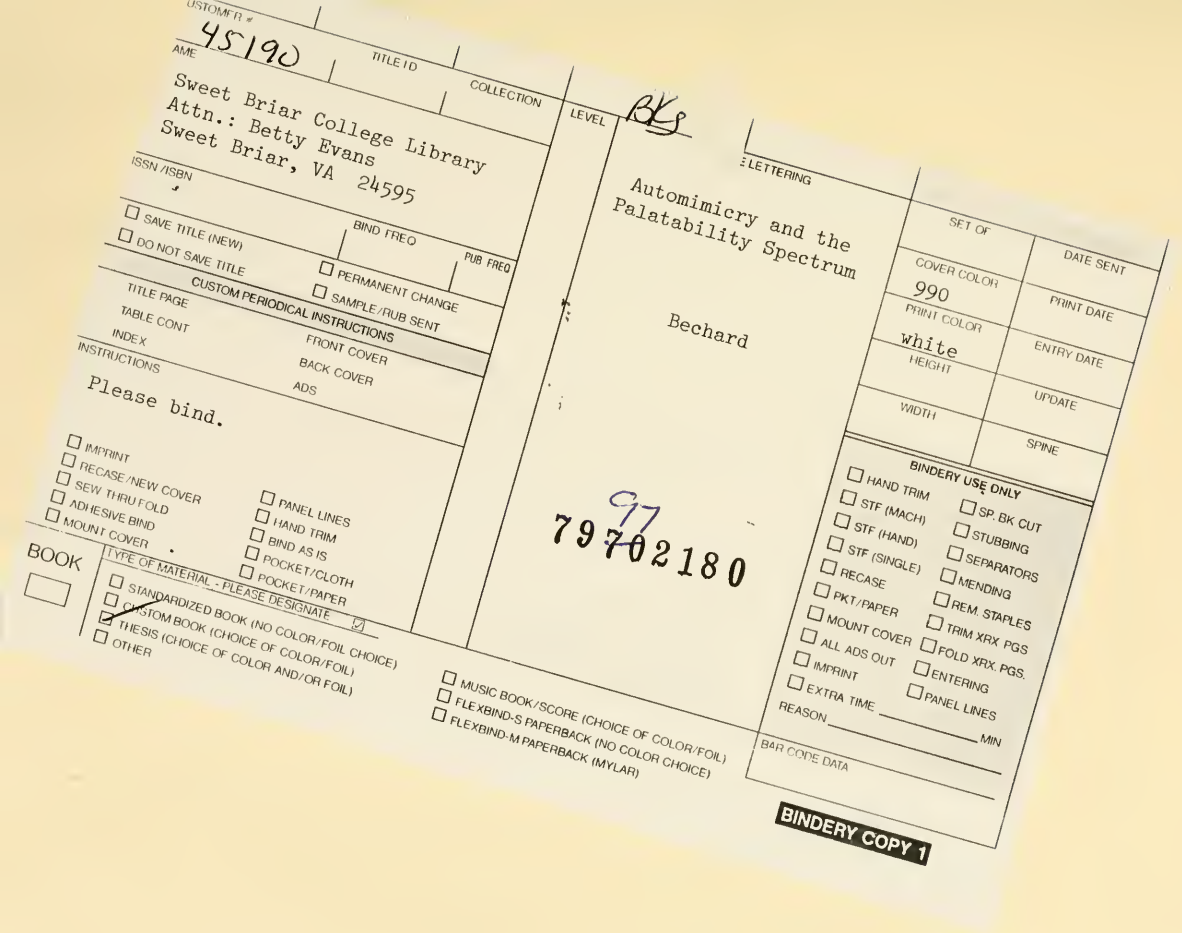

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Automimicry and the Palatability Spectrum

Submitted in partial fulfillment of the requirements for Honors in Biology

Sweet Briar College
Sweet Briar, Virginia

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April 15, 1996
"I pledge..."

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Introduction

Mimicry

Mimicry in insects is the imitation by one species of the distinctive coloration, appearance, or behavior of a different species. There are several types of mimicry, most of which rely on at least one of the species involved being unpalatable. Unpalatability can result from several different things, including noxious chemicals and physical defenses such as spines or toughness. In insects, it is usually the result of a chemical sequestered in the body that makes that insect either [illegible]l or poisonous to a predator. In most cases, an insect tha[illegible]alatability as a defense against predators is brightly colored to w[illegible] predators that it is noxious. For example, the caterpillars of the moth *Euchromia lethe* are bright yellow and have stinging hairs along their body that cause much discomfort to an animal that touched them (Edmunds 1974).

The first major type of mimicry involves a palatable mimic with coloration and markings similar to an unpalatable model. This is called Batesian mimicry. When a predator has eaten one or more of the unpalatable models and has learned to avoid that particular color pattern, the mimic gains protection from the predator. For example, Lady-bird beetles (Coccinellidae) are a common, unpalatable beetle in the Philippines. They are bright red with black spots. They are mimicked by a common roach of the genus *Prosoplecta*. This roach is edible to any insectivorous animal, but because it resembles the noxious Lady-bird beetle, it gains a large amount of protection (Wickler 1968).

Mullerian mimicry is similar to Batesian in that the model and the mimic have similar colors and markings, but in Mullerian mimicry, all of the species involved are unpalatable. The two butterflies *Hirsutis megara* and *Lycorea ceres* are a very good example of Mullerian mimicry. They are both highly toxic to birds and are avoided by them. These two butterflies look a lot alike, having similar color and markings (Brower 1969). A predator only has to eat a few of either of the species in order to learn to avoid that specific coloration. In this way, mimicry is beneficial to individuals of both species.

A third, and probably less well known, type of mimicry is automimicry. Unlike Batesian and Mullerian mimicry, which involve two or more species, automimicry involves a single species having a range of

palatability. In this "palatability spectrum" (Brower *et al.* 1967), each individual has a different amount or kind of a toxic substance in its body. Some of the members of the species may be completely palatable, while other individuals may contain enough toxin so that only a small fraction of an individual will make a predator ill (Brower 1984; Brower *et al.* 1972; 1970).

Case study: automimicry in the monarch butterfly

Automimicry can best be discussed by looking at the monarch butterfly, *Danaus plexippus.* Monarchs are the classic examples because their chemical ecology is probably the most studied of all butterflies.

Chemical ecology of the monarch butterfly

Monarch larvae feed on various species of milkweed, Asclepiadeceae. Many milkweed plants produce and store chemicals called cardenolides that act as a defense for the plant against herbivores such as caterpillars and cows. Cardenolides are very toxic to most animals, affecting mainly the heart. They cause the heart to beat slower and stronger, and in high enough doses, they can cause the heart to stop beating altogether. Another effect that cardenolides have on many vertebrates is the activation of the vomiting center in the brain. In the blue jay, the dosage necessary to cause vomiting is roughly equal to half the amount required to kill it (Brower 1984). This allows the animal to get rid of the cardenolides before it ingests a lethal amount. Even though cardenolides cause major heart irregularities and vomiting in some animals, most herbivores avoid them simply because they taste bitter. In most cases, bitter substances are toxic to animals, so many animals have inborn responses to bitterness, avoiding it if there is other food available. For this reason, most herbivores will avoid plants containing cardenolides (Brower 1984, Brower 1969).

Monarchs, however, are not only able to ingest the cardenolides without becoming sick, they use the cardenolides manufactured by the milkweed for their own defense against predators. The monarch larvae store the cardenolides in all their tissues except the fatty tissue. When the larva pupates into an adult, the cardenolides are stored in the wings, exoskeleton, and hemolymph (Brower *et al* 1982).

Some birds that eat the butterflies with a high cardenolide content will quickly become sick and will usually avoid any further contact with them. In laboratory experiments, when naive blue jays were given noxious monarchs, they all vomited within 18 minutes. After that, the birds refused any more monarchs. In one study, two out of six birds starved to death before they would eat any more monarchs. The other four accepted the offered palatable monarchs after a period of starvation, but they tasted the butterfly before they would eat it, rolling it around in their beaks until they were sure that the butterfly was safe to eat (Brower 1969). The palatable monarchs were produced by rearing caterpillars on cabbage, which does not contain cardenolides (Brower *et al.* 1967).

The monarch butterfly comes up against a problem when using cardenolides as a means of predator defense. Monarchs use many species of milkweed as a food source, but different species of milkweed contain different kinds and different concentrations of cardenolides. For example, the milkweeds *Asclepias curassivica* and *A. eriocarpa* are very toxic, containing large amounts of extremely potent cardenolides, while the species *Asclepias syriaca* is less toxic, containing a less potent variety of cardenolides. Finally, *Asclepias fascicularis* contains an immeasurable amount of cardenolides (Brower *et al.* 1982, Martin *et al.* 1992). The cardenolide content of the milkweed is very important because the palatability of the adult monarch is the direct result of the amount and type of cardenolides found in the larval foodplant. In palatability bioassays done on blue jays by Brower *et al.* (1968), the potency of the cardenolides in monarchs reared on different foodplants was compared. A standard of cardenolide potency called the ED 50, or Emetic Dose 50, is the minimum amount of a butterfly that can cause emesis, or vomiting, in fifty percent of the birds being force fed the butterfly. Cardenolide ED 50s can range anywhere from .305 g butterfly to .009g butterfly (Martin *et al.* 1992). The smaller the amount it takes to cause fifty percent of the birds to vomit, the greater the potency of the cardenolide.

Palatability spectra in wild populations of the monarch butterfly

This variation in the cardenolide content of the monarch food plant is what causes the palatability spectrum so characteristic of

automimicry. When a monarch larva feeds on a palatable milkweed, it obtains little to none of the chemicals necessary for the successful defense against predators. It must rely on its coloration as its main defense mechanism: it acts as a perfect mimic of toxic individuals of its own species. If a predator has had experience with the noxious variety of monarch, or even with the monarchs of moderate unpalatability, it will usually leave the palatable member alone because it cannot tell palatable from unpalatable. Many predators can tell different species apart by their visual appearance (in the case of the monarch, it is orange with black markings) and with experience, learn to leave butterflies with those colors alone.

In wild populations, the frequency of palatable and unpalatable monarchs varies widely. In North America, there are two distinct populations, a Western population and an Eastern population. The western population migrates to California every fall while the eastern population migrates to Mexico (Fink and Brower 1981; Brower and Moffit 1974). These two populations have very different palatability spectra because the distribution of food plants in the two areas are very different.

The California population contains a high percentage of palatable monarchs, but those that are noxious are extremely toxic, with a low ED 50 ranging between .009 g butterfly and .072 g butterfly (Martin *et al* 1992). Figure 1 shows the distribution of palatable and unpalatable monarchs in a sample from California.

The Massachusetts population of monarchs (Figure 1) has a greater number of individuals with cardenolides, but the average ED50 of the toxins is higher, around .152 g butterfly (Martin et al 1992), meaning that it would take a larger number of toxic Massachusetts butterflies than toxic California butterflies to cause a bird to vomit. In Massachusetts, most of the monarchs have some cardenolides, but only enough to make them bitter as opposed to emetic (Brower and Moffit 1974). The Massachusetts butterflies generally feed on *A. syriaca,* which is the most common milkweed in the eastern United States (Fink and Brower 1981).

Automimicry in other butterflies

These palatability spectra are not just found in the monarch butterfly. There is now good evidence of palatability spectra in a range of

butterfly species. For example, the queen butterfly, *Danaus gilippus,* shows a palatability spectrum. Like the monarch, the queen feeds on milkweed, many of which contain cardenolides. Because of the great variation among milkweeds, the queen also has a palatability spectrum that depends on the host plant of the larvae (Ritland 1994). Another example of a butterfly that shows a palatability spectrum is the checkerspot butterflies in the genus *Euphydryas.* Instead of storing cardenolides like the monarch or the queen, the checkerspot butterfly sequesters iridoid glycosides from its larval food plant, *Chelone glabra* (Scrophulariaciae) (Bowers 1990, 1980).

Although palatability spectra have been found in several species of butterflies, there have been no field experiments measuring the effect of variation in spectra on predation level in the wild. The experiment I did was attempting to measure this, making it the first field experiment of its kind.

This Study

Questions

The purpose of this experiment was to determine how variation in the palatability spectra affects the effectiveness of automimicry within a species. I addressed two main issues. First, what degree of protection did one palatable individual of a species get in populations with different levels of unpalatability? Second, what degree of protection did the entire population get with the same levels of unpalatability?

Original methods

In field experiments with wild birds as predators and pastry discs as "prey", I planned to test three different palatability spectra (Figure 2). In all of these spectra, 25% of the prey were palatable and 75% had some range of unpalatability. The first palatability spectrum showed classic Batesian mimicry, with 25% of the prey being palatable and 75% highly unpalatable. The second spectrum had 25% of the prey in each of four different palatability levels, ranging from completely palatable to very unpalatable. The third palatability spectrum consisted of 25% palatable, 50% slightly unpalatable, and 25% moderately unpalatable. Each spectrum was originally to be tested at two different sites, with the first site having brown as the models and automimics and green as the control, and the second with green as the models and automimics and brown as the control. The project was designed in this manner to insure that there was no pre-existing color preference that would confuse the results, and also to insure that the results from one site could be reproduced at a second site.

Artificial prey were made out of a mixture of flour and lard, dyed with edible food dye, and offered to the wild birds on feeding tables. This approach has been used in many experiments testing theories about prey defense and predator behavior (Edmunds and Dewhirst 1994, Greenwood *et al.* 1989, O'Donald and Pilecki 1970). For the first half of the experiment, pastry baits dyed brown were laced with varying amounts of ground monarch butterfly powder, as determined by pilot study 2. The butterfly powder was made from monarch butterflies collected from the Mexican overwintering sites in January, 1983, dried and ground into a fine powder.

The butterfly material was provided by Dr. L. P. Brower (University of Florida). Green pastry baits that had no monarch powder and that were completely palatable were used as a control species. For the second half of the experiment, green pastries were going to be laced with the butterfly powder from the same butterflies in the same amounts, and brown was to be used as the control .

Predictions

For spectrum one, I predicted that the population would have a large amount of protection. This population is an example of classic Batesian mimicry, with a palatable mimic and a very unpalatable model. In a laboratory study done by Jane Van Zandt Brower(1960) using starlings attacking palatable and unpalatable mealworms, prey populations with over 60% highly unpalatable models had a very low degree of predation. For this reason, both the population and the individual palatable member of the population will have a low predation rate and many more control prey will be taken than models or automimics.

Spectrum two had 25% palatable, and 25% each of slightly unpalatable, moderately unpalatable, and highly unpalatable. This population will still gain protection when compared to the control species, but the protection will not be as great as that of spectrum one. That is, more control prey will be taken than models and their automimics, but the ratio will be closer to 1 than spectrum 1. Furthermore, I predict that the birds will start to taste discriminate between the models and their automimics. Taste discrimination is when birds peck a prey to "taste" it before they actually eat it. In some cases the birds will simply taste and not kill, but in other cases, the birds will kill the prey first and then taste it for its level of palatability (Fink and Brower 1981). If the birds begin taste discriminating when faced with spectrum 2, the individual palatable member of the population should have an increased risk of predation compared to the unpalatable models.

The third population will also gain a certain degree of protection as a population, but because the majority of the population is either palatable or very mildly unpalatable, the population will have less protection than the other two. The ratio of control prey to models and automimics attacked should be closest to 1 for this example. In this case,

the low amount of cardenolide in the population will not be enough to keep the birds from eating the baits, even in the presence of an alternate prey species. Again, the birds should taste discriminate between the palatable and the unpalatable member, giving the palatable automimics an increased risk of predation relative both to the unpalatable models and to the automimics of spectra 1 and 2.

Pilot Studies: Methods and results

Before the actual experiment was started, three pilot studies were conducted. The first pilot study was run from October 24, 1995 until November 16, 1995. The purpose of this pilot study was to determine the colors for the experimental pastry baits. For my experiment I needed two colors that were eaten in approximately equal amounts, one for the cardenolide-laden prey and automimics and one for the palatable, alternate prey.

After the birds were accustomed to coming to the feeding table for seed, pastry baits of five different colors (light green, dark green, brown, red, and white) were placed outside on a feeding table near the woods behind Guion Science Center at Sweet Briar College. Pastry baits were about 1 cm in diameter and half gram in weight. Groups of 20 of each color were scattered on the table two times per day, weather permitting, for two hours. After the required time period, the pastries were picked up and the numbers of each color taken by the birds was recorded. The study was run until a minimum of 100 pastry baits were eaten.

From these trials, dark green and brown were chosen for the experimental prey colors, because they were eaten in almost equal numbers (Table 1).

The second pilot study was to determine the amount of powdered butterfly to place in the pastries for the different palatability levels. Powder from the ground up monarch butterflies from the Mexican overwintering population was mixed into brown pastry, that was then divided into 25 individual pastries and placed outside with 25 palatable brown pastries and 50 palatable green control pastries. The pastries were placed on the feeding table for the birds for one hour per day between November 18, 1995 and December 12, 1995, weather permitting, and the number of each "species" eaten was recorded. The table was observed at all times. The amount of butterfly powder in the pastries was doubled whenever it was apparent that the birds were not discriminating between the two colors.

Birds discriminated between browns and greens when there were 22.4 g of butterfly powder in 1000 g of pastry (Table 2). The pastries

started out with .28g of butterfly per 1000g of pastry. With this concentration, the birds took more brown than greens, so the concentration was doubled to .56 g of butterfly per 1000g of pastry. At this point, the birds were still taking a high proportion of browns compared to greens. At 1.12 g of butterfly per 1000g of pastry, the birds took more green than brown prey. At 2.24 g butterfly per 1000 g pastry, the birds took almost twice as many greens as browns. At this point, the semester was over, and there was no more time to continue the pilot study. The birds were showing a clear preference for the greens, but not at the desired 10:1 ratio, so I doubled the concentration of butterfly powder to 4.48 g per 1000g of pastry and called this my high concentration of the actual experiment. The concentration of powder added to the moderately unpalatable pastries was determined by halving the amount added to the highly unpalatable pastries and the amount added to the slightly unpalatable pastries was determined by quartering the original amount of butterfly powder.

During pilot study 2, the numbers of brown palatable and unpalatable prey was also counted for pilot study 3, to determine if the birds could visually discriminate between them. My research depended on the birds not being able to visually distinguish between the models and automimics. In order for me to distinguish between them, however, there had to be some sort of mark on the pastries that I could see. I placed a dot of yellow color made out of yellow food coloring, flour, and water, on the undersides of the brown palatable pastries. While the pastries were out for pilot study 2, the sites were continuously observed and the bird's behavior was recorded. The birds did not flip the pastries over and look for the yellow color on the bottom.

Observations of the birds during the pilot studies also allowed me to identify and define the range of bird behavioral responses to the pastries. The behaviors I identified as relevant included picking up and dropping pastries, flying away with pastries, pecking but not eating, and eating. At the Guion feeding site, the majority of the birds present were sparrows of different species, juncos, tufted titmice, white breasted nuthatches, house finches, and purple finches. The birds that fed on the pastries were the tufted titmice and the nuthatches. Very rarely at this site did any

other species eat a pastry, usually preferring the seed that was always out to attract the birds.

Experimental Attempt 1: Methods and Results

Methods

The pastries used for these experiments were made of flour and lard in the proportions of 1.5:1. They were colored with edible food dye, pressed out with a cookie press, and then shaped into small disks 1 cm in diameter, 1/4 cm in thickness, and a weight of .56g (weight based on N=50 pastries, standard deviation=0.08g). The birds were trained to take the pastries by placing white and red pastries out for the birds for 3-4 days before the experiments. The fresh pastries interspersed with black oiled sunflower seed, were placed out everyday until the birds were consistently eating the pastries. At this time, the actual experiments were started.

Each palatability spectrum was tested until a minimum of 100 experimental "prey" had been attacked and for at least one week. Fifty experimental prey in the predetermined proportions and fifty palatable control prey of a second color were placed out on the bird feeding tables for a maximum of one hour once a day. Daily test periods lasted until approximately one half of the pastries had been taken. Each site was tested at the same time every day. During this period, the sites were observed continuously and the number of unpalatable models, palatable automimics, and palatable control prey taken by the birds was recorded. Sites were located at least a quarter mile apart in order to reduce the probability that the birds would overlap between sites.

I used a comprehensive data sheet to record the date, time, site number, palatability spectrum being tested, temperature, and weather conditions. For each visit to the feeding table, I recorded the species of the bird, umber and colors of all pastries sampled, and the behaviors associated with it. For example, on February 5, 1996 I ran an experiment on site 2, palatability spectrum 1 (Figure 3). On this day, I had a total of six green pastries taken and one brown unpalatable pastry. The first visit to the table was by a chickadee who pecked and then ate a green pastry. The second visit was by a sparrow who ate one green pastry. At the end of

the experiment, the number I retrieved ("in") was counted and subtracted from the number I put out to get the number eaten.

Results from Attempt 1

The first experiment was run from An 13-21 at the house of Reuben Miller, Waugh's Ferry Road, Amherst, Va. Birds took 189 green palatable pastries, 52 brown unpalatable pastries, and 17 brown palatable pastries (table 3). At this site, the majority of the birds eating were titmice, who accounted for well over 75% of the pastries eaten. There was a strong color preference for green from the beginning of the experiment, with the birds taking many more greens than browns even before they had exposure to the unpalatable pastries. There was no noticeable difference in the treatment of brown palatable and brown unpalatable pastries. I placed brown unpalatable:palatable pastries out in a 3:1 proportion and the birds ate them in a 3.06:1 ratio.

Reasons for changing experimental design

The results from site one suggested that the experiment needed to be modified. The birds did not seem to notice when they did eat the pastries with the cardenolides in them. As figure 4 shows, the birds started out eating next to none of the brown pastries, but as time went on, a higher proportion of the removed prey were brown. This is in direct conflict with what was predicted for this experiment. The birds should have started off eating equal numbers of browns and greens, and then stopped eating the brown pastries because they were nasty, or because the birds were taste discriminating. It was therefore concluded that the cardenolides in the butterfly powder were neither potent enough nor in high enough concentration for the birds to be affected.

The butterfly powder had been spectroassayed in Lincoln P. Brower's lab on January 8, 1983 for cardenolide content using the methods described in Brower *et al.* 1972. There were approximately 72 µg of cardenolide per .1g of butterfly powder. The "highly unpalatable pastry" contained 4.48 g butterfly per 1000g pastry, or .32 µg cardenolide per 0.1g of pastry. Since a blue jay emetic unit of Mexican butterfly contains 320 µg (Martin *et al.* 1992), it would take approximately 176 half-gram pastries to make one blue jay vomit (table 4). Brower and Fink (1985)

summarizes a study in which the amount of pure cardenolides necessary for birds to taste reject was tested. With digitoxigenin, a cardenolides with an ED50 near to that of the Mexican butterflies, the rejection level was 96 μg per .1g of bread, 300 times the concentration in the pastries. Even when the more potent cardenolides were tested, the concentration at which taste rejection occurred was much higher than the concentration in my pastries. Therefore my experiment was started using a different kind of butterfly powder.

Experimental Attempt 2: Methods and Results

Methods

For the second attempt of this experiment, the basic methods remained the same. The most important change was the use of monarch butterflies that were reared on *A. humistrata*, a very potent milkweed. The second change was that the size of the pastry prey was reduced.

Butterflies that were reared on *A. humistrata* have an emetic potency of 57.1 μg cardenolide per emetic unit (Martin *et al.* 1992). The *A. humistrata* butterfly powder was assayed on April 12, 1996 in Lincoln Brower's lab and contained 351 μg cardenolide/.1g butterfly. For the highly unpalatable pastries, .84 g of butterfly powder as added to 75 g of pastry, producing pastry with 39.3 μg of cardenolide per gram. This makes each highly unpalatable pastry, weighing 0.25g, contain approximately 0.17 emetic doses (table 4). For the moderately unpalatable pastries, enough butterfly powder was added to make each pastry .09 of an emetic dose, and for the slightly unpalatable pastries, the amount of powder added made each pastry worth .04 of an emetic dose.

The size of the pastries was changed because the smaller birds appeared to have some difficulty eating the original pastries. The weight of the pastries was reduced to .25g (Based on N=50 pastries; standard deviation=.05g). The diameter of the pastries was also reduced. The new pastries were .75cm in diameter and .25cm in thickness.

Another change made due to time constraints was the reduction of the number of palatability spectra being tested. The third palatability spectrum was cut from the experiment, leaving palatability spectrum 1 with 75% of the brown highly unpalatable and 25% of the brown completely palatable, and palatability spectrum 2 with 25% of the browns

in each of 4 palatability levels, ranging from completely palatable to highly unpalatable.

Even though there was a strong color preference in experiment 1, brown and green were still the colors used for the experiments. There had been no initial preference at the Guion site during pilot studies 1 and 2 (Tables 1 and 2), indicating that the preference could be limited to the individual birds at site 1. In addition, there was not enough time to run a second pilot study to select new color choices. To insure that any color preferences did not skew the data, each palatability spectrum was supposed to be tested twice at two separate sites, once with brown as the unpalatable pastries and once with green as the unpalatable pastries.

Sites 2 and 3 were used for this study. Site 2 was located in the backyard of Dr. Linda Fink, 3 Woodland Rd, Sweet Briar College, and site 3 was located at the house of Karla Faulconer, Farmhouse Road, Sweet Briar College. Site 2 was run from January 30, 1996 until February 18, 1996. Site 3 was run from January 30 and February 10, 1996. It was decided after the completion of site 2 that no more experiments would be done because of time constraints and conflicting class schedules.

Results from attempt 2

Palatability spectrum 1 was tested at site 2 (table 5). At this site, a total of 92 greens were sampled to the 16 browns sampled. Of the brown pastries sampled, 13 were actually taken from the table: 6 palatable and 7 unpalatable. At site 3, palatability spectrum 2, a total of 94 green pastries were sampled and 14 browns, of which 10 were taken from the table (3 palatable and 7 unpalatable) (table 6). There was a significant difference between the number of greens to browns eaten at the two sites (X^2=68.35, degrees of freedom=1, X^2=crit=3.84, $p<0.001$). Because the birds started out eating large numbers of greens and no browns before they had the chance to learn that some of the brown pastries were unpalatable, I attributed this difference to a color preference on the part of the birds, not to a difference in the palatability of the pastries.

Bird Behavior

Over the course of the experiment, at least 10 different bird species visited the table, but some ate only seed. The most abundant and frequent visitors to the table were the sparrow species; these were not responsible for much of the pastry feeding. During this experiment, each species of bird behaved in a different manner, both on their approach to the feeding table and with their reactions to the cardenolides in the pastries. The most common bird handling pastries at sites 2 and 3 was the blue jay (table 7). Out of a total of 67 visits in which at least 1 pastry was taken, the blue jays accounted for 36. They came to the feeding table either by themselves or in groups of 2 or 3, taking between 3 and 6 pastries at a time. On 3 visits I could determine that the brown pastries the birds ate were models because when the total eaten was counted for the day, there were no automimics taken from the table. On 2 of these encounters, the blue jays showed the expected response when eating an unpalatable pastry by shaking their heads, bill wiping, and fluffing up (Brower 1969) (at site 1, where the pastries were not potent, the blue jays never ate any of the brown unpalatable pastries). Blue jays also showed the clearest color preference. It was very rare for me to see a blue jay eating a brown pastry. Of the 36 visits made by blue jays at sites 2 and 3 in which a total of 142 pastries were sampled, there were only 11 brown pastries sampled, and of those 11, only 5 were eaten (45%). In comparison, 134 green pastries were sampled by blue jays with 86 of them eaten (64%). At site 1, blue jays ate only green pastries, never any brown.

The second and third most common birds attacking pastries were the sparrows (which could not always be identified to species) and the tufted titmouse, with 14 and 11 visits respectively in which pastries were handled. Neither had as strong of a color preference as the blue jay, but they were definitely biased against the brown pastries. The sparrows sampled 11 greens and 3 browns. The titmice sampled 7 greens and 4 browns. The sparrows were more likely to visit and eat seed, but they did eat pastries, usually pecking at them until they were gone. The titmice tended to flit around the table, land, grab a single pastry, and fly off to a nearby bush. Of the 11 pastries the titmice sampled, the birds flew away

with 10 and ate only 1 at the table (I saw a second pastry being eaten at a tree).

Discussion

Testing the predictions

When I started this experiment, I was originally going to use three palatability spectra, one that was very potent, one with a moderate potency, and one that was even less potent. Because I ran out of time, I had to cut the third palatability spectrum from the experiment. However, because I ran the first site with a butterfly powder that was not potent, I inadvertently had three palatability spectra. Palatability spectrum 1 was run at site 2 with *A. humistrata*-raised butterflies as the cardenolide source. This spectrum had a majority of the brown pastries highly unpalatable. Palatability spectrum 2 was run at site 3, again with *A. humistrata*-raised butterflies as the cardenolide source. This spectrum was moderately nasty, with a range of palatability. Finally the third palatability spectrum was run at site 1 with Mexican butterflies as the cardenolide source. This spectrum was not nasty at all, and therefore could be considered a third palatability spectrum, even though it was technically the same palatability spectrum as site 2 (75% "highly unpalatable", 25% palatable).

The results of this experiment were inconclusive, with no strong patterns emerging from the data. However, some trends suggest that this experiment would work it I had more of time. One of the more important trends that could be seen, but not quantified, was how the blue jays treated the unpalatable pastries. On three different occasions, a blue jay ate a brown pastry that I knew was unpalatable. On two of these occasions, the blue jays showed the typical cardenolide response of bill wiping, head shaking, and feather puffing. When I saw birds eat a brown pastry, I tried to watch them so that I could see what they attacked next. I saw two of these three blue jays return to the table, avoid the brown pastries, and eat only greens or seed. The way the blue jays treated the brown unpalatable pastries after eating one supported my predictions. I originally predicted that the birds would eat the brown unpalatable pastries and learn that they were bad. After that I expected the birds to avoid the browns, which is what the blue jays did in the two instances that I observed.

There was an interesting trend in the ratio of palatable to unpalatable brown pastries taken by the birds at the three sites. At each

site I placed the pastries out in a ratio 3:1, unpalatable to palatable. IF the birds were not discriminating, I would expect them to take the brown pastries in the 3:1 ratio. They did so at site 1 when the butterfly powder was not potent. At site 2, although only 13 brown pastries were taken, which is too few to analyze statistically, a trend can still be seen. Of the 13 browns taken, 6 were palatable and 7 were unpalatable, a ratio of 1.17:1. This suggests that the birds may have been discriminating between the palatable and unpalatable brown pastries. At site 3, the birds took the pastries in a 2.33:1 ratio; the overall palatability of the brown pastries at site 3 was intermediate between that at sites 1 and 2.

These ratios follow the original prediction I made about how the unpalatable pastries would be treated by the birds. I originally said that the birds would do the most discrimination against the highest unpalatability, which in this case was site 2. At this site, the ratio of brown palatable:unpalatable taken was closest to 1, suggesting that the birds were discriminating between the two palatabilities. I also predicted that at the palatability spectrum with medium unpalatability, in this case site 3, the birds would discriminate, but not as much. Here the ratio was 2.33:1, which is farther from 1 than that of site 2. Finally, the least nasty palatability spectrum, site 1, had a ratio of brown palatable: unpalatable of 3:1, which is what would be expected if there were no discrimination.

When the ratio of brown to green prey eaten from sites 2, 3, and 1 were compared, there were few differences between them. However, at site 1, where monarchs from the Mexican overwintering sites were used to make the butterfly powder, the birds, over time, began to eat more of the browns, with a ratio of green:brown of 2.79:1. This is probably because the powder in them was not potent enough for the birds to notice in small amounts, so they had begun to overcome the color preference by eating brown pastries. For site 3, the ratio of greens to browns was 9.4:1. This shows that the birds were eating many more greens than browns. Most of this is due to the color preference, but the aversion to brown pastries would have been enforced whenver they did eat a brown unpalatable, because the browns were very potent. Finally, site 2 had a ratio of brown to green taken of 7.08:1. Again, this is farther from 1:1 than that of site 1, but it is lower than that of site 3.

These results support the original predictions that the birds would eat more green than brown pastries, even when the color preference is taken into account. When the brown pastries had Mexican butterflies in them and therefore were not very potent, the birds learned to eat them, and the number of browns to greens eaten was gradually approaching 1:1. However, when there were *A. humistrata*-raised butterflies in the pastries, the numbers of browns eaten was much lower. Birds showed a color preference with all three palatability spectra, but if the birds did not learn to discriminate further against the brown pastries, all of the spectra would look like that used at site 1, with the numbers of brown pastries taken approaching closer to that of green pastries over time.

When sites 2 and 3 are compared, they do not follow my original predictions that there would be a greater percentage of greens eaten at the site with more highly unpalatable pastries. Site 2 had the greatest percentage of highly unpalatable pastries placed out, but the birds ate a smaller proportion of the greens than at site 3. One possible explanation for this is that each site had different proportions of bird species, and each species treats the models and automimics in a different manner.

Problems with the study

The color preference was the greatest difficulty encountered during this experiment. At all three sites, the birds seemed to prefer green pastries over brown pastries even before they had time to learn that the brown pastries had cardenolides in them. On the first day of each experiment, the birds took many more green than brown pastries (tables 3-5). At this point in the experiment, the birds had never had access to unpalatable pastries, so they could not have known that the brown pastries were unpalatable. This points to a clear pre-existing color preference.

Green and brown were chosen as the experimental colors on the basis of the first pilot study. During this study, the birds ate green and brown pastries in approximately equal numbers (Table 1). This piece of information is in direct conflict with what was found during the actual experiment. One explanation could be that during the pilot study the birds treated light green and dark green as the same color, even though to my eyes, they were very different. If the results are looked upon in this light,

greens were eaten about twice as often as the browns in the first pilot study, suggesting the color preference. The major flaw with this explanation is that in pilot study 2, where the birds had access to only dark green and brown pastries, they did not show a color preference. The birds did exactly what I expected of them, which was to take approximately equal numbers of the two colors in the beginning of the experiment, then gradually learn that the brown ones were unpalatable and stop eating them. This points to no pre-existing color preference at the pilot study site. A possible explanation why the birds did not show a color preference at the guion pilot study site, but did at the other three sites, could be due to differences in the bird species found at each site. At the pilot study site, the majority of the pastries were taken by white-breasted nuthatches and tufted titmice. At site 1, tufted titmice were the main pastry predators. At sites 2 and 3, the blue jays took the majority of the pastries. This difference in the concentration of the bird species could explain why there were color preferences at the three experimental sites, but not at the Guion site.

If I were to do this experiment again, I would run another color pilot study. In this pilot study, I would choose colors that could not be confused by the birds, such as red, white, green, yellow, blue, and brown. The two colors chosen for this experiment would then be tested by themselves as different sites than the original pilot study, to insure that they are really being taken in equal numbers. However, because of the possible variation in bird species between sites, there still may be a color preference. Therefore, the best way to deal with color preferences is, as I originally planned, to alternate the colors used for the controls and models.

For this experiment I used two colors typical of cryptic insects. Although the original reasons the colors were chosen had nothing to do with the fact that they were cryptic colors, I think that it was an important aspect in the design of the experiment because some birds learn to discriminate against bright, warning colors faster than they do cryptic colors (Guilford 1990). If I had used one bright color such as red, and a cryptic color such as brown, the birds would have been more likely to discriminate against the red pastries simply because many chemically

defended species use red as a warning color, and the birds learn to associate that color with bad tasting or nasty insects.

The original plan had been to collect data on at least four sites during January, running two sites per day, and then to run two more sites, one at a time during February. Each site was observed continuously every time pastries were placed out. It took approximately two hours to set up, run, and take down each trial. Running two sites per day was manageable during January when I had no other classes, but not during the Spring semester when I had classes all day.

This timeline was destroyed when Virginia experienced a record-breaking winter storm that unloaded two feet of snow in the area. I was snowed in for a week, and was unable to run any experiments except at site 1 where I was staying. Although the bad weather delayed the data collection, it was very helpful in getting birds to eat the pastries. Because their natural food sources were buried underneath two feet of snow, many birds in the area depended on the bird feeders for food. During this time, they ate anything I put out for them, and they quickly learned to eat the pastries, even though they were a new type of food.

The next experiments were delayed further while I evaluated the results from site 1, decided to use *A. humistrata*-reared butterflies, and obtained them from Lincoln Brower. As a result of these problems, I only collected data on 2 spectra and 3 sites instead of 3 spectra and 6 sites.

Another problem was that I was unable to get close enough to the bird tables in two of the three sites because they were far away from convenient observation sites where the birds would not be scared away from the table. This led to problems in identifying the colors of some prey taken, and in seeing how many of the pastries were being removed by the birds. This problem was significant when the numbers actually taken by the birds, as recorded by my tallying the remaining pastries after each trial, were compared to the numbers I observed being taken by the birds. At site 1 was able to see 87% (205 out of 234) of the pastries taken. At site 2, I was able to see 75% (80 out of 105) of the pastries taken and at site 3, I saw 82% (85 out of 104) of the pastries taken. At site 1, the bird table was situated right outside of a window where I could watch all the activity. At site 2, I was 20 feet away from the table, and at site 3, 10 feet away. A chi-square test was run in order to determine if the

differences among sites in the number of pastry removals I observed and missed observing were significant. There was a significant difference found (X^2=7.22, degrees of freedom=2, X^2crit=5.00, p<0.05).

This problem could be remedied using a blind such as those used by hunters and birders. These blinds can easily be found in sporting good catalogs. Placing the blinds next to the bird tables would allow me to see exactly what the birds were doing during each visit to the table. Another way to fix this problem would be to place the bird table directly outside a window, allowing me to sit inside and watch the birds without disturbing them. A third possible solution would be to place a video camera next to the table in order to record all of the bird activity at the table.

A related problem was that I had to watch the bird table continuously and I was unable to observe what the birds did after eating or flying off with a pastry. This type of information is extremely valuable because many reactions to cardenolides occur minutes after the ingestion of the drug. This could be remedied by having a second observer present to watch the birds after they flew away from the table.

Implications of this study

Most studies involving mimicry ignore the natural variation; the prey is either completely palatable or completely unpalatable. There is no in between. Even in other studies done with automimicry, this can be seen. For example, a mathematical model of automimicry was published by Brower, Pough, and Meck in 1970, giving a formula that determined the automimetic advantage of a species when different factors were manipulated. This model only had two palatability levels. The models were considered highly unpalatable and the automimics were completely palatable. This is not accurate because in natural population of monarchs and other butterflies, there is a range of palatability, not just two levels.

During this study, I not only saw how the cardenolides affected the insect populations, but how the birds responded to the different palatability spectra. All of these observations were based on limited sample sizes, and no statistical analysis were done, but they did seem like real differences. For example, blue jays consistently avoided the brown pastries from the start of the experiment, especially after they tasted an unpalatable brown one. Sparrows on the other hand, were less

discriminatory. They showed a color preference against the brown pastries, but they seemed more willing to try the different colors than the blue jays were.

By studying the variation in cardenolide content, I was able to gain some insight as to why there is still predation on chemically defended prey species. For example, suppose there is a chemically defended butterfly species that shows the characteristics of automimicry. At a certain site, the larvae of the butterflies feed on four different food plants that are present in equal numbers, each containing a different amount of the chemical the butterflies sequester. Plant A contains none of the chemical, Plant B contains just a little bit, Plant C contains still more, and finally Plant D contains the highest amount of the chemical. In this example, the amount of chemical in the food plant would be directly related to the amount of chemical in the butterfly.

Predators of this butterfly would have to avoid the highly unpalatable butterflies. If they were taste discriminating, they would only eat the butterflies with low amounts of the chemical in them, those that fed on either Plant A or Plant B. This would cause selection pressures against butterflies that fed on these two plants, and the numbers of that butterfly subset would go down. However, those that contained more of the chemical would have much greater protection, and their numbers would increase in relative proportion to the entire population.

Suppose that oviposition preference of the female butterfly is determined genetically, so that a female will deposit the majority of her eggs on the same foodplant she fed on as a larvae. The butterflies that ate either Plant C or Plant D would live to reproduce more than the other two subgroups within the species, and more of the larvae in the next generation would be feeding on C or D. This would affect the predators: because there would be fewer palatable butterflies of that species available as a food source, the predators would turn to alternate food sources.

This would decrease the selection pressure against butterflies on plants A and B. As time went by, more of the palatable butterflies would survive, the proportion of palatable butterflies would increase, and predators would begin to eat the butterflies again.

This example shows that the palatability spectra themselves may change, especially for stationary prey species. One problem with this theory is how do the predators know when the spectra change? This can be answered when the time span involved in the cycle is looked at. These changes in the relative numbers within the population do not happen immediately, but over a period of years. During this time span, the predators are reproducing and therefore providing new, naive individuals who sample the changing prey population. They can respond either by avoiding a population of very toxic butterflies or by learning to taste discriminate between the nasty ones and the palatable ones. A single member of a predator population will probably be exposed to only one palatability spectrum in an area, unless it is a long lived species, but the offspring of that member and their offspring will have access to the changing numbers within the prey species.

Conclusion

This field experiment had a lot of kinks that had to be worked out. Although I was unable to draw firm conclusions, the results are encouraging. Mixing powdered butterflies into pastries is a good design. Wild birds will feed on colored pastries and will decrease their attacks on a color that is partly unpalatable. It is possible to watch individual birds and measure the differences in the responses of different species. With more time and a field assistant, I feel certain that the experiment will work, and the predictions about the three palatability spectra can be tested.

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Works Cited

Bowers, M. Deane. 1990. Recycling plant natural products for insect defense. In Insect Defenses. Evans, David L. and Schmidt, Justin O. eds. Albany, State University of New York Press. pp. 353-386.

Bowers, M. Deane. 1980. Unpalatability as a defense strategy of western checkerspot butterflies (*Euphydryas scudder,* Nymphalidae). Evolution 35(2): 367-375.

Brower, Jane Van Zandt. 1960. Experimental studies of mimicry: The reactions of starlings to different proportions of models and mimics. The American Naturalist XCIV(877): 271-282.

Brower, L. P. 1969. Ecological chemistry. Scientific American 220(2): 22-29.

Brower, L. P. 1984. Chemical defence in butterflies. In The Biology of Butterflies. Orlando, Academic Press. 109-133.

Brower, L. P., Brower, J. V. Z. and Corvino, J. M. 1967. Plant poisons in a terrestrial food chain. Proceedings of the National Academy of Sciences 57(4): 893-898.

Brower, L. P., Fink, L.S. 1985. A natural toxic defense system: Cardenolides in butterflies versus birds. Experimental assessments and clinical applications of conditioned food aversions. 433: 171-188.

Brower, L. P., McEvoy, P. B., Williamson, K. L. and Flannery, M. A. 1972. Variation in cardiac glycoside content of monarch butterflies from natural populations in eastern North America. Science 177: 426-429.

Brower, L. P. and Moffitt, C. M. 1974. Palatability dynamics of cardenolides in the monarch butterfly. Nature 249(5454): 280-283.

Brower, L. P., Pough, F. H. and Meck, H. R. 1970. Theoretical investigations of automimicry, I. Single trial learning. Proceedings of the National Academy of Sciences 66(4): 1059-1066.

Brower, L. P., Ryerson, W. N., Coppinger, L. L. and Glazier, S. C. 1968. Ecological chemistry and the palatability spectrum. Science 161:1349-1351.

Brower, L. P., Seiber, J. N., Nelson, C. J., Lynch, S. P. and Tuskes, P. M. 1982. Plant-determined variation in the cardenolide content,thin-layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus plexippus* reared on the milkweed, *Asclepias eriocarpa* in California. Journal of Chemical Ecology 8(3): 579-633.

Edmunds, M. 1974. Defense in animals. New York, Longman Group Limited.

Edmunds, M. and Dewhirst, R. A. 1994. The survival value of countershading with wild birds as predators. Biological Journal of the Linnean Society 51: 447-452.

Fink, L. S. and Brower, L. P. 1981. Birds can overcome the cardenolide defence of monarch butterflies in Mexico. Nature 291(5810): 67-70.

Greenwood, J. J. D., Cotton, P. A. and Wilson, D. M. 1989. Frequency-dependent selection on aposematic prey: some experiments. Biological Journal of the Linnean Society 36:213-226.

Guilford, Tim. 1990. The evolution of aposematism. In Insect Defenses. Evans, David L and Schmidt, Justin O. eds. Albany, State University of New York Press. pp. 23-62.

Martin, R. A., Lynch, S. P., Brower, L. P., Malcolm, S. B. and Van Hook, T. 1992. Cardenolide content, emetic potency, and thin-layer chromatography profiles of monarch butterflies, *Danaus plexippus*, and their larval host-plant milkweed, *Asclepias humistrata*, in Florida. Chemoecology 3:1-13.

O'Donald, P. and Pilecki, C. 1970. Polymorphic mimicry and natural selection. Evolution 24(2): 395-401.

Ritland, David B. 1994. Variation in palatability of Queen butterflies (*Danaus gilippus*) and implications regarding mimicry. Ecology 75(3): 732-746.

Wickler, W. 1968. Mimicry in plants and animals. Hampshire, BAS Printers Limited.

Figure 1: This shows the range of palatability found in the Massachusetts migrant monarch population and the California population in cardenolide per butterfly. In the Massachusetts population, the majority of the butterflies have a moderate amount of cardenolides. In the California population, the majority of the butterflies have a low level of cardenolides. (From Fink and Brower 1981).

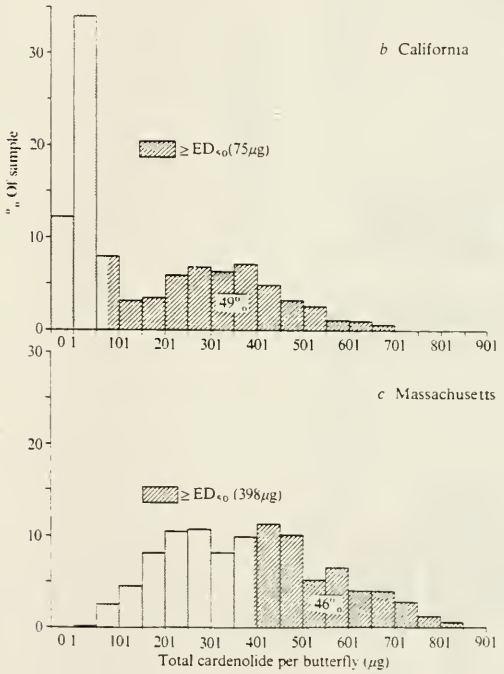

Figure 2: Three palatability spectra that were originally going to be used for the experiment. Because of time constraints, spectrum 3 was cut from the experiment.

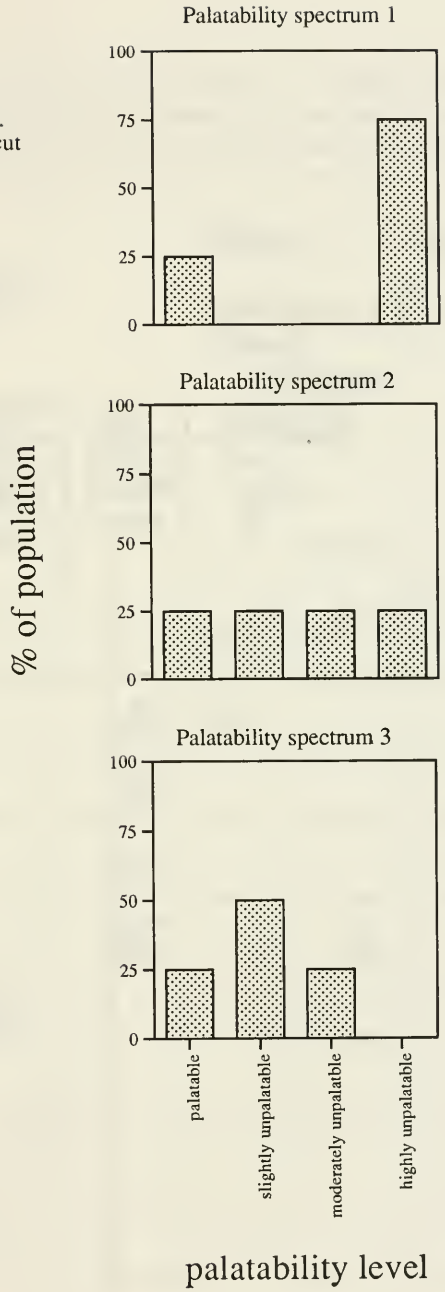

Figure 3: Copy of the data sheet I used to collect data at the experiment sites. These data sheets allowed me to record the time of the experiment, the date, the temperature, what site I was testing, and the weather. In addition to these, the total number of each color and palatability placed out, taken in, and taken by the birds was recorded, along with observations on feeding behaviors.

DATE: 2/5/96 DAY 3 Temp.: 20°F

TIME START: 2:15 Weather: Cold, Sunny
END: 3:18 Cond. Windy

SITE No.: 2

PALAT Spec.: 1 BUP

	OUT	IN	EATEN
	60g	44g	6g
	37BUP	36BUP	18UP
	13BP	13BP	Ø

Brown Green

VISIT#	Sp.	PE.	Re	FW	PUD	E	O	Sp	PE	Re	FW	PUD	E	O
1								CH	1				2	
2								SP					1	
3								SP					1	
4								BJ			2		1	
5	SP					1								
6														
7														
8														
9														
10														
11														

Table 1: Results from pilot study 1. 20 pastries per color were offered simultaneously for 2 hours each day. Table values indicate the number of pastries removed by the birds. Dark green and brown were chosen for the experimental prey because they were eaten in equal numbers.

	Day										
color	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**total**
Red	0	0	0	0	0	1	6	0	9	7	23
White	3	2	0	0	0	0	9	1	11	15	41
Lt. Grn	0	0	1	0	0	0	8	1	4	5	19
Dk Grn	1	0	1	0	3	3	3	0	7	6	24
Brown	0	0	0	0	1	1	3	3	10	6	24

Table 2: Summary of results from pilot study 2 determining the concentrations of butterfly powder to use in the actual experiments. Pastries with varying amounts of butterfly powder were placed out for the birds in order to determine at what concentration the birds could detect and discriminate against cardenolides. This pilot study started out with the low concentration of .28g butterfly per 1000g pastry. This was doubled whenever it was apparent the birds were not discriminating against the pastries with the cardenolides in them. When there were 2.24g butterfly per 1000g pastry, there was a significant difference in the number of green taken vs. the number of brown taken (X^2=10.86, degrees of freedom=1, X^2crit=3.84, p<0.001). Each day 25 brown unpalatable (BUP), 25 brown palatable (BP), and 50 green (GRN) pastries were placed out for the birds and the number taken within one hour was recorded.

		# Out	# Taken	Brown:Green
		.28g b'fly /1000g pastry		63:47=1.34
Day 1	BUP	50	14	
	BP	50	8	
	GRN	100	16	
Day 2	BUP	50	21	
	BP	50	20	
	GRN	100	31	
		.56 g b'fly/1000g pastry		15:19=.79
Day 3	BUP	50	6	
	BP	50	9	
	GRN	100	19	
		1.12g b'fly/ 1000g pastry		52:59=.88
Day 4	BUP	50	13	
	BP	50	18	
	GRN	100	29	
Day 5	BUP	50	10	
	BP	50	11	
	GRN	100	30	
		2.24g b'fly/ 1000g pastry		90:140=.64
Day 6	BUP	50	17	
	BP	50	17	
	GRN	100	26	
Day 7	BUP	50	8	
	BP	50	12	
	GRN	100	32	
Day 8	BUP	50	5	
	BP	50	8	
	GRN	100	39	
Day 9	BUP	50	12	
	BP	50	11	
	GRN	100	43	

Table 3: Summary of results from site 1, palatability spectrum 1 (75% highly unpalatable, 25% palatable), showing numbers of pastries sampled, taken, picked up and dropped (PUD), pecked, flown with (FW), and eaten by birds. Each day 50 green, 12-13 brown palatable, and 37-38 brown unpalatable were offered to make a total of 100 pastries available to the birds at each trial.

Site 1, Palatability spectrum 1, Mexican butterflies

	sampled	taken (palatable, unpalatable)	PUD	Pecked	FW	Eaten	didn't see
Day 1							
Green	21	21	0	0	5	0	16
Brown	1	1 (0,1)	0	0	1	0	0
Day 2							
Green	45	45	0	0	25	14	6
Brown	7	7 (1,6)	0	0	4	0	3
Day 3							
Green	34	34	0	0	18	16	0
Brown	12	12 (2,10)	0	0	2	0	10
Day 4							
Green	24	24	0	0	17	0	7
Brown	16	15 (7,8)	0	1	15	0	0
Day 5							
Green	21	21	0	0	21	0	0
Brown	14	12 (3,9)	1	1	7	0	5
Day 6							
Green	19	18	0	1	13	5	0
Brown	11	10 (1,9)	1	0	6	0	4
Day 7							
Green	27	26	0	1	21	3	2
Brown	14	12 (3,9)	0	2	12	0	0
Total							
Green	191	189	0	2	120	38	31
Brown	75	69 (17,52)	2	4	47	0	22

Table 4: Calculations for determining the blue jay emetic dose per pastry and the number of pastries that would make one blue jay vomit for both Mexican butterflies and *A. humistrata* -raised butterflies. If I used the Mexican butterflies, it would take 176.8 pastries to make 1 blue jay vomit. Each pastry would contain .6 % of an emetic unit. If I used the *A. humistrata*-reared butterflies, it would only take 5.83 pastries to make one blue jay vomit. In this case, each pastry would contain 17 % of an emetic unit.

A. Mexican butterflies

Pastries per ED_{50}

$$\frac{72\ \mu g\ cardenolide}{.1g\ butterfly} = \frac{720\ \mu g\ cardenolide}{1\ g\ butterfly} * \frac{4.48\ g\ butterfly}{1000g\ pastry} = \frac{3.23\ \mu g\ cardenolide}{1g\ pastry}$$

$$\frac{3.23\ \mu g\ cardenolide}{1g\ pastry} * \frac{.56g}{pastry} = \frac{1.81\mu g\ cardenolide}{pastry}$$

$$\frac{320\ \mu g\ cardenolide}{ED_{50}} * \frac{1\ pastry}{1.81\ \mu g\ cardenolide} = \mathbf{176.8\ \frac{pastries}{ED_{50}}}$$

ED_{50} per pastry

$$\frac{ED_{50}\ units}{320\ \mu g\ cardenolide} * \frac{1.81\ \mu g\ cardenolide}{pastry} = \mathbf{.006\ \frac{ED_{50}\ units}{pastry}}$$

B. *A. humistrata* raised butterflies

Pastries per ED_{50}

$$\frac{351\ \mu g\ cardenolide}{.1g\ butterfly} = \frac{3510\ \mu g\ cardenolide}{1\ g\ butterfly} * \frac{.84\ g\ butterfly}{75\ g\ pastry} = \frac{39.3\ \mu g\ cardenolide}{1\ g\ pastry}$$

$$\frac{39.3\ \mu g\ cardenolide}{1\ g\ pastry} * \frac{.25g}{pastry} = 9.8\ \mu g\ cardenolide\ per\ pastry$$

$$\frac{57.1\ \mu g\ cardenolide}{ED_{50}} * \frac{1\ pastry}{9.8\ \mu g\ cardenolide} = \mathbf{5.83\ \frac{pastries}{ED_{50}}}$$

ED_{50} per pastry

$$\frac{ED_{50}}{57.1\ \mu g\ cardenolide} * \frac{9.8\ \mu g\ cardenolide}{pastry} = \mathbf{.17\ \frac{ED_{50}\ units}{pastry}}$$

Table 5: Summary of results from site 2, palatability spectrum 1(75% highly unpalatable, 25% palatable). Only days on which pastries were taken are shown on this table. PUD=Picked up and dropped, FW=Flown with.

Site 2, Palatability spectrum 1, *A. humistrata butterflies*

	sampled	taken (palatable, unpalatable)	PUD	Pecked	FW	Eaten	didn't see
Day 2							
Green	10	9	0	1	2	6	1
Brown	1	1 (1,0)	0	0	0	1	0
Day 3							
Green	7	6	0	1	1	4	1
Brown	1	1 (0,1)	0	0	0	1	0
Day 4							
Green	14	13	0	1	3	5	5
Brown	3	2 (0,2)	1	0	0	1	1
Day 5							
Green	6	6	0	0	4	2	0
Brown	1	1 (1,0)	0	0	1	0	0
Day 6							
Green	27	27	0	0	6	15	6
Brown	3	2 (0,2)	1	0	0	1	1
Day 7							
Green	18	18	0	0	3	7	8
Brown	4	3 (1,2)	0	1	1	0	2
Day 8							
Green	13	13	0	0	3	10	0
Brown	3	3 (3,0)	0	0	1	2	0
Total							
Green	95	92	0	3	22	49	21
Brown	16	13 (6,7)	2	1	3	6	4

Table 6: Summary of results from site 3, palatability spectrum 2. Only days on which pastries were taken are shown on this table.
PUD=Picked up and dropped, FW=flown with

Site 3, Palatability spectrum 2, *A. humistrata* butterflies

	Sampled	taken (palatable, unpalatable)	PUD	Pecked	FW	Eaten	didn't see
Day 1							
Green	25	24	0	1	4	15	5
Brown	4	2 (0,2)	2	0	2	0	0
Day 2							
Green	26	26	0	0	6	14	6
Brown	0	0 (0,0)	0	0	0	0	0
Day 4							
Green	4	4	0	0	3	1	0
Brown	4	4 (1,3)	0	0	2	1	1
Day 6							
Green	40	40	0	0	16	21	3
Brown	5	4 (2,2)	0	1	0	1	3
Total							
Green	95	94	0	1	29	51	14
Brown	13	10 (3,7)	2	1	4	2	4

Table 7: Total number of observed visits to the feeding tables at sites 2 and 3 combined for each bird species and their observed behaviors. The blue jay was the most common visitor to the tables.

Species	#visits	#pastries handled per visit	#Pastries							
			PUD	Green Peck	FW	Eat	PUD	Brown Peck	FW	Eat
Blue jay *Cyanocitta cristata*	36	2-13	0	3	42	86	4	2	0	5
House Sparrow *Passer domesticus*	14	1	0	0	1	10	0	0	0	3
Tufted titmouse *Parus bicolor*	11[a]	1	0	0	6	1	0	0	4	0
Carolina Chickadee *Parus carolinensis*	2	1-2	0	1	2	0	0	0	0	0
White-breasted Nuthatch *Sitta carolinensis*	2	1	0	0	1	0	0	0	1	0
Red-belliedWoodpecker *Melanerpes carolinus*	1	1	0	0	0	0	0	0	1	0
Rufous-sided Towhee *Pipilo erythrophthalmus*	1	1	0	0	0	0	0	0	0	1

a. I saw one of the titmice that flew with a pastry eat it in a tree. This is recorded in the text.