reply to Tullberg et al. (2000), Alatalo & Mappes (2000) suggest that the conflicting findings of Tullberg et al. (2000) result from a difference in predator experience. Tullberg et al.’s (2000) great tits encountered all unpalatable prey in their first experiment, perhaps causing the birds to “eventually use them equally irrespective of prey dispersal” (Alatalo & Mappes, 2000, pg F2). Unfortunately, in their reply Alatalo & Mappes (2000) only reanalyzed the aposematic solitary and aggregated prey from their 1996 study. The fact that there is a benefit of aggregation for the aposematic prey stimuli does not address the issue addressed by Tullberg et al.’s (2000) paper: Is aggregation necessary for aposematism to first evolve from a cryptic and palatable organism or from an intermediate form? The existence of an aggregation benefit for forms that are already aposematic does nothing to shed light on whether gregariousness plays a significant role in the initial evolution of aposematism. This question is far from resolved.

Although Alatalo & Mappes (1996) and Tullberg et al. (2000) used the same predator, the same visual prey stimuli, and the same general procedures, Tullberg et al. (2000) is not a strict replication of Alatalo & Mappes (1996). Therefore, the reason why they obtained different, conflicting results may lie in the experimental differences in prey combinations presented to the predators. As aposematism is taxonomically widespread and presumably evolved under selection from a range of taxonomically and ecologically distinct predators, it is also important to examine these questions with multiple appropriate predators. Additionally, artificial prey stimuli designed to be more biologically relevant would elicit more natural predatory behaviors and strategies, while remaining evolutionarily novel so they would not elicit innate behaviors and preferences.

Dietary Wariness: Taste-aversion learning, Neophobia, and Dietary conservatism

Dietary wariness is a suite of behavioral phenomena in which predators show transient hesitance to approaching novel food items (neophobia) as well as reluctance to incorporate new foods into the diet, even after neophobic responses have disappeared (Marples, Quinlan, Thomas, & Kelly, 2007). Wariness may occur after predators have learned to avoid aversive foods (Schlenoff, 1984) or without such experience (Marples & Kelly, 1999; Smith, 1977).

Taste-aversion learning

Aversion learning is a phenomenon in which an animal learns to avoid foods that have been associated with illness or another aversive experience, like an unpleasant taste (Bernstein, 1999). Taste-aversions are learned quickly, often after only one aversive experience, and the learned association decays very slowly (Bernstein, 1999; Krebs & Davies, 1993). Krebs and Davies (1993) discuss a European starling (*Sturnus vulgaris*) that learned to avoid an aposematic caterpillar after only one trial. This bird refused to investigate this species again a full year after its single experience with the prey, even though it had not encountered the species in the meantime (p87). Often the foods that induce taste-aversions are novel, which increases the rate of aversion learning, although novelty is not necessary for this type of learning to occur. Studies in rats (*Rattus norvegicus*) have found that the effects of taste-aversion learning can occur even following a long delay between the food experience and the onset of illness, possibly up to 12h after eating the food (reviewed by Bernstein, 1999).

Taste-aversion learning could have contributed to the evolution of aposematism because an animal that learned to avoid a noxious prey after one encounter would avoid similar prey in the future, and mutations that make the unpalatable prey distinguishable from other palatable prey species would be favored by selection (Guilford, 1992; Servedio, 2000; Wiklund & Jarvi, 1982). Likewise, predators that learn quickly to avoid unpalatable or aversive prey will incur fewer metabolic costs of ingesting toxins, thereby enjoying increased fitness.

Generalization of learned aversions to novel stimuli is also important for understanding aposematism and mimicry (Pavlov, 1960). Avian predators have been shown to generalize their learned taste-aversions to novel prey (Schlenoff, 1984). When blue jays (*Cyanocitta cristata*) learn to avoid unpalatable seeds of a particular novel color (i.e. yellow), they subsequently avoided other novel colored seeds. This avoidance of novel colors did not occur when the trained seeds were palatable. Such generalization following taste-aversion learning would provide evolutionary opportunities for unpalatable prey to evolve conspicuousness and may even provide an opportunity for the evolution of Batesian mimicry.

Other predators also have innate aversions to novel foods. Rats are well known to exhibit strong food neophobia, which appears to be due to their physical inability to vomit (Bernstein, 1999). Thus, it seems likely that rats which more readily learned food-aversions experienced higher fitness than rats that did not learn food aversions as quickly and became ill more often. Naïve great kiskadees (*Pitangus sulphuratus*) will avoid coral snake patterns and also generalize this innate avoidance to similar ringed patterns (Smith, 1977). To my knowledge, there have been no empirical studies investigating how predator taste-aversions and generalizations might influence aposematic evolution, although there have been mathematical models that attempt to do so (Servedio, 2000).

Neophobia

Neophobia is the fear of anything new, and in animal behavior, neophobia describes a transient tendency to avoid unfamiliar foods, objects, or situations (Mallet &

Joron, 1999; Speed, 2001). Neophobia could be important to the initial evolution of aposematism for several reasons. Krebs and Davies (1994) observed that either conspicuousness or unpalatability must have evolved first in the ancestors of aposematic species. If a conspicuous mutant appeared in a cryptic and palatable population, it would be quickly detected and attacked, unless its predators displayed neophobia when they encountered it. However, neophobia is a transient phenomenon; as the predator encounters more and more of the novel prey, it becomes less unfamiliar and more familiar, reducing the predator's avoidance of the prey (Mallet & Joron, 1999). Indeed, if many prey are sufficiently distinct to be perceived as novel from each other, then predators may cease being neophobic because everything is novel, and "novel" is no longer so startling or alarming.

Unfortunately, there has been little work done on neophobia specifically contributing to the evolution of aposematism. Speed (2001) created a theoretical model in which a virtual predator with varying psychological characteristics preyed upon a virtual population of prey with varying social organizations. Speed found that, whether prey are solitary or aggregated in the environment, neophobia is an important psychological characteristic that leads to the evolution of aposematism when predators forget warning signals. Servedio (2000) did not include neophobia in her model of aposematic evolution, but notes that neophobia might allow conspicuous individuals to reach a frequency in which green beard selection could play a substantial role in aposematic evolution. Clearly, empirical studies are needed to test whether neophobia could play an important role in aposematic evolution.

Dietary Conservatism

Dietary conservatism is a long lasting reluctance to consume new foods, even after the food is no longer eliciting neophobic responses (Marples & Kelly, 1999; Marples et al., 2007; Marples, Roper, & Harper, 1998; Thomas, Marples, Cuthill, Takahashi, & Gibson, 2003). Some birds, depending upon the species and individual experience, will avoid consuming novel foods for 10 min up to longer than 15 weeks after the initial presentation of the food (Marples & Kelly, 1999). Dietary conservatism appears to progress through four stages, from strictly visual inspection to full acceptance of the food in the diet (Marples & Kelly, 1999).

The avoidance of novel prey that results from dietary conservatism may facilitate the evolution of aposematism. With birds that avoid attacking a novel prey item for extended periods of time, dietary conservatism can selectively favor novel conspicuous prey despite the prey's increased visibility (Thomas et al., 2003). Birds often selectively attack familiar prey even when the novel morph is fully palatable regardless of the specific colors employed by the novel prey, suggesting that novelty specifically elicits dietary conservatism (Schlenoff, 1984). More work must be done to discern the potential contributions of dietary conservatism to the evolution of aposematism.

Chapter 2: Experiments

In this thesis, I conducted three experiments that tested these two mechanisms (gregariousness and dietary wariness) and how they may facilitate the initial evolution of aposematism. First, I determined whether gregariousness facilitated the evolution of aposematism via a cryptic and unpalatable intermediate prey phenotype. I tested this by conducting a replication of Alatalo & Mappes' (1996) Novel World experiment with cryptic and conspicuous artificial moth stimuli as prey and blue jays (*Cyanocitta cristata*) as predators. Second, I confirmed that the low relative predation rate on aposematic (conspicuous and unpalatable) moths in experiment 1 was due to learned avoidance of unpalatable prey stimuli rather than differences in the prey stimuli detectabilities. If "conspicuous" prey are easier to detect than "cryptic" prey when all prey are palatable, then predation on conspicuous prey will be higher than predation on cryptic prey. Finally, I determined the effects of prior experience (aversion learning) and dietary wariness on predator foraging decisions when encountering novel cryptic and conspicuous prey. When presented with novel prey, avoidance learning predicts that predators with experience with unpalatable prey should avoid prey that resembles the learned item, while predators without such experience should not avoid prey. Dietary wariness predicts that all predators, regardless of their prior experience with unpalatable prey, will avoid novel prey until neophobia subsides and also will exhibit hesitance to consume novel prey for an extended period of time.

General Methodology For All Experiments

Subjects

Twenty adult hand-raised blue jays (*Cyanocitta cristata*) of unknown sex aged 1 – 11 yrs were divided into two groups of 10. One group of 10 jays participated in experiment 1, the second group of 10 jays participated in experiment 2, and all 20 jays participated in experiment 3. Jays were maintained no lower than 80% of their ad libitum feeding weight to ensure sufficient motivation in training and testing procedures on a diet of Lafaber's cockatiel pellets, turkey crumbs, and mealworms (*Tenebrio molitor*). Jays were individually housed in wire cages measuring 48 x 40 x 38 cm and provided fresh water ad libitum.

Four jays, maintained on ad libitum food and water, were designated as companion jays. One of the four companion jays was placed in a cage in the testing room and provided with fresh water during training and testing trials to make the subjects more comfortable and more exploratory. The companion was returned to its home cage during non-testing periods and the identity of the companion jay used on a particular day was rotated. As food was not provided during trials, the companion jay was rotated daily to ensure that each jay did not go longer than one day's sessions (2 – 8 h) in a row without ad libitum food. The companion jays and subjects were all housed in the same home room within their individual home cages while they were participating in the study.

Moth Stimuli

Artificial digital moths, very similar to those used by Bond and Kamil (1998) were created by selecting a small piece of grayscale pixilated cryptic background, shaping it into a wing-like shape in Adobe Photoshop Elements, and making it a symmetrical, 23 x 23 mm^2, 2-winged moth (Figure 1). To choose the moths for the experiments, I created 60 moths of varying appearance, printed them on a random portion of the cryptic background used in the experiments, and ranked them in degree of crypticity by eye. I chose five cryptic-looking moths and five conspicuous-looking moths and paired them together based on relative feature similarity by eye (Figure 2). Only these five pairs of moths and a highly conspicuous training moth (Figure 3) were used in this study. Moths and backgrounds were printed on white copy paper using an Epson Stylus Color 640 inkjet printer using Office Depot® Brand Model 405 black ink (no color ink loaded in the printer, 1440 dpi x 720 dpi). Any white paper edges were removed, and the paper was taped onto the pre-training or experimental apparatus using 3M Scotch® permanent double-sided tape such that the moth was situated directly above a food well (see Pre-Training and Training below).

Palatable and Unpalatable Food Pellets

Palatable food pellets were manufactured using the jays' regular cockatiel pellet and turkey crumb diet (Purina TestDiet® 5TVF Precision Pellets). These palatable pellets were used in all pre-training and training procedures and in all three experiments. Unpalatable pellets consisted of palatable pellets spiked with 2.2% quinine sulfate and 4.3% ground mustard powder, prepared by Purina TestDiet®. More dilute concentrations of quinine and mustard proved to be ineffective at deterring blue jays from ingesting the spiked food (J. Dykema, unpublished data). When jays sampled these unpalatable pellets, they exhibited headshake responses, a typical “disgust” response in chickens and other birds (e.g., Marples et al., 2007), and spat out the food. Unpalatable pellets were used only during experiment 1.

Pre-training

Jays were trained to peck through sheets of paper for food reward in their home cages. Jays were first allowed to obtain three palatable food pellets in a 3cm diameter food well drilled into the center of a 9 x 9 x 2 cm^3 block of wood. A piece of corrugated paper was glued to the underside of the block using Elmer’s Glue-All™ Multi-Purpose Glue (non-toxic) to hold food in the well. Jays were then trained to peck through progressively smaller holes in a 9 x 9 cm^2 piece of white paper attached on top of the block with double-sided tape for food reward until they were pecking through a thin slit made with a razor blade directly above the food well. Once jays were pecking through plain white paper readily, I presented the block with a 9 x 9 cm^2 piece of paper with a highly conspicuous training moth (Figure 3) printed in the center of the grayscale pixilated background, also slit with a razor blade above the food well. Once jays had probed three of these training moths, pre-training was complete. All jays readily pecked and probed through the paper and moths, and this pre-training was completed on the same day it began.

Training

The jays were tested in a rectangular room measuring 4.4 x 2.7 m^2. A speaker placed in the southeast corner of the testing room broadcast white noise to mask outside noise. A wire cage placed in the southwest corner of the room housed the companion jay during testing periods. The testing room was lighted with two fluorescent fixtures. A Panasonic WV-BL200 closed circuit camera was mounted in the center of the ceiling and sent a signal to a Sony Trinitron television and Sharp VC-A410 VCR outside the testing room in the holding area. All trials were recorded on videotape but all scoring was completed during the trial.

Before the jays were trained, they were habituated to the testing room and familiarized with general experimental procedures. The jays were carried from their home cage and placed in a holding cage outside the testing room. From this holding cage, the jays entered and exited the room through a porthole that the experimenter could open and close with a small sliding door located on the east wall of the testing room. Before each trial, the lights were turned off in the holding area and the jay entered the testing room through the porthole, in which a small perch and a food dish containing mealworms and palatable pellets were placed in the center of the room. Jays were allowed to move about the room freely for 60 - 120 minutes. At the end of the habituation session, the lights were turned on in the holding area, the lights in the testing room turned off, and the porthole opened. If the jay did not return to the holding cage on its own, the experimenter entered the room and gently encouraged the bird to return. Each jay received one habituation session per day until they ate all the provided food on two consecutive sessions. Eight of the jays were habituated without the experimental apparatus during a previous study, while the remaining 12 jays were habituated with the apparatus in place.

The experimental apparatus for experiments 1 and 2 consisted of a 2.43 m^2 board of 1.9 cm thick plywood with 1,444 holes (3 cm diameter) drilled 6 cm apart (center to center) in a linear array (38 x 38 holes) placed in the center of the room. The experimental apparatus for experiment 3 was similar, except that it was 1.215 m^2 and contained 361 holes in a 19 x 19 array. Paper could be adhered atop the board using double-sided tape, and the board was placed on flat sheets of corrugated paper to hold the food pellets in the holes.

Jays were then trained to peck at the training moth (Figure 3). Twenty individual training moths were positioned on the cryptic background directly above randomly determined food wells (using a random number generator). The rest of the apparatus was covered with sheets printed with only background patterning (no moths), and each piece of paper was slit (1 - 2 cm long slit) with a razor blade above each food well. In food wells below each moth I placed three palatable pellets. Jays were allowed to hunt for 60 minutes per day and training was completed when a jay probed at least 10 of the 20 training moths on two consecutive training sessions.

Testing

During each trial, the jay entered the testing room via the holding cage and porthole, and encountered one pair of moths, one cryptic and one conspicuous, consistently throughout an experiment, so they could improve their detection of the specific phenotypes. Moths or moth aggregations were placed in predetermined random locations (using a random number generator) that were unique for every trial. During a trial, a jay was allowed up to 1 h to hunt, had the opportunity to attack both cryptic and conspicuous moths in any order, and was allowed to revisit previously attacked moths as well. Jays received one test trial per day, and trials were conducted between 800 h and 1600 h 5 - 7 days per week. After a trial was completed, the jay was carried back to its home cage and fed.

During each trial I recorded the number of each moth phenotype that was attacked, the order in which moths were attacked and all relevant behaviors (headshake response, bill-wiping, spitting out pellets, swallowing, pecking, etc.). Once the trial was completed I also recorded the number of pellets removed (eaten) from each attacked moth to estimate insect "death," since being attacked does not necessarily translate into death for an insect (Wiklund & Jarvi, 1982). I designated moths with zero or one pellet removed from the food well as "surviving" the attack, while moths which lost two or three pellets received extensive damage and were "killed".

For each trial, I found relative predation rate by calculating the proportion of moths that were attacked for each moth phenotype. For example, if a jay pecked five cryptic and palatable moths in a trial, the cryptic and palatable relative predation rate was 5/16, or 0.3125. I also found the relative kill rate by calculating the proportion of attacked moths that were killed for each moth phenotype. For example, if a jay killed two of five attacked cryptic and palatable moths in a trial, then the relative kill rate was 2/5, or 0.4. I then transformed the proportional data using an arcsine square root transformation and performed Shapiro-Wilk's test of normality and Levene's test of homogeneity of variance (SPSS) on the transformed data. The arcsine square root transformation is used to normalize proportional distributions. Therefore, I can assume the data are sampled from a normal distribution. Additionally, parametric tests such as ANOVA are reportedly robust to violations of normality, so I placed higher weight on the Levene's test in the decision to apply parametric or non-parametric statistics.

Experiment 1: Effects of unpalatability on predator food choices.

I repeated Alatalo & Mappes' (1996) experiment to determine whether gregariousness is necessary for aposematism to evolve under blue jay predation. Specifically, I wanted to determine the effects of gregariousness, prey appearance, and prey palatability on relative predation rates over time.

Methods

The 10 jays were randomly divided into two groups: Solitary and Gregarious. Jays hunting the Solitary moths encountered moths that were distributed solitarily, while jays hunting the Gregarious moths encountered moths that were grouped together in same-phenotype groups of four (Figure 4). Each jay was randomly assigned to hunt one pair of moths such that all five moth pairs (Figure 2) were hunted by both a Solitary and a Gregarious jay. In each trial, jays encountered 32 moths: 16 Cryptic-Palatable, eight Cryptic-Unpalatable, and eight Aposematic (Conspicuous-Unpalatable). All 16 unpalatable moths concealed three unpalatable pellets, while the 16 palatable moths concealed three palatable pellets (Figure 4). Jays were allowed to hunt for 1h or until all palatable moths had been attacked. This occurred in 1% of trials.

A repeated-measures mixed-groups factorial ANOVA was performed to determine whether moth sociality and moth phenotype influenced relative predation rates as the trials progressed. I conducted a repeated measures ANOVA on cryptic and unpalatable relative kill rates to determine whether they survive attacks differently when they are Solitary or Gregarious. I also calculated the distance traveled (in potential moth locations) from each palatable moth pecked and from each unpalatable moth pecked for all Gregarious trials to determine whether jays preferentially attack moths within a palatable cluster or not using a paired t-test. If jays do attack palatable moths within a cluster more often than unpalatable moths, then this suggests a predator strategy of win-stay-lose-shift in which predators move short distances when the most recent attack yielded palatable prey and they move longer distances when the most recent attack yielded unpalatable prey.

Results

Relative predation rates differed among the three moth types. Cryptic and palatable moths (0.582, 0.024 SEM) were attacked most often, and aposematic moths (0.323, 0.029 SEM) were attacked least often (Figure 5; $F_{2,16} = 14.164$, $p < 0.001$).

Relative predation rates did not differ significantly between solitary and gregarious moths (Figure 6; $F_{1,8} = 2.713$, $p = 0.138$), although Solitary moths (0.508, 0.025 SEM) appeared to be attacked slightly more often than Gregarious moths (0.366, 0.025 SEM). Relative predation rates also did not differ significantly overall across trials (Figure 7; $F_{9,71} = 1.637$, $p = 0.123$).

Relative predation rates across trials did differ significantly between the different moth types (Figure 8; $F_{18,144} = 2.256$, $p = 0.004$). Relative predation rates remained relatively steady for aposematic moths, but increased for the two cryptic moth types. No other interactions were significant ($p > 0.1$).

Relative kill rates by jays also differed between the three moth types. Cryptic and palatable moths (1.047, 0.051 SEM) were killed more often than the two unpalatable moth morphs (cryptic unpalatable 0.462, 0.058 SEM; aposematic 0.376, 0.061 SEM; Figure 9; F2,14 = 32.156, $p < 0.001$).

Relative kill rates also differed across trials (Figure 10; $F_{9,63} = 3.189$, $p = 0.003$). Moths Sociality had no effect on relative kill rates ($p > 0.1$) and none of the interactions were significant (all, $p > 0.5$).

Jays moved away from many aggregations of prey before consuming all moths in the group; of 176 moth clusters which were attacked, only 31 (17.6%) were completely depleted. Jays also moved significantly further away after attacking an unpalatable moth than after attacking a palatable moth. Jays traveled 6.53 (0.61 SEM) locations away from palatable moths and 13.07 (1.19 SEM) locations away from unpalatable moths ($t_{80} = 4.122$, $p < 0.0001$).

Discussion

Solitary and Gregarious cryptic and unpalatable moths had equivalent relative predation rates on trial one, demonstrating that gregariousness per se did not lower predation for individual cryptic and unpalatable moths. This is in contrast to Alatalo & Mappes (1996) but in agreement with the findings of Tullberg et al. (2000).

Relative predation rates did not differ significantly between Solitary and Gregarious moths, although Gregarious moths of all phenotypes tended to have lower predation risk than did Solitary moths, despite a group's increased detectability (Riipi et al., 2001). This suggests that when prey are protected by either crypsis or unpalatability, aggregating together may extend a survival benefit. If so, the benefit may be due to a dilution effect, particularly for palatable prey, as jays often moved away from aggregations before consuming all moths in the group. Despite the somewhat lower predation for Gregarious prey, the overall pattern did not change from Solitary prey. Cryptic and palatable prey were attacked most often, aposematic prey were attacked the least, and cryptic and unpalatable prey experienced an intermediate rate of attack.

Jays killed palatable and unpalatable moths at different rates, with palatable moths killed significantly more often. This confirms that jays find the unpalatable pellets truly unpalatable. This is consistent with prior experiments that used quinine and mustard to manipulate unpalatability (Rowe & Guilford, 1996; Speed, Alderson, Hardman, & Ruxton, 2000), and with findings that blue jays will avoid unpalatable foods (Schlenoff, 1984).

Predation on aposematic moths did not change significantly between trial one and trial 10, suggesting two possibilities. The jays may have learned quickly (during the first part of the first trial) that conspicuous moths yielded unpalatable foods, and subsequently avoided them. However, these moths that are conspicuous to human eyes may in fact be cryptic to avian eyes, a possibility that cannot be resolved with this study. Therefore, I conducted a second experiment to determine whether the jays were quickly associating conspicuousness with unpalatability or whether the moths were actually cryptic.

Experiment 2: Relative visibility differences of cryptic and conspicuous moths.

In this experiment, I tested whether the detectability of cryptic and conspicuous moths differed. If cryptic moths are really more difficult to detect than conspicuous moths, then on trial one, jays should attack significantly more conspicuous moths than cryptic moths. However, since cryptic moths are more abundant in the environment (see below), jays should learn to better detect cryptic moths over time, and the relative predation rate should increase as trials increase. Predation on conspicuous moths should remain constant throughout the experiment.

Methods

This experimental protocol was identical to experiment 1 with only the following two exceptions. First, all moths concealed three palatable pellets, and second, 10 new jays, naïve to the moth stimuli used in experiment 1, were allowed to hunt for up to 1 h or until they had attacked 16 out of 32 moths, whichever came first.

To examine the initial and learned effects of grouping and effects of the two moth types (cryptic and palatable, conspicuous and palatable) on relative predation rates, I compared relative predation rates in all trials using a repeated measures ANOVA. To determine whether jays were attacking the moths at different rates, I compared the relative predation rates between the two moth types during trial one and trial 10 using paired t-tests.

Results

Conspicuous moths were more readily detected than were cryptic moths. Relative predation rates on conspicuous moths (0.979, 0.039 SEM) were significantly higher than attacks on cryptic moths (0.658, 0.016 SEM; Figure 11; $F_{1,8} = 8.1088$, $p = 0.022$). No other main effects or interactions were significant (all $p > 0.1$). In trial one, conspicuous moths (0.926, 0.149 SEM) were attacked more often than cryptic moths (0.519, 0.060 SEM; $t_9 = -2.691$, $p = 0.025$) demonstrating that conspicuous moths are easier to detect than cryptic moths. In trial 10, predation on the two moth types (conspicuous 0.838, 0.159 SEM; cryptic 0.768, 0.038 SEM) did not differ ($t_9 = -0.355$, $p = 0.731$). Over time, jays learned to detect cryptic moths and attacked the two moth types at equivalent rates (Figure 12).

Discussion

Jays attacked conspicuous moths more often than cryptic moths on trial one. This suggests that in experiment one, jays were associating conspicuousness with unpalatability quickly and that conspicuous moths are more readily detected by the jays.

I found very different responses to conspicuous moths in experiments 1 and 2. One hypothesis for this finding is that there were fundamental differences between the jays that participated in the two experiments, likely based on the moth palatability in the two experiments. Therefore, I compared the relative predation rates on cryptic-palatable prey between the two experiments on each of the 10 trials using independent t-tests. Relative predation rates on cryptic palatable prey differed only on trials five ($t_{18} = -5.415$, $p < 0.001$) and six ($t_{18} = -5.534$, $p < 0.001$), when jays from experiment 1 attacked cryptic palatable moths significantly less often than jays from experiment 2. Relative predation rates did not differ between the two sets of jays on any other trial (all, $p > 0.1$). This finding suggests that although predation on the conspicuous moths differed between the two sets of jays, the jays still attacked cryptic and palatable moths at equivalent rates. This suggests that the reason for the different relative predation rates on conspicuous prey is due to the palatability of these moths in the different experiments rather than fundamental differences between the groups of jays. However, as these experiments were not conducted simultaneously in time and there are other differences between the experiments, including the number of cryptic palatable moths available each trial, this inference should be accepted cautiously.

Also, these different responses to conspicuous prey, especially given similar relative predation rates on cryptic and palatable prey, suggest that jay experience may result in different subsequent responses to novel prey. However, dietary conservatism has been studied in several bird species, and even in naïve birds, experience with one food type results in subsequent avoidance of all other food types for an extended period of time (Marples & Kelly, 1999). Additionally, blue jays that were fed unpalatable novel colored sunflower seeds subsequently avoided other novel colors of seeds, whereas jays that were fed palatable novel seeds did not avoid other novel seeds later (Schlenoff, 1984). I tested between these alternative hypotheses in experiment 3.

Experiment 3: Effects of prior experience on palatable novel cryptic and conspicuous prey acceptance.

Prior aversive experience and dietary wariness have been demonstrated to influence predatory decisions when encountering novel prey. When presented with novel prey, avoidance learning predicts that predators with experience with unpalatable prey should avoid prey that resembles the learned item, while predators without such experience should not avoid prey (Bernstein, 1999). Dietary wariness predicts that all predators, regardless of prior experience with unpalatable prey, will hesitate to attack and consume novel prey for an extended period of time ranging from several minutes to several weeks (Marples & Kelly, 1999).

A previous study on predator generalization found that birds which have learned to avoid unpalatable food of a particular color (ex. red) will generalize their food avoidance to other novel colored foods (ex. blue) (Schlenoff, 1984). Blue jays did not avoid any foods following experience with palatable novel prey. These results, on the whole, are inconsistent with either the aversion learning or the dietary wariness hypotheses, and suggest that predators may utilize multiple mechanisms when making predatory decisions. However, Schlenoff (1984) did not evaluate conspicuousness of the food. As the novelty of an object will inherently fade with time, conspicuousness may be a more ecologically relevant prey characteristic than novelty for predators to generalize avoidance. In this experiment, I tested these three non-mutually exclusive alternative hypotheses, aversion learning, dietary conservatism, and novelty generalization.

Methods

All 20 jays used in experiments 1 and 2 participated in experiment 3. Experiment 3 trials were initiated 6 - 15 weeks after a jay completed its previous experiment. In this experiment jays encountered one of the five pairs of moths to which it was completely naïve. To increase the likelihood that the jays would detect the moths in a short amount of time, the experimental apparatus was one-quarter the size of the previous experiments (1.215 m^2, with 361 holes) and to ensure the jays did not exert anti-apostatic selection on rare morphs (Lindstrom et al., 2001), jays were allowed to hunt 20 moths, 10 cryptic and 10 conspicuous, in pre-determined random locations, each of which again concealed three palatable pellets. Since I was interested in the initial response to novel prey following experience with or without unpalatable food, the jays were given two trials over two days. Jays were allowed to hunt for 1h or until they had attacked 10 of the 20 moths, whichever came first.

To determine whether jays from experiment 1 and 2 attacked novel cryptic and conspicuous moths differently, I compared the cumulative attacks from trials one and two on cryptic and conspicuous moths between jays from experiments 1 and 2 using χ^2. Also, I compared the number of jays that attacked a cryptic or a conspicuous moth first in trial one using Fisher’s exact probability test.

Results

Jays with prior experience with unpalatable prey attacked significantly more cryptic prey than jays with no experience with unpalatable prey. Jays from experiment 1, which had experience with unpalatable prey, attacked 122 cryptic moths and 78 conspicuous moths in this experiment. Alternately, jays from experiment 2, which did not have experience with unpalatable prey, attacked 80 cryptic moths and 120 conspicuous moths (Table 1; $\chi^2_1 = 17.64$, $p < 0.001$). Among birds from experiment 1, eight pecked cryptic moths first and two pecked conspicuous moths first. By contrast, among birds from experiment 2, one pecked a cryptic moth first and nine pecked a conspicuous moth first (Table 2). These were significantly different rates (Fisher exact test, $p = 0.006$).

Discussion

In this study, jay behavior did not support the aversion learning hypothesis, the dietary wariness hypothesis, or the novelty generalization hypothesis exclusively. Rather, it appears that jays used a variety of mechanisms to make their foraging decisions in this experiment based on their prior experience. Jays that did not experience any unpalatable prey (experiment 2) attacked conspicuous moths significantly more often than they did cryptic moths. These results are inconsistent with both the dietary conservatism and the dietary wariness hypotheses, but consistent with the aversion learning and the novelty hypotheses. Instead of avoiding unfamiliar novel prey, jays in this study selected the most easily detected prey type, which is consistent with the findings of experiment 2. Jays that had experience with unpalatable cryptic and conspicuous prey (experiment 1) attacked significantly more novel cryptic moths than novel conspicuous moths. The fact that prior experience with aversive cryptic prey did not generalize into avoidance of novel cryptic prey suggests several possibilities.

First, as jays from experiment 2 had more experience with palatable cryptic prey (only two-thirds of cryptic moths were palatable in experiment 1), perhaps they did not experience unpalatable cryptic prey often enough to learn the aversive association. However, as the unpalatable and palatable cryptic moths were visually identical and cryptic moths were the only source of palatable food in the experimental room, it seems unlikely that even extended experience with the prey would result in learned aversion to cryptic moths. This possibility could be tested by providing predators with extensive experience with palatable and unpalatable visually identical cryptic prey.

Another possible explanation is that the unpalatable cryptic prey in this experiment were not as memorable as unpalatable conspicuous prey. Predators have been shown to learn to avoid conspicuous unpalatable prey significantly faster than they learn to avoid cryptic unpalatable prey, and they must sample fewer prey individuals before learning the aversive association (Gittleman & Harvey, 1980). Presumably, since only a third of cryptic moths in experiment 1 were aversive, learning to avoid these cryptic prey and then generalizing this learned avoidance to novel cryptic prey would likely require rare and exceptional circumstances. It is likely that the crypticity of the original prey inhibited aversion learning and generalizing to avoid novel cryptic prey.

The final possibility is that just as predators appear to have a toxicity-dependent generalization threshold (Darst & Cummings, 2006) they may also have a conspicuousness-dependent generalization threshold. With such a threshold, only prey above a certain conspicuousness level would be avoided by predators with avoidance learning experience. Jays that experienced unpalatable cryptic and conspicuous moths only avoided novel conspicuous moths in this experiment, supporting this possibility. However, my results cannot distinguish between this hypothesis and the hypothesis that predators simply do not learn to avoid cryptic unpalatable prey well. To test between these hypotheses, experienced predators would need to simultaneously choose between several alternative prey that vary in conspicuousness. If supported, this final hypothesis has interesting implications for how predator psychology has favored the evolution and maintenance of mimicry.

General Discussion

In this thesis I have experimentally found that gregariousness is not a necessary factor in driving selection for aposematism via a cryptic and unpalatable intermediate ancestor. The results support recent experimental (Tullberg et al., 2000) and phylogenetic (Sillen-Tullberg, 1988) evidence. Although simply being unpalatable would benefit a cryptic unpalatable mutant, gregariousness of cryptic unpalatable forms did not provide an additional survival benefit. Although I and others have found that gregariousness is not a necessary evolutionary precursor to aposematism, we must bear in mind the fact that aposematism is a widespread evolutionary phenomenon that has likely evolved multiple times in distantly related taxa, and as such, it is likely have evolved via different pathways in different taxonomic groups. Future research on how aposematism evolves must focus on phylogeny to determine how aposematism arose in particular taxonomic groups (Harlin & Harlin, 2003; Lindstrom, 1999). Only when we understand how an organism evolved its aposematism will we be able to ask what selection mechanisms, including predation, influenced the process.

I also found that my artificial moth stimuli were effective artificial prey for the study of predatory behaviors. The moths resembled extant prey species that many predators naturally hunt, and their appearance (size, color, crypticity, etc.), as well as the appearance of the background, can be easily manipulated to study how prey appearance affects predation strategies. While these particular stimuli utilize disruptive coloration and background matching for camouflage, different stimuli could be created to utilize different camouflage strategies. Additionally, conspicuous luminance or brightness contrast, rather than color contrast, is known to be effective stimulus to facilitate avoidance learning in color-blind invertebrates (Prudic, Skemp, & Papaj, 2007). I have shown that grayscale prey, with no known color contrast, also facilitates avoidance learning in vertebrate predators with sophisticated color vision. The digital prey used here have no three-dimensional component that could confound a prey detection experiment (Tullberg et al., 2000). Insectivorous birds, like the blue jay, are likely to learn to hunt these types of prey readily, which would facilitate experimental study of predation in the laboratory as well as more natural settings. Also, the reward provided for each stimulus was manipulated easily; one could simply place more or less food, or different types of food, in the well below the stimulus. This system may also prove useful for studying optimal foraging when prey vary in appearance or in relative reward.

Finally, my work showed that predators experienced with aversive prey can generalize their avoidance to novel conspicuous prey, while not doing so to novel cryptic prey. In contrast, predators with no experience with unpalatable prey more often attacked the stimulus that was most readily detected, the conspicuous prey. Whether this observation results from a reduced ability to associate cryptic coloration with unpalatability (Gittleman & Harvey, 1980) or from a conspicuousness-dependent generalization gradient remains to be seen. Future research should investigate these possibilities. Nevertheless, it is clear that novelty is not the only basis for stimulus generalization (Schlenoff, 1984). Conspicuousness hastens avoidance learning (Gittleman & Harvey, 1980) and slows the reversal of this learned avoidance (Roper, 1994). Again, as aposematism is an evolutionary phenomenon with a historical basis, we must not exclude other prey characteristics as important factors for a predator’s foraging decisions. Hawks appear to selectively attack the odd colored prey from a group (Mueller, 1971), and chicks seem to use color rather than background contrast as a cue for avoidance learning (Gamberale-Stille & Guilford, 2003).

Further research must be conducted comparing predation strategies between wild-caught and hand-reared adults. The extent to which my results were significantly affected by my use of hand-reared adult blue jays is unknown. Wild jays may behave very differently than hand-reared jays, even with similar experience with unpalatable foods.

Although much research on aposematism is theoretically driven by the apparent paradoxical evolution of unpalatability and conspicuousness from a cryptic and palatable ancestor, much of the research that is actually conducted is disconnected from this unmistakably historical question (Harlin & Harlin, 2003). For example, Mappes, Marples, & Endler (2005) specifically discussed potential resolutions to the evolutionary question of aposematism, but do not suggest the use of evolutionary tools to address them. This is occurring despite the increasing availability of appropriate tools, including molecular techniques and comparative methods. Recent reviewers (Harlin & Harlin, 2003; Lindstrom, 1999) have also argued for the consideration of phylogenetic history in experimental and theoretical treatments of aposematic evolution: “without a phylogenetic hypothesis, we are at a loss as to which questions to ask” in aposematism (Harlin & Harlin 2003, pp 206). I am convinced that the next step is phylogenetic exploration to shed light on this fundamental evolutionary question concerning aposematism.

Figures and Tables

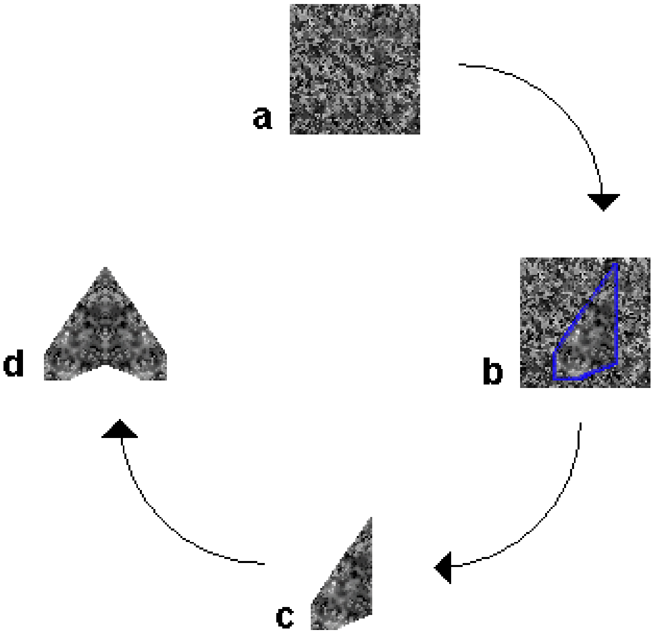


Figure 1: Creation of moth prey stimuli. From a sample of background patterning (a), a wing-shaped portion of the pattern was selected (b). Using this wing-shaped portion of background pattern (c), a symmetrical 2-winged moth was created (d) using Adobe Photoshop Elements 3.0.

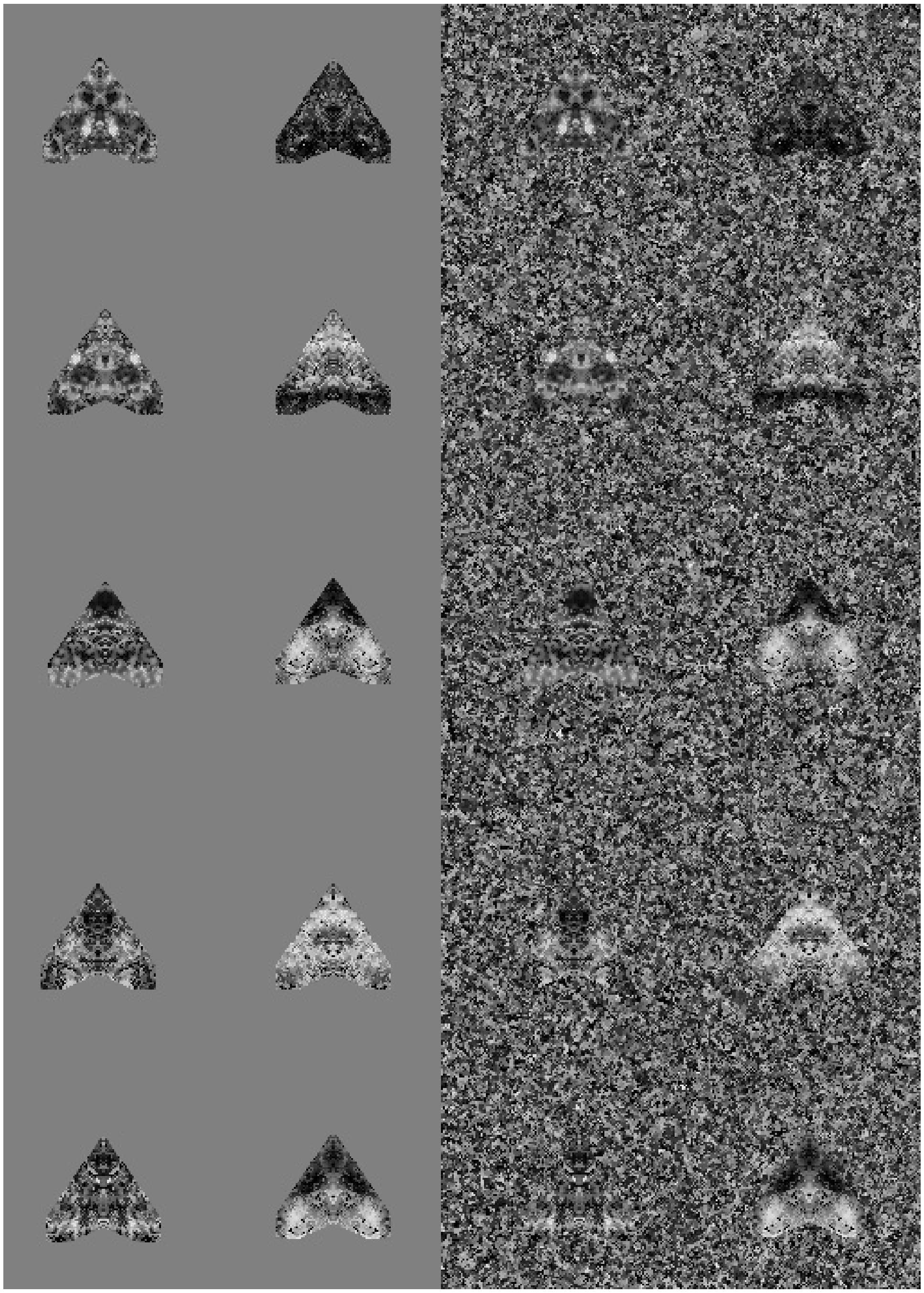


Figure 2: Cryptic and Conspicuous Moth Pairs used in the three experiments. Moths are presented here on a flat gray background and on the speckled background used in the experiments Cryptic moths are on the Left on each background, and Conspicuous moths are on the Right on each background.

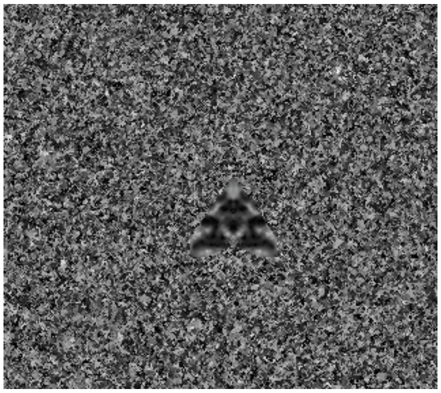


Figure 3: The training moth used in training procedures.

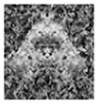
		Moth Sociality			
		Solitary n=5		**Gregarious n=5**	
Moth Type	**Cryptic-Palatable**		**16x1=16**		**4x4=16**
	Cryptic-Unpalatable		**8x1=8**		**2x4=8**
	Aposematic		**8x1=8**		**2x4=8**

Figure 4: Experimental Design in Experiment 1. Subjects were randomly assigned to one of the Moth Sociality conditions (Between Groups), and encountered all three Moth Types (Within Groups) simultaneously in each of ten trials. The total number of moths was constant across groups, but Solitary moths were displayed in groups of one, and Gregarious moths were displayed in groups of four, as depicted in the figure. For example, Solitary Cryptic-Unpalatable moths were presented to jays in eight groups of one moth each, for a total of eight Cryptic-Unpalatable moths available per trial. Similarly, Gregarious Cryptic-Unpalatable moths were presented to jays in two groups of four moths each, for a total of eight Cryptic-Unpalatable moths available.

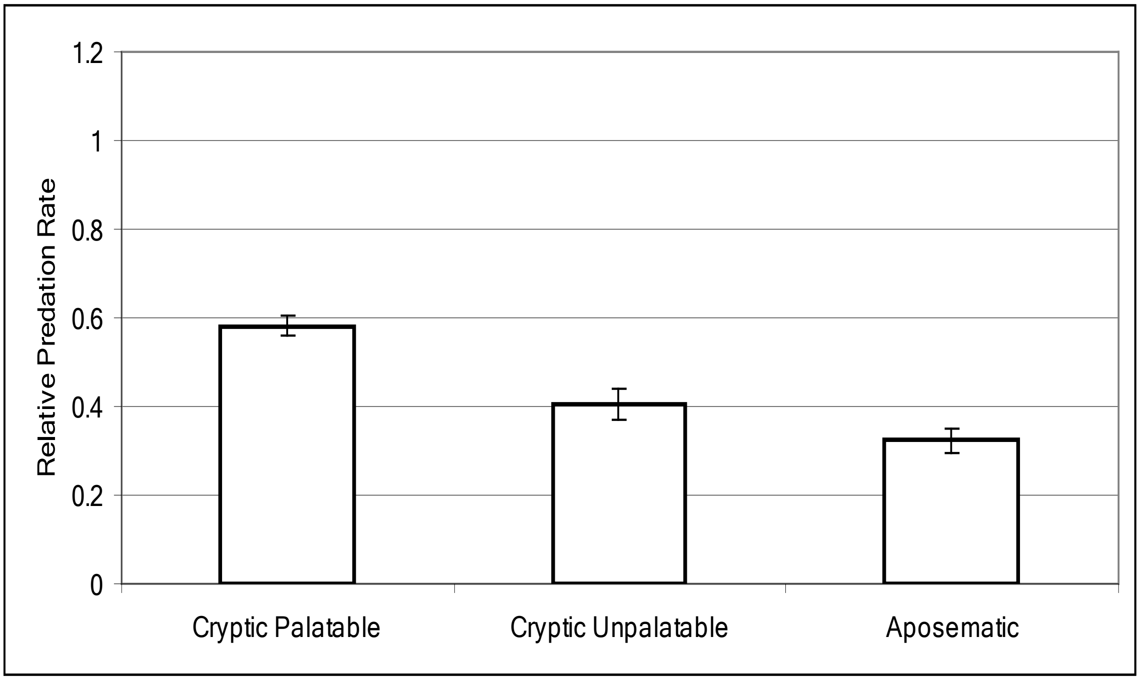


Figure 5. Overall relative predation rate ± SEM among the three moth types in experiment 1. Relative predation rate is the arcsine square root transformed proportion of moths that were attacked in each trial, calculated separately for each moth phenotype. Cryptic and palatable moths were attacked most often, and aposematic moths were attacked least often.

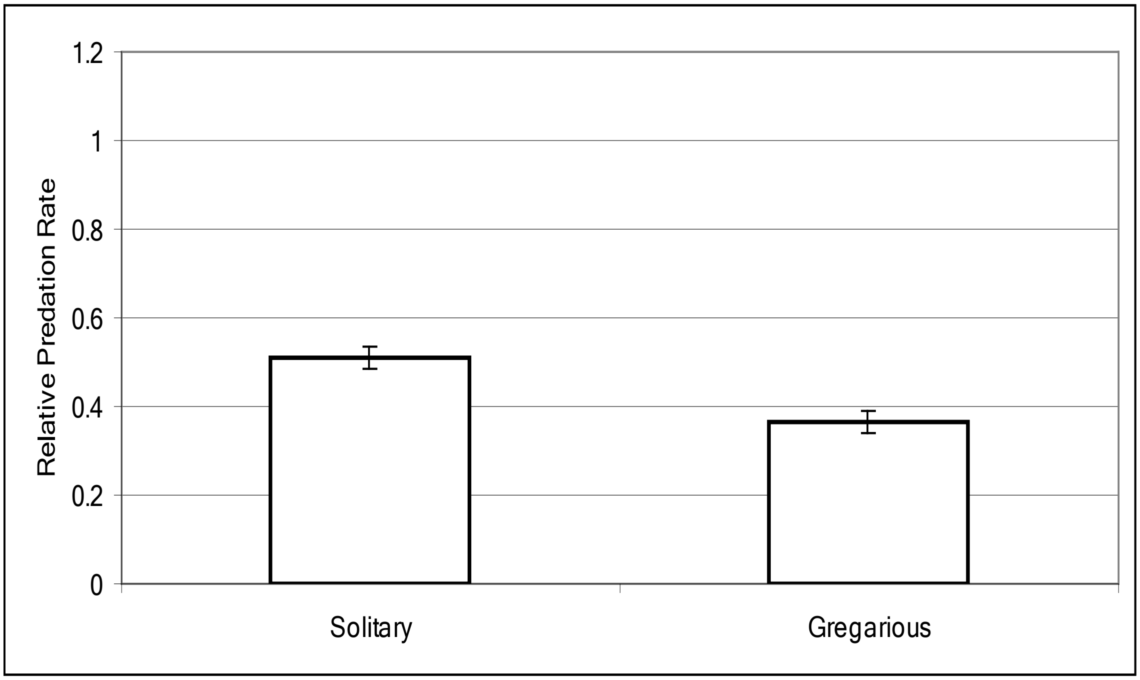


Figure 6. Overall relative predation rate ± SEM on all solitary and gregarious moths in experiment 1. Relative predation rate is the arcsine square root transformed proportion of moths that were attacked in each trial, calculated separately for each moth phenotype. Predation did not significantly differ between solitary and gregarious moths, although solitary moths appeared to be attacked slightly more often.

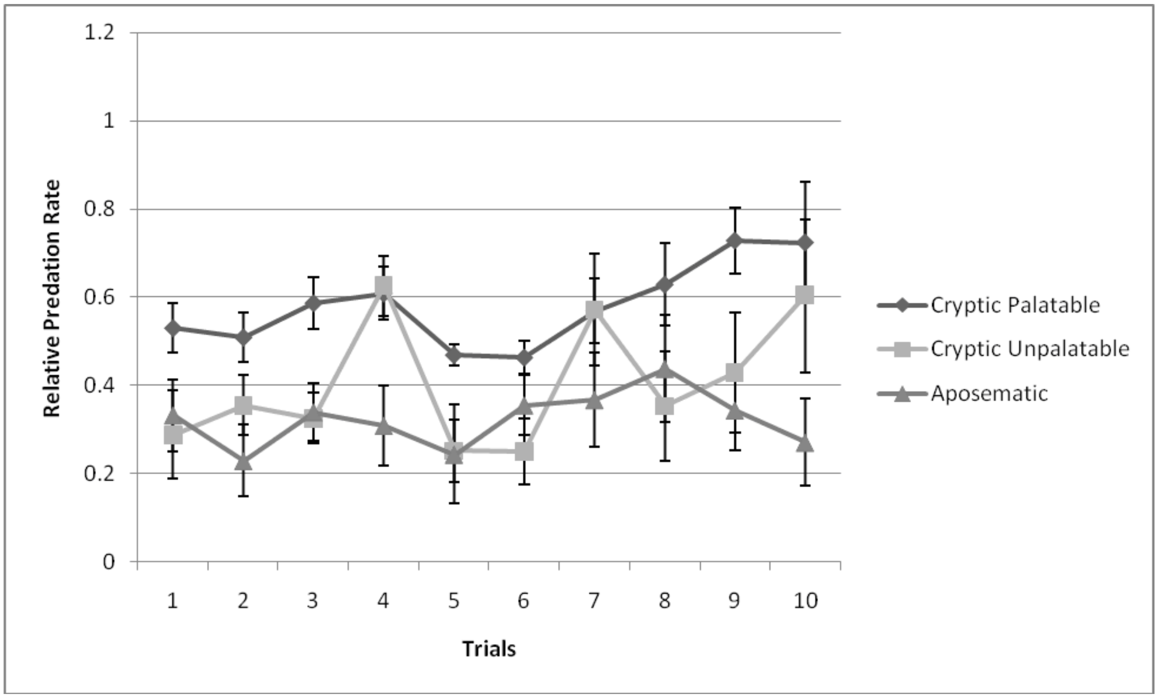


Figure 7: Overall relative predation ± SEM of cryptic palatable, cryptic unpalatable, and aposematic moths in experiment 1. Relative predation rate is the arcsine square root transformed proportion of moths that were attacked in each trial, calculated separately for each moth phenotype. Predation differed significantly between the three moth types. Cryptic palatable moths were attacked most often and aposematic moths were attacked the least.

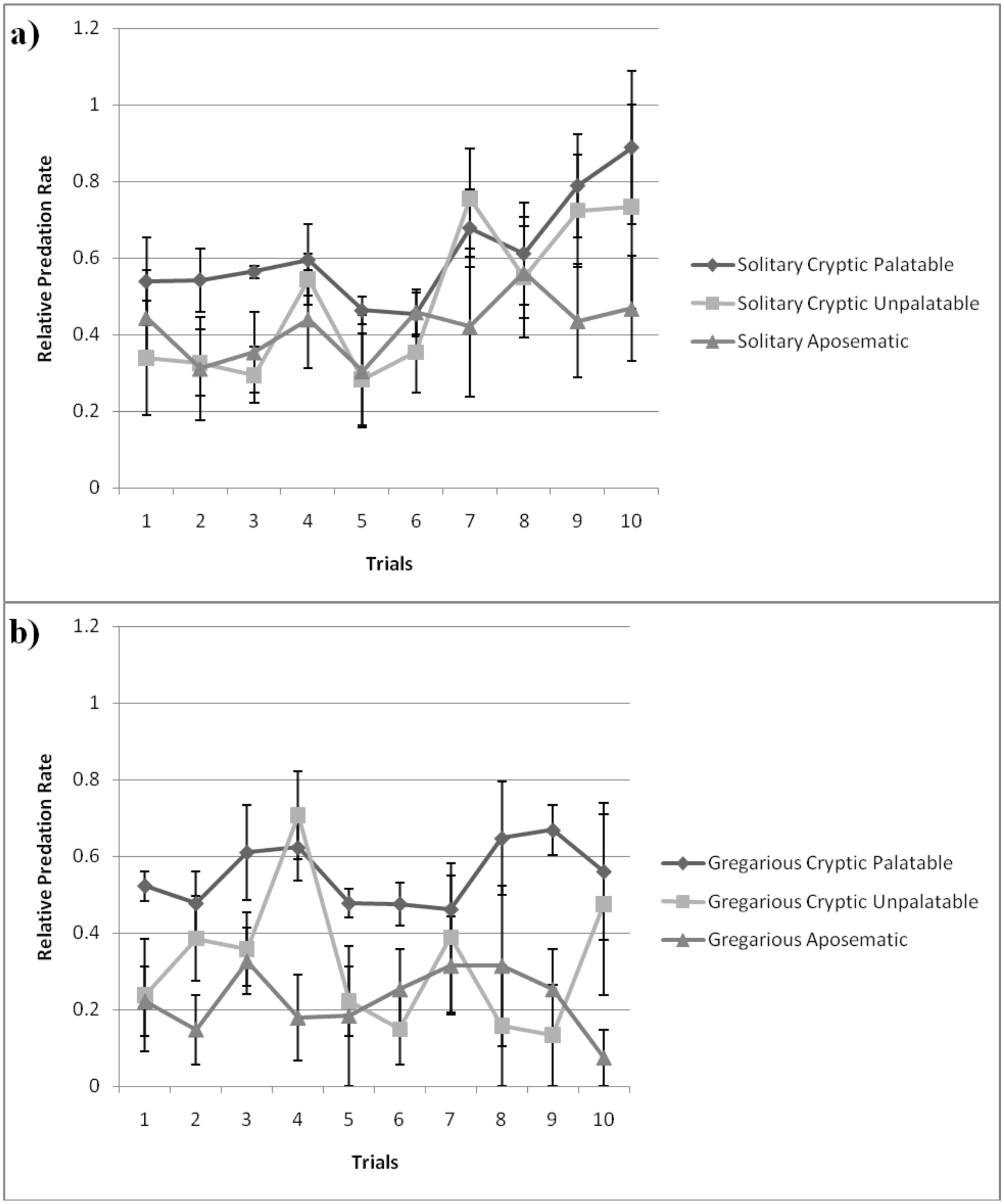


Figure 8: Relative predation rate ± SEM of cryptic palatable, cryptic unpalatable, and aposematic solitary (a) and gregarious (b) prey in experiment 1. Relative predation rate is the arcsine square root transformed proportion of moths that were attacked in each trial,

calculated separately for each moth phenotype. Relative predation rates did not differ between the moth sociality treatments, although predation on solitary moths of all three phenotypes were slightly higher than predation on gregarious moths.

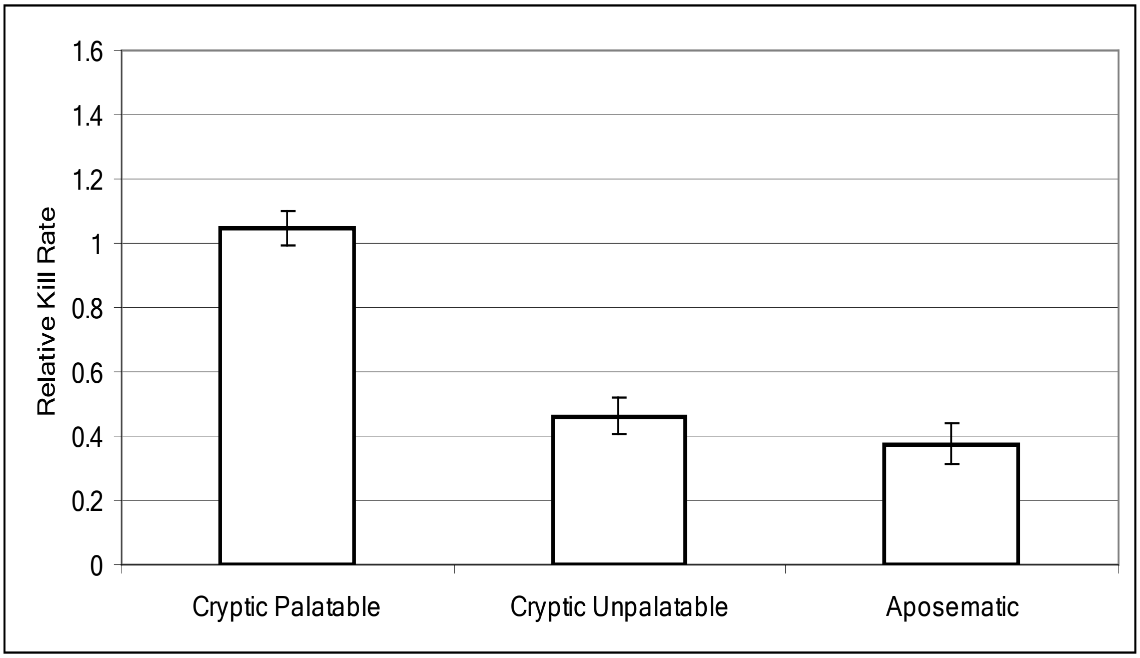


Figure 9. Overall relative kill rate ± SEM among the three moth types. Relative kill rate is the arcsine square root transformed proportion of attacked moths that were killed, in each trial, calculated separately for each moth phenotype. Cryptic palatable moths were killed significantly more often than the two unpalatable moth types.

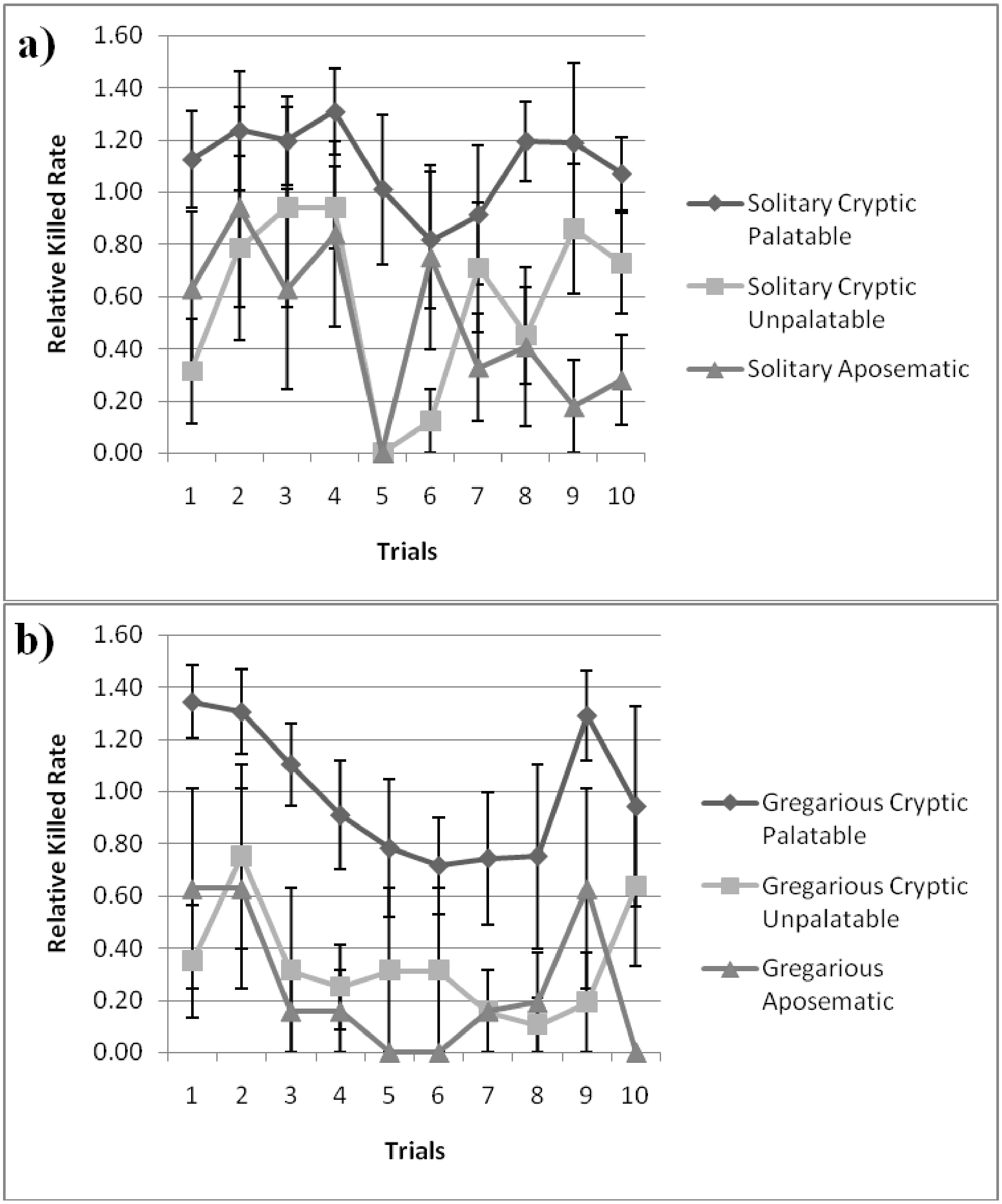


Figure 10. Relative kill rate ± SEM of cryptic palatable, cryptic unpalatable, and aposematic solitary (a) and gregarious (b) prey in experiment 1. Relative kill rate is the arcsine square root transformed proportion of attacked moths that were killed, in each

trial, calculated separately for each moth phenotype. Cryptic palatable moths were killed more often than the two unpalatable moths. Relative kill rates did not differ between solitary and gregarious treatments.

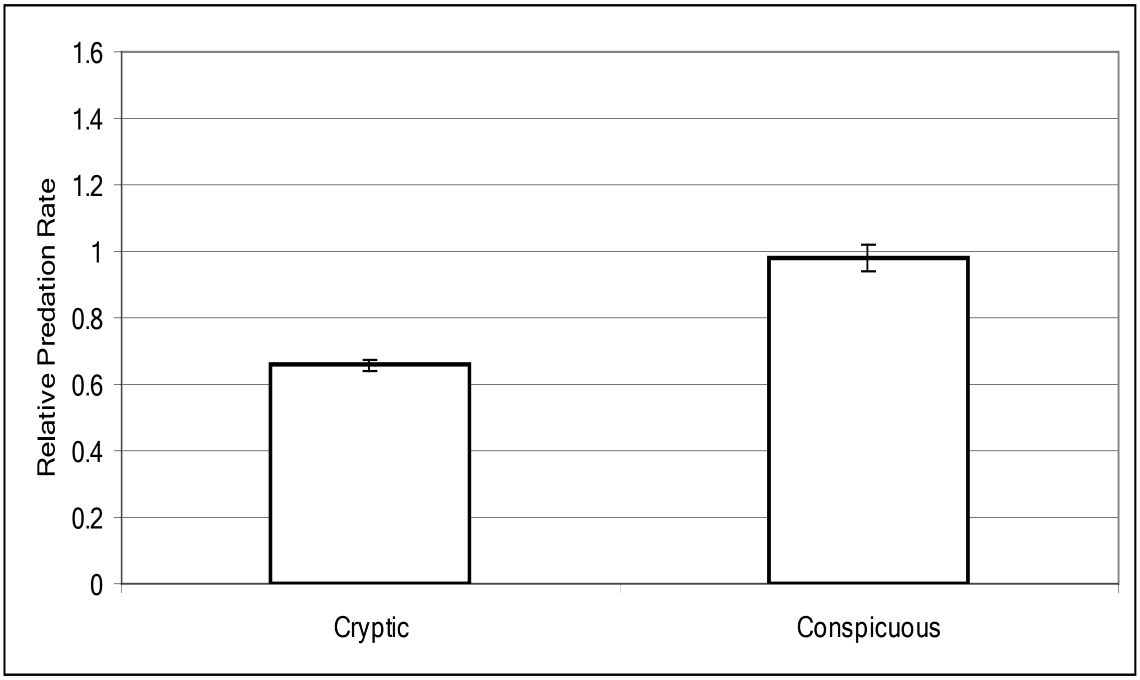


Figure 11. Overall relative predation rate on cryptic palatable and conspicuous palatable moths in experiment 2. Relative predation rate is the arcsine square root transformed proportion of moths that were attacked in each trial, calculated separately for each moth phenotype. Conspicuous moths were attacked significantly more often than cryptic moths in both solitary and gregarious conditions.

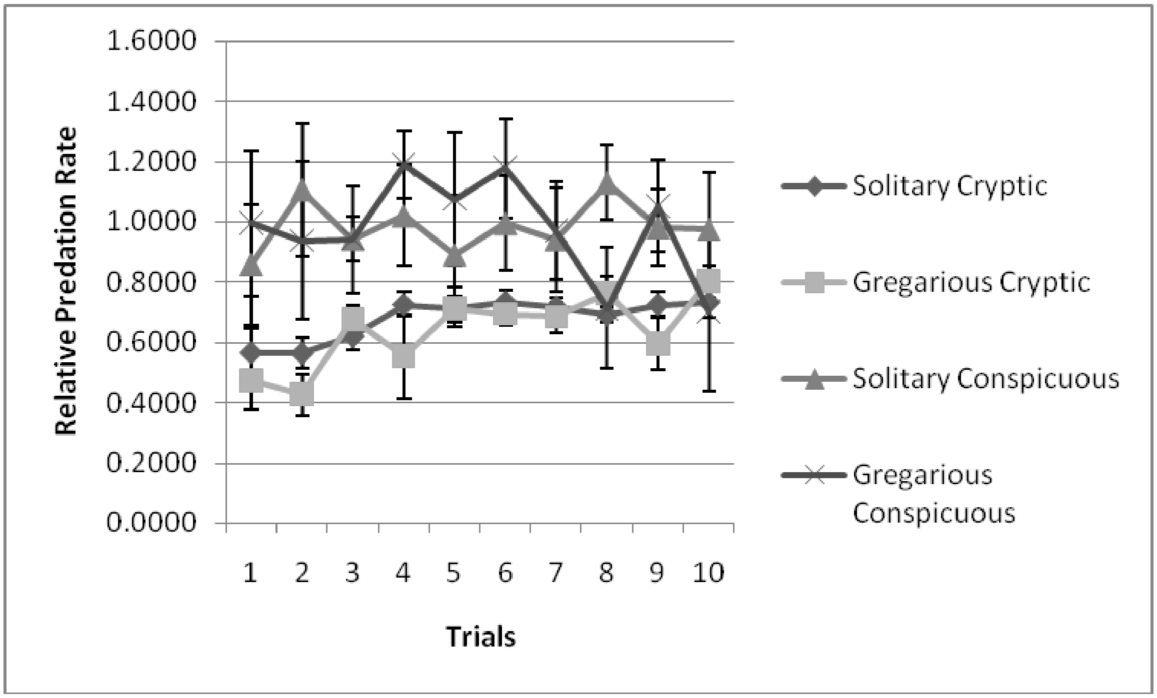


Figure 12: Relative predation rates across trials ± SEM for solitary and gregarious cryptic and conspicuous moths in experiment 2. Relative predation rate is the arcsine square root transformed proportion of moths that were attacked in each trial, calculated separately for each moth phenotype. Jays attacked significantly more conspicuous moths than cryptic moths in trial 1, but not in trial 10.

Table 1: Cumulative number of attacks to cryptic and conspicuous moths by all 20 jays in both trials of experiment 3. All jays attacked 10 moths in each of their two trials. Data from jays used in experiments 1 and 2 are tabulated separately. Jays from experiment 1, that did experience unpalatable cryptic and conspicuous moths, attacked significantly more cryptic novel moths. In contrast, jays from experiment 2, that did not experience unpalatable prey, attacked significantly more conspicuous novel moths.

	Cryptic	Conspicuous
Experiment 1 Jays	122	78
Experiment 2 Jays	80	120

Table 2: First moth pecked by all 20 jays in trial one of experiment 3. Data from jays used in experiments 1 and 2 are shown separately. Jays from experiment 1, that did experience unpalatable cryptic and conspicuous moths, first attacked cryptic novel moths significantly more often than they first attacked conspicuous novel moths. In contrast, jays from experiment 2, that did not experience unpalatable prey, first attacked conspicuous novel moths significantly more often than they first attacked cryptic novel moths.

	Cryptic	Conspicuous
Experiment 1 Jays	8	2
Experiment 2 Jays	1	9

References

Alatalo, R. V., & Mappes, J. (1996). Tracking the evolution of warning signals. *Nature*, *382*(6593), 708-710.

Alatalo, R. V., & Mappes, J. (2000). Initial evolution of warning coloration: comments on the novel world method. *Animal Behaviour*, *60*(1), F1-F2.

Bernstein, I. L. (1999). Taste aversion learning: a contemporary perspective. *Nutrition, 15*(3), 229-234.

Bond, A. B., & Kamil, A. C. (1998). Apostatic selection by blue jays produces balanced polymorphism in virtual prey. *Nature*, *395*(6702), 594-596.

Brower, L. P., Brower, J. V. Z., & Corvino, J. M. (1967). Plant Poisons in a Terrestrial Food Chain. *Proceedings of the National Academy of Sciences of the United States of America, 57*(4), 893-898.

Darst, C. R., & Cummings, M. E. (2006). Predator learning favours mimicry of a less-toxic model in poison frogs. *Nature, 440*, 208-211.

Darwin, C. (1881). *The Descent of Man and Selection in Relation to Sex*. London: John Murray.

Dawkins, R. (1976). *The Selfish Gene*. Oxford: Oxford University Press.

Farrell, B., Dussourd, D. E., & Mitter, C. (1991). Escalation of plant defense: do latex and resin canals spur plant diversification? *The American Naturalist*, *138*(4), 881-900.

Fisher, R. A. (1958). *The genetical theory of natural selection* (2nd ed.). New York: Dover Publications, Inc.

Gagliardo, A., & Guilford, T. (1993). Why Do Warning-Colored Prey Live Gregariously. *Proceedings of the Royal Society of London Series B-Biological Sciences, 251*(1330), 69-74.

Gamberale-Stille, G., & Guilford, T. (2003). Contrast versus colour in aposematic signals. *Animal Behaviour, 65*(5), 1021-1026.

Gamberale, G., & Tullberg, B. S. (1996). Evidence for a peak-shift in predator generalization among aposematic prey. *Proceedings of the Royal Society of London Series B-Biological Sciences, 263*(1375), 1329-1334.

Gamberale, G., & Tullberg, B. S. (1998). Aposematism and gregariousness: the combined effect of group size and coloration on signal repellence. *Proceedings of the Royal Society of London Series B-Biological Sciences, 265*(1399), 889-894.

Gittleman, J. L., & Harvey, P. H. (1980). Why are distasteful prey not cryptic? *Nature, 286*, 149-150.

Guilford, T. (1990). Evolutionary Pathways to Aposematism. *Acta Oecologica-International Journal of Ecology, 11*(6), 835-841.

Guilford, T. (1992). Chapter 16: Predator Psychology and the Evolution of Prey Coloration. In M. S. Crawley (Ed.), *Natural Enemies: The Population Biology of Predators, Parasites and Diseases* (pp. 377-394). Oxford: Blackwell Scientific Publications.

Harlin, C., & Harlin, M. (2003). Towards a historization of aposematism. *Evolutionary Ecology, 17*(2), 197-212.

Hatle, J. D., & Salazar, B. A. (2001). Aposematic coloration of gregarious insects can delay predation by an ambush predator. *Environmental Entomology, 30*(1), 51-54.

Joron, M. (2002). Aposematic Coloration. In R. T. Carde, & Resh, V. H. (Ed.), *Encyclopedia of Insects* (pp. 39-45). New York: Academic Press.

Krebs, J. R., & Davies, N. B. (1993). *An Introduction to Behavioural Ecology* (Third ed.). Oxford: Blackwell Science Ltd.

Lindstrom, L. (1999). Experimental approaches to studying the initial evolution of conspicuous aposematic signaling. *Evolutionary Ecology, 13*(7-8), 605-618.

Lindstrom, L., Alatalo, R. V., Lyytinen, A., & Mappes, J. (2001). Strong antiapostatic selection against novel rare aposematic prey. *Proceedings of the National Academy of Sciences of the United States of America, 98*(16), 9181-9184.

Lindstrom, L., Alatalo, R. V., Mappes, J., Riipi, M., & Vertainen, L. (1999). Can aposematic signals evolve by gradual change? *Nature, 397*(6716), 249-251.

Mallet, J. (1999). Causes and consequences of a lack of coevolution in Mullerian mimicry. *Evolutionary Ecology, 13*(7-8), 777-806.

Mallet, J., & Joron, M. (1999). Evolution of diversity in warning color and mimicry: Polymorphisms, shifting balance, and speciation. *Annual Review of Ecology and Systematics, 30*, 201-233.

Mappes, J., & Alatalo, R. V. (1997). Effects of novelty and gregariousness in survival of aposematic prey. *Behavioral Ecology, 8*(2), 174-177.

Mappes, J., Marples, N., & Endler, J. A. (2005). The complex business of survival by aposematism. *Trends in Ecology & Evolution, 20*(11), 598-603.

Marples, N. M., & Kelly, D. J. (1999). Neophobia and dietary Conservatism: Two distinct processes? *Evolutionary Ecology, 13*(7-8), 641-653.

Marples, N. M., Quinlan, M., Thomas, R. J., & Kelly, D. J. (2007). Deactivation of dietary wariness through experience of novel food. *Behavioral Ecology, 18*(5), 803-810.

Marples, N. M., Roper, T. J., & Harper, D. G. C. (1998). Responses of wild birds to novel prey: evidence of dietary conservatism. *Oikos, 83*(1), 161-165.

Mitter, C., Farrell, B., & Wiegmann, B. (1988). The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? *The American Naturalist, 132*(1), 107-128.

Mueller, H. C. (1971). Oddity and specific searching image more important than conspicuousness in prey selection. *Nature, 233*, 345-346.

Pavlov, I. P. (1960). *Conditioned reflexes; and investigation of the physiological activity of the cerebral cortex*. New York: Dover Publications.

Poulton, E. B. (1890). *The Colours of Animals: Their meaning and use especially in the case of insects* (2nd ed.). London: Kegan Paul, Trench, Trubner, & Co. Ltd.

Prudic, K. L., Skemp, A. K., & Papaj, D. R. (2007). Aposematic coloration, luminance contrast, and the benefits of conspicuousness. *Behavioral Ecology, 18*(1), 41-46.

Reader, T., & Hochuli, D. F. (2003). Understanding gregariousness in a larval Lepidopteran: the roles of host plant, predation, and microclimate. *Ecological Entomology*, *28*(6), 729-737.

Riipi, M., Alatalo, R. V., Lindstrom, L., & Mappes, J. (2001). Multiple benefits of gregariousness cover detectability costs in aposematic aggregations. *Nature, 413*(6855), 512-514.

Roper, T. J. (1994). Conspicuousness of Prey Retards Reversal of Learned Avoidance. *Oikos, 69*(1), 115-118.

Rowe, C., & Guilford, T. (1996). Hidden colour aversion in domestic chicks triggered by pyrazine odours of insect warning displays. *Nature, 383,* 520-522.

Ruxton, G. D., & Sherratt, T. N. (2006). Aggregation, defense and warning signals: the evolutionary relationship. *Proceedings of the Royal Society B-Biological Sciences, 273*(1600), 2417-2424.

Schlenoff, D. H. (1984). Novelty: a basis for generalization in prey selection. *Animal Behaviour, 32*(3), 919-921.

Servedio, M. R. (2000). The effects of predator learning, forgetting, and recognition errors on the evolution of warning coloration. *Evolution, 54*(3), 751-763.

Sillen-Tullberg, B. (1988). Evolution of gregariousness in aposematic butterfly larvae: A phylogenetic analysis. *Evolution, 42*(2), 293-305.

Skelhorn, J., & Ruxton, G. D. (2006). Avian predators attack aposematic prey more forcefully when they are part of an aggregation. *Biology Letters, 2*(4), 488-490.

Smith, S. M. (1977). Coral-snake pattern recognition and stimulus generalization by naive great kiskadees (Aves: Tyrannidae). *Nature, 265,* 535-536.

Speed, M. P. (2001). Can receiver psychology explain the evolution of aposematism? *Animal Behaviour, 61,* 205-216.

Speed, M. P., Alderson, N. J., Hardman, C., & Ruxton, G. D. (2000). Testing Mullerian mimicry: an experiment with wild birds. *Proceedings of the Royal Society B-Biological Sciences, 267,* 725-731.

Thomas, R. J., Marples, N. M., Cuthill, I. C., Takahashi, M., & Gibson, E. A. (2003). Dietary conservatism may facilitate the initial evolution of aposematism. *Oikos, 101*(3), 458-466.

Tullberg, B. S., Leimar, O., & Gamberale-Stille, G. (2000). Did aggregation favour the initial evolution of warning coloration? A novel world revisited. *Animal Behaviour, 59*, 281-287.

Wiklund, C., & Jarvi, T. (1982). Survival of distasteful insects after being attacked by naive birds: A reappraisal of the theory of aposematic coloration evolving through individual selection. *Evolution, 36*(5), 998-1002.