Anti-predator adaptations and strategies in the Lepidoptera

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General Abstract

This thesis examines visual anti-predator strategies employed by the Lepidoptera. I examine key aspects of pattern and behaviour and how they relate to the reduction of an individual's predation risk.

Symmetrical patterns have been found to be easier to remember and pick out, suggesting that symmetry is beneficial to aposematic displays. This suggests that symmetry may be maladaptive in cryptic patterning and asymmetry beneficial. In Chapter one, I report the results of a field experiment using artificial prey and wild birds to investigate how asymmetry and symmetry affect the efficiency of cryptic patterning to reduce predation. I found that asymmetry does not affect predation rate, in agreement with previous work. Yet, there is still the problem of how to mesh this with the potentially conflicting conclusions of symmetry studies.

Chapter two examines aspects of the intimidation hypotheses of Lepidopteran eyespots. These address the generally larger and more centrally placed spots found on Lepidopteran wings and state that they startle or intimidate predators, providing time for escape. While it is agreed that eyespots intimidate or startle predators, the mechanism has not been agreed. There are two competing lines of thought 1) that 'eyespots' intimidate because they resemble the eyes of the predators' own predators and 2) that it is the conspicuous colouration of the pattern that induces the startle or avoidance behaviour. The first experiment utilised artificial prey with differing 'directions of gaze' in a field setting. If purely conspicuous patterns direction of gaze should have no influence on prey survival. The results indicate that patterns imitating staring or upward gazes provide the greatest protection, suggesting that in some cases eyespots may be being perceived as eyes and not simply as conspicuous patterns. I wanted to see if it would be possible to find a way in which to measure or quantify the reaction of an animal to 'real' eyes, in order to compare it to the reaction to eyespots. Recent trials investigating human reactions to eye contact suggested a computer based method may be possible. In this second experiment we examined whether the direct gaze of a predator might produce a measurable effect in human subjects. I was not able find any effect, but it is unclear as to whether this is due to problems with the experimental set up.

In Chapter three I investigate a factor often over looked in the study of crypsis, that of the behavioural adaptations that can enhance its efficiency. The larvae of the early thorn moth (*Selenia dentaria*) masquerade as twigs, using both colouration and behaviour adaptations. I compared the angle at which the larvae rested, to the angle at which real twigs deviate from the main stem. The results found that the larvae showed variation in their angle of rest and do not appear to match the angle of real twigs on the host tree. This result suggests that perfectly matching the angles of real twigs is not necessary to twig mimicry.

While carrying out this experiment it was noticed that a breeze appeared to increase larval activity and induced a 'swaying' behaviour. This led me to examine whether mimic species may utilise the visual 'noise' produced by windy conditions to camouflage movement. Firstly, a small 'proof of concept' pilot was carried out, followed by a larger study using 2 different twig mimic species. The study involved measuring movement and swaying behaviour in 3 conditions (still air, wind setting 1 and 2). The results suggest that cryptic and mimetic lepidopteran species may use windy conditions to camouflage their movements and that some species may employ specialised 'swaying' behaviours. Cryptic species are limited in opportunities to move between foraging sites without increasing detection by predators, therefore, any adaptation that might reduce detection is extremely advantageous.

In Chapter four I examine how conspicuousness and colouration are affected by living in a group, particularly in relation to other group members. A field experiment using groups of artificial prey, with differing densities and group sizes was used to explore the effect of group size and density on the predation risk and detectibility of cryptic prey. My results show that, as expected, larger groups are more likely to be detected, but that the increase is much slower than a linear increase. This suggests that groups must increase considerably in size before any individual group member will suffer increased predation risk.

The second experiment examines the 'oddity effect' and how it affects predation. This hypothesises that when confronted by grouped prey, predators can increase their kill rate by concentrating their efforts on capturing unusual or 'odd' prey, a strategy that reduces the 'confusion effect'. A field experiment was conducted with groups composed of differing proportions of two artificial

cryptic prey types. Groups with odd individuals did not suffer an increase in conspicuousness and were not attacked more often. However, once located and attacked the groups did suffer a greater predation rate. Odd individuals were predated at a greater rate than normal individuals and the rate did not change as more or less odd individuals were added to the group. A computer based 'game' was used to further investigate the oddity effect. The results from the initial run of the game appeared to show strong evidence for the oddity effect, with a further significant increase in this effect when attention is split between foraging for prey and scanning for predators. To be confident of this result the experiment was repeated with the 'odd' and 'normal' seed patterns reversed. The new data set strongly suggested that much of the effect seen in the previous experiment was due to a difference in pattern visibility between the two seed patterns. Nevertheless, the results indicated that selecting odd seeds is quicker than selecting normal seeds. The results from both the field and computer trials suggest that preference for odd prey may improve predator foraging speed and efficiency.

Chapter five investigates whether cryptic and non-defended prey could reduce their predation risk by grouping with aposematic and defended prey. This was tested using artificial prey in a field setting. My results show that undefended non-aposematic prey can benefit by grouping with aposematic prey with no evidence that predation rates for aposematic prey were adversely affected by this association. If confirmed this might illuminate the origins of Batesian mimicry.

I have investigated a range of anti-predator adaptations and strategies in the Lepidoptera and in particular pattern elements and use of crypsis and aposematic displays. These anti-predator strategies are important in that they modify predation rate and so directly influence the evolution of species. While I have been able to provide evidence for some current hypotheses, in many respects my results demonstrate that there is still a lot to learn about visual anti-predatory strategies.

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Author's Declaration

I declare that the work recorded in this thesis is entirely my own and is of my own composition. Patrick Costello and Alison Shand aided with data collection for Chapter 1, Patrick Costello aided in collecting data for chapter 2 (Section 2.1) and Kevin Mahon aided with data collection for chapter 5. No part of this thesis has been submitted for another degree.

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Introduction

No organism (from the smallest bacterial cell to the blue whale) lives or acts in isolation. We interact with a host of other species every hour of every day and, along with the physical environment, it is the sum of those interactions that acts to shape life on Earth. We can broadly characterise these interactions into three categories. The first and arguably the most widespread, since all organisms experience it to some degree, is that of competition. Competition can occur as both an inter-species and intra-species effect, and is where individuals compete for a share of a limited resource. That resource can be anything from space within a habitat, to food or a mate. Those organisms that compete successfully are the most likely to survive and pass on their genetic information to the next generation, making competition a vital component of evolutionary change.

Secondly there are those interactions that involve two or more species, where the relationship benefits one or more of those species. Where only one party benefits, but the other suffers no effect (positive or negative) the relationship is described as commensalism and where all parties benefit from the relationship it is termed mutualism. In some instances these relationships are obligatory, but this is not always so.

Finally, there are those interactions in which one party is exploited or eaten by another. These parasite-host and predator-prey interactions are characterised by the dichotomy of the costs and benefits, with the negative effects all resting on the host/prey end of the equation. A very simple food chain will have plants at its base which are fed on by organism A, which is predated on by organism B, which in turn is predated on by organism C, but it is very rare that such a simple food chain is found in the natural world. More often organisms are part of a larger food web, with each organism being party to multiple interactions, feeding on and being predated on by multiple other species.

In such complex communities there is often intense competition between predators for the various prey species. That competition ensures that only those predators that are able to find and capture prey the most efficiently are the most likely to survive to pass on their genes. This ensures that any adaptation or specialisation that increases a predator’s ability to hunt and capture prey is

maintained within the population. This selection for ever greater efficiency has led some predators to evolve specialised adaptations to hunt one particular prey type. For instance the aye-aye (*Daubentonia madagascariensis*) has a highly specialised long, thin, third digit which is not used during locomotion, but is used almost exclusively in the extraction of grubs from tree trunks (Lhota et al., 2008) or the peregrine falcon (*Falco peregrinus*) that can accelerate to speeds well in excess of 300km/h when diving to strike its prey (Baumgart, 2011). Yet, still there are some grubs that are able to escape the aye-aye's sensitive probing finger and some birds that are able to evade the peregrine falcon's dive, and those individuals are the ones that are most likely to contribute their genes to the next generation. This is the so often mentioned 'evolutionary arms race', with prey evolving more elaborate and diverse defences against their predators and predators evolving new mechanisms to combat them. This dynamic between predator and prey is an important and powerful evolutionary force, leading to adaptations on both sides of the predator-prey relationship constantly shifting and changing over evolutionary time. However, while we understand the importance of the predator-prey relationship we still do not understand many of the intricacies of how certain defensive or predatory mechanisms work or what steps led to their development. To fully understand these we need to ask very specific questions about the mechanisms involved, and one of the best ways to answer those questions is through field and laboratory experiments using real organisms. To do this we need to select our study organism carefully.

The ubiquitous nature of the Lepidoptera has led them to be intensively studied, beginning with the earliest naturalists and biologists. The anatomy and the morphology of both the immature and adult stages have been extensively studied and due to their availability are often used as experimental subjects for anatomy and physiology studies (Ed. Capinera, 2008). The Lepidoptera are also of interest in ecological studies, where the segregation of habitat, dietary requirements and morphology between life stages has provided fertile ground for study. They also make an effective 'prey' organism in studies investigating predator-prey interactions as they are preyed upon by a wide variety of organisms, from insects and arachnids to mammals and birds, which between them have a diverse range of hunting techniques. For example avian predators are predominantly visual predators and so most are well equipped to hunt using

sight with sensitive tetrachromatic colour vision to search out and target prey (Finger and Burkhardt, 1994). Therefore the most effective anti-predator adaptations a Lepidopteran will have against avian attack is likely to be visual.

Predators that use sight to hunt are known to use shape, colour and pattern to form search images to increase foraging efficiency (Pietrewicz and Kamil, 1979). However, there are also a number of ways that colouration and pattern can be utilised by prey organisms to reduce their chances of being predated. These fall into three broad categories; those that reduce predation by avoiding detection entirely by predators, by being detected but advertising their unsuitability as prey (Ruxton et al., 2004) and by being detected but misclassified as something non-edible (Skelhorn et al., 2010b). All three strategies described are found within the Lepidoptera and in some cases a single species may use different strategies at different points within their lifecycle(Gamberale and Tullberg, 1996).

The first group describes any type of crypsis or camouflage. In its simplest form crypsis can take the form of background matching: this is where an organism's colouration matches the background colouration of its habitat. An example of this is the white fur of the arctic fox (*Vulpes lagopus*) in winter, which blends in its snowy habitat. This basic type of crypsis can be further enhanced by adding countershading and/or disruptive patterning. Countershading, where the ventral surface of an organism is a lighter colour than the rest of the body, is an extremely common feature of animal colouration (Ruxton et al., 2004). Disruptive patterning is a pattern that breaks up the body outline and often includes pattern elements than appear to run over the true body edge or create false body outlines (Stevens and Merilaita, 2009). Both countershading and disruptive patterning hinder a predator's ability to detect or recognise an organism by disguising the organism's true outline and have been found to enhance crypsis and reduce predation by avian predators (Fraser et al., 2007; Rowland et al., 2007; Stevens and Merilaita, 2009).

The next two groups both assume that the predator will detect the organism but that it will choose not to attack. The first is arguably another, more complex, form of crypsis. In this case the prey's colouration or patterning is used to mimic the appearance of an inedible model such as a pebble, twig or bird

faeces. This type of cryptic patterning is called ‘Masquerade’ and when successful causes predators to misidentify as the prey as its inedible model and disregarded them (Skelhorn et al., 2010b). This effectively allows the prey animal to ‘hide’ in plain sight.

Finally we have the use of aposematic or warning colouration. Unlike the previous types of colouration and patterning, aposematic patterning does not hide or disguise the prey animal at all. In fact, it does quite the opposite using conspicuous behaviour, odour, sound or colouration (e.g. red, yellow, black or orange) to announce unprofitability (usually due to toxicity or distastefulness) (Cott, 1940; Poulton, 1890). Aposematic displays are generally very conspicuous, a trait which is thought to 1) enable predators to easily distinguish defended prey from undefended prey and 2) impose costs that only defended prey can afford, such as increased detection rates (Sherratt and Beatty, 2003). In some cases organisms will use a combination of conspicuous signals to startle and deflect predators. For example the peacock butterfly (*Inachis io*) hibernates as an adult with their wings closed hiding their large conspicuous wing spots. However, if disturbed they flick their wings open and closed several times, flashing the spots and making a hissing noise (Blest, 1957; Wiklund et al., 2008).

Effective aposematic signalling provides great advantages for survival, but secondary defences such as toxins can be costly to produce. So why go to the expense of developing secondary defences yourself when, by mimicking characteristic patterns and behaviours of those that do, you can benefit without them? It is not even completely necessary for mimics to perfectly match all aspects of the model’s patterning, with even imperfect mimicry providing some protection (Kikuchi and Pfennig, 2010). This type of mimicry is called Batesian mimicry. A commonly used example of this is the relationship between some colubrid snakes of the *Pliocerus* genus (non-venomous and rear-fanged) and the *Miccrurus* coral snakes (venomous and front fanged) that live in many of the same areas of Central America. Where they do co-habitat it has been found that the patterning of the non-venomous colubrid snakes more closely resembles their venomous model the coral snake (Greene and McDiarmid, 1981). However, dishonest signals like those of Batesian mimics can change the effectiveness of the warning signal. It has been shown that as the ratio of mimics to models

increases, the effectiveness of the warning signal is reduced and predation rates for both groups increase (Ruxton et al., 2004).

Conversely, when two or more unpalatable or defended prey species share similar characteristics and/or patterning it can benefit both species by strengthening a predator's association between that pattern and unprofitability(Mallet and Barton, 1989). This type of mimicry where all parties are generating an honest signal of secondary defences is called Müllerian mimicry, and its most celebrated example is that of the *Heliconius* butterflies of South America. Here we find groups of heliconiine species, together with some species from other lepidopteran groups, which all resemble one another in some way (Brower, 1996).

As we can see, colour and patterning are important factors in determining predation rates. However, how well these strategies work can also be affected by whether an organism lives singly or as part of a larger group. Being part of a larger group has a number of benefits, with the presence of the other group members diluting the predation risk and making it difficult for predators to either pick out an individual from the group or approach unseen(Krause and Ruxton, 2002). However, a large group is unlikely to be able to remain as cryptic as a single animal. In fact, there is a trade off between the dilution of risk and increased conspicuousness, with increasing group size easing detection by predators (Jackson et al., 2005).

Despite all we do know about predator-prey interactions there is still much to be answered. Through a series of lab and field experiments I have attempted to look at some of the outstanding questions. There is still much discussion over asymmetry and its affect in cryptic patterning. In Chapter 1 I report on the results of a field experiment examining whether asymmetry can enhance cryptic patterning. To do this I used artificial baits of varying levels of asymmetry and monitored the predations, with the view that any benefit of asymmetry should be seen in an increased survival rate for those baits. From there I wanted to look at one of the most common symmetrical pattern elements found in Lepidopteran aposematic displays; the eyespot. How eyespots are interpreted by predators is an important question still being discussed today and in chapter 2 I report on two experiments I conducted in an attempt to provide evidence to answer this

question. If eyespots are being interpreted as eyes we might expect that changing the apparent direction of the 'eyes' might have an effect on their ability to reduce predation rates. With this in mind I conducted an experiment using artificial baits with the central circle of the 'eyespot' off centre so as to appear to be gazing in different directions. In experiments with humans it has been found the direct gaze of another person is automatically processed by the brain. Therefore for my second experiment I wanted to examine if the direct gaze of another species is able to elicit a similar response and whether there is any difference made between the binocular gaze of a predatory species to that of a prey species. To do this a computer trials were designed using the Stroop test as a basis for measuring attention and reaction times. If a measurable response was found this may lead to a way of determining if eyespots are being reacted to in a similar manner to real eyes.

There are often behavioural adaptations that enhance aposematic displays, such as the startling flashing of eyespots utilised by some Lepidopteran species. However, comparatively little has been done to examine behavioural equivalents that enhance camouflage. In Chapter 3 I look at how behaviour is used to enhance masquerade in two Lepidopteran species with twig mimic larvae. I test early thorn (*Selenia dentaria*) to assess whether they adapt their resting position to better match their food plant. The results from this experiment may go towards understanding how 'perfect' mimicry must be in order to effectively reduce predation risk. I then used both early thorn (*Selenia dentaria*) and peppered moth (*Biston betularia*) larvae to assess whether they are able to use behavioural adaptations to camouflage their movement. The ability to move between habitats or find new food resources without increasing predation risk would represent a considerable benefit for species that must otherwise remain still to maintain their masquerade defence.

In Chapter 4 I wanted to examine the effect of group composition on predation. How does group, size, density and composition of your group affect your chances of predation? Further does being different from the majority of your group affect, both your own chance of being predated, and the predation rate for the group as a whole? Could I find any evidence for the Oddity Effect? To investigate this I designed field experiments using groups of sunflower seeds with compositions designed to mimic these scenarios. These were set out and the

local wild bird population was allowed to remove seeds at will for a set period of time. In this way I was able to compare predation rates between the different group types. I then wanted to examine if dividing attention between prey selection and scanning for predators could change the way the Oddity Effect was felt. To do this a computer game was designed so that I could use human volunteers to act as predators and allow for more parameters to be measured than was possible in the field experiments. Finally, in Chapter 5 I investigate the effects of associating or grouping with aposematic species when using crypsis. Here again sunflower seed groups were used as baits, with some baits made aposematic with additives to change the colour and make the baits distasteful.

Chapter 1. Importance of asymmetry in the crypsis of model moths.

Symmetry is found throughout the animal kingdom in the body plans of almost all multi-cellular life, from the bilateral symmetry we can see in ourselves to the radial symmetry found in the phyla Cnidaria and Echinoderamata. It is often thought that developmental instabilities caused by stress can be seen in the adult animal in the form of asymmetrical development of patterns and/or form, making symmetry an honest and potentially important signal of individual quality (Ciuti and Apollonio, 2011). The effect of symmetry on camouflage or warning patterns has also been explored using a diverse range of subjects such as pigeons(Delius and Nowak, 1982), humans (Attneave, 1954) and honey bees (Horridge, 1996). These studies have shown that patterns that include lines of symmetry are more easily detected, learnt and reproduced than those with asymmetry. Consequently symmetry & asymmetry are of interest as pattern elements in the study of crypsis and aposematism.

Aposematic patterns are conspicuous warning displays intended to advertise unpalatability and deter predators (Ruxton et al., 2004) Therefore, any pattern element that may make them more easily identified, recognised and/or memorised should increase effectiveness. A number of studies have examined the effect of symmetrical pattern elements in aposematic displays, but with differing results. Forsman & Merilaita (1999) were able to show that domestic chicks (*Gallus gallus domesticus*) were able to learn to avoid unpalatable artificial prey faster when the pattern consisted of 2 spots of equal size than when the pattern was asymmetrical in spot size. In a later study (Forsman and Herrstrom, 2004) it was also shown that symmetry in shape, pattern and colour enhanced the innate avoidance behaviour of chicks to conspicuous palatable prey. These results suggested that conspicuous prey would be under strong selection pressure to maintain symmetrical signals, as they increase innate avoidance and the rate of aversion learning by predators, both of which have strong fitness benefits.

However, since publication it has been suggested that the way in which the size of the stimuli asymmetries & spot areas were calculated in Forsman and Herstrom’s (2004) study may have confounded overall size differences with

asymmetry differences. This meant that the threshold for discrimination between symmetrical and asymmetrical stimuli by the birds was in fact between 20% & 32% (Swaddle and Johnson, 2007) rather than around 7.5% as reported. Also when recalculated it was found that the asymmetrical stimuli used in the size asymmetry trials had a spot area which was on average 7% smaller than the symmetrical stimuli (Swaddle and Johnson, 2007) and as other studies have indicated that conspicuous wing spots are more effective with increased size (Stevens et al., 2008a) this makes it difficult to determine that differences were caused by asymmetry alone.

Both of the previous studies mentioned were lab based with the Forsman & Merilaita (1999) study allowing chicks to select prey from a large number of artificial stimuli & Forsman & Herstrom's (2004) study using a 2-way forced choice design. Even taking in to account some of the possible problems with Forsman & Herstrom's study they provide some evidence that symmetry speeds learning of patterns and that asymmetry in colour and shape increases predation.

However, a later field study conducted by Stevens et al. (2009b) found that there was no benefit to symmetry, with asymmetry in shape, size and position conferring no extra survival cost. The reasons for the difference in results are uncertain, although it may be that the design of the lab studies and in particular, the 2-way forced choice test, may not provide results representative of the decision making processes used by avian predators in the wild, who will often encounter prey sequentially and make decisions to accept or reject, rather than decisions about choosing one of two alternatives to accept.

Crypsis, unlike the aposematic patterning we describe above, is used to reduce detection by matching the colours, patterning and texture of the background an organism is sitting against(Ruxton et al., 2004). Predators which use vision to locate cryptic prey must rely on noticing subtle differences in colour, shade, pattern or texture between their prey and the background(Endler, 1978; Ruxton et al., 2004). As very few natural substrates or backgrounds contain the type of bilateral plane of symmetry typical of most animals, symmetry is though to be a strong visual cue to their presence. In field experiments using artificial moth-like stimuli, Cuthill et al. (2006a; 2006b) found that symmetry reduced the

effectiveness of both disruptive and back-ground matching patterns with symmetry incurring a significant fitness cost.

However, as the previous investigations into this phenomenon have used entirely artificial patterns, we propose to use the patterning of the moth species the peach blossom moth (*Thyatira batis*) (Figure 1–1). The peach blossom moth is widely distributed across the UK and has been recorded within a short distance of the test area (Butterfly Conservation, 2012). This allowed us to be confident that the patterning was representative of the natural prey species in the area. This patterning is assumed to be cryptic and does not include any aposematic colouring. However the pinkish spots provide a strong and simple pattern element which allows easy modification of the pattern. By using the patterning of a real moth species and modifying it to take it from positionally symmetrical to very asymmetrical we plan to test the hypothesis that asymmetry is beneficial in cryptic patterning using more ecologically-realistic targets than previous studies.

Figure 1–1. Peach blossom moth (*Thyatira batis*).

1.1.1 Materials and Methods

This experiment was conducted over two seasons and at 3 sites. The first season between 25th May & 30th Sept 2010 used mixed deciduous woodland at Dawsholm Park, Glasgow (55°89'61.75"N, 4°31'59.98"W). The second season between April 26th and July 23rd 2011 used two smaller sites, with the first at the Glasgow Botanic Gardens, Glasgow, UK (55°52'53.57"N, 4°17'28.02"W) and the other at Kelvingrove Park, Glasgow, UK (55°52'24.92"N, 4°16'49.50"W). In both seasons different trees and trials were used to ensure that the same areas were not used for consecutive trials. For the second season the two sites were used alternately to ensure that each site was free from the artificial stimuli for a minimum of 72 hours prior to Day 1 of any run.

Artificial baits were made using modified images of the Peach Blossom moth (*Thyatira batis)*. The baits were designed with 3 levels of asymmetry plus controls of symmetrical baits with and without spots. There were 8 treatments in total (Figure 1–2) consisting of triangular stimuli 41mm wide at the base and 35 mm tall printed on Canon matt photographic paper (MP-101).

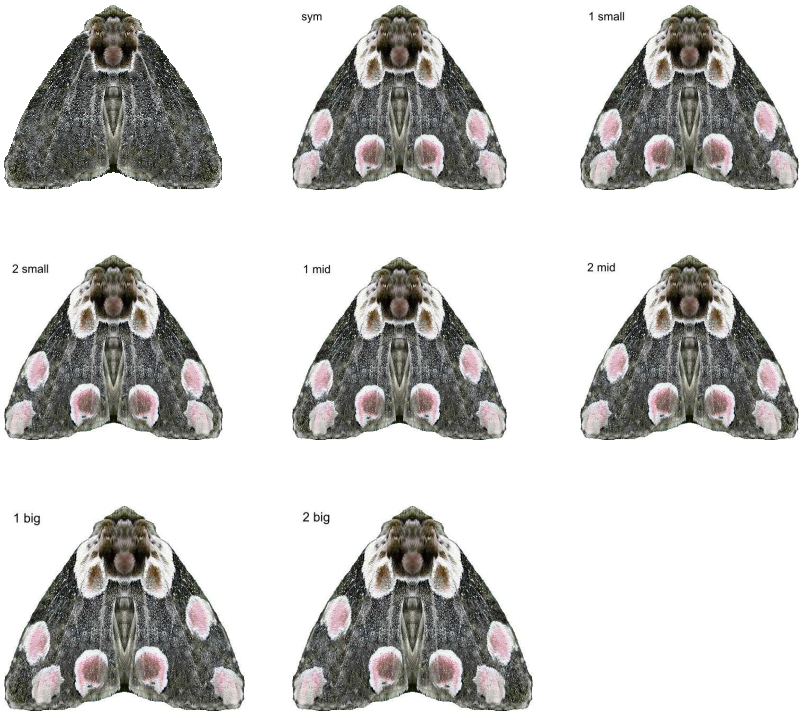


Figure 1–2. Asymmetrical stimuli (not actual size)
(From L→ R: Control without spots; Control with spots; Small asymmetry 1; Small asymmetry 2; Mid asymmetry 1; Mid asymmetry 2; Big asymmetry 1; Big asymmetry 2.)

Of the 8 stimuli, 2 were designed to act as controls, both with fully symmetrical designs but one with spots and one without. This enabled us to control for any effect of the spots and allowed us to compare the effect of symmetry with the asymmetrical treatments. The 6 remaining stimuli were the treatment groups with 3 levels of asymmetry. Each pair of stimuli had one which had the asymmetrical element on the left and another on the right, which allowed us to control for laterality. This is important as many species show lateralisation and a preference for one side over another ((Magat and Brown, 2009)). To give a measure of the relative levels of asymmetry we measured the distance between the centres of the 2 spots on the outside edges of each stimulus and calculated the percentage difference between the two sides. The small asymmetry stimuli were found to be 80% asymmetrical, the medium stimuli are 150% asymmetrical and the large stimuli 175% asymmetrical, all of which are well above the levels of asymmetry known to be detectable (Swaddle and Johnson, 2007). With these stimuli we hoped to be able to discern whether there was any effect of asymmetry and the degree of asymmetry needed.

The edible component of each bait consisted of a mealworm (frozen overnight, then thawed) pinned vertically to the centre of the underside of each stimuli. The mealworm is pinned to the underside with only the tip projecting, rather than on the surface, as it is important that there is no other source of asymmetry other than the printed pattern.

On each of 14 days 72 trees (i.e. 9 replicates of each of the 8 baits) were selected at random with a minimum gap of 10m between them. Only trees without lichen covering the trunk and that were of at least 0.9m in circumference were selected. Different sections of the wood were used in rotation with the same sections of woodland not used in consecutive trials and care taken to ensure that no tree was used more than once. Stimuli were assigned randomly to a tree and attached a minimum of 1.5m up from the base using dressmaker's pins. Randomisation of the allocations was achieved by assigning the stimuli to tree using a random number generated by the Excel function RAND() and using the SORT function to put them in ascending order to be placed on trees 1-72.

The experiment was conducted over 48hrs with the baits put out on the morning of day 1 and checked for survival at 2, 4, 24 and 48hrs. Avian predation was taken to be indicated by complete or almost complete disappearance of the mealworm. Non-avian predators such as slugs were indicated by the slime trails left behind and spiders and harvestmen by the mealworms being hollowed out leaving the empty exoskeleton. While the treatment groups were not watched a number of different bird species were observed close to or in the immediate area including blackbirds (*Turdus merula*), bluetits (*Cyanistes caeruleus*), bullfinch (*Pyrrhula pyrrhula*), carrion crow (*Corvus corone*), house sparrows (*Passer domesticus*), magpie (*Pica pica*), robin (*Erithacus rubecula*), rock pigeon (*Columba livia*), starling (*Sturnus vulgaris*) and wood pigeon (*Columba palumbus*). Every time the site was visited the date & time of arrival and departure was noted. Weather conditions and temperature at 9am, 12pm and 5pm were taken from the Met Office website for each day of the experiment.

The collected data was analysed in SPSS and a Cox proportional hazards regression used to accommodate the censored data and the varying predation risk throughout the day (Cox, 1972; Klein J.P. & Moeschberger, 2003; Lawless, 2002). Effect sizes are given by the odds ratio (Exp(B)), which is the ratio of the probability of predation in one treatment compared to the probability of predation in another treatment. We compared all treatments to the symmetrical control, so that the Exp (B) values given are the likelihood of predation when compared with this treatment.

The data were analysed at both the individual stimuli level (8 treatments) and with the stimuli grouped so that the mirrored stimuli from each asymmetry grading (small, medium & large) were analysed together giving 5 treatment groups.

1.1.2 Results

In total we had 23.6% of our stimuli predated by birds and 76.4% censored stimuli (not eaten by birds), with the majority of stimuli taken within the first 24hrs after placement. None of the stimuli types differed significantly in survival rate from the symmetrical control (Control/No spots, Wald statistic (WS) = 0.178, p = 0.67, Exp (B) = 1.04; 1S, WS = 2.26, p = 0.13, Exp (B) = 0.86; 2S, WS = 2.95, p = 0.09 Exp (B) = 1.17; 1M, WS = 0.84, p = 0.36, Exp (B) = 0.92; 2M, WS = 0.59, p = 0.44, Exp (B) = 0.93; 1B, WS = 0.36, p = 0.85, Exp (B) = 1.02; 2B, WS = 0.39, p = 0.53, Exp (B) = 0.94). The survival rates for all the stimuli are for the most part tightly grouped, with no obvious pattern of difference between the symmetrical and asymmetrical stimuli (Figure 1–3).

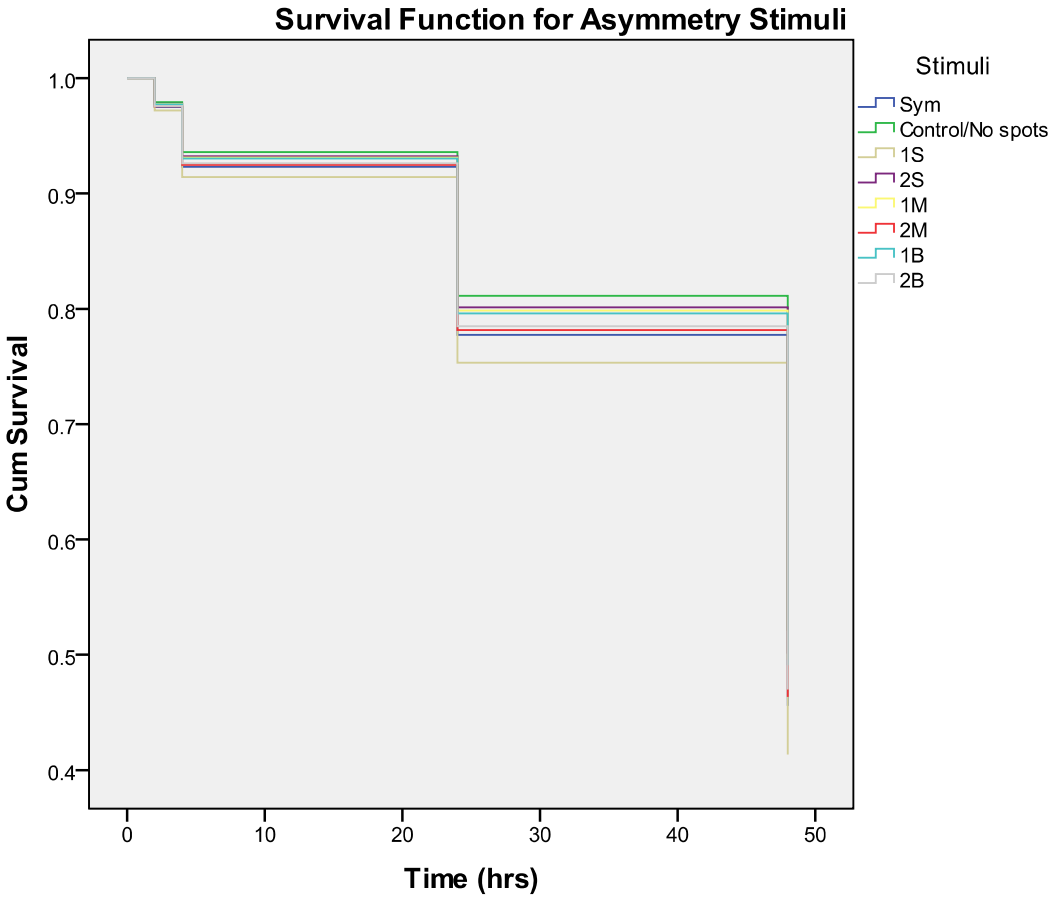


Figure 1–3. Asymmetry stimuli, cumulative survival probability - for the 8 stimuli types surviving avian predation over time.

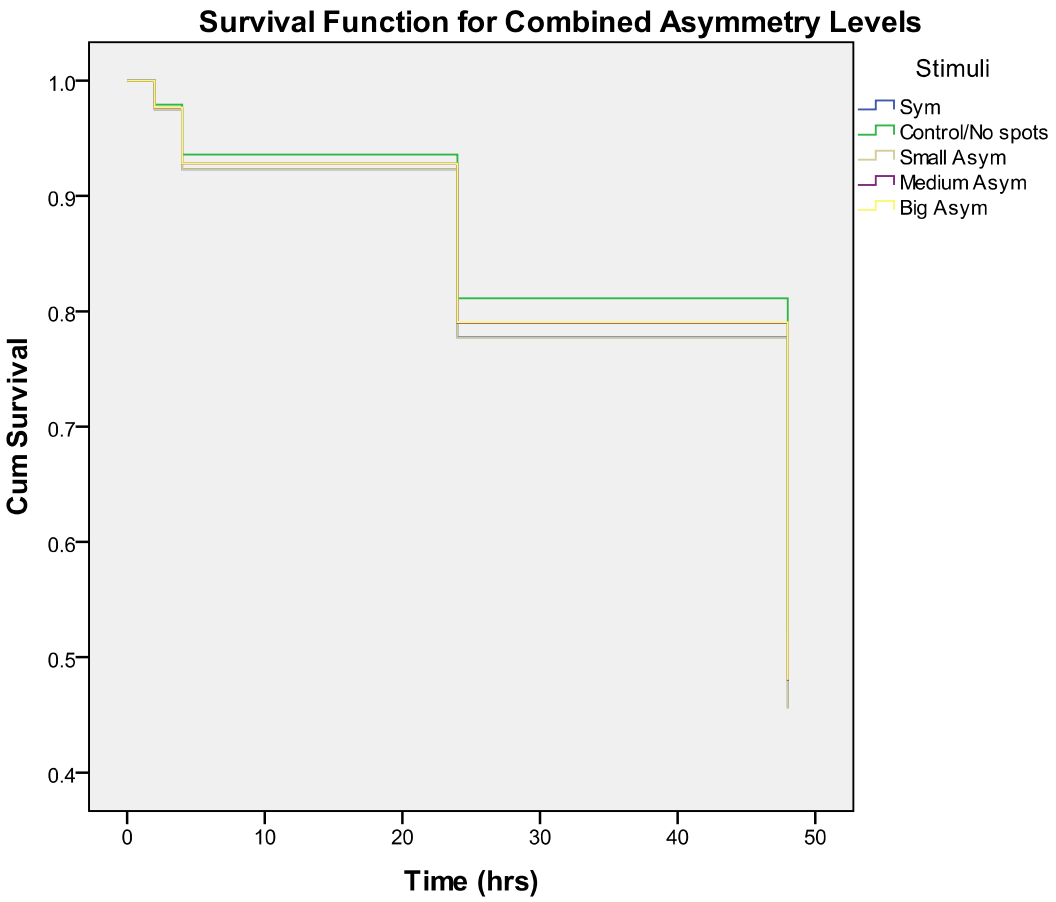


Figure 1–4. Combined asymmetry levels, cumulative survival probability - for the 2 control and 3 levels of asymmetry stimuli surviving avian predation over time.

When the stimuli were grouped by the extent of the asymmetry they show (small, medium & large asymmetry) no significant difference was found between the different levels of asymmetry (Control/No spots, Wald Statistic (WS) = 0.72, p = 0.4, Exp (B) = 1.07; Small asymmetry, WS = 1.87, p = 0.17, Exp (B) = 0.89; Medium asymmetry, WS = 1.14, p = .29, Exp (B) = 1.07; Large asymmetry, WS = .001, p = .97, Exp (B) = 1.00) (Figure 1-4). Therefore, we conclude that there is no effect of stimulus type on survival probability to the end of the 48 hour test period.

1.1.3 Discussion

Our analysis found no significant difference in survival rate between any of the treatment types. To increase our power to find even a weak effect we combined data from the asymmetry pairs e.g. small 1 & 2 were combined in to one treatment. Here, again, we found no significant difference between the stimuli and again no clear trend or pattern can be seen in the graphed results. From these results it appears as though asymmetry of wing pattern has no effect on

the survival rate of the stimuli we used. However, we should also point out that we had a much lower avian predation rate than those reported in some earlier studies. In Stevens et al's 2009 paper they described censored (number of stimuli not taken by birds) rates of 27% and 17.3% which are considerably smaller than the 76.4% we had.

There are a number of possibilities that might help explain this. It may in part be due to longer trial durations used in some of the previous studies, with Stevens et al. conducting trials over 48hrs (2008a), 72 hrs (2009a; 2009b) and 96 hrs (2009b). However, we would argue that by extending the duration of the trials to achieve a greater predation ratio we risk losing a degree of realism. We may have been harsher in our judgment of whether a stimulus had been predated by 'other' predators and so censored a larger percentage of the stimulus.

There may also have been a problem with identifying which predator was responsible for bait being removed. On a number of our planned checks we observed the common wasp (*Vespula vulgaris*) cutting up and removing the mealworm baits. Since both in this and previous field studies the complete or almost complete removal of the mealworm bait was used as a signifier of avian predation, this behaviour by the wasps made determining whether a bait has truly been removed by a bird extremely difficult. However, as wasps are likely to locate prey by scent rather than by vision and should not be influenced by the pattern on the stimuli, as long as the placement of the stimuli and baits is sufficiently randomized, wasps may increase the average number of baits removed, but should not affect the overall outcome. This added noise would make detection of visually-mediated choices by avian predators more difficult to detect. We suggest that future fieldwork using these techniques should be carried out during the winter months when wasps are dormant, which would allow us to be confident that this potentially confounding factor had been removed.

There are a number of other factors we might want to consider and changes that we might want to make to the experimental design before we would continue with any further tests. For example in this and previous studies the stimuli and treatments have all been printed out on to card and pinned flat on to a tree, but

for the majority of moth and butterfly species the naturally adopted resting position is not flat against the substrate. This may be important as the angle at which a pattern is viewed will change how much of the pattern as a whole is seen and its symmetry. Perhaps by using a more 3 dimensional stimuli and bait it would be possible to more accurately mimic the natural conditions in which the patterns would be encountered. This may also change the way our stimuli are seen, as there is a chance that by presenting our stimuli flat we have inadvertently created paired eyespots where in a more natural position only one of the spots would be visible at any time (Appendix i for further discussion). It has been argued previously that only large levels of asymmetry benefit camouflage and that the developmental changes and sizable mutations this would require are statistically unlikely (Dawkins, 1976, 1996), however what we have described above may be a way to work around these constraints.

This raises a couple of possibilities; the first is that if when in a natural resting position the peach blossom moth only has one wing fully visible at any time, its patterning will always be asymmetrical; the second is that it maybe that the pattern can act as either aposematic when viewed directly from above and the paired symmetrical ‘eyespots’ are visible or cryptic when viewed from any other angle where only one spot is visible and the pattern is asymmetrical.

We chose, in this instance, to test our hypothesis in a field rather than lab setting as this adds a degree of realism to the setting that we could not replicate in the lab. However, there are several problems with this approach that limit how we can interpret that data. The stimuli we used are not ‘real’ moths and so we can not be sure that the birds interacting with them as they would a real moth. It would be interesting to test this possibility with a laboratory study perhaps with birds only able to approach a 3-dimensional stimulus from either the side or head on and comparing their willingness to feed.

A potential middle ground between the lab and field studies would be an aviary study using wild caught birds. An aviary would allow us greater control over the environment, while still retaining some of the realism of a field experiment. We would be better able to exclude non-avian predators and be certain that any bait taken was indeed removed by a bird. We would also be able to observe a

bird's behaviour before removing the bait, do they observe the bait from a distance first and from what angle do they approach?

In conclusion, our study found no effect of asymmetry on predation rates, but there is much that can be done to examine this further. Our results agree with those recent studies that have found no survival advantage of symmetrical over asymmetrical markings, so it may be that response to symmetry is something that only occurs in the simplified visual domain of laboratory test arenas. As pattern symmetry is widespread throughout the animal kingdom the most parsimonious explanation might be that, rather than having functional importance in signalling, symmetry reflects underlying developmental or genetic constraints.

Chapter 2. Effectiveness of Lepidopteran Eyespots in deterring predators.

2.1 Is apparent direction of gaze important?

It is well known that prey organisms use a variety of patterns and markings to reduce their risk of predation. These markings can take the form of camouflage, mimicry and/or conspicuous warning colours(Cott, 1940; Ruxton et al., 2004). A pattern which has produced quite considerable interest and debate is that of paired circular 'eyespots' most commonly found on tropical fish and lepidopteran species. Until recently these markings have not been well defined within the literature and so for the purposes of this report we will use the definition given by Stevens in his 2005 review.

"… approximately circular marking on the body of an animal, composed of colours contrasting with the surrounding body area, often comprised of concentric rings and occurring in bilaterally symmetrically pairs." (Stevens, 2005)

There are two main hypotheses for how 'eyespots' may provide protection from predation. The first is the 'deflection hypothesis' which suggests that eyespots draw attention and attacks away from vital areas allowing prey to survive attacks. This hypothesis seems to fit particularly well in the case of species with smaller more peripheral spots such as the squinting bush brown (*Bicyclus anynana*, see Figure 2–1)(Stevens, 2005). The second is the 'intimidation hypothesis' where generally larger and more centrally placed spots startle or intimidate the predator which slows or halts its attack long enough to allow escape(Stevens, 2005). Examples of this kind of eyespot can be found on the european peacock butterfly (*Inachis io,*Figure 2–1), where the eyespots are continuously visible while the butterfly rests with its wings open, or the eyed hawk-moth (*Smerinthus ocellata*) which will reveal the eyespots from behind its forewings when threatened. It is this second hypothesis which has led to the most debate; as although it is agreed they elicit a startle response(Blest, 1957; Vallin et al., 2005; 2007), it has not been agreed which aspects of the markings cause the reaction. Here we have two competing lines of thought, the first is that 'eyespots' intimidate because they resemble the eyes of the predators' own

predators and the second stating that it is the conspicuous colouration which induces the startle or avoidance behaviour. In this second interpretation the patterns are intimidating simply by being novel (Coppinger, 1969, 1970; Marples and Kelly, 2001) and conspicuous (Blest, 1957) , rather than through misidentification.

Figure 2–1. Examples of two types of eyespots.
(L) Squinting bush brown (*Bicyclus anynana*) (Image © 2005 Antónia Monteiro/University at Buffalo) with small peripheral spots; (R) european peacock butterfly (*Inachis io*) (Image © Lynne Kirton) with large more central spots.

In an attempt to understand whether it is eye mimicry or conspicuousness which provides protection from predation Stevens et al have conducted a series of experiments (Stevens et al., 2009a; Stevens et al., 2009b; Stevens et al., 2008a; Stevens et al., 2007; Stevens et al., 2008b) using artificial moth stimuli and wild living birds, an approach that differed considerably from previous work which has used real butterflies
(Ruxton, 2005; Vallin et al., 2007). In the 2007 paper Stevens found that stimuli with highly contrasting patterns survived better than those with low contrasting patterns. However, it was also found that patterns of concentric circles with components of equal width, traits which could be interpreted as more eyelike provided significantly better protection. It is also important that results differed dependent on whether the background colour was midway between black and white on a ratio or linear scale, with arguably more 'eye-like' stimuli apparently providing better protection when a linear scale was used. Nevertheless, when looking at the results from the series of experiments as a whole Stevens et al have concluded that the results provide evidence to support that it is conspicuousness rather than eye mimicry that elicits the avoidance reaction. However, this does not concur with that of some earlier work (Jones, 1980)

where the results were determined to be showing eye mimicry is key to the avoidance response (Appendix ii for full comparison).

In Stevens et al 2009 paper they touched on the possibility that the apparent direction of gaze may be a possible contributing factor to predator avoidance. It has been shown that a number avian species are able to react to and follow human gaze (Bugnyar et al., 2004; Hampton, 1994) and in a recent study it has been shown that wild-caught European starlings (*Sturnus vulgaris*) are sensitive to a predators' direction of gaze, with a direct gaze resulting in increased time to feeding resumption, reduced feeding rate and a reduced amount of food consumed overall (Carter et al., 2008). Work with species as diverse as domestic chickens, jewelfish and mouse lemurs (*Microcebus murinus*) has suggested that eye contact or direct gaze is avoided (Coss, 1978, 1979; Gallup et al., 1972; Lill, 1968; McBride et al., 1963) showing that it may represent an aversive event. These results suggest that if eyespots are being responded to as eye mimics and not as conspicuous patterns, we may be able to manipulate predation rate by changing the apparent direction of the 'eyespots' gaze. With this in mind we plan to test the null hypothesis that apparent direction of gaze has no influence on prey survival, and the alternative hypothesis that an apparent 'staring' or 'straight' gaze will work to reduce predation risk. For the null hypothesis to be rejected we would require that there be a significant difference in survival rates between the different treatments. For the alternative hypothesis to be accepted we would have to find that there is a significant reduction in predation risk for those stimuli with the 'staring' or 'straight' gaze in comparison to the other stimuli.

2.1.1 Materials & Methods

This experiment was conducted in mixed deciduous woodland across 3 sites within the city of Glasgow, Strathclyde, UK. The data was collected over 2 seasons with the a short pilot study of 4 trials carried out between 25th June 2009 & 9th July 2009 and the large data set collected between the 4th October 2009 & 15th January 2010 at Pollok Country Park (55°49'53"N, 4°18'28"W, see Figure 2–2). The second data set was collected from two smaller sites at the Glasgow Botanic Gardens, Glasgow, UK (55°52'53.57"N, 4°17'28.02"W, see Figure 2–2) and Kelvingrove Park, Glasgow, UK (55°52'24.92"N, 4°16'49.50"W, see Figure 2–2) between April 26th and July 23rd 2011. The two sites were used alternately to ensure that each site was free from the artificial stimuli for a minimum of 72 hours prior to Day 1 of any run. We specifically chose to carry out the main body of this experiment in winter when wasps would not be active. This was due to both wasps and birds being capable of entirely removing the bait from the stimuli and so making it impossible to know which had been responsible. However, we would not expect wasps to vary in their preference between stimuli. By carrying out the experiment in winter we were able to remove this potentially confounding factor.

Figure 2–2. Locations of experimental sites.
(L: Pollok Country Park & R: Glasgow Botanic Gardens & Kelvingrove Park)

Artificial baits were made using printed 'moth' shapes on Canon matt photographic paper (MP-101). There were 7 treatments in total (Figure 2–3) consisting of triangular stimuli 44mm wide at the base and 36mm tall. The stimuli were given a background colour of grey to ensure they were of a lighter

colour than the trees they would be placed on. This is important as previous work by Stevens et al. (2008a) has shown that eyespots on background matching prey may actually increase predation.

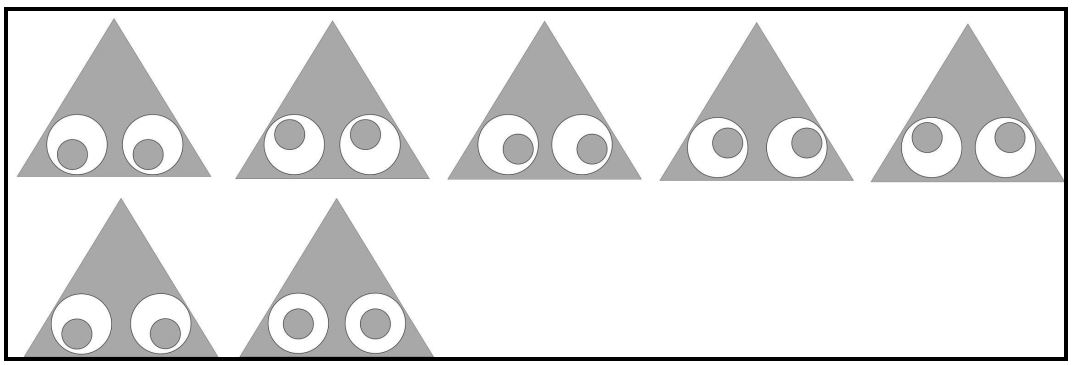


Figure 2–3. Eyegaze stimuli (not actual size).
(From left to right stimuli 'eyes' are looking; down & left (DL); up & left (UL); down & right (DR); up & right (UR); up & out (UO); down & out (DO); straight (S).)

Artificial stimuli with a very basic design were decided upon as this allowed us to control the levels of contrast and area of ink used. The stimuli designs all use the same area of ink and had the same contrast, with the only difference between them the direction of gaze. These 7 stimuli were used to attempt to mimic all possible gaze directions; from the potentially threatening direct gaze, looking away, unfocused or not resembling eyes at all. If these stimuli are perceived as eyes then we might expect that the 'straight' stimuli would most closely resemble direct eye contact or an intense stare of the kind which is thought to elicit a fear response. The DL & DR and UL & UR are designed to resemble eyes looking away. The UO & DO are designed to resemble a less direct gaze or unfocused gaze which may not be perceived as eyes at all. These stimuli are reasonably similar to the stimuli used by Stevens et al.(2008a).

Our methodology follows the same general procedure as Cuthill et al. (2005) with the edible component of each bait consisting of a mealworm (*Tenebrio molitor* larvae) frozen overnight, then thawed and pinned vertically to the centre of each stimuli. Meal worms were not used if they were frozen for longer than 48 hours or reused or refrozen. The mealworms were pinned to the front of the stimuli to ensure that avian predators (who are unlikely to have encountered anything similar before) could recognise that they contained an edible component.

At the start of each trial 70 trees (i.e. 10 replicates of each bait type) were selected at random with a minimum gap of 10m between them, with only trees with little or no lichen covering the trunk and of at least 0.9m in circumference were used. Baits were assigned randomly to a tree (1-70) prior to arriving at the site and attached a minimum of 1.5m up from the base using dressmaker's pins. Randomisation of the allocations was achieved by assigning baits a number generated by the Excel function RAND() and using the SORT function to put them in ascending order to be placed on trees 1-70. To aid relocation of the stimuli at the 2009/2010 season at Pollok Country Park site three mountain bike trails running through the experimental area were used as guide to pin out, with the three tracks used sequentially (Figure 2–4) which ensured that there was a minimum of 6 days between the use of each trail. For the 2011 season the two sites (Botanical Gardens & Kelvingrove Park) were used alternately which ensured a minimum of 72 hours between the use of each site. The minimum distance between each stimuli and the rotation of different trails between trials are features designed to reduce the likelihood of any one bird encountering multiple stimuli. It was also decided that, as this area is a popular recreation area and likely to be particularly busy at the weekends, to reduce disturbance levels weekends were to be avoided as Day1 of any trial.

Every time the site was visited the date & time of arrival and departure was noted. Weather conditions and temperature at 9am, 12pm and 5pm were taken from the Met Office website for each day of the experiment.

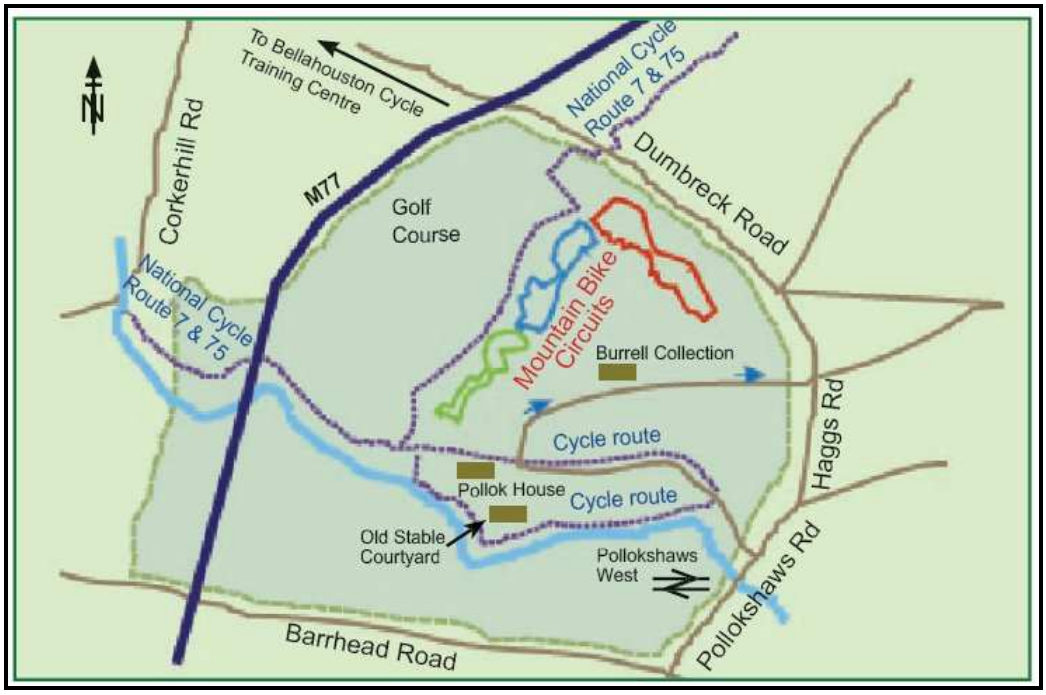


Figure 2–4. Pollok Country Park - Mountain bike tracks (Image from http://www.flickr.com/photos/defmech/154929385/)

The experiment was conducted over 48hrs with the baits put out on the morning of day 1 and checked for bait survival at 2, 4, 24 and 48hrs. Avian predation was taken to be indicated by complete or almost complete disappearance of the mealworm. Non-avian predators such as slugs were indicated by slime trails left behind and spiders by the baits being sucked dry leaving only hollow exoskeletons. Harvestmen were also seen eating the baits but similar to the spiders would eat the insides leaving the exoskeleton. Once all data had been collected the total number of each stimuli used across all trials was calculated along with survival and predator type. The predation of the bait by avian and non-avian predators or the 'survival' of the bait to the end of the trial, were all treated as censored values in the survival analysis.

The survival analysis was conducted in SPSS and a Cox proportional hazards regression used to accommodate the censored data and the varying predation risk throughout the day (Cox, 1972; Klein J.P. & Moeschberger, 2003; Lawless, 2002). Significance was then tested using the Wald statistic and effect sizes are given by the odds ratio (Exp(B)), which is the ratio of the probability of predation in one treatment to the probability of predation in another treatment.

2.1.2 Results

Although 260 of each stimulus were put out some were dislodged, lost or damaged due to weather and therefore not included in the final analysis so that there was a minimum of 254 included for each treatment.

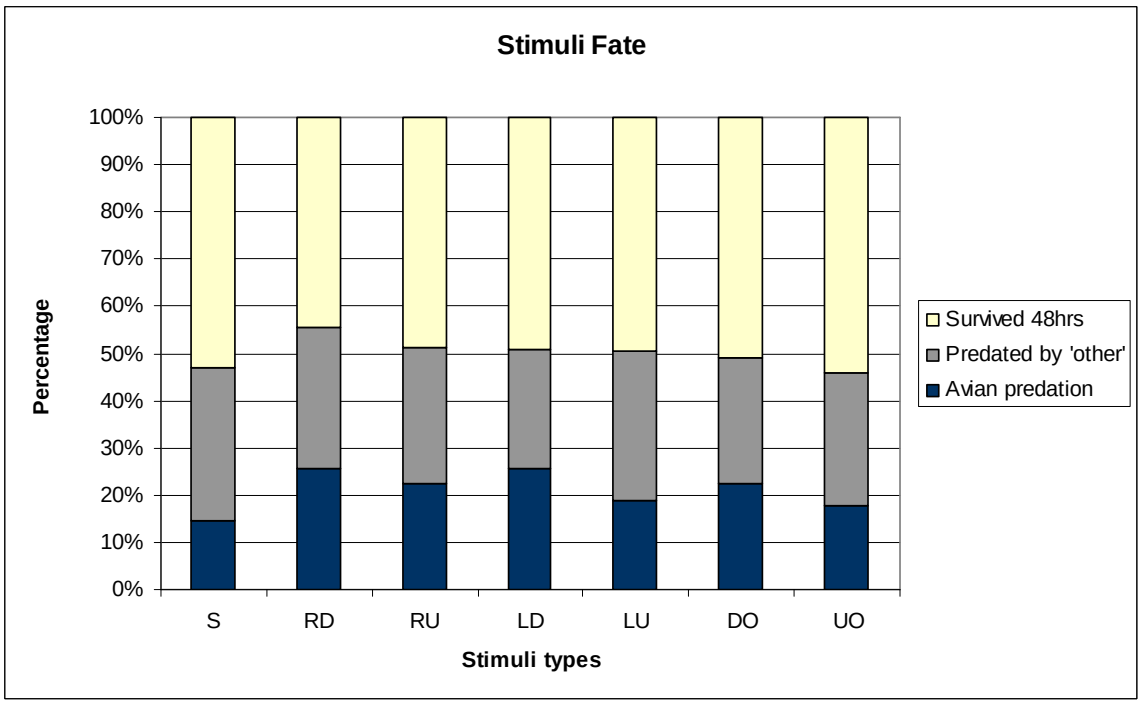


Figure 2–5. Graph depicting the fate of different stimuli types.
(S = straight, RD= Right & Down, RU = Right & Up, LD = Left & Down, LU = Left & Up, DO = Down & Out, UO = Up & Out)

Looking at the percentage of the total stimuli that where predated by avian or 'other' predators or survived to the end of the 48hrs, we can see here that roughly 50% of each stimuli type survived to the end of the 48hr trial (Figure 2-5).

The survival analysis carried out examined each stimulus type when compared to the stimulus with an apparent 'straight' or 'direct' gaze. It was found that survival rates for the downward looking stimuli LD, RD and DO are significantly different than the S (straight) stimuli with p-values of <0.05 (LD <0.000; RD <0.000; DO <0.019). The upward looking stimuli LU, RU and UO do not differ significantly from the S stimuli survival rate.

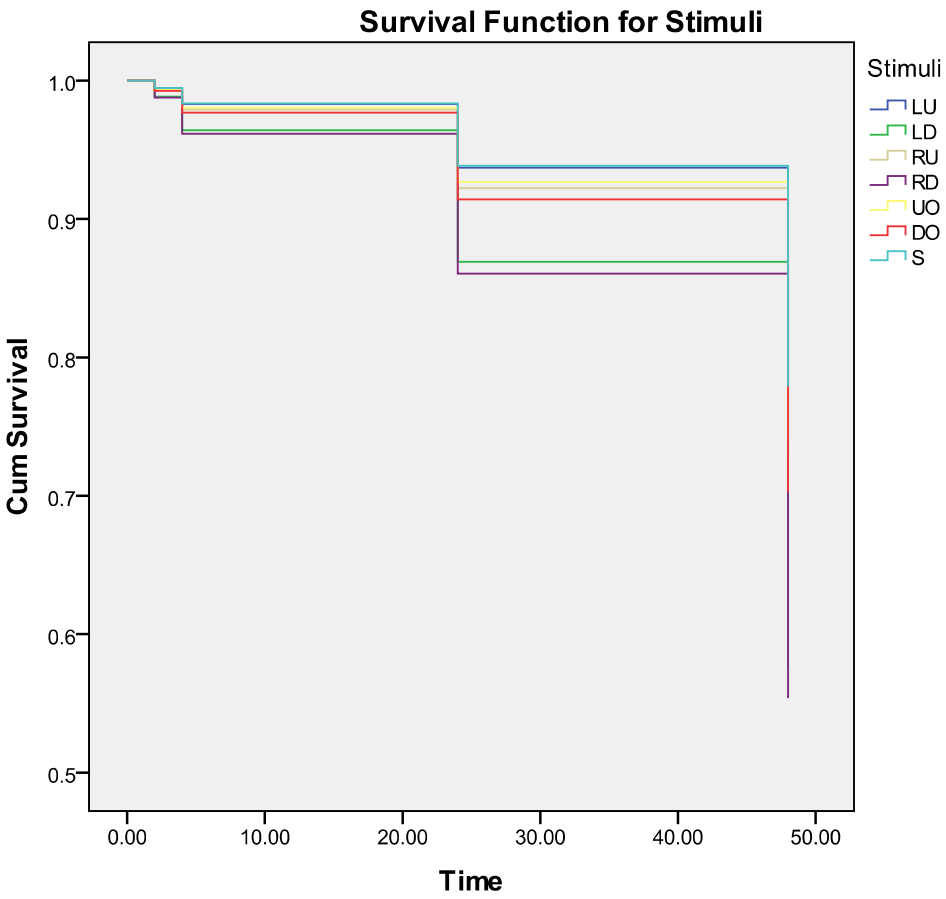


Figure 2–6. Cumulative survival probability
- per each stimuli type surviving avian predation through time.

By examining the probability of each treatment surviving avian predation as a function of time (Figure 2-6) we can see that the 'staring' (S) stimuli had the best survival rate of all the stimuli types and that the survival rates for the upward gazing stimuli (UO, LU and RU) do not differ significantly from the S stimuli. However, the downward gazing stimuli (LD, RD & DO) have a significantly reduced survival rate when compared with that of the S stimuli, with the RD and LD stimuli suffering the greatest levels of predation.

2.1.3 Discussion

For this experiment we are only interested in those stimuli that were predated upon by birds, since other predators are not expected to be affected by the treatments. From (Figure 2–5) we can see that only a small proportion of the stimuli were taken by birds, with the majority with of the stimuli either surviving to the end of the 48hr trial (50%) or predated by non-avian predators such as slugs, harvestmen and spiders (29%). The results of the survival analysis shows us that the all stimuli with patterns designed to give the impression of a downward gaze (LD, RD and DO) suffered a greater predation rate than the stimuli with the direct staring gaze (S) (Figure 2–6). On the other hand, the stimuli with the upward gazes (LU, RU and UO) showed no significant difference in predation rate when compared to direct gaze stimuli. As all the stimuli used were identical in contrast and area of colour, these results would indicate that the apparent direction of the printed eyes was affecting the predation rates, with patterns imitating a staring or upward gaze providing a greater degree of protection from predation than patterns imitating a downward gaze.

One obvious contrast between our study and some previous studies carried out by other workers was that we experienced considerably smaller predation ratios with only 21% of the stimuli we used being predated by birds. Other studies such as Stevens et al. (2009b) had the censored data (not eaten by birds) for two experiments totalling 27% and 17.3% respectively. This difference may in part be due to longer trial durations used in some of the previous studies, with Stevens et al. conducting trials over 48hrs (2008a), 72 hrs (2009a; 2009b) and 96 hrs (2009b). However, we would argue that by extending the duration of the trials to achieve a greater predation ratio we risk losing a degree of realism. We must also consider what might be a ‘normal’ predation rate for a wild population. Although unable to locate any studies in the UK that look at natural predation rates for lepidopteran populations we were able to compare our results with a study examining the predation rates of lepidopteran larvae in a Neoptropical environment (Koh and Menge, 2006). This study found that predation rates were anywhere between 30-60%. However, this was not limited to avian predation and included any predatory attack. Nevertheless, our results fall within these levels and with some of the previous studies showing what appear to be considerably higher predation rates. Obviously this is not the most ideal of comparisons and

there are obvious differences between the neotropical forest used in this study and the highly disturbed temperate woodland used in the UK studies. It is possible that predation rates experienced may be considerably higher in a neotropical environment or that the distribution of avian and other predators differ considerably. For a true picture of what 'natural' predation rates are we really to carry out a similar study within the UK.

The large central eyespots that our stimuli are intended to mimic are, as discussed previously, thought to be startling when suddenly revealed during an attack. This means that having the eyespots in full view, motionless and in the same position for a number of days is a highly unnatural situation, with the potential for neo-phobic or startle reactions to be overcome due to continued exposure.

We had anticipated that the staring stimuli would have a greater survival rate, but these results seem to also indicate a possible difference between the way the upward and downward stares are being perceived, with the staring and upward looking stimuli both apparently conferring a greater protective value. To provide a greater understanding of this result, we feel that future work may have to look at the angle and height at which birds are first encountering the stimuli. The stimuli were attached to trees at a minimum of 1.5m, a height which would mean that the majority of birds would be approaching the stimuli from above. In this case the upward looking stimuli although not apparently staring directly at the birds may have appeared more vigilant and therefore more threatening than the downward looking stimuli. Whatever the mechanism, this result would lend support to the hypothesis that it is similarity to eyes which give eyespots their aversive qualities and not conspicuous colouration or contrast. However, this is not to say that conspicuous colouration has no part to play as having highly contrasting and conspicuous markings is likely to enhance and add to any effect.

It would be interesting in future investigations to look more closely at the apparent difference between the upward and downward looking stimuli. A potential improvement would be to find some method to control the direction from which the stimuli are approached and may require an aviary experiment. Would we for instance still have the same result if the stimuli were approached

from below? The use of motion sensitive photography would allow us to determine the angle at which birds approach the stimuli and perhaps also determine how long if at all birds observe the stimuli before attacking. Any delay in attack, a long observation period or repeated visits before the stimuli is attacked might give us a greater insight into how the patterns affect predation.

It is also generally assumed that eyespots are startling due to their resemblance to the predator's own predators, with staring stimuli most closely resembling an actively hunting predator. However, our results might hint at some aspect of implied vigilance, with predators perhaps less willing to attack an apparently vigilant prey? We may also want to investigate whether all eyes are equally startling i.e. predator vs. non-predator eyes. Birds of prey and big cats have often been noted to have highly conspicuously coloured irises surrounding a dark and strongly contrasting pupil and although this has been touched on to some extent by Stevens et al. (2009), it may be of interest to examine in more detail whether this type of eye is any more startling or aversive than the eyes of non-predatory species. Another potential avenue of investigation is to look at the evidence that "bigger may be better", with larger eyespots potentially providing greater protection. The theory behind this hypothesis is that larger eyes may be used to estimate the size of the animals, with larger eyes indicating a larger animal and potential more dangerous predator. Again, this has been touched on by Stevens et al. (2008b), but has also been suggested by work with reptiles (Burger et al., 1991) which were shown to flee more quickly in response to a large rather than a small eye.

We chose, in this instance, to test our hypothesis in a field rather than lab setting as this adds a degree of realism to the setting that we could not replicate in the lab. However, there are several problems with this approach that limit how we can interpret that data. The stimuli we used are not 'real' butterflies and so we can not be sure that the birds interacting with them as they would a real butterfly. The larger, more eye like, eyespots that we are attempting to investigate are the type that are usually revealed suddenly or flashed at an attacker in order to startle, but our eyespots were on continuous display and motionless and therefore not being seen as they would in nature.

A potential middle ground between the lab and field studies would be an aviary study using wild caught birds. An aviary would allow us greater control over the environment, while still retaining a degree of realism when compared to lab studies. We would be better able to exclude non-avian predators and be certain that any bait taken was indeed removed by a bird. We would also be able to observe a bird's behaviour before removing the bait. Do they observe the bait from a distance first and from what angle do they approach? A further improvement would be to better mimic the behaviour of the model butterflies with eyespots that are suddenly revealed when a bird approaches.

As can be seen from the above there is still a large amount of work that needs to be done to disentangle the effects of conspicuousness and eye mimicry. What do predators use to identify eyes, if they do at all? There is of course the possibility that eyespots are effective because they are conspicuous and that eye mimicry increases their effectiveness in certain circumstances.

In conclusion, while there still much more worked needing to be done our findings that the apparent direction of gaze affects predation rates, is evidence that at least in some cases eyespots may be being perceived as eyes and not simply as a conspicuous pattern.

2.2 Does the direct gaze of a predator have any costs to prey?

Aversion to two facing eyes can be found throughout the animal kingdom, with species of fish, mammals and birds all shown to show some kind of innate aversion or flight response to their presence (Coss, 1978, 1979; Hampton, 1994; Jones, 1980; Stevens, 2005). In almost all of these experiments the greatest responses have been elicited from paired horizontally orientated stimuli, but have varied from simple black dots or concentric circles to a real human gaze. The apparent ubiquity of this response would seem to indicate that aversion to gaze is perhaps an important and primitive defensive mechanism. In fact eyespots are well known to be used by both Lepidopteran and fish species to intimidate or startle predators allowing them time to escape, although the mechanism is still being debated and not all researchers argue that intimidation is achieved by mimicry of a potential predator's eyes (see Stevens, 2005).

Primates have been shown to have a strong bias towards looking at the eyes when presented with faces of their own and other primate species (Emery, 2000). Humans also show a strong response to the gaze of another with evidence that people are both more generous and more honest when being 'watched' by the image of a pair of eyes (Bateson et al., 2006; Haley and Fessler, 2005), a reaction which appears to be entirely unconscious. An interesting aspect is that it is not necessary for the image used to be photographic or realistic as, Haley & Fessler were able to elicit a response using a stylised image. It also appears that we find it extremely difficult to ignore a direct gaze, with a recent paper (Conty et al., 2010) suggesting that we find it almost impossible to refrain from processing a direct gaze even when concentrating on another task.

PET scans have been used to investigate brain activation when carrying out discrimination tasks between eye-contact and no eye-contact conditions. It was found that the amygdala showed significant activation, with the left active in both conditions and the right during the eye-contact condition(Kawashima et al., 1999). The amygdala is responsible for some of our more primitive and instinctual responses such as the fight or flight response. That eye-contact is able to activate this area of the brain may suggests that it has an important and perhaps primitive role. For the most part experiments investigating human

reactions to direct gaze have concentrated on its importance in social interactions. Here I would like to look at its link to predator avoidance. An obvious difference between predatory species (including humans) and the prey species is that for the most part predatory animals will have their eyes at the front of their head to enable them to more accurately judge distance, while prey animals tend to have eyes set on either side of their head for a wider angle of view. We intend to investigate whether the direct gaze of another species is able to elicit a similar response to that of a human gaze and whether there is any difference made between the binocular gaze of a predatory species to that of a prey species.

2.2.1 Materials & Methods

This experiment was based around the classical Stroop test. The Stroop test requires participants to identify the colour of the ink in which a string of letters is printed. It has been found that it typically takes a participant longer to respond when the letter string represents a colour word that is incongruent with the ink colour, than when neutral signs are used (Figure 2–7). This effect is known as Stroop interference(Stroop, 1935). The cause of this delay is thought to occur due to skilled readers automatically processing the incongruent word (Macleod, 1991) and therefore acting as a powerful distracter from the task of identifying the ink colour.

Figure 2–7. Colour word and neutral signs.

Top line: Example of incongruent colour and word combinations
Bottom line: Example of neutral signs.

Our study is based on a previous study looking at the distracting effect of direct human eye gaze and its effect of increasing Stroop interference in participants (Conty et al., 2010). We wanted to use a similar methodology to compare the effect of the direct gaze of other species when compared to that of the humans. We therefore have three main categories of images, human, predatory animals and prey (non-predatory) animals (Table 2–1).

IMAGE LISTS				
HUMAN	**PREY**	**PREDATOR**	**WORD LIST**	
Female 1	Goose	Bear	book	narrow
Female 2	Buffalo	Domestic Cat	cloud	plant
Female 3	Chicken	Domestic Dog	dream	sea
Female 4	Cow	Tiger	elbow	shelf
Male 1	Deer	Eagle	floor	stone
Male 2	Goat	Lion	gesture	tray
Male 3	Pig	Wolf	hat	wind
Male 4	Sheep	Crocodile	middle	window

Table 2–1. Word list & images used.

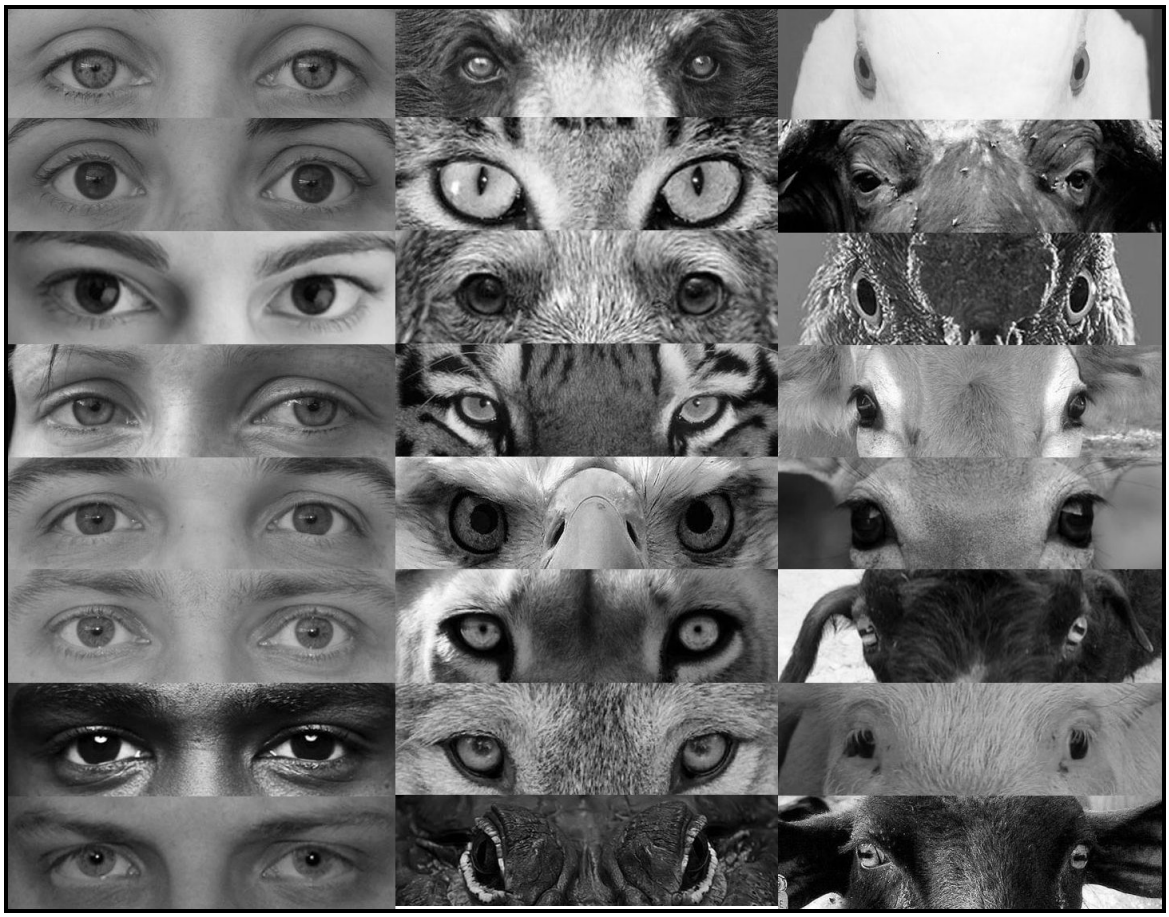


Figure 2–8. Stimuli used in phase 3.
(Order/identification as seen in Table 2–1)

For the human images we have 4 male and 4 female images all of adults with neutral expressions. The predatory animals were picked from those that are historically thought of as dangerous to humans and the non-predatory animals are those that might be considered human prey. All images were cropped so that only the eyes were visible and the inter-pupilary distance was normalised so that all species have the same distance between the eyes regardless of species. A neutral image (Figure 2–8) was also created using a gradient between a dark and

light grey colour sampled from an average eye image. The darker parts of the gradient correspond to the position of the pupils in the eye images.

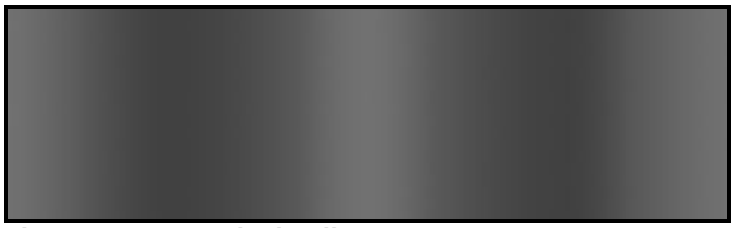


Figure 2–9. Neutral stimuli

A word list of 'neutral' words unrelated to predation or the images was created which would accompany the images (Figure 2–9).

2.2.1.1 Procedure

Volunteers were naïve to the purpose and had either normal vision or corrected to normal vision. Volunteers were met & had the test explained to them. For ethical and data protection reasons data on sex and age of the participants was not collected.

Phase 1 (learning):

The volunteers needed to become proficient at using the right key for each colour so each volunteer had a practice period of 128 trials to get used to the set up & associating describing the ink colour of neutral words (e.g WIND) to the appropriate keyboard response (coloured labels on keyboard to mark keys Red = 4, Green = 5, Orange = 1, Purple = 2).

Phase 2 (Classical Stroop):

To get the baseline of their response to the Stroop test each volunteer was given the classical Stroop test with incongruent colour words and the neutral strings (i.e. XXXX) presented randomly and in equal proportions over 96 trials.

Phase 3 (Test):

Finally there was the test period of 160 trials where the Stroop test was performed but with eye images appearing (0.5° of visual angle) just above the

string (Figure 2–10). The images were of 4 types (human, predator, prey & a neutral image), with 8 images in each category. These will were presented in a random order that was changed for each participant. A total of 34 volunteers were tested using the custom written software.

At the beginning of the test period the volunteers are told that the images are not informative to the task and that they should ignore them & avoid reading the strings. They were explicitly told to focus on the ink colour. In every trial, the stimulus display was preceded by a fixation cross centred on the letter string.

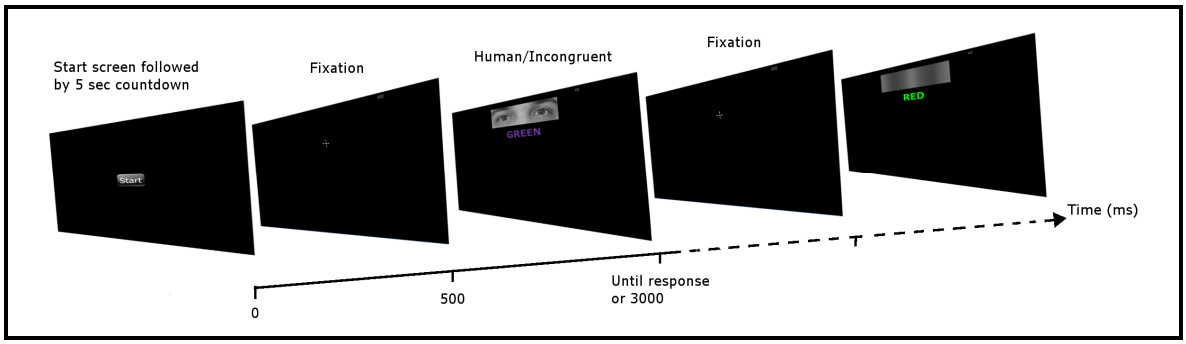


Figure 2–10. Time course of phase 3 and example stimuli.
(Image author's own)

2.2.1.2 Data Analysis

As only correct responses would be included in an analysis, we wanted to check that we had a high percentage of correct answers. Also any outlier reaction times (RTs) exceeding 2 standard deviations (SD) above the mean (for each individual) were rejected. The data was then checked to ensure that it showed normal distribution using the Shapiro-Wilk test.

A Univariate General linear model was used to analyse the RTs of the test period with Image Type (Human, Predator, Prey and Neutral) and Type of String (incongruent/neutral) as the within-subject factors and the interference score (RT difference for the incongruent minus neutral strings) of the training period as a covariate.

2.2.2 Results

The percentage of correct responses is very high with 97.9% correct responses recorded. Outlier reaction times (RTs) exceeding two standard deviations above

the mean were rejected which resulted in an average of less than 5% of the total number of responses per subject and string condition being removed from the data set.

String Condition/Image	Human	Pred	Prey	Neutral
No. Neutral Xs	266	289	291	309
No. Incongruent	206	230	182	204

Table 2–2. No. of each image/word string conditions used.

Table 2-2 shows the number of each String condition (Incongruent word/colour or Neutral Xs) and image combination used. Problems with the randomisation method we used which were not found until after the test period was completed (conditions were assigned to each subject randomly without reference to how many times needed to be used), combined with the removal of outliers, has meant that some combinations of image/string conditions were used more than others. The average Interference Score across all subjects was found to be 134ms and this increase in RT between the two conditions is evidence of the Classical Stroop effect.

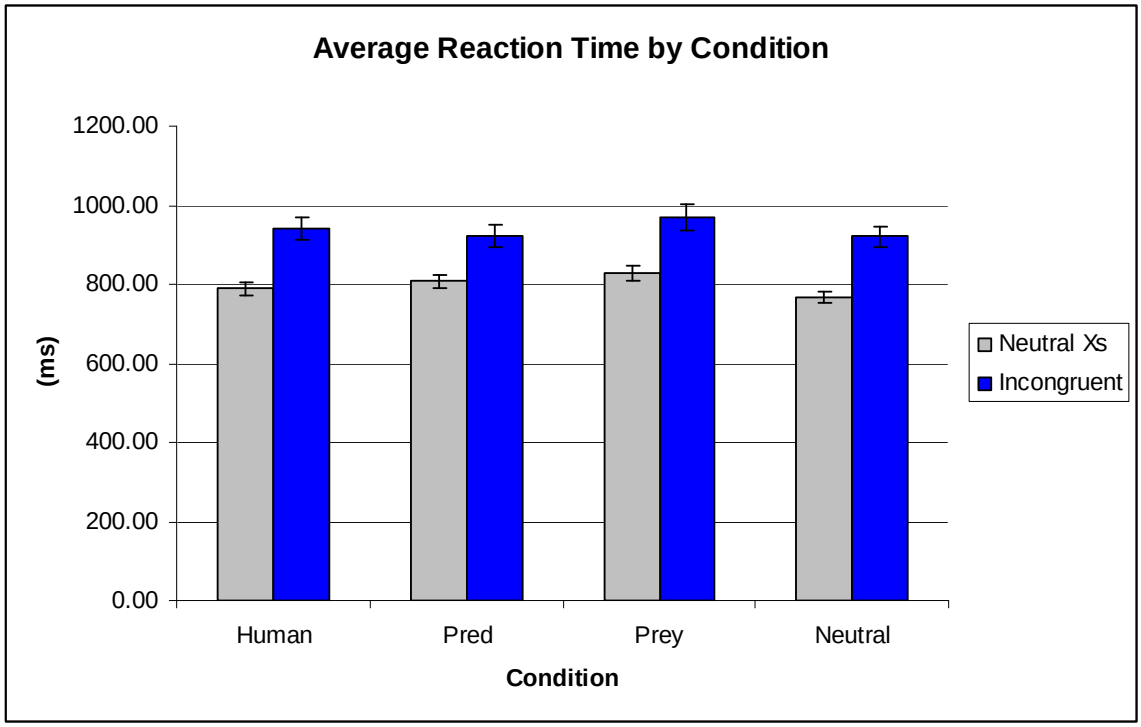


Figure 2–11. Average reaction time for each image/string condition with SE.

Figure 2-11 records the average RT for each condition and the standard error. We can see that the incongruent conditions against show the delaying effect of the classical Stroop test.

We checked for normal distribution using the Shapiro-Wilk Test as this is more appropriate for small sample sizes (< 50 samples) but can also handle sample sizes as large as 2000. For this reason, we used the Shapiro-Wilk test as our numerical means of assessing normality. The data we used was the square root of the average RT per subject, per condition.

The Incon-Human, Incon-Pred and NeutralX-Neutral conditions were found to significantly deviate from a normal distribution (Incon-Human, W= 0.94, d.f. = 37, p = 0.03; NeutralX-Human, W= 0.98, d.f. = 37, p = 0.76; Incon-Pred, W= 0.93, d.f. = 37, p = 0.03 ; NeutralX-Pred, W= 0.96, d.f. = 37, p = 0.18; Incon-Prey, W= 0.96, d.f. = 37, p = 0.18; NeutralX-Prey, W= 0.99, d.f. = 37, p = 0.88; Incon-Neutral, W= 0.12, d.f. = 37, p = 0.17; and NeutralX-Neutral, W= 0.92, d.f. = 37, p = 0.015). Although not all of our data is normal, we have used a General Linear Model to analyse our data and they are known to be robust in dealing with non-normal data distributions.

From the analysis we found no significant interactions for any of the conditions tested and thus there is no evidence that the subjects were distracted differentially by the different types of image (GLM, F = 0.131, p = 0.94)

2.2.3 Discussion

As expected out participants showed the classical Stroop interference. However, the type of eyes above the strings had absolutely no effect on the size of the Stroop effect. This does not match the results reported in Conty et al's (2010) study, where it was found that direct human eye contact significantly increased the size of the Stroop effect. In this study neither the human, animal or neutral image had any significant effect.

As mentioned previously (Chapter 2.1), direct gaze has a measurable effect in other species with species as diverse as domestic chickens, jewelfish and mouse lemurs (*Microcebus murinus*) finding that eye contact or direct gaze is avoided (Coss, 1978, 1979; Gallup et al., 1972; Lill, 1968; McBride et al., 1963) and may represent an aversive event. There is also work that shows that this effect can be seen across species with a number of avian species able to react to and follow human gaze (Bugnyar et al., 2004; Hampton, 1994). In a recent study it

was shown that wild-caught European starlings (*Sturnus vulgaris*) are sensitive to a predators' direction of gaze, with a direct gaze resulting in increased time to feeding resumption, reduced feeding rate and a reduced amount of food consumed overall (Carter et al., 2008).

With this evidence and what we know about the effect of the gaze of other humans has (Bateson et al., 2006; Haley and Fessler, 2005) it may be too early to determine that there definitely no effect to be found here. It is possible that the effect we were looking for is too small to be found with this sample size or that some aspect of our experimental set up meant that we were unable to replicate the previous study's results and it would be prudent to attempt to repeat this experiment to be sure that this is not the case.

Eyespots are a common component of the aposematic or startling displays and in particular within the Lepidoptera (Ruxton et al., 2004; Stevens, 2005) and there is debate as to whether these eyespots are intimidating due to their resemblance to the eyes of the predators' own predators or that it is their conspicuous colouration that induces the startle or avoidance behaviour. In this second interpretation the patterns are intimidating simply by being novel (Coppinger, 1969, 1970; Marples and Kelly, 2001) and conspicuous (Blest, 1957) , rather than through misidentification. If we are able to accurately measure the response to real eyes, we might be able to use this approach to test whether humans respond to Lepidopteran eyespots in the same way.

Chapter 3. Masquerade and cryptic behaviour in Lepidopteran larvae.

3.1 How does resting position influence crypsis?

Animals have evolved many ways to avoid detection by predators. One of the most widespread and common across multiple taxa is the use of camouflage to evade visually hunting predators(Ruxton et al., 2004). The most basic form of camouflage is background matching, where an animal will match the colours, patterning and texture of its body to the background. A good example of this would be the pacific tree frog (*Hyla regilla*). This species has differing proportions of either a green or brown morph (Figure 3–1) depending on the season, with the morph matching the background colour most closely found in greater numbers(Wente and Phillips, 2003). However, this kind of cryptic body colour does impose constraints and in particular limits habitat choice, as against any other background it loses any benefits.

Figure 3–1. Pacific tree frog (*Hyla regilla*) - green & brown colour morphs. (Image © Wikimedia Commons)

There are however more types of camouflage than just background matching. There are many examples for instance of animals which use mimicry, with species such as the dead leaf mantis (*Deroplatys desiccate*) and the bird dropping spider (*Celaenia excavata*) closely resembling inedible components of their environment. This type of specialised camouflage is known as ‘masquerade’ and works to reduce detection rates in a different way from crypsis (Skelhorn et al., 2010b), with animals benefiting not from remaining unseen by their predators, but by being misidentified as inedible and disregarded.

Masquerade appears in fact to be a fairly common strategy within the larvae of UK moth species, with a number of species known to be twig or bird dropping mimics. In laboratory experiments carried out by de Ruiter (1952), using the twig mimic species the canary-shouldered thorn (*Ennomos alniaria*) and the august thorn (*Ennomos quercinaria*), they were able to show that jays (*Garrulus glandarius*) were unable to discriminate between the larvae and the twigs of their host plants. However, in this experiment the twigs and larvae were presented to the jays scattered across the floor of the experimental cages and not as they would be encountered in the wild.

What we have not mentioned yet are the behavioural adaptations that are an integral part of both the cryptic and masquerade strategies. With crypsis it has been shown that remaining still is an integral aspect of the strategy (Ioannou and Krause, 2009) and it is not hard to see that the same may be true if masquerading as a rock or bird dropping.

In a more recent study by Dockery et al. (2009) the behavioural tactics employed peppered moth larvae (*Biston betularia*) to masquerade as twigs were investigated. Like many twig mimics, *B. betularia* (as well as employing similar colour and shape patterns as the target model), will hold their bodies rigid and motionless while angled out from the main branch. Dockery et al wanted to investigate whether the larvae were consistent in the angle at which they held themselves and how this related to two of their known food plants: hawthorn (*Crataegus monogyna*) and birch (*Betula pendula*). To do this they allowed larvae to settle into position within their rearing tubs. Once settled they were removed with the twig and placed against a plastic protractor to measure the angle. What they found was that the angle at which the larvae held themselves compared to the branch they were resting on varied and tended to be more acute than that found from either food plant. It was suggested that the variation in resting angle may be a phenotypic plasticity allowing the larvae to match themselves to a number of different possible food plants, but that overall matching the angle seemed unlikely to be critical to the masquerade strategy.

The early thorn moth (*Selenia dentaria*) is another species whose larvae use twig mimickry and are commonly found in the UK on Common Hawthorn (*Crataegus*) and other deciduous trees. As with *E. alniaria* and *E. quercinaria* (de Ruiter,

1952), they hold their bodies rigid and at an angle from the branch, supporting themselves only by one pair of prolegs and the anal claspers (Figure 3–2). To provide themselves with additional support they have also been seen to spin a silk thread between their head and a nearby twig or leaf.

Figure 3–2. Resting positions *S. dentaria*. (Image author's own)

What we hope to do is replicate some of the measurements taken by Dockery et al. but using *S. dentaria* rather than *Biston betularia*. We plan to modify some of the measuring techniques used to see if we can find a method that might introduce less disturbance and examine whether the position of the hawthorn branch or twig they are resting on has any effect.

The aims of our study:

1. to determine if the early thorn caterpillars show consistency in their angle of rest;

2. to determine if the angle of rest is affected by the positioning of the hawthorn branches they rest on (either (180˚) vertical or (90˚) horizontal);

3. to determine if the angle of the hawthorn twigs relative to the branch and the resting angle of caterpillars are similar;

4. to determine whether they match their direction of resting angle to that of the hawthorn branches they are resting on & whether the position of the branch will affect this;

As with Dockery et al. (2009) we would expect that if the larvae show a consistency in their angle of rest it would suggest that this may be under genetic control rather than showing behavioural flexibility. We would also expect that if the larvae are able to behaviourally match their angle of rest to the host plant that positioning would suggest that this is an important aspect of the masquerade. However, if there are consistent differences this might suggest that either it is not important or that there may be other associated costs making matching the angle unprofitable. Further to this, we wanted to see whether the larvae consistently matched their direction to that of the twigs on the hawthorn branch, as this would seem to be an important aspect in keeping up the pretence of being a twig.

3.1.1 Materials and Methods

The early thorn (*Selenia dentaria)* larvae used were obtained from John Delf at Liverpool Hope University. The larvae were obtained at the 1st instar stage and were only used once they reached 4th or 5th instar. The larvae were kept in 1000ml plastic food boxes with holes pierced into the lids to allow for air circulation, with 8-9 larvae per box. At total of 22 larvae were used. Fresh leaves & twigs of Common Hawthorn (*Crataegus monogyna*) were provided every two days in a clean box and the larvae transferred using an artist's brush.

To investigate the resting behaviour 6 clamp stands were set up with 6 hawthorn branches of 15-20cm in length, picked without conscious bias from the same area that the feed stock was taken. Three clamp stands had the branches set vertically with the cut end at the top and the remainder of the branches set at a 90° angle from the vertical. These were chosen as not all branches project straight out from the main trunk, with some likely to droop down as is characteristic in a number of willow species.

The larvae were moved from their tub using an artist's brush and placed on to the branches. They were left for a minimum of 45 minutes to allow them to

settle. Once settled those that were determined to be exhibiting the desired behavioural response, i.e. with the body rigid and still and held at an angle from the Hawthorn branch were carefully photographed using a digital camera (Panasonic DMC-TZ7) so as not to touch or disturb the larvae. Once photographed the larvae were returned to their tubs. Since no larvae were photographed more than once in 24 hours and the larvae were free to settle anywhere they chose each picture was regarded as independent.

The pictures were then sorted into those where the larvae were resting on vertical (180˚) or horizontal (90˚) Hawthorn branches (Figure 3–3).

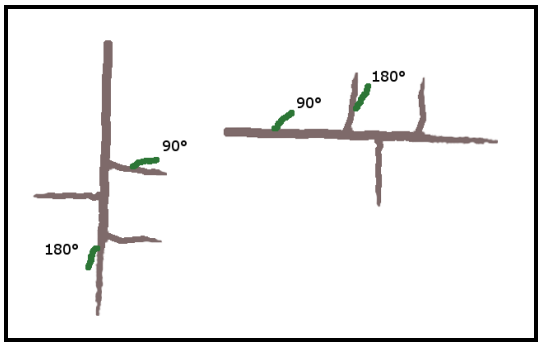


Figure 3–3. Defining resting on 90°and 180°twigs. (Image author's own)

The digital photographs were then used to determine the angle of the larvae. This was done using the 'measure tool' of the image manipulation program GIMP 2.6 which measures angles and distances (Figure 3–4).

Figure 3–4. Measuring angle in GIMP 2.6.
(The angle was measured from a point equidistant between the last 2 prolegs along a line as close to the middle of the larvae as possible and along the plane of the twig.) (Image author's own)

In order to maximise consistency in measurements all measurements were taken by the same individual. The first image used was measured 10 times in order to assess whether the measurements were consistent. The difference between the

highest and lowest measurements was 1.24°. For the rest of the pictures two measurements were taken 24 hours apart and averaged to provide the final figure, with the mean difference between any two measurements coming to 0.51° and well within acceptable limits.

The Hawthorn used in the larvae photographs were then used to make up part of the twig angle sample, along with an equal number of samples taken from more branches collected from the same site. In each case the angle that the Hawthorn twigs protruded from the main stem was measured using a transparent plastic 180° protractor. Two measurements of each twig were taken 24hrs apart and the average taken. The mean difference between the two measurements was calculated to be less than 1°. The greater difference between measurements for the Hawthorn angles, as compared to the larval angles of rest, is likely to have been caused by a reduction in accuracy when measuring with a plastic protractor compared to digital measurements taken with a software package.

A second set of data was collected alongside the angle data. Here we assessed whether the larvae were matching their direction of rest to the Hawthorn twigs on the branch. The larvae were either scored as either not matching (Figure 3–5, A) or matching (Figure 3–5, B). As with the angle data this was separated into two categories depending on whether the larvae were resting on horizontal (90°) or vertical (180°) branches.

Figure 3–5. Direction of resting postiton.
A is an example of a larva which is resting in the opposite direction from the Hawthorn twigs. B is an example of a larva which is matching the direction of the twigs. (Image author's own)

Mann-Whitney U tests were used to determine if there were significant differences between the resting angles of the larvae on the 90° and 180°

branches and the combined resting angles of the larvae (on the 90° and 180° branches) as compared to angles taken from Hawthorn.

3.1.2 Results

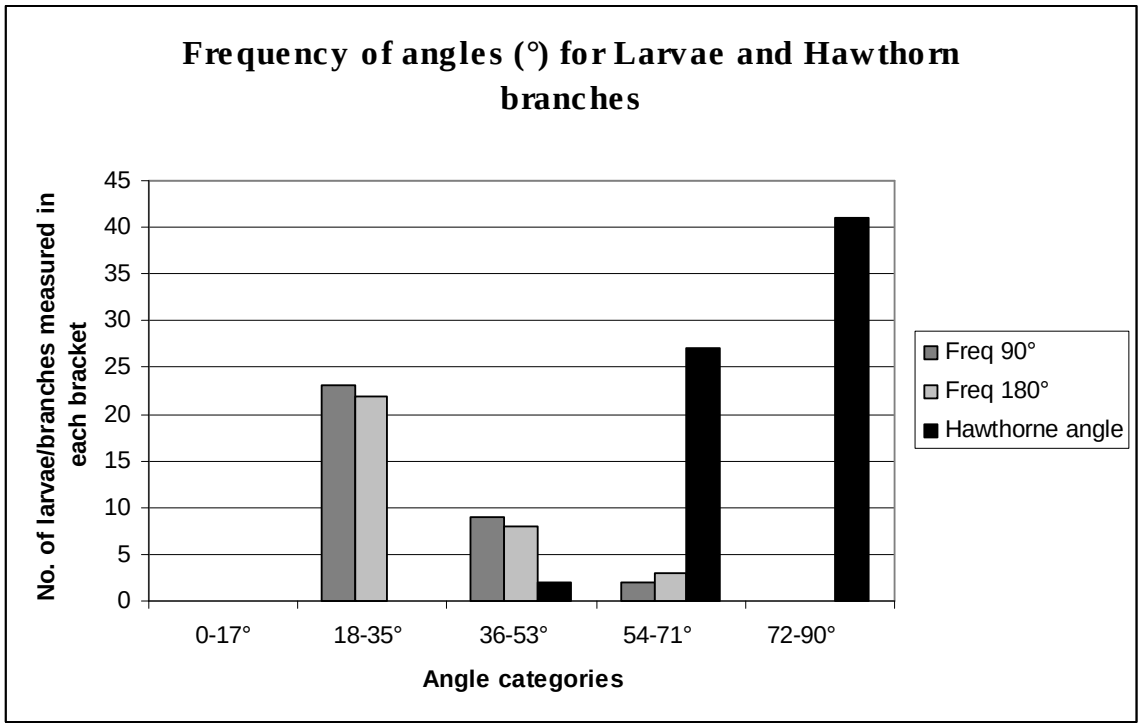


Figure 3–6. Frequency of angles (°)

The above figure has the frequency of angles measured from each group (90°, 180° & Hawthorn twigs) separated into 5 brackets of equal size.

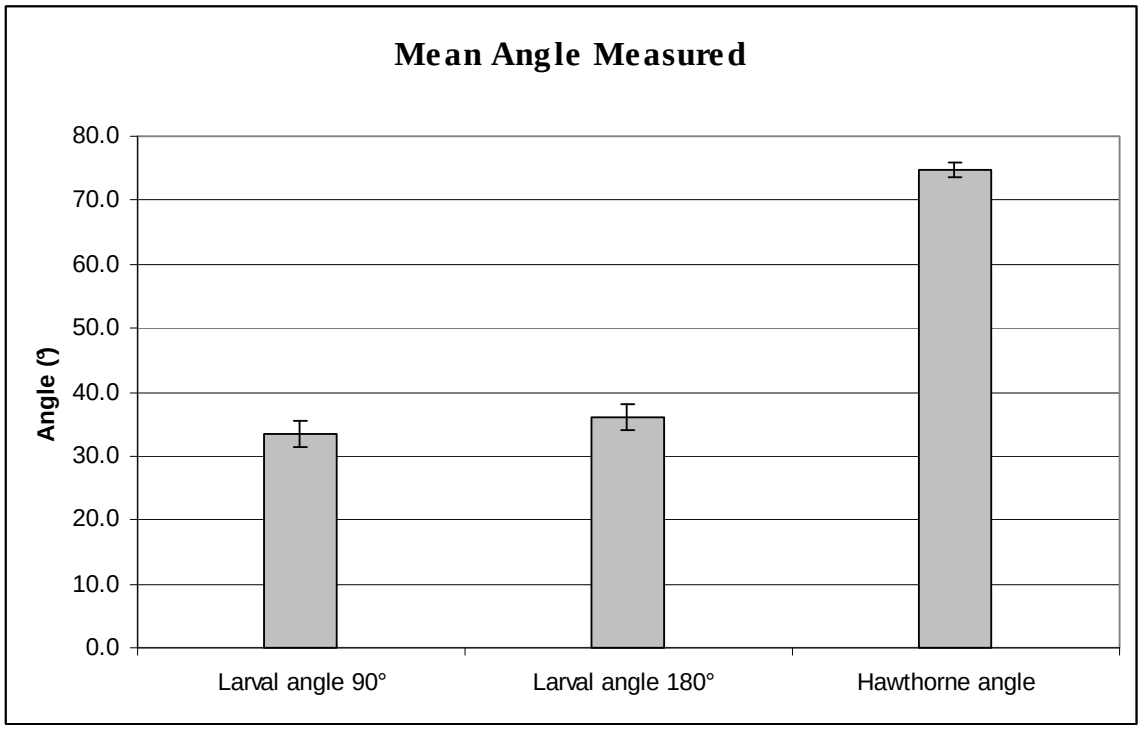


Figure 3–7. Angles of larva and hawthorn (means and variance)

Figure 3–7 shows mean angle for each category and shows that the Hawthorn angles measured are clearly considerably greater the larvae (Coefficients of variance: Larval 90°, CV = 34.9; Larval 180°, CV = 31.2; Hawthorn, CV= 14.5).

After analysis it was found that there is no significant difference between the resting angles of the larvae on the 90° and 180° branches ($N = 67$, $U=672$, $p = 0.164$). Inspection of the data suggests that larvae rest at more acute angles to the branch than those made by twigs. However, when the data was combined and compared to angles taken from Hawthorn we were found a significant difference between the larvae and Hawthorn ($N = 137$, $U= 4647$, $p < 0.0001$).

Count		90°\180°		Total
		90°	180°	
Y/N	0	3	22	25
	1	32	12	44
Total		35	34	69

Table 3–1. Cross-tabulation - Matching Y(1)/N(0) * 90°\180°

We see that there are a greater number of larvae mis-matching their direction of rest on the vertical (180°) branches (Table 3–1). The results of the Chi-squared test carried on the data collected allowed us to determine that the number of larvae on the vertical (180°) branches mis-matching their direction of rest is significantly different from that expected by chance alone (Chi = 23.52, $p<0.0001$)

3.1.3 Discussion

Figure 3–6 provides the frequency of the angles measured for each group and Figure 3–7 the means and variance. From these we can see that there appears to be variation in both the angles adopted by the larvae and the hawthorn sample. However, the larvae in the 180° & 90° groups appear to rest at a more acute angle relative to the branch and show greater variance than the hawthorn twigs measured.

Our results support the null hypothesis that there is no difference between the resting positions of the 180° & 90° groups. Having determined that there was no difference, we combined the data to compare against the angles taken from hawthorn. This analysis confirmed that there is a significant difference between the two sample sets and that the larvae do not matching their angle of rest to the hawthorn twigs.

The results presented in Figure 3–6 and Figure 3–7 agree with the results Dockery et al, (2009)found when using *B. betularia* and suggests that matching the exact angle of twigs on the host tree is not necessary for the benefits of twig mimicry to be felt. This also concurs with the results of Skelhorn et al, (2010b) which found that birds with experience of hawthorn branches took longer to

attack the twig mimic species Brimstone moth, (*Opisthograptis luteolata*), and the Early thorn moth, (*S. dentaria*) larvae even when presented to them singly with no branch. This would suggest that while positioning on the branch may add to the effectiveness of mimicry, it is possible to benefit even without perfectly matching the twig angle. Finally we examined the direction larvae faced when resting and found that the orientation of the Hawthorn branch had a significant effect on whether larvae matched their direction to that of the hawthorn twigs. It was found that those larvae resting on the 180° (vertical) were significantly less likely to match the direction of the host branches twigs.

Therefore while we found no evidence to show that there is a relationship between the orientation of the hawthorn branch and the angle of rest, it does appear to have an effect on the direction the larvae face. In fact it suggests that a significantly larger number of larvae mis-matched their direction (Table 3–1) compared to the hawthorn twigs when the hawthorn branch was held at 180° (vertical). To summarise, we found that larvae do not match their angle of rest to match the angle of the host branch's twigs, with the Hawthorn twigs found to protrude from the main branch at a considerably more obtuse angle than the larvae. Further, it was found that when branches were orientated at a 180° (vertical) angle, resting larvae were significantly less likely to match the direction in which they sat to that of the branch's twigs.

There are a number of scenarios which could account for our results. It may be physically difficult for the larvae to maintain an angle of rest that matches that of the more obtuse angle of the Hawthorn. If more energy is expended in maintaining the greater angle, but without a corresponding decrease in predation pressure it is unlikely that this would be selected for in the population. Again, a difference in energy expenditure may account for the differences between 180° & 90° groups in the direction the larvae faced. The larvae are known to use a silk thread to secure and suspend themselves at the preferred angle and this would not be possible when facing downwards. However, even on 90° branches this is not always possible, so it is unclear as to whether the extra energy need to maintain the body position would be any more prohibitive.

Alternatively, we could look to the mechanism that the larvae use to orientate themselves on the branch. It may be that this mechanism is upset or confused by having the branch at 180°. However both of these explanations seem doubtful, as it is not unlikely or uncommon to find branches that droop down towards the ground, making these short comings particularly disadvantageous. A potential problem which might have bearing on our results is that the hawthorn we had access to was taken from managed parks and woodland which are likely to either have current or past trimming regiment. Therefore, it is possible, that trimming has affected branching patterns on these trees and they do not give an accurate representation of 'normal' twig angles.

Another option may be that there is a greater disadvantage to matching the orientation of the twigs when the branch is in this position. In this orientation, in order to match the direction of the twigs as they emerge from the main branch, the larvae must face downwards towards the ground. As their main predators are birds and are likely to attack from above, it may be that by facing downward they would miss cues as to the presence of predators and leave themselves vulnerable to attack (perhaps by not freezing or remaining still). To investigate this further it would be of interest to look at how much more predation mis-matched larvae experience. This could be done with a field experiment using artificial prey. Assuming it was found that there is a greater predation rate when mis-matched we could then look at the mechanism by which the larvae orientate themselves. For instance it if it is not the twigs on the branches in their environment that they use, perhaps it is light. In this case we could easily test this by either placing the larvae on the branches in darkness or by manipulating from which direction light hit the branches.

In summary, *S. dentaria* show variation in their angle of rest and do not appear to match the angle at which twigs emerge from the host branch. Their angle of rest is also not affected by the orientation of the host branch (180°or 90°). However, the degree to which the larvae match their direction of resting angle to that of the host branch is affected by the orientation of the branch. These results suggest that perfect mimicry is not necessary to reduce predation rates and there is likely to be a point at which other considerations constrain any further adaptation towards it. In a recent study examining imperfect mimicry in Syrphidae species (Penney et al., 2012) a strong relationship was found between

body size and mimetic fidelity. This suggested that smaller and therefore less profitable prey species had reduced predation pressure limiting the selection for perfect mimicry. While the Lepidopteran species we used are generally considered to be overall very good twig mimics, they do appear to be at their most convincing in the final and largest instars. Perhaps to further investigate this we should examine how mimetic fidelity changes with instar and body size.

3.2 Concealing movement. How does movement influence crypsis?

Crypsis is a common and widespread adaptation found across widespread taxa, which is used to reduce detection by both predators and prey (Ruxton et al., 2004; Stevens and Merilaita). Crypsis in its simplest form is colouration which matches the background the organism is viewed against, such as the white fur of the snowshoe hare (*Lepus americanus*) (Figure 3–8).

Figure 3–8. Snowshoe hare (*Lepus americanus*) (Image © Shayroy4)

Figure 3–9. Duvaucel's gecko (*Hoplodactylus duvaucelii*) (Image © Steve Reekie)

This can be added to with disruptive camouflage where pattern components break up the outline of the organism (Ruxton et al., 2004; Stevens and Merilaita, 2009), such as that found on duvaucel's gecko (*Hoplodactylus duvaucelii*) (Figure 3–9). The function of crypsis is to increase the chance that other organisms will remain unaware that the cryptic organism is present. A related but separate (Skelhorn et al., 2010a) condition is that of masquerade. Organisms using this strategy take on the appearance of an inanimate object such as a leaf, stone, twig or bird dropping. This leads them to be misidentified as the inanimate object it has modelled itself on and hence disregarded (Skelhorn et al., 2010b).

Masqueraders are in fact hiding in plain view. Masquerade is about avoiding being recognised, whereas crypsis is about avoiding being identified as an entity at all.

The benefits associated with crypsis and masquerade are however likely to come with associated costs. If you want to be a convincing stone you must have the associated behavioural traits, which means in this case that any movement may give the game away. In fact there is evidence to show that once a distant predator has been detected camouflaged prey will 'freeze' (Broom and Ruxton, 2005; Eilam, 2005). Although a long standing belief, with a large body of anecdotal evidence, there has in fact been little data collected from controlled experiments to support the importance of stillness to crypsis. However, in a recent paper Ioannou and Krause (2009) were able to test this hypothesis with the use of three-spined sticklebacks and their chironomid larvae prey presented against a red (cryptic) or white (conspicuous) background. The effects of both background matching and motion were compared as to their effect on detection rates. Ioannou and Krause were able to show that for crypsis to be effective background matching needs to be coupled with remaining motionless and that movement significantly increases detection by predators. This restriction on movement of course has a significant impact on an organism's ability to perform a number of tasks including foraging for resources, mate finding, and adopting a preferred microclimate in response to changing environmental conditions.

However, for those organisms using masquerade there may be some contexts in which movement may occur without reducing the effectiveness of masquerade. Examples may be founding in leaf and twig mimics, such as stick insects and mantids both of which are known to walk with low amplitude swaying movements which may be a form of movement concealment (Edmunds and Brunner, 1999; Robinson, 1966), where the swaying movement may enhance resemblance by imitating the effect of wind on the leafs or twigs(Bedford, 1978; Cott, 1940). The mantid *Hierodula patellifera* is a well-documented leaf mimic, a strategy used to reduce detection by both potential prey and predators. In a recent study it was found that both in the field and under laboratory conditions walking and swaying were more frequently observed in windy conditions and increased with wind velocity (Watanabe and Yano, 2009). In the same study, lab experiments also found that the discovery rate of the mantids by predators was

significantly lower on swaying versus fixed leaves. These results suggest that the mantids were actively responding to the changes in wind condition and that swaying may be an adaptive behaviour to reduce predation.

In a recent study it has been found that foraging birds change their behaviour in windy conditions and become much less sensitive (shows as a reduced tendency to flush) to potentially threatening movement with increasing wind speed(Carr and Lima, 2010). The authors suggest that this may be a learned behaviour that serves to avoid the cost of repeated false alarms, with the increased experience of non-threatening wind-blown debris in windy conditions causing habituation. This may introduce a situation which predators can use to increase their chances of remaining undetected.

It is likely that the increase in the background movement introduced by windy conditions will complicate detection of prey and/or predators for visual organisms. It has been shown that the lizard *Amphibolurus muricatus* is less accurate in responding to targets which more closely mimic background movement (Woo et al., 2009). Windy conditions may therefore provide an opportunity for cryptic and masquerading organisms to move without incurring greater risk of detection.

The larvae of the early thorn moth (*Selenia dentaria)* are twig mimics commonly found in the UK on Common Hawthorn (*Crataegus*) and other deciduous trees. When allowed to settle on a branch, they will assume a rigid posture pointing out from the main branch in a similar manner to a twig. In our previous experiences with this species we had noted that under windy conditions larvae appeared to sway back and forth as if mimicking a twig moving with the wind. Although anecdotal in nature, it presented an interesting possibility that this may be a behavioural strategy.

It has previously been considered that in some cryptic species such as mantids and crickets the swaying back and forth motion could be explained as an attempt to determine distance (Kral, 2009; Poteser and Kral, 1995), with mantids needing to judge striking distances to capture prey and the crickets to judge distances before a jump. However, our moth larvae have no need to

accurately judge distances and so any swaying behaviour may be more convincingly attributed as a behavioural adaptation for masquerade.

We wanted to carry out a small pilot study to see if we could replicate the behaviour we had previously identified and to examine whether the swaying motion was in fact produced by the larvae themselves or could be explained by other factors such as the larvae magnifying the movement of the branch. By firmly clamping and immobilising the branch on which the larvae are sitting we can be certain the any swaying behaviour we find is not due to the transfer of movement from the twig to the larvae. We also wanted to investigate whether there was any effect of wind on the time taken by the larvae to settle. After the initial confirmation we want to see if we could replicate our previous results using a larger sample size and improve on the experimental design by using a more standard 'twig' that could be clamped more firmly to prevent any movement transfer. To test whether this behaviour was limited to *S. dentaria* or could potentially be found in other twig mimic species we wanted to carry out the same tests on the larvae of the peppered moth (*Biston betularia*), which is also a twig-mimic. Further to this we wanted to determine if this change in behaviour is triggered visually or mechanically. Are the larvae responding to the visual cue of the leaves and twigs around them moving or is it a response to the air current passing over the hairs?

3.2.1 Materials & Methods

3.2.1.1 Larvae

For the initial pilot experiment the early thorn (*Selenia dentaria*) larvae were obtained from John Delf, formerly of Liverpool Hope University. The larvae were obtained at the 1st instar stage and were only used once they reached 4th or 5th instar. A total of 12 larvae were used for this experiment, with each caterpillar used a maximum of once in 24 hrs.

For main study we used two species of lepidopteran larvae. Peppered moths (*Biston betularia*) were obtained from Dr Hannah Rowland from the University of Glasgow and early thorn (*Selenia dentaria*) obtained from Glasgow Museums Research Manager for Natural History, Richard Sutcliffe. The larvae were

obtained at the 1st instar stage but were only used once they reached the 5th instar. In total 20 early thorn and 18 peppered moth larvae were used.

All larvae were kept in 1000ml plastic food boxes with holes pierced into the lids to allow for air circulation, with 8-9 larvae per box while the larvae were developing. Larvae were transferred using an artist's brush to a new box with fresh leaves & twigs every two days. White willow (*Salix alba*) were provided for the *B. betularia* and Common Hawthorn (*Crataegus*) for the *S. dentaria*. Once at the correct instar for use in tests, the larvae were put into separate numbered boxes.

3.2.1.2 Pilot - Experimental Set up

To investigate the resting behaviour 2 clamp stands were set up with hawthorn branches of 15cm in length and a minimum of 1cm in diameter (Figure 3–10). This length and diameter were chosen as once clamped they were unlikely to move in a breeze. All leaves were removed prior to the experiment as they increase drag (Vogel, 1989) and the likelihood of the twigs moving in the breeze.

Figure 3–10. Clamp & twig set up (Image author's own)

To provide the 'wind' a portable office fan (Tefal 12" Supercooling Oscillating Table Fan) was set up at a distance of 1m. The larvae were moved from their tub using an artist's brush and placed on to the branches and the timer started. For the first 10 minutes the larvae were scored as to whether they were 'swaying' and 'travelling' every minute and thereafter every 5 minutes until an

hour had elapsed. ‘Swaying’ was described as a side to side movement of the body and ‘travelling’ was when the larvae was moving along the twig or changing direction.

The larvae were scored like this under 3 different conditions (in a randomised order for each individual). The first was with no breeze or air movement. The second was at setting 1 on the fan and the third at setting 2. Using a SILVA ADC Summit anemometer, we measured the average wind speed over 1 minute at a distance of 1m of for the 2 fan settings used. Setting 1 on the fan was found to have an average speed of 1m/s over 1 minute and setting 2 was found to have an average of 1.5m/s. The caterpillars’ activity scores were collated into 3 time periods 0.5-5 minutes, 6-25 minutes and 30-60minutes. The scores were then converted into a proportion of the total number of scorings and graphed.

3.2.1.3 Full Study - Experimental Set up 1

To investigate the larvae’s behaviour under the different wind conditions two clamp stands were set up with 1cm diameter dowelling to act as an artificial branch. This diameter of dowelling was chosen as once clamped it did not move in a breeze. To ensure the larvae stayed on the artificial twig and did not escape on to the rest of the experimental set up, a thick layer of petroleum jelly was painted on to the dowelling. It was found that this needed to be around 2 mm thick and painted on to at least 4 cm length of dowelling to be effective at preventing larvae from moving out with the experimental area. The dowelling was marked with 1cm intervals using marker pen and a video camera was set up above to record the trials (Figure 3–11).

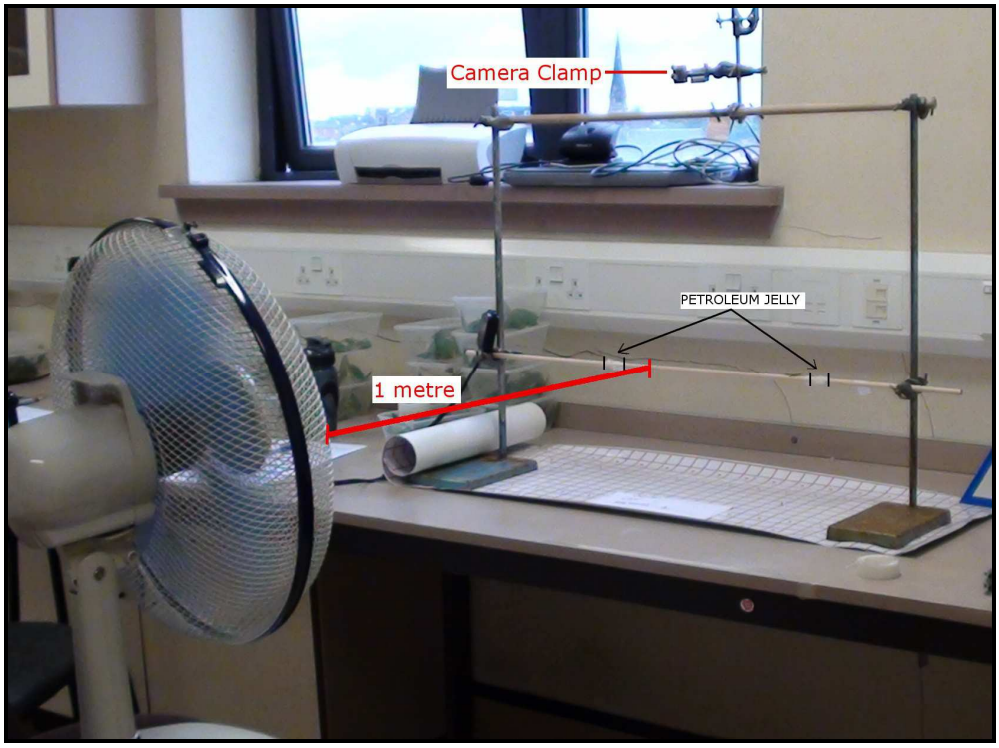


Figure 3–11. Clamp & artificial 'twig' set-up for experiment 1 (Image author's own)

To provide the 'wind' the same portable fan (Tefal 12" Supercooling Oscillating Table Fan) was set up at a distance of 1m. Once selected for use in the experiment the larvae were kept one to a box to aid individual identification and each larva was submitted to each of the 3 treatments in a random order. During each trial the larva was scored at 30 seconds, 1 minute and then at 1 minute intervals until 30 minutes had elapsed. The larvae were scored as to whether they were 'swaying' and/or 'travelling'. 'Swaying' was described as a side to side movement of the body and 'travelling' was when the larvae was moving along the twig or changing direction. We used 20 early thorn and 18 peppered moth larvae which were scored under 3 different wind speed conditions. The first was with no breeze or air movement. The second was at setting 1 on the fan and the third at setting 2.

1.1.1.4 Experimental Set up 2

In this case we wanted to investigate whether the increased activity shown by larvae in the pilot study in response to air movement was caused by visual or mechanical cues. For this we modified the set-up from the first experiment by isolating the dowelling rod and clamps within a clear plastic enclosure and attaching small twigs and branches to the front (Figure 3-12).

Figure 3–12. Experiment 2 set up, enclosed clamp & dowelling (Image author's own)

When turned on the desk fan produced air currents that moved the branches and leaves, and while the larva would be able to see the movement from within the enclosure it would not be able to feel the air current itself (because of the isolating plastic box). For this test we did not used 3 air speeds as in the previous condition, but only used setting 2. Only *S. dentaria* larvae were used.

3.2.1.4 Experimental set up 3

For the final set-up we wanted to examine whether there may be some other mechanism responsible for the increased activity we had observed that we had not so far taken in to account. To do this we wanted to investigate both visual and mechanical stimulation of the caterpillar separately and so modified the experiment set-up again. We kept the clear plastic enclosure and branches, but obscured the view through the plastic with newspaper. This meant that during the experimental period the larvae were isolated from both the air current and the visual cue of the moving branches. Only *S. dentaria* larvae were used.

In every experiment, between every trial the dowelling rod was wiped to remove any silk thread that might have been left by the previous larvae. For each experiment a Kruskal–Wallis one-way analysis of variance was used to test the percentage score for each caterpillar to highlight any significance differences between the three wind speeds.

3.2.2 Results

3.2.2.1 Pilot

As the larvae we were using were in the final instars prior to pupation, some of the larvae began to pupate before we had finished our tests. From the 12 larvae used we were able to get 8 separate measurements at the 'No Breeze' and Setting 1 conditions and 6 for setting 2.

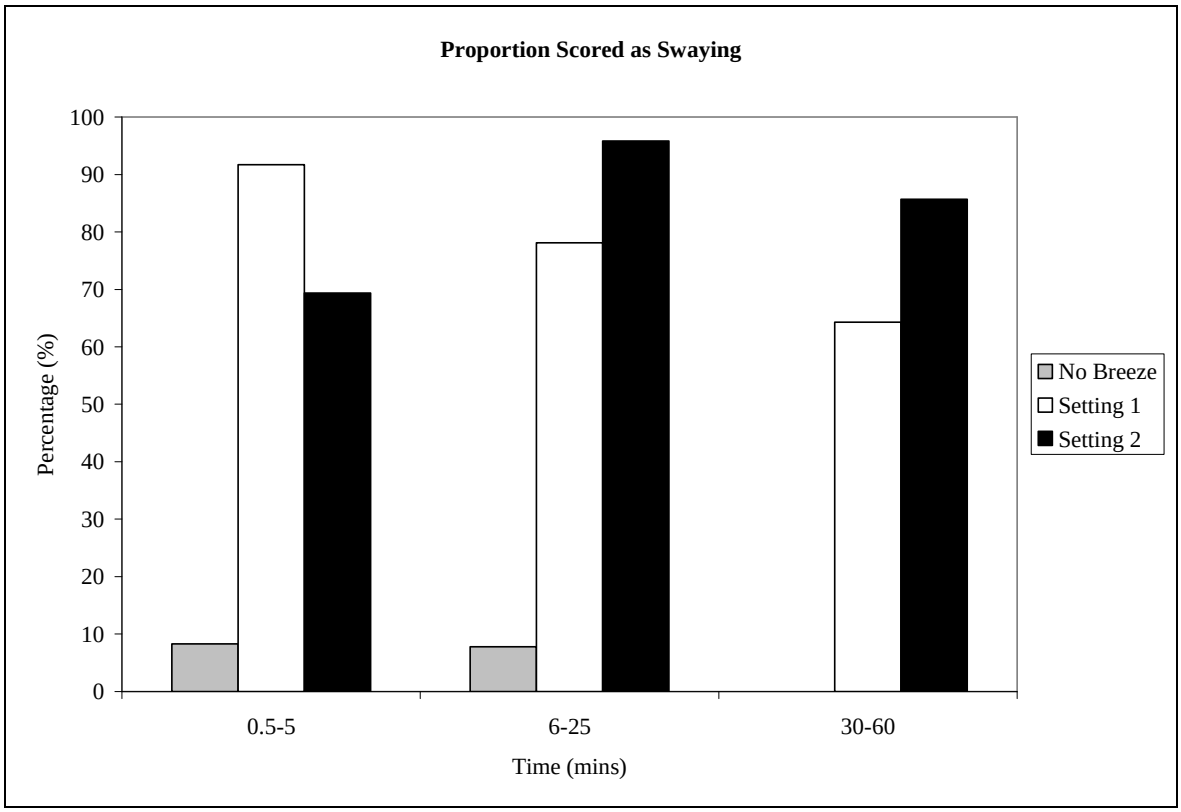


Figure 3–13. Proportion of larvae were scored as 'swaying'.

The larvae were scored as swaying or not and travelling or not at every census time point, 'Swaying' described as a side to side movement of the body and 'travelling' when the larvae was moving along the twig/dowelling or changing direction. We plot the percentage of caterpillar-timepoints at which swaying was observed, segregated into three time intervals (0.5-5, 6-25 and 30-60 minutes) and three wind speeds. We see from Figure 1-13 that it appears that the proportion scored as swaying decreased over time, but increased with wind speed but did not show a strong consistent trend over time.

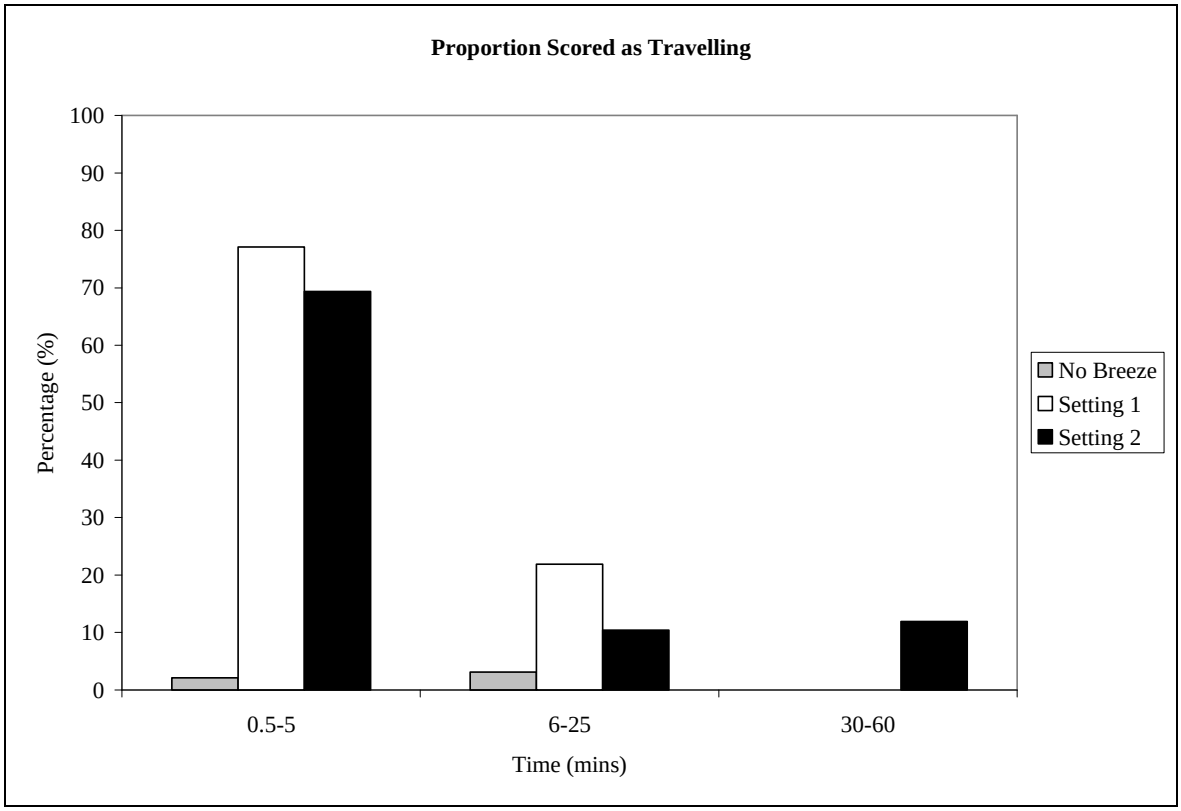


Figure 3–14. Proportion of larvae scored as 'travelling'.

Figure 1.14 suggests that travelling was more frequent at higher wind speeds, but the incidence of travelling declined over time.

3.2.2.2 Full Study - Experiment 1

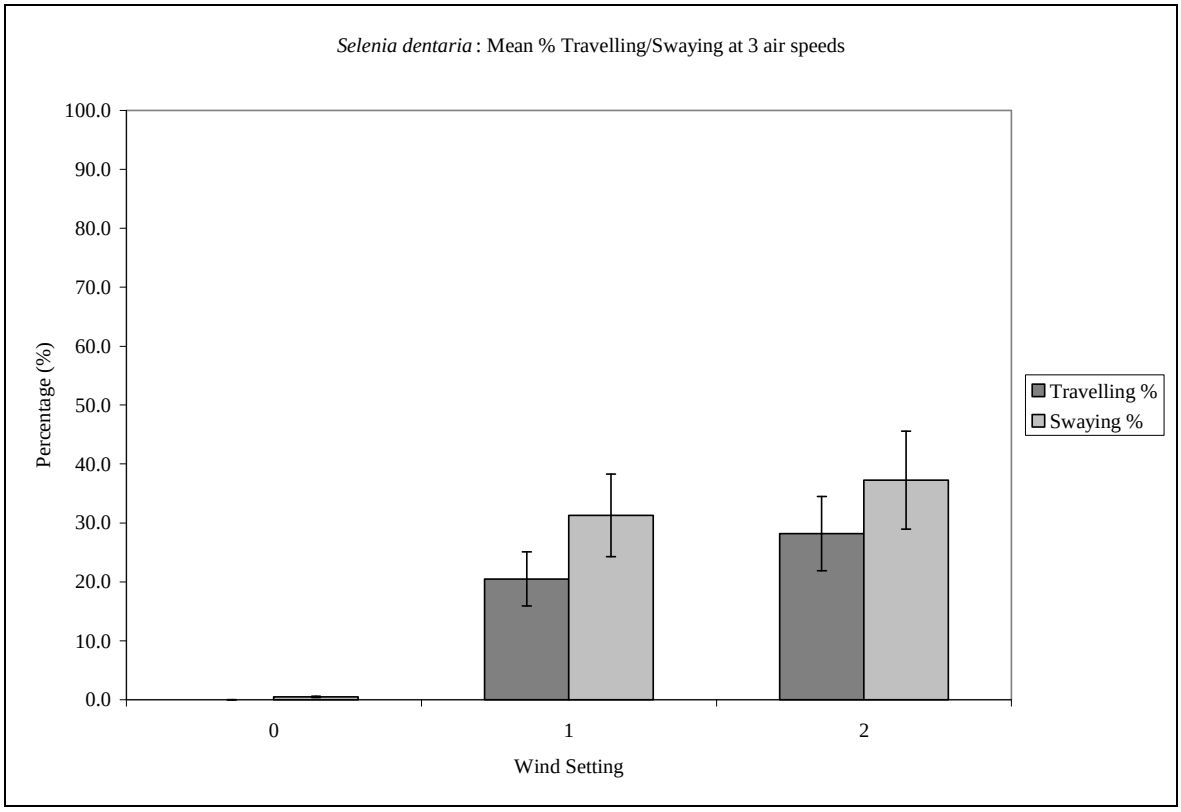


Figure 3–15. Mean percentage 'Travelling' and 'Swaying' (S. dentaria). Percentage that S. dentaria larvae were scored as 'Travelling' and 'Swaying' at 3 air speed settings.

We found a significant difference in the behaviour of larvae in an air current when compared to those in still air, with all larvae remaining stationary in still air after being placed on the dowelling rod. However, once an air current was introduced both 'travelling' and 'swaying' behaviour increased, with both showing a significant difference between air speed settings (Travelling, Kruskal-Wallis chi-squared = 38.9177, df = 2, p<0001; Swaying, Kruskal-Wallis chi-squared = 41.3214, df = 2, p<0001). The mean time to travelling and swaying commencing after application of an air current was found to be less than 5 minutes in all cases. The larvae were not continuously active after this point and had all settled by the 20 minute mark (Time 1st scored travelling, Set1 = 4.6(SE ±1), Set2 = 3(SE ±0.7); Swaying, Set1 = 2.2(SE ±0.5), Set2 = 2.5(SE ±0.5). Last scored travelling, Set1 = 12.2(SE ±2.7), Set2 = 14.4 (SE ±3.2); Swaying, Set1 = 16.8(SE ±3.8), Set2 = 19.6(SE ±4.4). It was also noted that swaying began before or concurrently with the larvae travelling along the dowelling and continued for a time after it had stopped.

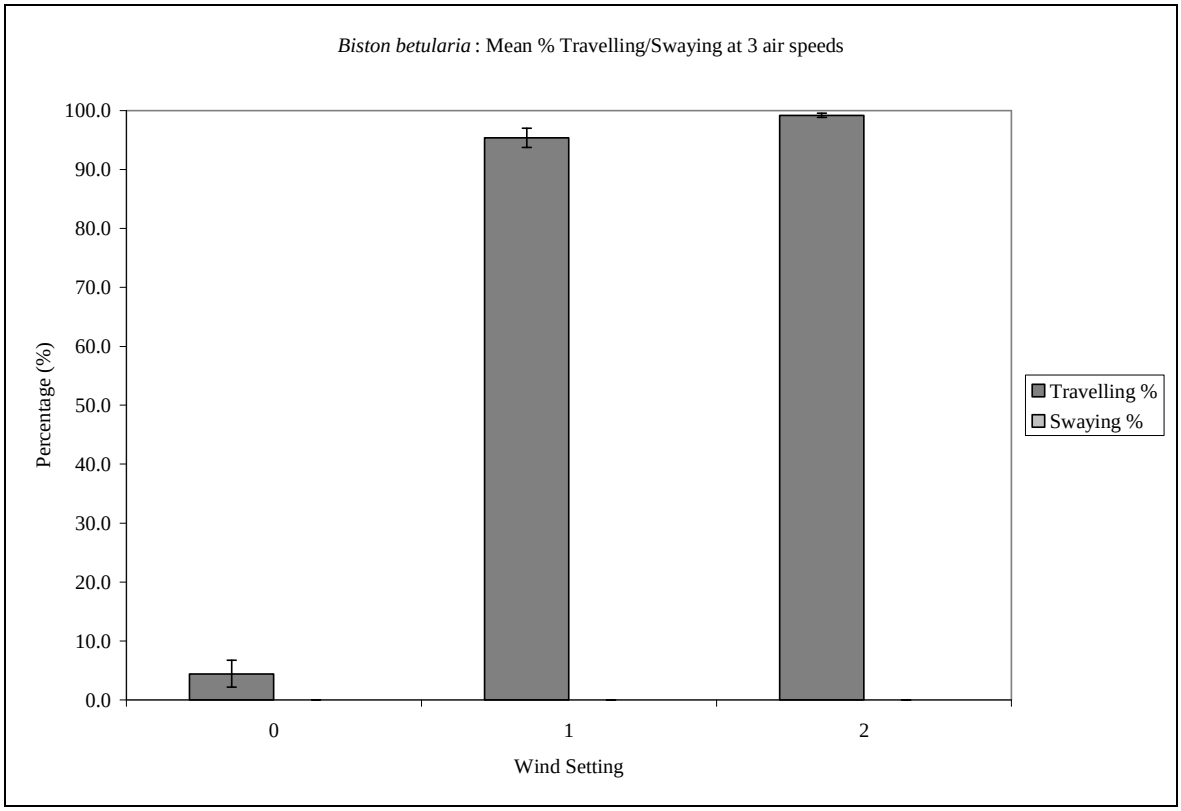


Figure 3–16. Mean percentage 'Travelling' and 'Swaying' (*B. betularia*). Percentage that *B. betularia* larvae were scored as 'Travelling' and 'Swaying' at 3 air speed settings.

The *B. betularia* larvae were found to show no 'swaying' behaviour in any of the conditions tested, but did exhibit a significant amount of 'travelling' behaviour at both setting 1 and 2 of the fan. In fact at both setting 1 and 2 the larvae spent the majority of the trial moving up and down the dowelling rod. In all cases travelling was found to commence quickly after the trial had begun (1st scored travelling, Set0 = 3.3(SE ±1.5), Set1 = 0.6(SE ±0.1), Sett2 = 0.5(SE ±0.0); Last scored travelling, Set0 = 7.6 (SE ±3), Set1 = 30(SE ±0), Set2 = 30(SE ±0)). As with the *S. dentaria*, a significant difference was found between airspeed settings settings (Kruskal-Wallis chi-squared = 35.659, df = 2, p<0001), with the *B. betularia* larvae almost continuously active for the duration of the 30 minute test period.

3.2.2.3 Full Study - Experiment 2

When isolated from air currents, but able to view the movement the air produced in leaves and twigs set up between the fan and enclosure, the *S. dentaria* larvae spent 0.3 % (SE ±0.1) of their time travelling compared to 28.2% (SE±6.3) found without the barrier. Although a small amount of 'swaying' was seen this was very much reduced from the non-isolated condition with 21.4% (SE±6.18) compared to the previous 37.3 (SE±8.3). Both swaying and travelling were found to differ significantly between the Isolated condition and the non-isolated condition (Exp1) (Travelling, Mann-Whitney $U = 1$, $p<0.001$; Swaying, $U = 21$, $p<0.001$).

3.2.2.4 Full Study - Experiment 3

When isolated from air currents and unable to see the effects of the air currents the larvae behaved as they did when no air currents were being generated with very little 'swaying' or 'travelling' behaviour (% time travelling = 0.3 SE ±0.3; swaying = 1.6 SE ±1.09).

Both travelling and swaying were found to be significantly different when compared against the original non-isolated and blinded test (Exp1) (Travelling, $U = 1$, $p<0.001$; Swaying, $U = 5$, $p<0.001$).Travelling was not found to be significantly different between the isolated (Exp2) and the isolated and blinded larvae (Exp3) (Travelling, $U = 72$, $p = 1$). Swaying behaviour was found to be significantly different (Swaying, $U = 37$, $p<0.001$) between the isolated and isolated and blinded conditions.

3.2.3 Discussion

The results of our first experiment show that, as expected for a species that relies upon masquerade to reduce predation, under still air conditions *S. dentaria* larvae remain very still. However, in conditions that mimic a natural breeze, the larvae became active moving around the artificial twig before settling in to the typical resting 'twig' like pose before the end of the test period. Also of note is that the *S. dentaria* larvae displayed a characteristic side to side swaying movement which began either concurrently with or just before the larvae began to move along the artificial twig. We do however have to

consider the possibility that the swaying is not generated by the larvae themselves. As described in the methods section we took a number of precautions to ensure that the twigs in the pilot study and the dowelling rod in the main study remained still throughout the experiment and so we feel confident that movement is not being transferred in this way. Further, the larvae stopped swaying when they eventually settled on a fixed position on the substrate. The air currents the larvae were tested in are the equivalent of 1 on the Beaufort Scale (light air) and considerable lower than what might be expected on an average British summer's day, making it unlikely that the larvae were forced to move due to discomfort caused by the strength of the air current. This suggests some sort of adaptive behaviour particularly as early thorn larvae are twig mimics for which movement against a still background potentially introduces a greater risk of discovery (Ioannou and Krause 2009).

The behaviour we have described may be evidence that *S. dentaria* uses the visually confusing movement of leaves and branches moving around in windy conditions to camouflage their movement. Seen in other cryptic and mimetic species such as leaf mimics and stick insects (Edmunds and Brunner, 1999; Robinson, 1966) the swaying movement is thought to be a form of cryptic movement designed to blend in with background movement of leaves and branches. What we propose is that by moving in windy conditions and using a swaying-like movement to mimic the movement of background vegetation larvae are able to move between foraging locations without incurring the costs we would normally associate with a cryptic or masquerading species being active during daylight hours. The fact that the caterpillars eventually stopped swaying when they stopped moving suggests that this behaviour acts to reduced dangers associated with movement on their host-plant, rather than being a more generalised mimicking of visual movement in the background.

Of course if an increase in background noise can increase crypsis it also has implications for species that use visual signals for intra-species communication and some recent studies have suggested this. For example some lizard species that use body movements for territorial displays and signalling, have been found to modify their signalling movements in response to increased vegetation movement in windy conditions (Ord et al., 2007; Peters et al., 2007). Similarly, insects that use vibrations to signal potential mates have been found to

predominately signal in still wind conditions and are less successful in the presence of wind induced vibrations (McNett et al., 2010). These results strongly suggest that wind can adversely affect detection not only by unintended recipients (such as predators) but by intended targets.

When we then tested *B. betularia* under the same conditions it was found that while both *S. dentaria* and *B. betularia* remained still in the still air conditions, *B. betula* was considerably more active than *S. dentaria* in the 'windy' conditions and did not show any 'swaying' behaviour. In fact the *B.* betula larvae we tested spent almost the entirety of the trial periods walking up and down the artificial twig. It is difficult to say why this difference should exist as both species are typical twig mimics, with both strongly resembling the inedible twigs of their host plant species. It may be possible that differences in host tree species flexibility, leaf shape and branch distribution may produce considerable differences in their movement in windy conditions. Theses differences are likely to change the effectiveness of cryptic movement and other anti-predator strategies. It may also be possible that the slightly larger body size of *B. betularia* in comparison to *S. dentaria* makes holding the typical 'twig' pose more difficult in more open and wind blown positions, but this seems unlikely when the air currents we used in this test were well below the average wind strength you might expect over a British summer. Finally, it may be that *B. betularia* is pickier in their selection of resting site and while the air currents were available to camouflage their movement they are unwilling to settle until a more suitable site than the artificial twig we provided.

In the second and third experiments we conducted we wanted to investigate whether the larvae were using physical or visual cues to determine when to use the cryptic movement strategy. The travelling and swaying scores from the experiments in which the larvae were either isolated from the air movement by a perspex sheet (Exp2) or isolated and blinded to the visual effects of the sir currents, differed significantly from the equivalent wind speed setting for the non-isolated or blinded first experiments (Exp1). When compared against each other the isolated and isolated and blinded results, not significantly different from each other for travelling, did differ for the swaying results, with the isolated individuals only showing greater swaying than the isolated and blinded tests.

Our results suggest that the larvae are most likely using mechanical stimulation from air currents passing over their body as a physical cue to conditions, rather than visual or other cues to determine air conditions. The cues are therefore most likely from air currents disturbing hairs and bristles on the larvae's body surface. However, the percentage swaying scores for Experiment 2 where the larvae were isolated from air currents but able to see their effects on leaves and twigs outside the enclosure, were considerably greater than expected. This may indicate that perhaps the swaying behaviour has some visual component to it. However, when examined further it was noted that the majority of the unexpected result came from 1 outlier (larva 8) and if this one data point is removed the results become considerably closer to what we saw in the isolated and blinded experiment.

It is also possible that the slight increase in travelling and swaying seen in the isolated conditions when compared to the still air conditions may be due to our experimental set-up not perfectly sheltering the larvae from all the air movement generated. In both experiments it was noted that small air movements were still evident near the top opening of the enclosure and from some corner joints. While very small it is possible that this was detected by the larvae and may in fact explain a problem we had with larvae repeatedly dropping off the twig. This behaviour is consistent with dropping as an anti-predator behaviour noted in other lepidopteran species. In this instance small air vibrations from the wings of predatory insects such as bees or wasps can cause larvae to freeze and drop off their host plant (Tautz, 1978; Tautz and Markl, 1978; Tautz and Rostás, 2008).

A useful development from our study might be to examine the effect on predation rate of being cryptic and remaining stationary against a moving background. In Ioannou and Krause's 2009 paper they found that remaining motionless was an important component in preventing detection in cryptic organism, but their study was carried out against a static background. It would be interesting to see if there was a corresponding cost to remaining still when against a moving background. For instance if you are a twig or leaf mimic in windy conditions, but do not mimic the movement are you more easily spotted? We suggest that this could be tested with the use of video or computer graphics which either have a static or swaying stimuli in front of a moving background of

vegetation. To test for a difference in survival human volunteers (or birds trained to peck a screen, (Dittrich et al., 2010; Taylor et al., 2002) could be timed to see how quickly and accurately they can pick out the different stimuli.

In conclusion our results suggest that twig-mimicking lepidopteran species may be using wind generated movement of the leaves and twigs of their host plants to camouflage their own movements. There is also evidence that in some cases larvae may utilise specialised ‘swaying’ movements to further camouflage their movements. As cryptic species are generally limited in their opportunities to move between sites due to the increased risk of detection by predators, any adaptation that reduces predation while allowing movement between foraging sites could be extremely advantageous.

Chapter 4. Effects of grouping and group composition on crypsis.

4.1 Does group size or density have an effect on predation rate?

There are many examples throughout the natural world of organisms aggregating and living together in groups. There are many advantages and disadvantages to living in a group with the comparative weights of each likely to change depending on availability of food, concentration of predators and environmental factors. The adage ‘safety in numbers’ is particularly true for prey species, with group living commonly cited as achieving a reduction in individual predation risk. A number of mechanisms can contribute to this affect, from the simple dilution of risk with increased numbers, to sensory confusion of predators and collective vigilance making it difficult for predators to either pick out an individual from the group or approach unseen (Krause and Ruxton, 2002).

However, group living does not come without its potential disadvantages as there is evidence to suggests that as group size increases detection rate by predators also increases, leading to a higher rate of predatory attacks. The increase in detection rate leads to the trade-off between detection and survival of attacks. Evidence for this trade-off was found in a recent study investigating the effect of grouping on risk of parasitism by parasitoid wasps. Here it was found that despite large groups of leaf mines attracting a greater number of parasitoids, an individual’s risk of parasitism declined with increasing group size(Low, 2008).

What may prove to be particularly informative is the relationship between group size and predation rate for species which rely on crypsis as their main defence, as here increased conspicuousness due to larger group size may have a particularly detrimental effect. Using human ‘predators’ searching for prey groupings on computer screens (Jackson et al., 2005) found evidence of increased ease of detection with group size, but with the effect quickly

saturating, a result which is in agreement with previous bird trials (Riipi et al., 2001) and subsequent trials using *Daphnia* (Ioannou and Krause, 2008).

Another possible influencing factor in predator detection rates is prey density (i.e. inter-individual distance) within a group, a factor which until recently very little was known about. A recent paper used *Daphnia magna* to examine the effect of density (Ioannou et al., 2009) and found that the denser groups, as well as denser areas within groups were more conspicuous to predators and therefore were the target of a greater number of attacks.

Since the previous studies generally used simplified laboratory environments (with the exception of (Low, 2008), we wanted to explore the effects of group size and density on predation risk felt by relatively cryptic prey in a more natural setting. Using wild birds predating on artificial prey we hoped to investigate how these two factors might interact to influence detectibility. Are there particular combinations of these factors that might either maximise or minimise individual predation risk?

4.1.1 Materials & Methods

This experiment was conducted at Festival Park in the centre of Glasgow (Latitude 55°51'14.57"N & Longitude 4°17'21.67"W) between March 17th and April 14th 2009 (Figure 4–1).

Figure 4–1. Experiment site (White and Red line = 10m)

Each trial took place over 1 hour with 18 different treatments per trial and a total of 45 trials over the experimental period. The treatments consisted of either Black or Striped sunflower seeds in group sizes of 5, 15, or 30 seeds spread over an area of 4cm, 10cm or 20cm diameter (Figure 4–2) for example groups). To ensure that the seeds from each group were spread over the desired area circular cardboard 'stencils' cut to the correct diameters were used.

Figure 4–2. Example seed groups.
30 striped sunflower seed groups spread over 10cm diameter (Left) and 4cm (Right) diameter areas. (Image Author's own)

The 18 treatments were set out in a different randomly generated order each day and assayed for predation 1 hour later in the same order. The groups were set out with a minimum of 10m between them and after the experimental period had elapsed the remaining seeds from each treatment were counted and totals recorded. To ensure the remaining seeds were found quickly on return to the site, markers (golf tees) were placed 2m from each group. In order to more easily and quickly find the seed groups a stick was placed at the edge of the seed group and a golf tee placed at the other end (Figure 4–3). Once the stick is removed we believe the tee was sufficiently distant from the seed group as to prevent it being used as a cue. When retrieving the seeds the coloured golf tee is more easily found than the seeds and when placed next to the tee the stick points directly at the seed group.

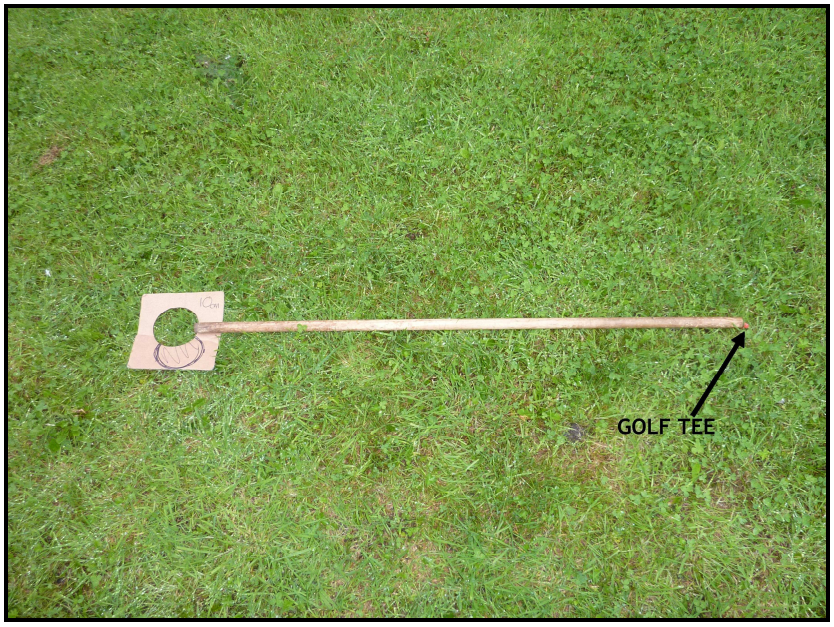


Figure 4–3. Method for locating seed groups.
(Image Author's own)

When collecting in the seeds groups it was important to use the same amount of search effort for each group. To ensure this if seeds were missing the search was continue for 5 minutes after the last seed was found.

As part of separate study we have attempted to independently assess the accuracy of our seed counting and retrieval technique. Randomly sized groupings of seeds were placed out at different sites. A second experimenter then immediately collected in the seeds using the same technique as used in the main study. In each case the collector did not know the total number of seeds.

Of the 15 trials, only one inaccurate reading was recorded. This result allows us to be confident in the analysis of the main study that missing seeds are not an artefact caused by the accuracy of the human collection method.

As the trials took place in a public park we must also be aware that each trial is likely to have been subjected to differing levels of disturbance and that there is the possibility that some groups were preyed on by something other than avian predators. While we can not rule it out entirely we know that from previous unpublished trials that consumption of experimental seeds by mammals, such as grey squirrels (S*ciurus carolinensis*), is extremely rare. It is also unlikely that predation by ants or other insects would have any effect due to the timing of the

trials in early spring and by having the test period during the day we minimised the likelihood of rodent predation.

The data recorded for each trial included the date, the time at which the treatments were placed out and collected in and the time at which the site was left. The temperature and weather conditions for Glasgow were recorded for 9am and 12pm from the MET office website (http://www.metoffice.gov.uk/education/archive/uk/).

4.1.2 Results

The results gathered for Day; Order; Seed Type; Group size; Group spread and average Temperature were analysed to determine if any had a significant effect on predation rates. 'Day' is the date on which the trials took place and the 'Order' is the order in which the 18 groups were set out. The 'Seed Type' could be either Black or Striped sunflower seeds with 'Group Size' describing the number of seeds in each group (5, 15 or 30). 'Group Spread' is the area in which the seeds were distributed with spreads of 4, 10 or 20cm in diameter. The 'Temperature' is the average temperature calculated for each day based on data obtained from the MET Office. A full set of temperature data was not always available, however, this was taken in to account in the analysis.

Using a Logistic Regression it was found that Day, Order, Seed Type and Group Spread did not significantly affect the likelihood of a group being attacked, although Group Spread came close to statistical significance ($p = 0.07$). However, it was found that an increase in Group Size ($p = 0.001$ and average Temperature $p = 0.001$)did significantly increase the chance of at least one individual in the group being predated.

TEMPERATURE ℃	5-7 ℃	7-9 ℃	9-12 ℃
Total No. Groups in temperature range	126	306	195
No. of groups predated	9	59	38
Probability of Group Predation	7.1%	19.3%	19.5%

Table 4–1. Probability of group predation over 3 temperature ranges

The lowest temperature range has a much lower group predation rate than the two higher temperature ranges (Table 4–1). Where temperature data was not available the group data was not included in the analysis and so only 35 of the full 45 trials were used.

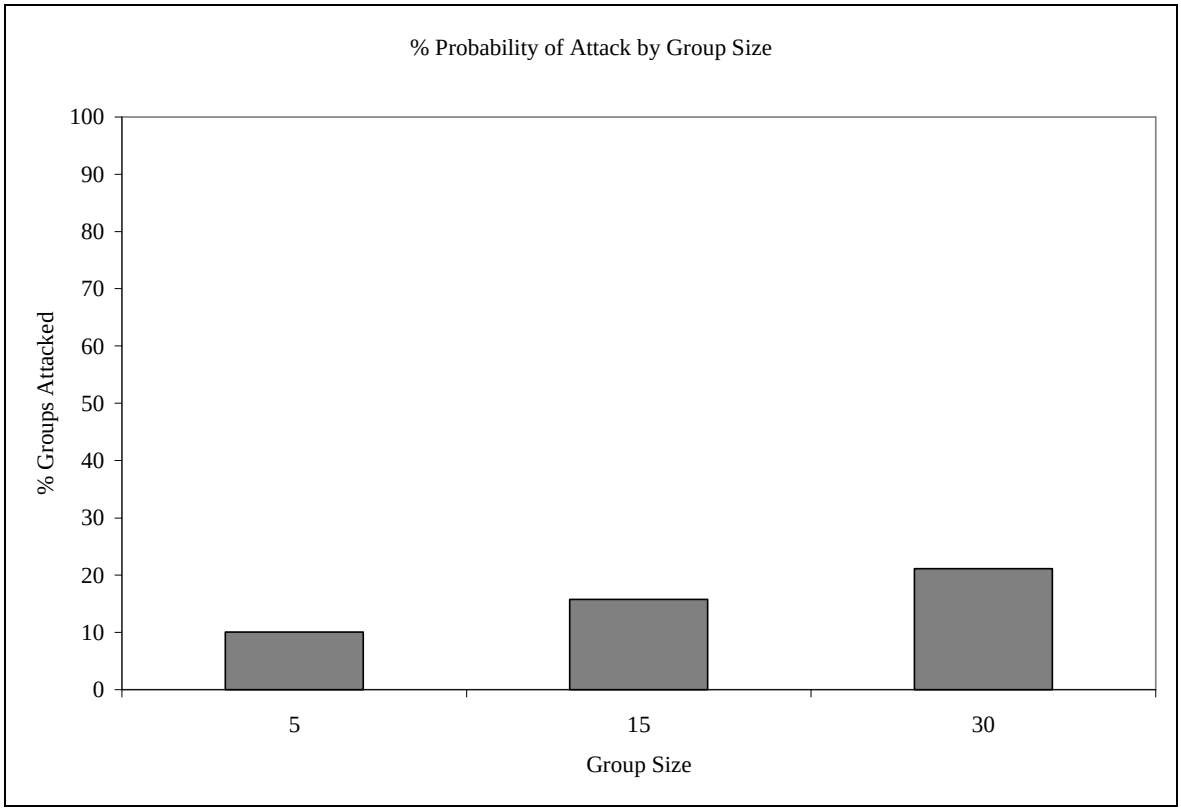


Figure 4–4. Group size - Probability of attack (Per individual; Group 5 = 0.04%, Group 15 = 0.06%, Group 30 = 0.08%)

The percentage probability of attack increases with Group Size at both the group and individual level (Figure 4–4).

Group Spread (cm)	Total No.	No. attacked	%
4	267	33	12.36
10	266	40	15.04
20	267	52	19.48

Table 4–2. Group spread - Probability of attack

The percentage probability of attack increases with Group Spread (Table 4–2).

Group Spread	4	10	20
Group Size	**Number of attacks**		
5	8%	13.2%	9%
15	13.3%	10.3%	23.3%
30	15.7%	21.6%	26.1%

Table 4–3. Number of groups attacked by group size and spread.

The percentage probability of attacks on each combination of Group Size and Spread shows that the largest group size with the largest group spread has the highest probability of being attacked (Table 4–3).

We analysed the data using a GLM to predict the arc sin transform of (Remaining/Group Size) to investigate the factors affecting an individual's risk of attack. From this we found that can see that the only factor that had a significant effect was day (Seed type, F= 2.7, d.f = 1, p = 0.1; Group Size, F= 0.41, d.f = 1, p = 0.67; Spread, F= 1.6, d.f = 1, p = 0.21; Day, F= 7, d.f = 1, p< 0.0001).

4.1.3 Discussion

A logistic regression analysis was used to investigate which factors had a significant affect on detection rates, where a group is described as being detected when at least one 'individual' has been removed. We found that larger groups are more likely to be detected; a result which is in agreement with previous studies (Ioannou and Krause, 2008; Jackson et al., 2005; Low, 2008; Riipi et al., 2001). An unexpected result was that of increased detection rate for those trials carried out on days with higher average temperatures (Table 4–1), as it is generally accepted that avian predation risks are higher when temperatures are lower (Macleod et al., 2005a; Macleod et al., 2005b; McNamara et al., 1994). However, this might be explained by ground conditions on warmer days. The drier soil conditions on these days may have made it more difficult to access invertebrate prey in the top soil, therefore making the sunflower seed 'prey' more attractive.

To examine the role of group size further we looked at the probability of any group size being attacked. We found that although the probability of any particular group being attacked does increase with group size, that increase is much slower than a linear increase (Figure 4–4). This suggests that groups would have to increase considerably in size before individual group members would be at any increased risk of attack due to increased detectibility.

To investigate the effect of density on detectibility we spread each group size over 3 different area sizes. Although group spread was not found to be statistically significant, it was approaching significance and should be considered in further studies (Table 4–2) shows that the probability of being attacked at different group spreads and although the effect is not a strong one it suggests

that the risk of attack increases with the increase in area over which a group is spread. Looking at the number of attacks on each combination of group size and group spread we find, perhaps unsurprisingly, it indicated that the effect of group spread is stronger at larger group sizes (Table 4–3).

A GLM was used to investigate an individual's risk of attack rather than that of the group. We found that none of the factors measured had any affect on individual risk, bar day (a factor of no interest here). It is reasonable to suggest here our ability to pick out individual differences in risk is low because the vast majority of individuals survived each trial. We know that this is not due to the bait being unattractive, as the sunflower seeds used are commonly used in birdfeed. However, the use of sunflower seeds may explain why predation of one individual within the group does not always lead to all group members being eaten. The sunflower seeds used require manipulation to get at the edible seed inside the seed shell and so it is likely that a predator may move to an area with more cover to carry this out. Then, if disturbed or distracted a predator may move on rather than go back to the same group.

As with any study we must be aware of the risk of experimental error when evaluating our results. During pilot trials for this study it became clear that the count of individuals predated from each group could become easily confounded without thorough searches of test areas to ensure all remaining seeds are located and counted. Without consistently thorough searches we could not be confident that we were not measuring search effort rather than predation. However, due to the results of our small scale counting and retrieval test and that temperature was found to have a significant effect, (a factor unlikely to influence searching ability) we can be reasonably certain that this is not the case here.

As the trials took place in a public park we must also be aware that each trial is likely to have been subjected to differing levels of disturbance and that there is the possibility that some groups were preyed on by something other than avian predators, this is particularly likely as grey squirrels were seen in the area. However, it is unlikely that predation by ants or other insects would have any effect due to the timing of the trials in early spring and by having the test period during the day we minimised the likelihood of rodent predation.

Future studies of group size and density would greatly benefit from a much larger sample size, particularly if we want to be able to see the effects at an individual level. Another possible avenue of investigation is suggested by the fact that there appeared to be no predator preference shown to either the striped or black sunflower seeds used. We have taken this idea further by using them in mixed groups and manipulating the proportion of each seed type and using them to investigate whether there is any effect of prey ‘oddity’ on predator preference (See Chapter 4).

4.2 Field test of the effect of ‘oddity’ within a group on predation risk.

It is well known that prey species form groups to defend themselves from predator attack, with group members benefiting from the accumulation of many different anti-predator mechanisms(Krause and Ruxton, 2002). The ‘confusion effect’ is a well-documented phenomenon found across a wide range of predatory taxa (Jeschke and Tollrian, 2007), which effectively reduces a predator’s attack to kill ratio. It does this by limiting their ability to single out and successfully attack one individual within a group, with the effect further enhanced with visually similar prey (Krakauer, 1995; Tosh et al., 2006).

The effectiveness of grouping at reducing predation has led to speculation as to how predators might overcome this problem. A well documented candidate is the ‘oddity effect’. This hypothesises that when confronted by grouped prey predators can increase their kill rate by concentrating their efforts on capturing unusual or ‘odd’ prey. These ‘odd’ prey types stand out of the crowd with the effect of reducing or potentially overcoming the ‘confusion effect’ entirely (Landeau and Terborgh, 1986; Ohguchi, 1978). While this is an attractive idea, and one which has historically been used to explain cases where rare or ‘odd’ individuals appear to have been preferentially targeted, there have been comparatively few studies which have been able to conclusively demonstrate this effect. There are also possible confounding issues to be looked at such as whether ‘odd’ individuals are in fact just more highly sought after prey items, or whether they are more conspicuous within the environment regardless of other group members. There may also be other complicating factors such as differences in defence capabilities (Mathis and Chivers, 2003) and prey density (Allen et al., 1998) both of which have been shown to modify prey selection.

An often-cited study of the oddity effect is that of Landeau & Terborgh (1986) where the interactions of largemouth bass (*Micropterus salmoides*) and groups of silvery minnows (*Hybognathus nuchalis*) were used. They were able to show that by including one or two ‘odd’ individuals in a group of 8 prey successful capture of both the odd and normal prey was greatly increased, and that this effect disappeared as the number of ‘odd’ individuals was increased to 50%. There was also the suggestion that the oddity effect may be confined to small group sizes,

but more recent studies have not been able to find any evidence to support this (Krakauer, 1995; Ruxton et al., 2007). However, they were able to confirm that the oddity effect disappeared as the 'odd' phenotype increased to 50%(Ruxton et al., 2007).

Another well known and related concept is that of apostatic selection where predators show a preference for the more common prey type (Endler, 1991) with anti-apostatic selection describing a preference for rare morphs. From this we can see that the description given for anti-apostatic selection is very similar to that of the oddity effect. The main differences are those of scale: with apostatic selection generally considered at population level and the oddity effect within smaller groupings where prey are viewed simultaneously by predators. Further reasoning for this separation is that the oddity effect is generally invoked as a method of mediating the effects of the confusion effect, an effect generally assumed to require aggregations of moving prey. However, Krakauer's (1995) paper which is often cited as theoretical evidence for the confusion effect, found evidence of both the confusion and oddity effects within a static prey system. We would therefore like to expand upon this and investigate whether predators of slow moving or sedentary prey aggregations might be benefiting from the oddity effect.

Recent studies investigating the diversity of fish assemblages inhabiting coral reefs have provided support for the idea that predators may reduce species diversity within a system by targeting rare species (Almany et al., 2006; Almany and Webster, 2004). In these cases it was found that although not more vulnerable to predators due to appearance or colouration, rare prey species suffered consistently greater predation. Work investigating prey choices in wild bird populations has found that they switch prey types in response to changes in morph frequency to maintain anti-apostatic selection (Allen and Weale, 2005). These studies suggest that anti-apostatic selection and the oddity effect are mechanisms by which predators can be strong influences on diversity at both the species and community level and provide credence to those proposing that frequency-dependent selection such as apostatic selection or the oddity effect may be a major force driving evolution with the power to cause the divergence or convergence of phenotypic traits(Greenwood, 1985). There are also potentially important implications for rare prey species conservation as there

may be a tipping point at which predators will switch prey preferences to already dwindling populations.

It is for these reasons we feel that it is important to investigate and understand the circumstances under which anti-apostatic selection and/or oddity effects occur. We propose to use wild birds as our predator assemblage, as to our knowledge no previous study exploring the effect of oddity has used wild-living predators. We have elected to use groups of inert prey in the form of either black or striped sunflowers seeds to avoid any confounding factors generated by within-group interactions between mobile prey. Previous studies within the experimental area have found that the resident wild bird population does not show a preference when presented with same-sized groups of each morph. This provides us with the opportunity to use mixed groups to look for any effect of oddity. We hope to answer 3 questions:

1) Do ‘odd’ individuals within a group suffer greater predation risk than their ‘normal’ group mates?

2) Does the presence of ‘odd’ individuals increase the predation risk of other individuals within the group?

3) Is there any evidence for the oddity effect in groups of stationary prey?

4.2.1 Materials & Methods

This experiment was conducted at Festival Park in the centre of Glasgow (Latitude 55°51'14.57"N & Longitude 4°17'21.67"W), from the 4th-9th June 2009.

Figure 4–5. Study site
(White and Red line = 10m, © Google Earth)

Each trial took place over 1 hour with a total of 20 (5 group sets) treatments per trial. The treatments consisted of groups of 30 sunflower seeds spread over an area of 10cm in diameter with differing proportions of black seeds mixed with striped seeds. There were 3 test groups; 29 striped plus 1 black; 25 striped plus 5 black; 20 striped plus 10 black plus a control with all striped seeds (Table 4–4 and Figure 4–6). To ensure that the seeds from each group were spread over the desired area a circular cardboard 'stencil' cut to the correct diameters was used and seeds spread from a height of at least 10cm to ensure an even spread.

Group	No. Striped Seeds	No. Black Seeds	Total
Control	30	0	30
1	29	1	30
2	25	5	30
3	20	10	30

Table 4–4. Treatment groups

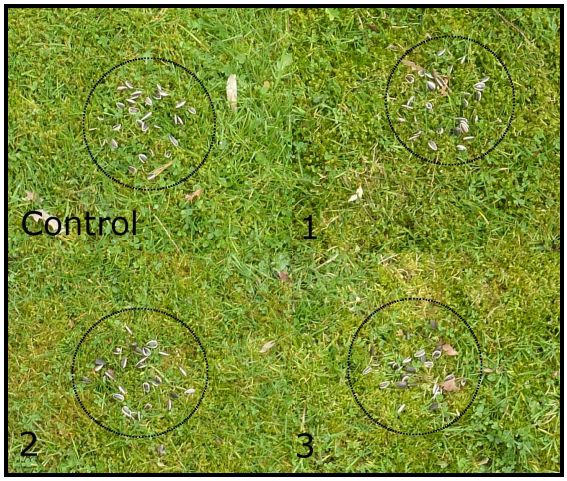


Figure 4–6. Treatments L-R: Control, Group 1, Group 2, Group 3. (Image Author's own)

The 20 replicates were set out in a random order, with a minimum of 10m between them. They were left for 1hr after which time the remaining seeds from each treatment were counted and totals recorded. To ensure the remaining seeds were found quickly on return to the site, markers (golf tees) were to be placed 2m from each group.

In order to more easily and quickly find the seed groups a stick was placed at the edge of the seed group and a golf tee placed at the other end (Figure 4–7). Once the stick was removed we believe the tee was sufficiently distant from the seed group as to prevent it being used as a cue. When retrieving the seeds the coloured golf tee was more easily found than the seeds and when placed next to the tee the stick pointed directly at the seed group.

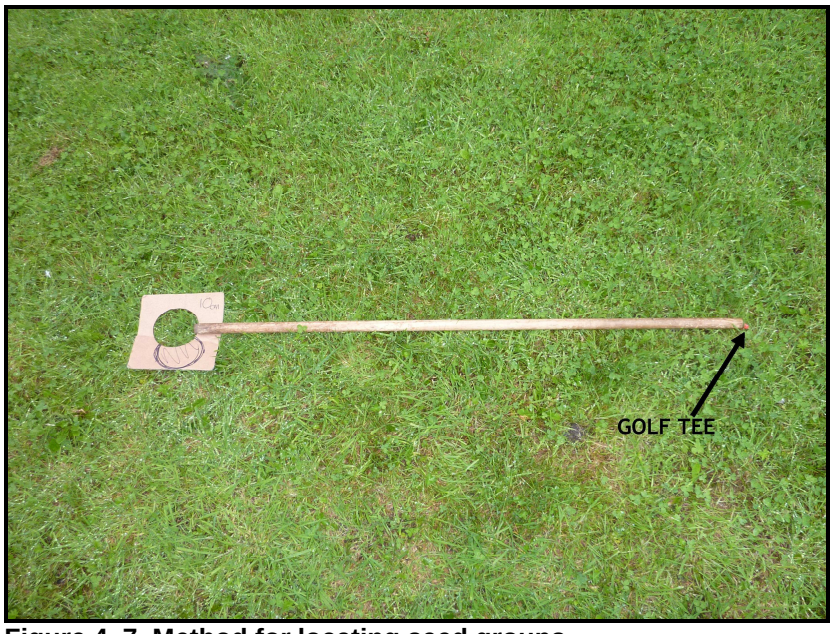


Figure 4–7. Method for locating seed groups.

When collecting in the seeds groups it was important to use the same amount of search effort for each group. To ensure this if seeds were missing the search was continued for 5 minutes after the last seed was found.

The data recorded for each trial included the date, time at which the treatments were placed out and collected in and the time at which the site was left. The temperature and weather conditions for Glasgow were recorded for 9am and 12pm from the MET office website (http://www.metoffice.gov.uk/education/archive/uk/).

4.2.1.1 Pilot study:

To ensure that the two seed types used were equally visible and of a similar level of difficulty to 'collect' we conducted a small pilot study. 600 seeds were put out in 20 groups varying in total number between 25 & 35, and proportion of black and striped seeds from around a third to two thirds black. Groups were made up in advance and on the day of testing one experimenter selected a bag at random and spread the seeds across a 10cm diameter area as described for the main study. A second experimenter (the same responsible for collection in the main study) would then immediately collect as many seeds as they could find using the same techniques used in the main study.

Of the 600 seeds put out only 3 black and 3 striped were not found. This allows us to say that any differences found in the main study are due to difference in predation and not due to bias introduced by the collection method.

4.2.2 Results

Analysis of the effect of group on the percentage of all seeds in each group (black & striped) surviving to the end of the test period found that group had a significant effect on survival (Univariate Analysis of Variance, p = 0.018).

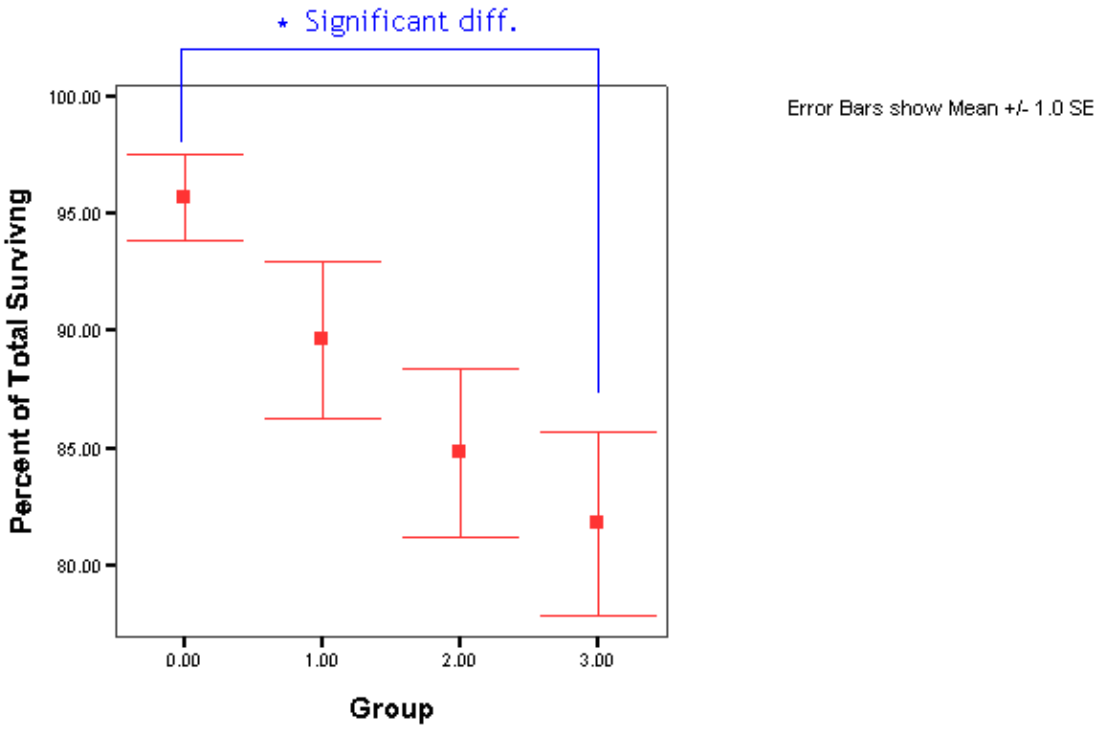


Figure 4–8. Percentage of individuals surviving per group.
(Groups: 0.00 = Control, 1.00 = 1 Black seed, 2.00 = 5 Black seeds, 3.00 = 10 Black seeds)

The only groups which show a significant difference in survival when compared were the Control (all striped) and Group 3 (10 black seeds) (Bonferroni test p = 0.18). The above graph shows us the relationship between the Control group and the 3rd treatment group (Figure 4-8). No significant difference was found between the percentages of black (odd) seeds to survive in each treatment group. (As there were no black seeds included in the control group this was not included in that analysis.)

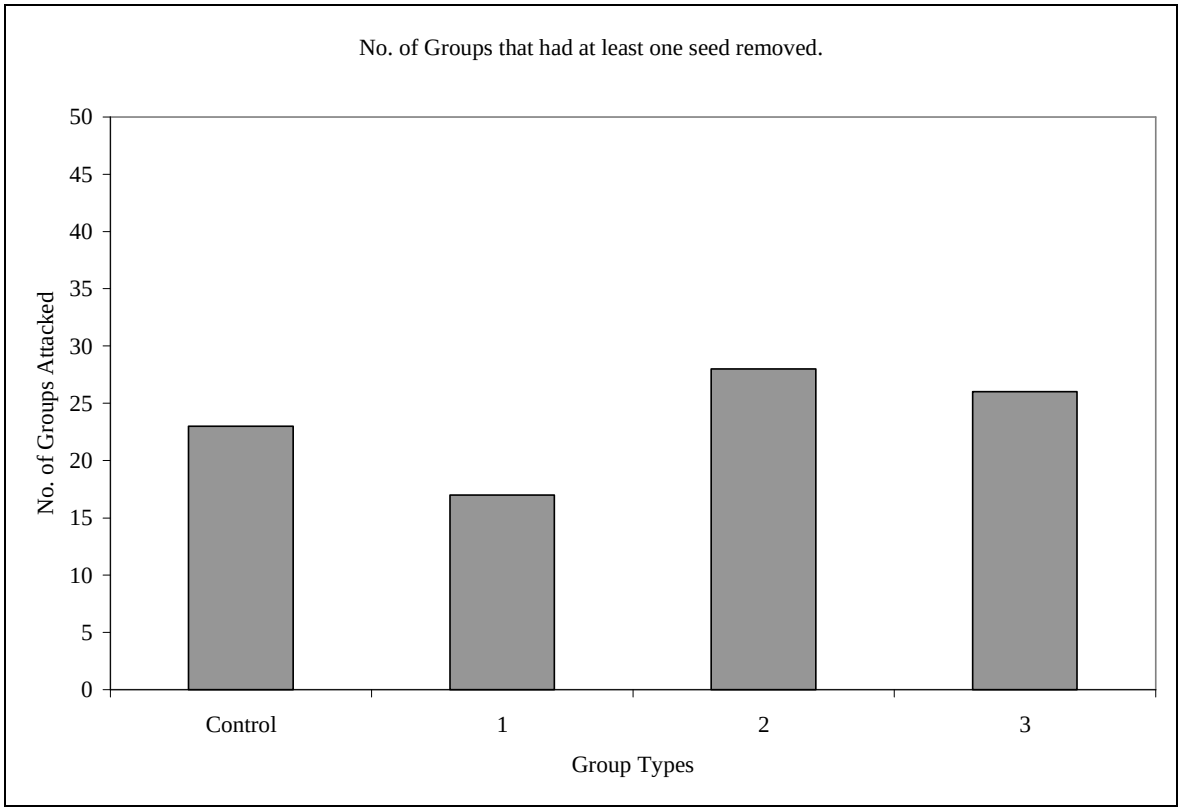


Figure 4–9. Number of Groups that have had at least 1 seed removed. 50 groups of each type were used. Chi^2 contingency table Group1 p>0.1, Group2 p>0.1, Group3 p>0.5. (Groups: Control = 30 Striped, 1 = 1 Black seed, 2 = 5 Black seeds, 3 = 10 Black seeds)

The above table (Figure 4–9) provides the number of groups from each group type that suffered predation of at least 1 seed. When we compared the number of predated vs. non-predated replicates of each group we found no significant difference between the treatment groups and the control in the number of groups predated. When we compared the number of striped and black seeds predated from each group we found that the number of black seeds eaten from each group is significantly greater than we would expect if seeds were being selected at random (Chi^2 values: Group 1 (1 Blk seed) $p<0.01$; Group 2 (5 blk seeds) $p<0.001$; Group 3 (10 Blk seeds) $p<0.001$)

4.2.3 Discussion

General Linear Models were used to analyse the data collected and to measure the effect of group on seed survival. Analysis of group effect on seed survival shows that group does have a significant effect on the total number of seeds to survive.

We found that only the 3rd treatment (33.3% 'odd') differs significantly from the Control (0% 'odd') (Bonferroni, $p = 0.18$), with the intermediate groups (3.3% &

16.6% ‘odd’) having no significant effect seed survival to the end of the trial. This result tells us that seeds in the 3rd treatment group had a significantly greater chance of being predated.

A previous investigation carried out by Landeau & Terborgh (1986) has found that groups with 1 or 2 ‘odd’ (12.5-25%) individuals in a group of 8 suffered greater risk, but when this was increased to 3 or 4 (37.5-50%) the effect disappeared. A subsequent study by Ruxton et al (2007) has confirmed this upper limit of 50%. This combined with our results may suggest that there may be a range where ‘odd’ individuals will increase the predation risk of individuals with a group.

We examined how group affected the predation of ‘odd’ (black) seeds and found that there was no significant difference between groups. This suggests that although groups with more ‘odd’ individuals may suffer greater predation the risk, the predation rate felt by ‘odd’ individuals is not influenced by the number of other ‘odd’ individuals in their group.

From our analysis we can be reasonably sure that treatment did not have any effect on the number of groups that suffered predation (Figure 4–9). This suggests that the presence of odd individuals does not have any effect on a group’s overall risk of predation, but does influence the way that risk is distributed between group members.

We then wanted to investigate whether predators showed any preference for ‘odd’ or ‘normal’ seeds. After analysis we were able to show that ‘odd’ seeds were removed at a rate greater than would be expected for random prey selection. This suggests that they are either more conspicuous against the substrate or in some way preferable and being actively selected. However, a previous study (unpublished Group experiment) using groups of either all black (‘odd’) or all striped (‘normal’) seeds found no difference in predation rates between the groups, suggesting that it is the group composition and their position within it that is driving the increase in predation risk.

Great care was taken to ensure that all seeds remaining after the trial period were located and counted. Despite this it is not possible to say that no seeds

were missed. This however, would only be problematic if we believed that one seed type was more likely to be missed than the other and from the results of the pilot study carried out we can be reasonably sure that there was no bias in collection towards either black or striped seeds.

In summary, although the presence of 'odd' individuals does not change the group predation risk (how likely the group is to be attacked) as a whole (1) 'odd' prey suffer a greater rate of predation than 'normal' conspecifics within the same group. This effect is not altered by the number of other 'odd' prey within that group. However, (2) the greater the number of 'odd' prey within a group the greater the predation risk felt by all individuals within the group (increased number removed from the group). Our results also suggest that (3) there is evidence of the oddity effect within this system despite using static prey, an observation which concurs with the previous theoretical work carried out by Krakauer (1995).

What we now need to consider is the reason for birds using this strategy with stationary prey. When confronted with a group of moving prey it makes sense that predators need to be able to quickly single out one individual for a successful attack, but with stationary prey it would seem that there should not be the same problems. The experimental area used in this study was within a public park and therefore subject to pedestrian traffic. It is likely that this limited the length of feeding bouts. Therefore, what we might be seeing here is the predator using the oddity effect to speed up prey selection which will allow them to minimise the time spent in the open and/or devote greater attention to scanning for predators. A recent paper by T. Waite (2008) has suggested that predators may use a 'unique choice heuristic'. He proposed that by using a general rule of thumb where odd prey items are given automatic preference, predators can skip the deliberating phase of the choice process thereby leaving more time to scan for danger.

What we need to examine next is the effect of time/predation pressure and how this affects prey choice and the oddity effect. Further to this it would be interesting to examine factors other than visual oddity or other aspects of visual oddity such as prey movement. There has for instance been some indication that having a different pace or gait may generate the oddity effect (Hatle et al.,

2002) and it may be interesting to look at whether non-visual predators take use the oddity effect with sound or smell. Examples might be found where there are groups which share a common scent. Overall there is still much that we need to look at with regards to the oddity effect, its ecological consequences and implications for the maintenance of multiple colour morphs within a population.

4.3 Computer based test of the effect of ‘oddity’ within a group on predation risk.

This experiment continues on from our previous study of the oddity effect where we looked to see if there was any evidence for the oddity effect when predators are faced with groups of stationary prey. Our results suggested that predators may be using the oddity effect to select prey. Therefore, we now need to consider why predators might use this strategy with stationary prey. When confronted with a group of moving prey it makes sense that predators need to be able to quickly single out one individual for a successful attack, but with stationary prey there would appear to be ample time to pick out and consume as many prey as wanted.

However, this view does not take in to account the risk the predator may face from its own predators. Therefore, what we may be seeing is a predator using the oddity effect to speed up prey selection, which then allows them to minimise the time spent in the open and/or to devote greater attention to scanning for predators. A recent paper by T. Waite (2008) in which he tested blue jays (*Cyanocitta cristata*) for their preference for oddity suggested that predators may use a ‘unique choice heuristic’. He proposed that by using a general rule of thumb, where odd prey items are given automatic preference, predators can skip the deliberating phase of the choice process thereby leaving more time to scan for danger. Although Waite’s experiment did not use cryptic prey we might expect to be able to see evidence of this.

For that reason what we wanted to examine in this study is the effect of time/predation pressure and how this affects prey choice and the oddity effect when searching for cryptic prey in static groups. Can preference for oddity be explained by the need to divide attention between multiple tasks?

4.3.1 Materials & Methods

A flash game was designed were human players take the part of the hunting predator. The player must attempt to gather as many of the stationary prey (seeds) as possible in the time allotted, with the number of seeds shown in the corner of the screen. Two types of seeds where used with a 3:1 ratio of 'normal' to 'odd' seeds.

In order to assess whether scanning for predators is a factor which contributes to the use of the oddity effect we have two scenarios. The first is as above where the human player is only required to collect as many prey as possible, however, the second requires that they must keep a look out for a 'predator' which will appear at the side of the screen (Figure 4–10). When the predator appears they must press the space bar to escape; if they fail to, they lose all the collected seeds.

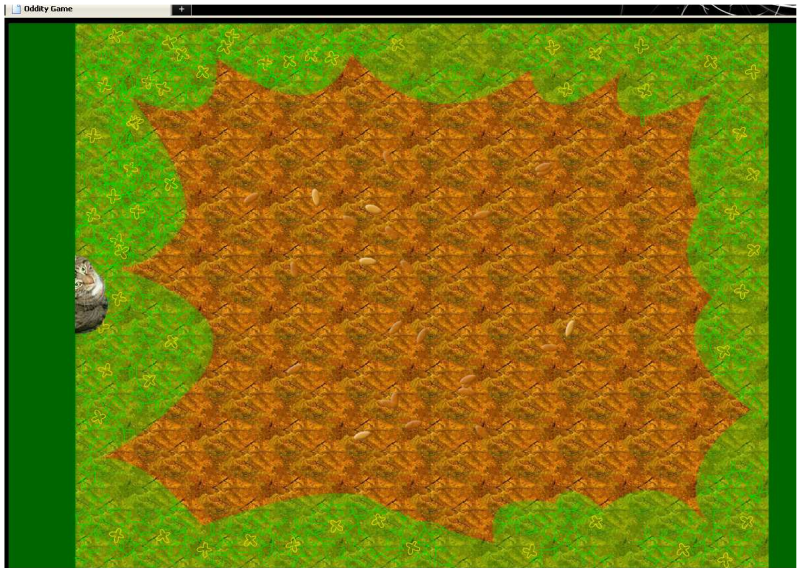


Figure 4–10. Screen print of Oddity Game

Players were recruited by email and from the /r/biology subreddit at www.reddit.com (an online social news and message board site) which asked them to take part and directed them to the game website. On the website front page was an explanation of the study and the rules of the games. Players were then asked to press a button to generate 10 games. The 10 games consisted of 5 non-predator games and 5 predator games which were generated in a random order. After each game was played the parameters and results were sent to a central database.

Parameter name	Parameter description
iDiameter	Diameter in pixels of seed spread (Area seeds are spread across)
iNormalSeed	Number of normal seeds.
iOddSeed	Number of odd seeds
strNormalSeedID	Seed id's (seed pattern 1-9)
strOddSeedID	Seed id's (seed pattern 1-9)
iPredatorTime	Time in seconds before predator appears/or before game ends if there is no predator
iSeedSize	Percentage value.100 would mean the seed would be the size it is currenlty, 50 would mean it was half the size, 200 would double the size etc.
iPredatorLurkTime	Number in seconds of the amount of time you want the predator to appear before it pounces. 3/4 of this time is spent appearing quietly around the edge, 1/4 is spent pouncing
bShowPredator	Can = either true(1) or false(0) depending on whether you want the predator to appear

Table 4–5. Game parameters (All modifiable parameters)

The above table (Table 4–5) lists all the modifiable parameters of the game and provides an explanation of each variable.

4.3.1.1 Pilot study:

To ensure that the two seed types used are equally visible and of a similar level of difficulty to 'collect' we conducted a pilot study using 12 volunteers. A number of seed designs were considered with each varying slightly in pattern and/or colouration and 3 were picked to be tested (Figure 4–11; All parameters Table 4–6. Parameters of Pilot Study) were kept to the same values we anticipated would be used in the full study. A seed diameter of 50 pixels was used as this produced 'seeds' that while of a large enough size to be easily selected when deliberately clicking on them was not so large as make random clicking across the screen a viable selection technique. The 3 to 1 ratio of normal to odd seeds within a group of 40 seeds was used as this produced a manageable group size to work with on the computer screen and ensured that the odd seeds would still be perceived as 'odd' within the group.

	Diameter	No. Seeds	Predator Time	Seed Id	SeedSize	ShowPredator
Pilot Study	450	40	10	8, 9 or 10	50	0

Table 4–6. Parameters of pilot study

The only difference between this and the full study is that there was only one seed type per game and no predator. Each volunteer played 4 games using each seed type, making a total of 12 games per person. The 12 games were presented

to each person in a random order to minimise the effect of learning on the results.

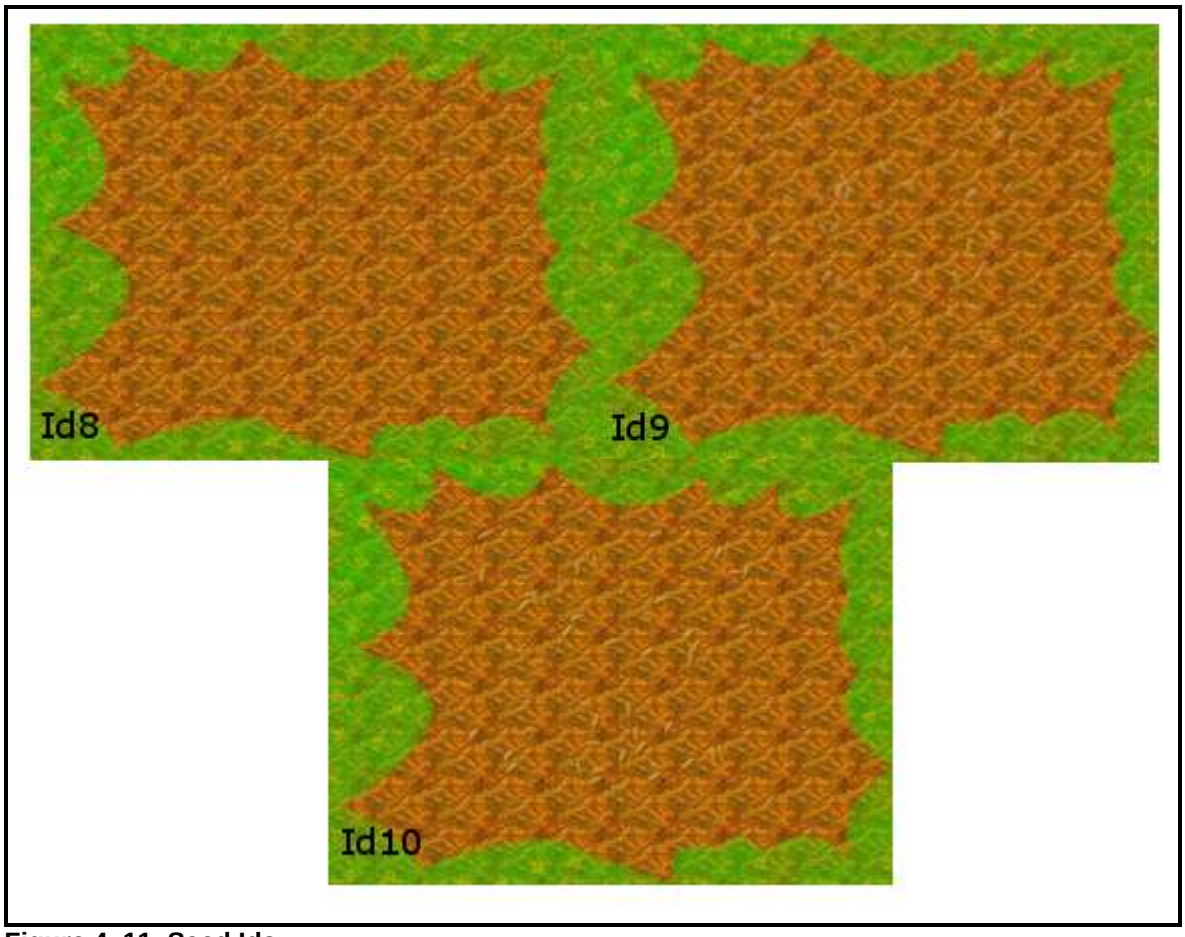


Figure 4–11. Seed Ids.

Seed Type	No. players	Total Seeds	Games Played	Mean Seeds
Seed 8	12	836	66	13±4.7
Seed 9	12	865	67	13±3.2
Seed 10	12	831	67	12±3.4

Table 4–7. Pilot results

The results of the pilot study suggested that the three seed types were comparable in visibility and difficulty to collect (Table 4–7). Using these results it was decided that seed types 8 & 9 (Figure 4–12) would be used in the full study.

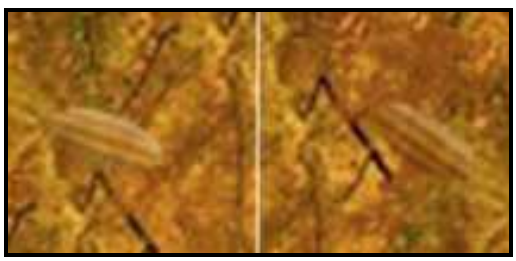


Figure 4–12. L-R: Odd Seed (9), Normal Seed (8).

4.3.1.2 Full Study Part 1

The parameters used in the full study are shown below in Table 4–8.

Full Study: Parameters							
Diameter	**No. Normal Seeds**	**No. Odd Seeds**	**Predator Time**	**Normal Id**	**Odd Id**	**Seed Size**	**Show Predator**
450	30	10	10	8	9	50	0 or 1

Table 4–8. Full study game parameters

Volunteers were recruited via email requests and internet message boards. They were given a brief outline of the game, expected duration, contact details for more information and the website address to go to if they wanted to take part. Once on the website the contact details etc were repeated and they were invited to press a button to generate ten games. The ten games consisted of five predator and five non-predator games presented in a random order. Before each game players were told which type of game they were about to play, with this message before each predator game (Supplementary Figure 1);

> "The aim of the game is to get as many seeds as you can in the time you are given, while keeping an eye out for the cat that wants to eat you. You collect the seeds by clicking on them with the mouse BUT if you see the cat you have two seconds to press the space bar..... if you don't you will be eaten and lose all your seeds. The seeds will not be easy to find so keep looking"

And this message before each non-predator game (Supplementary Figure 2);

> "The aim of the game is to get as many seeds as you can in the time you are given. You collect the seeds by clicking on them with the mouse. The seeds will not be easy to find so keep looking."

As the games were played the parameters used & data generated was stored in a central database. Table 4–9 defines and lists all the data collected from each game.

Data collected	Definition
GameTimeStamp	Time & Date game was played.
IPAddress	IP address of players computer
Total Seeds	Total no. of seeds collected
SeedOrder	The order in which seeds are collected e.g. normal, odd, odd, normal
SeedTimes	The time in seconds at which each seed was taken e.g. 1.325,4.75,5.333
CaughtByPredator	Whether the player was caught by the predator (only applies to predator games).

Table 4–9. List & definitions of data collected.

The only information kept besides game data is a time stamp for each game and the originating IP address. This information was used to identify and group games played by the same individual. Any games that could not be grouped in this way (for instance if two players at the same IP address played at the same time) were discarded. Once grouped, each set of games was given a unique 'Personal ID' e.g. ID1, ID2, ID3, etc.

4.3.2 Analysis

To look at the results gained from all games played we first looked at the ratio of odd to normal seeds taken. Using a Chi-squared Goodness of Fit table, we examined whether the ratio taken differs from the 3:1 ratio of normal to odd we would expect if there were no effect of oddity. This was then repeated to look at only the first seeds taken in each game.

Using unpaired t-tests we compared the time it takes for an odd or normal seed to be picked out from the group. This was done by looking both at the first seed taken in each game and across all seeds taken. We then compared the time taken to pick a seed at each position in the order taken (1^{st}, 2^{nd}, 3^{rd}, etc) and the average 'selection' time for the first 10 seeds from each game.

To examine if the type of seed picked first has any effect or predicative ability in regard to the rest of the seeds picked in that game, we examined the proportion of odd seeds picked when either an odd or normal seed are selected first. We then compared results between the predator and non-predator treatments. Once again we looked at the ratio of normal to odd seeds and the first seed taken from each game.

To carry out the comparison we will use the statistical package R to carry out a Generalized Linear Model. The model will compare the ratios between the two game types (predator & non-predator) with individual players taken into account as a random effect.

R Model input:
m1<lmer(cbind(Normal,ODD)~ShowPredator+(1|PersonalID),family=binomial)

4.3.2.1 Full Study Part 2: Reversing the seed patterns

Despite our pilot experiment showing that the two seed patterns we used were collected at a very similar rate was, there was always the possibility that the effect we observed in the full experiment was an artefact produced by differences in the visibility of the patterns rather than an effect of oddity.

Therefore after completing our initial 'Oddity Game' experiment we felt that in order to confirm our results we needed to carry out the experiment again but reversing the colour patterns. This would mean that the 'odd' pattern from our previous experiment would be used as the 'normal' pattern in this experiment and vice versa. If the results from this experiment agreed with our previous experiment we could be much more confident in our conclusions.

The methods used in this experiment matched exactly the methods used in the previous experiment bar the seed patterns used for the odd and normal seeds. In the initial experiment we used seed types 8 & 9 (Figure 4–13) with 8 used for the 'normal' seed pattern and 9 used as the 'odd' pattern. For this experiment we wanted to reverse the patterns so that 9 would be the 'normal' pattern and 8 the 'odd' pattern.

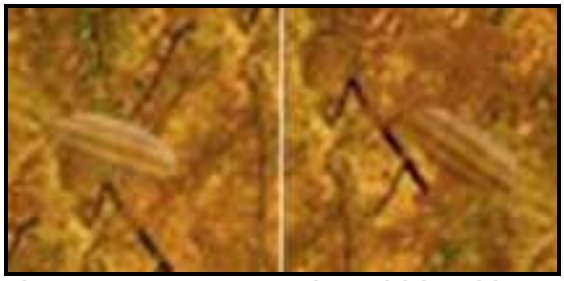


Figure 4–13. L-R: Normal Seed (9), Odd Seed (8).

4.3.2.2 Analysis

Again the analysis exactly matched that used in the previous experiment.

4.3.3 Results Part 1

4.3.3.1 Analysis of combined Predator & Non-predator treatments.

	Totals
Total no. of unique players	158
Total no. of games played	1225
Total no. of non-predator games played	607
Total no. of predator games played	618
Total no. odd seeds taken	5011
Total no. of normal seeds taken	5131
Total no. of seeds taken	10142

Table 4–10. Overview of basic figures & results

Once the raw data was received the data was cleaned up to remove any games in which no seeds were collected. This was done as these games would not add any weight to the ratio comparisons of normal and odd seeds. Table 4–10 provides the basic figures and totals produced from the final data set. We examined the ratio of normal to odd seeds for both the 1st seed taken in each game and across all seeds for each game (This includes all games played e.g. Predator and non-predator). The null hypothesis in this case is that the ratio of seeds picked would not differ from the 3:1 ratio in which the seeds were provided. We found that in both cases (all seeds & 1st seed) the seeds picked differed significantly from what we would expect had the seeds been picked randomly (Chi-squared Goodness of Fit, 1st Seeds $p = <0.001$; All seeds $p = <0.001$). In both cases odd seeds were taken approximately twice as frequently as expected.

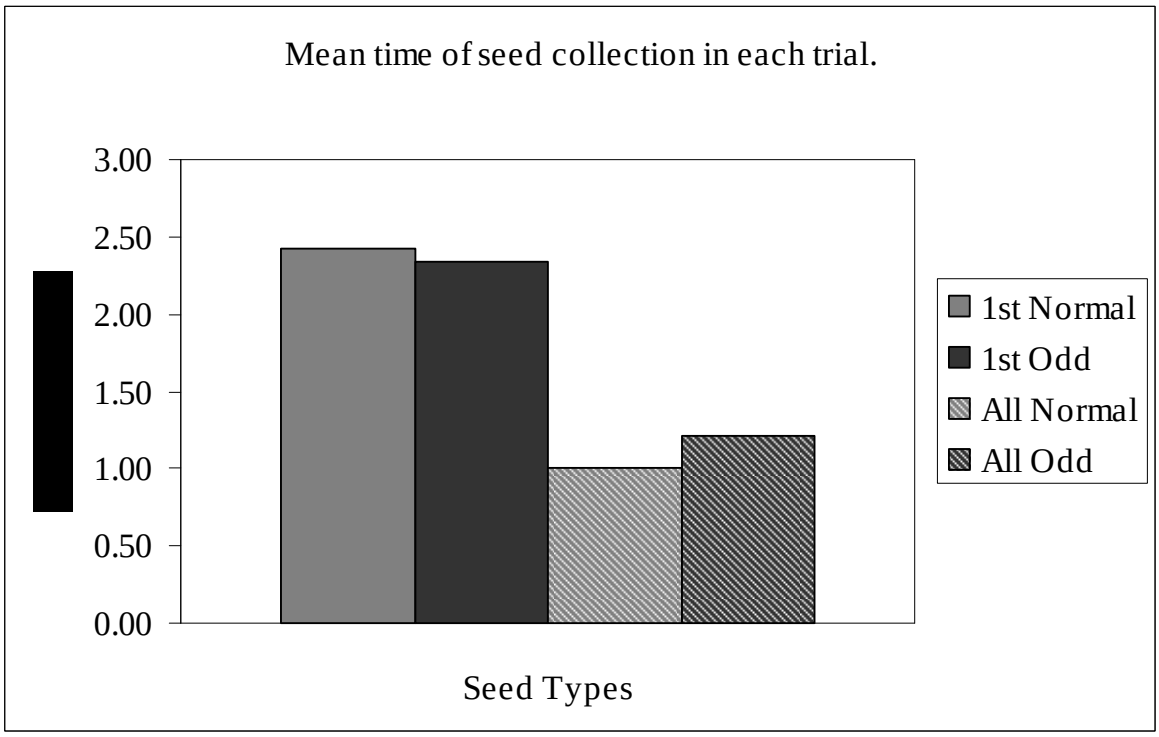


Figure 4–14. Time (in seconds) to pick seeds.

Our results show no significant difference between the two seed types when we look at only the data from the first seeds taken from each game. However, when we look at all the odd and normal seeds combined the time taken to pick a normal seed is significantly shorter than the time taken to pick an odd seed (Unpaired t-test, All seeds p<2.2e-16) (Figure 4–14).

Seed Position	No.Odd	Average time	No. Normal	Average time	P-value
1st	786	*2.344*	439	2.419	0.488
2nd	644	1.162	500	*1.014*	**0.002**
3rd	580	1.180	510	*0.939*	**<0.0001**
4th	542	1.039	486	*0.895*	**0.0003**
5th	501	1.063	465	*0.846*	**<0.0001**
6th	431	0.985	461	*0.859*	**0.0001**
7th	372	0.937	439	*0.840*	**0.004**
8th	311	0.879	387	*0.820*	0.080
9th	232	*0.772*	350	0.774	0.945
10th	174	0.833	300	*0.705*	**0.0003**

Table 4–11. Average time to pick odd & normal seeds for the first 10 seeds. (*Italics* = shortest time & bold indicates significance) & the results of Unpaired t-tests on each dataset.

We can see from this data that where a significant difference was found in the time taken to select a seed at a particular position, it was almost always faster to pick a normal seed. It can also be seen that the number of odd seeds collected at each position remains continually higher than the ratio (1 odd to 3 normal) they were provided in.

1st seed	No. Games	Average % Odd	Average % Normal
Odd	753	48.6	51.4
Normal	392	36.6	63.4
Total	1145		

Table 4–12. Average proportion of Odd & Normal seeds -collected from games when either an Odd or Normal is collected first.

With reference to whether an odd or normal seed was picked first we then looked at the average proportion of odd and normal seeds taken for the rest of the game. As the first seed in each game was not included in the proportion calculated, only those games in which 2 or more seeds were taken were included.

The results (Table 4–12) from this analysis show that both conditions show a preference for odd seeds, with a higher proportion of odd seeds than the (1:3) ratio in which they were provided. However, there is a difference in the strength of the effect between the two. The games in which an odd seed was selected first shows a stronger preference (Unpaired t-test, p-value < 2.2e-16) than the games in which a normal seed was selected first.

4.3.3.2 Comparison of Predator & Non-predator treatments.

Analysis of the first seeds taken in predator and non-predator games suggests that the chance that either an odd or normal seed will be picked first does not differ significantly between predator and non-predator games (Pearson's chi-squared p = 0.95).

Non-predator Games	All Seeds	Odd Seeds	Normal Seeds
Min	1	0	0
1st Qu.	5	3	1
Median	8	4	4
Mean	8.38	4.11	4.28
3rd Qu.	11	6	7
Max	22	10	15

Table 4–13. Summary of seeds collected in non-predator games.

The mean number of odd and normal seeds collected is around 4 per game and that the greatest number of odd seeds collected was 10 which would mean that all the available odd seeds in that game had been collected (Table 4–13).

The null hypothesis when comparing the predator and non-predator games is that there will be no difference in the number of odd seeds collected between the two game types. This necessitates that there is 'room for improvement' in the number of odd seeds collected in the predator games vs the non-predator games. As there was a strong effect of oddity prevalent in the non-predator games, with a number of players approaching the upper limit of 10 odd seeds, it was necessary to remove those players who would have little ability to increase the odd seeds collected in predator games from the dataset.

To provide the cut off point we took the mean number of odd seeds collected in the non-predator games (4.11 odd seeds) and added a quarter of that again (1.03 odd seeds) to give us the cut off point of 5.14 odd seeds. We then calculated the mean of the odd seeds collected by each player in their non-predator games, with any player found to have a mean greater than 5.14 removed from the data set. Of the 158 unique players this removed 33 leaving 125 (79.1%) and 911 games (74.4%). This data was then used in the GLM analysis.

	No. Trials	Odd Seeds	Normal Seeds
Non-Predator	449.00	1539.00	2011.00
Mean		3.43	4.48
Predator	462.00	1673.00	1945.00
Mean		3.62	4.21

Table 4–14. Mean Odd/Normal seeds in predator & non-predator treatments. (Using data minus players with >5.14 mean odd seeds in non-predator treatments.)

The means obtained from the adjusted data set (Table 4–14) suggests an increase in the ratio of odd to normal seeds in the predator treatments. Analysis confirmed a significant difference in the ratio of odd to normal seeds between predator and non-predator treatments, with more odd seeds removed in the predator treatment (Generalized Linear Model, $p = 0.0146$).

4.3.4 Results Part 2

4.3.4.1 Analysis of combined Predator & Non-predator treatments.

	Totals
Total no. of unique players	62
Total no. of games played	487
Total no. of non-predator games played	247
Total no. of predator games played	240
Total no. odd seeds taken	425
Total no. of normal seeds taken	4398
Total no. of seeds taken	4823

Table 4–15. Overview of basic figures & results

Once the raw data was received the data was cleaned up to remove any games in which no seeds were collected. This was done as these games would not add any weight to the ratio comparisons of normal and odd seeds. Table 4–15 provides the basic figures and totals produced from the final data set. The null hypothesis in this case is that the ratio of seeds picked would not differ from the 3:1 ratio in which the seeds were provided. However it was found that in both cases, all seeds & 1st seed taken in each game, the seeds picked differed significantly from what we would expect had the seeds been picked randomly. In both cases, odd seeds were taken at a much lower than expected frequency (Chi-squared Goodness of Fit, All seeds $p<0.0001$; 1st seed $p<0.0001$).

Our results show that when looking at the time taken to selects seeds, both for the 1st seeds collected and the data for all the seeds collected, it takes significantly longer to collect a normal seed (Unpaired T-test 1st seed p = 0.01058; All seeds p = <0.0001).

Seed Position	No.Odd	Average time	No. Normal	Average time	P-value
1st	10	*1.52*	477	1.90	***0.010***
2nd	40	*0.78*	446	0.95	***0.022***
3rd	43	*0.88*	434	0.90	0.848
4th	40	*0.75*	428	0.88	0.060
5th	46	*0.79*	412	0.90	0.208
6th	40	*0.82*	413	0.88	0.339
7th	40	*0.83*	391	0.87	0.576
8th	34	0.89	361	*0.84*	0.644
9th	37	*0.68*	307	0.81	***0.016***
10th	21	0.81	246	*0.79*	0.897

Table 4–16. Average time to pick odd & normal seeds for the first 10 seeds. (*Italics* = shortest time & bold indicates significance) & the results of Unpaired t-tests on each dataset.

We then went on to look at the average time taken to pick the first 10 seeds from each game and the results of the un-paired t-tests on the dataset for each position (Table 4–16). We can see from this data that although for almost all positions the odd seeds took less time to pick, the only significant differences were found for the 1st, 2nd and 9th seeds. It can also be seen that the number of normal seeds collected at each position remains much higher than the ratio of 1 odd to 3 normal seeds they were provided in.

With reference to whether an odd or normal seed was picked first we then looked at the average proportion of odd and normal seeds taken for the rest of the game. As the first seed in each game was not included in the proportion calculated, only those games in which 2 or more seeds were taken were included.

1st seed	No. Games	Average % Odd	Average % Normal
Odd	10	14.77	85.23
Normal	477	11.14	88.86
Total	487		

Table 4–17. Average proportion of Odd & Normal seeds - collected from games when either an Odd or Normal is collected first (Unpaired t-test: p = 0.4315).

The results (Table 4–17) from this analysis show that both conditions show a preference for normal seeds, with a higher proportion of normal seeds than the 1:3 ratio in which they were provided. However, no difference was found in the percentage of odd or normal seeds picked when either an odd or normal seed was selected first (Unpaired t-test, p-value = 0.4315).

4.3.4.2 Comparison of Predator & Non-predator treatments.

The analysis of the first seeds taken in each game suggests that the chance that either an odd or normal seed will be picked first does not differ significantly between predator and non-predator games (Pearson's Chi-squared test, p=0.340).

Non-predator Games	All Seeds	Odd Seeds	Normal Seeds
Min	2	0	1
1st Qu.	8	0	7
Median	10	0	9
Mean	9.84	0.81	9.03
3rd Qu.	12	1	11
Max	19	5	17

Table 4–18. Summary of non-predator games.

Table 4–18 provides a summary of the seeds collected in all the Non-predator games. From this we can see that the mean number of odd and normal seeds collected per game with only 0.81 odd seeds collected on average and 9.03 normal collected. As the maximum number of odd seeds collected was nowhere near ten (maximum available) we didn't feel that it was necessary to carry out any adjustments to the data set (as in the previous experiment, see Chapter 4, 4.3.3.2). There we were forced to remove the highest scoring players as they had collected either the maximum number of odd seeds available or very close to the maximum in the non-predator game, thus leaving little or no room for improvement in the predator games.

	No. Trials	Odd Seeds	Normal Seeds
Non-Predator	247	201	2229
Mean		0.8137652	9.03
Predator	240	224	2169
Mean		0.9333333	9.0375

Table 4–19. Mean Odd/Normal seeds in predator & non-predator treatments.

Table 4–19 shows us the means obtained from the adjusted data set and from this we can see that there appears to very little difference in the ratio of odd to normal seed in the two predator treatments. The analysis of our results found no significant difference in the ratio of odd to normal seeds between predator and non-predator treatments (Generalized Linear Model, p 0.178).

4.3.5 Discussion

In both the assessed whether the ratio of seeds collected from each game differed from the 3:1 ratio of normal to odd seeds in which they were supplied, with any deviation away from this ratio suggesting that the seeds were not taken randomly. When we examined the ratio of the total number of normal and odd seeds taken over all games and the first seeds taken in each game for part 1 (Odd = Seed Id 9, Normal =Seed Id 8) we found that both showed a highly significant difference with p = < 0.001. This suggested a strong bias towards picking odd seeds, both as the first seed and overall, with the ratio of normal to odd seeds more like that of a 1:1 ratio than that of the 3:1 ratio in which they were provided. This result confirms what was found in a previous study, which is that the oddity effect can be found in static systems as well as systems in which prey are in motion (a situation thought to induce the confusion effect in predators).

However, when we looked at the results for the part 2 (Odd = Seed Id 8, Normal =Seed Id 9) we again found a significant difference with p = < 0.001, but this time with a a strong bias towards picking the normal seeds, both as the first seed and overall, with the number of normal seeds selected far exceeding the 3:1 ratio in which they were provided. This result does not match that found in our part 1 and suggests that the seed pattern (seed pattern 9) used may have been responsible for the results found, rather than any effect of oddity.

We then examined the time it taken for a seed to be picked. For the first seed in each game this was taken to be the time from the start of the game until the first seed was picked and for all other seeds it was the time from the last seed taken until the seed was picked. We hypothesised that predators use the oddity effect to decrease the time taken to isolate and pick prey from a group, therefore for this to be true we would expect odd seeds to be picked faster than normal seeds. Looking at Part 1 first of all we found no significant difference was between odd and normal seeds the time taken for the first seed to be selected in each game (Figure 4–14). This suggested that despite there being considerably fewer odd seeds within the group, it does not take any longer for them to be selected. When we then analysed the results from all the seeds taken over all games the relationship changed with odd seeds now taking significantly longer to select than a normal seed ($p = < 2.2E-16$), with the mean odd time 0.25 seconds slower than the normal seeds. This might suggest a unique choice heuristic with odd seeds chosen preferentially it does not increase the time to select until the pool of odd prey begins to be depleted.

We then looked at the results for part 2 where the seed designs had been reversed for odd and normal the results seem to support our hypothesis that preferentially selecting odd seeds maybe an adaptation to speed up prey acquisition. For the first seed taken from each game it was found that there was a significant ($p = 0.01$) difference between odd and normal seeds, with the mean odd time found to be 0.38 seconds faster than normal seeds. We then analysed the time results for all seeds taken over all the games played & we found that once again there is a significant difference with the mean odd time 0.17 seconds faster than the mean normal time.

To look more closely at this we then compared the time taken to pick seeds at each position for the first 10 seeds in each game using Un-paired t-tests and comparing the average time. This showed that again there are significant differences between the odd and normal seed, but that almost all positions it would be quicker to pick a normal seed. However, despite the apparent disadvantage of taking longer to select, odd seeds continued to be selected preferentially in higher numbers than we would expect by chance alone. We again checked this against the results from the reversed pattern results and again we found a significant difference between the selection times (1^{st}, 2^{nd} &

9th seeds), with odd seeds taking less time to select than normal seeds (Table 4-11) . That it is still quicker to select the odd seeds in part 2 despite normal seeds being more numerous and more conspicuous adds weight to the hypothesis that a preference for oddity may reduce selection deliberation time.

Next we examined whether having an odd or normal seed being selected first had any relation to the total proportion of odd or normal seeds taken in each game. We hypothesised that those players that selected an odd seed first may continue to show an increased preference for oddity in their seed selection. Our results suggest that the first seed selected in each game does appear to have an effect or relationship with the seeds that are subsequently selected (Table 4-12). While both conditions show a higher than expected number of odd seeds picked, the effect appears to be stronger in those games where an odd seed is selected first. However, when we looked at the results for part 2 we that the first seed selected did not have any effect or relationship with the seeds that are subsequently selected (Table 4-17). Due to the confounding effect of the difference in conspicuousness between the seed patterns used we can not say whether there is any effect.

The second part of our experiment was to look at whether there was any difference between the predator and non-predator games. The purpose of this was to examine whether the oddity effect would be stronger in situations where attention is divided between prey selection and scanning for predators. Analysis of the first seeds taken from each game for both parts 1 and 2 showed no significant difference between the ratios of odd to normal seeds taken from the two sets of games. This result does not support our hypothesis, although due to what appears to be a very strong effect of seed pattern it might be that any other effect are effectively drowned out.

To examine this further we wanted to look at the ratio of all the seeds taken in each game. However, when looking at the summary statistics for part 1, the non-predator games (Table 4-13) it was realised that there were a number of individuals that removed a very high proportion of the odd seeds available in the control (non-predator) games and in some cases 100%. Obviously this would mean that for those players there is little or no room to increase the number of odd seeds they collect in the predator games. Since including these players in

the analysis could mask an increase shown by the rest of the players it was decided that they should be removed from this analysis (see methods for details). This was found to not be necessary for the part 2 results.

A model was then used to compare the ratios of odd to normal seeds across the two game types. For part 1 the analysis showed that there was a significant increase in the ratio of odd seeds taken in the predator games (Table 4–14). However, for part 2 where the seed patterns had been swapped round there was no significant change in the ratio of odd seeds taken in the predator games (Table 4–19). This suggests that the difference we found in part 1 is likely to be caused by a difference in the level of conspicuousness between the seed patterns and does not provide support for the hypothesis that a preference for odd prey may be a way to reduce selection time when attention must be split between prey selection and scanning for predators.

Previous studies that have examined the behaviour of foraging birds have suggested that they are limited by the amount of attention they can give to both foraging and scanning for predators and that this limit in attention may be a major cause of mortality in the wild (Dukas and Kamil, 2000). This may provide us with some insight as to why the oddity effect would still be evident in prey selection by predators from grouped but static prey, where we would expect them to suffer less from the confusion effect. This combined with knowledge that the preference for odd seeds continues even when apparently disadvantageous suggest that what we may be seeing is the effects of Waite's (2008) suggested 'odd choice heuristic'. This rule of thumb should dictate that oddity should always be selected preferentially, potentially short-cutting the deliberating phase of prey selection. This would allow a greater proportion of the limited attention a predator has to be put towards scanning for danger and thereby increasing survival.

The use of search images may also have a role to play here, with predators that using shape, colour and pattern to form search images to increase foraging efficiency (Pietrewicz and Kamil, 1979). Another paper by Dukas and Kamil (2001) examined the effect of dividing attention by searching for two distinct cryptic prey types. For this they used blue jays (*Cyanocitta cristata*) trained to search for two types of cryptic prey on a computer monitor. In the first

treatment cues were given to signal which prey item to search for and no cost was seen in switching between searching for each prey type. Conversely, in the second treatment where no cues where given and the jays had to divide their attention between searching for two prey types they paid for this with a lower detection rate. This might suggest that once a predator has locked in to using the odd search image there would be penalties for switching to a new search image.

In summary our results suggest that selecting odd individuals takes less time than selecting normal group members and supports the hypothesis that selecting odd prey may be an adaptation to reduce selection time. While in this case we use the term seeds when describing the on screen stimuli these results are equally applicable to lepidopteran larvae and other prey species that rely on crypsis. Unfortunately, due to the differences in conspicuousness we can not say anything further. If as we suspect the pattern used for the normal seed in this case was more conspicuous this may lend even more weight to the idea that a preference for oddity reduces selection deliberation time i.e. that despite normal seeds being more numerous and being more visible it is quick to pick an odd seed.

In future we need to find a better way to assess the conspicuousness of the seeds patterns we use. The results from both experiments along with the pilot test we carried out suggest that the context in which the seed patterns are viewed can strongly affect the predation rate. In this case we found that while the seeds patterns have very similar predation rates when presented in single pattern groups, when we then presented them as part of a mixed group the differences in conspicuousness became far more explicit. Therefore we propose testing the seed patterns in pairs with a 50:50 pattern mix. This should allow us to select 2 patterns which appear equally conspicuous when presented together. Our findings on the effect of group context on the conspicuousness of cryptic patterning, also has implications for crypsis in the field. It potentially points to a scenario in which two groups combine and despite both having previously suffered similar levels of predation, one phenotype may find that it now suffers a far higher percentage of the predation burden.

Chapter 5. Protection by association: Evidence for Aposematic commensalism

Many foragers hunt by sight, using shape, colour and pattern to form search images which increase foraging efficiency (Pietrewicz and Kamil, 1979). Most avian foragers are well equipped with sensitive tetrachromatic colour vision to search out and target prey (Finger and Burkhardt, 1994).

There are a number of ways that colouration and pattern are utilised by organisms to reduce predation and they generally fall in to three categories; (1) Crypsis, in which the prey reduces predation by avoiding detection by predators entirely; (2) aposematism, where the prey is easily detected but advertise their unsuitability as prey (Ruxton et al., 2004); and (3) masquerade, in which the prey may be detected but is misclassified as something non-edible (Skelhorn et al., 2010b). In the first category we include any type of crypsis or camouflage, such as background matching and/or disruptive patterning. The effect of these patterns is to hinder detection of an organism so that predators pass by without detecting their presence. In the case of disruptive patterning, this is achieved by breaking up an organisms distinctive outline (Stevens and Merilaita, 2009). The next two categories do not prevent detection of the organism, but change a predator's estimation of profitability so that they choose not to attack. This is achieved either by 'masquerade' where colouration or patterning mimic the appearance of something inedible such as a twig or bird faeces (Skelhorn et al., 2010b) or with the use of aposematic signals such as conspicuous behaviour, odour, sound or colouration to indicate unprofitability and advertise defences(Cott, 1940; Poulton, 1890). The conspicuousness nature of aposematic displays is thought to (1) enable predators to easily distinguish defended prey from undefended prey and (2) impose costs that only prey displaying an 'honest' signal of unsuitability can afford, such as increased detection rates(Sherratt and Beatty, 2003).

Effective aposematic signalling provides great advantages for survival and therefore, great opportunities for cheats. Why go to the expense of developing secondary defences when, by mimicking the characteristic warning signals of an

unpalatable or otherwise defended model organism, you can benefit without them? This type of mimicry is called Batesian mimicry. Nonetheless, it is not necessary for mimics to perfectly match all aspects of the model's patterning because even imperfect mimicry provides some protection(Kikuchi and Pfennig). However, dishonest signals, like those of Batesian mimics, can change the effectiveness of the warning signal. A number of studies have shown that, as the ratio of mimics to models increases, it reduces the effectiveness of the warning signal and predation rates for both groups increase(Ruxton et al., 2004).

To understand selection for the evolution of Batesian mimicry we must evaluate the fitness consequences of being non-mimetic. How does being in close proximity to aposematic prey affect undefended cryptic prey? One may predict that they would suffer greater predation, with the aposematic prey drawing the attention of predators to the location, which then turn to the more palatable and profitable prey once identified. Alternatively, non-signalling and palatable prey may benefit from their proximity to their aposematic neighbours. A laboratory study conducted by Mappes, Tuomi & Alatalo (1999) investigated this issue using wild caught birds presented with aposematic (unpalatable) and palatable prey in groups of either purely palatable, aposematic or mixed prey types. Their results suggest that palatable prey, in fact, benefit from a reduction in predation risk when grouped with aposematic prey through 'aposematic commensalism'. That is, palatable prey appear to benefit from being offered alongside unpalatable prey even when there was no strong similarity in appearance between the prey types. This might occur if predators avoid locations where they have had noxious experiences, or if an aversive experience causes a period of disinterest in food of any kind. Importantly, commensal relationships and associations between cryptic and aposematic species may be an important 'first step' towards the evolution of Batesian mimicry.

A parallel to this type of relationship, where one species benefits from association with another, may be found in botany. In this case, 'magnet species' (Molina-Montenegro et al., 2008) that are particularly attractive to pollinators do not divert most of the pollinators away from less attractive plants nearby, as may be expected. In fact, they appear in some cases to increase the number of visits those plants receive. This means that less attractive species can increase

their chances of being pollinated and, therefore, increase the number of seeds they produce, just by growing in close proximity to more attractive species.

Mappes' original research (Mappes et al., 1999) was conducted in a lab setting and under idealised conditions with prey items presented either singly or in pairs on a wooden plate. We, therefore, decided that it would be beneficial to generalise this study with a larger sample size and with the prey items presented in a more ecologically realistic setting to examine whether the observed effect would still be found in a less carefully controlled environment. Additionally, in the original experiment all 5 treatments, namely, (1) 1 aposematic prey item; (2) 1 palatable prey item; (3) 2 aposematic prey items; (4) 2 palatable prey items; (5) 1 palatable and 1 aposematic prey items were presented simultaneously, whereas, in nature predator-prey encounters are more likely to occur sequentially. The change from simultaneous to sequential presentation changes the predators' task from that of a comparison followed by a decision on whether to attack, to purely a choice between attacking or leaving without feeding. In a lab experiment like this, birds may also learn that whichever item they do not choose is removed, making the comparison stage a more important step in the process. We might also want to consider that predators may use the number of prey presented as a cue to the general availability of food, with a choice between two or more prey items indicating a greater availability of food. This may mean that when presented with an individual food item there is a greater perceived uncertainty in the future availability of food and therefore greater pressure to attack.

We further tested the hypothesis that non-conspicuous and undefended prey could reduce their predation rate by associating with aposematic prey by using groups of sunflower seeds (5 or 10 seeds) made either, conspicuous and unpalatable (UnP), or cryptic and palatable (P). The seeds were presented in mixed or single treatment groups within a field setting and the local wild bird assemblage was allowed to select and remove seeds, at will, over a set period of time. This allowed us to compare the survival rate of each seed/group type at the end of each trial.

5.1.1 Methods & Materials

This experiment was conducted between October 2010 and March 2011 in areas of park land in the centre of Glasgow, Scotland (Latitude: 55° 52' N, Longitude: 4° 15' W).

Groups of coloured seeds were left out at selected sites at 9am and their remaining numbers were checked at 4pm. There were 20 replicates of each of the 5 different treatments used (Table 5–1). This included 4 groups of homogenous colouring (i.e. all seeds within each group being of the same colouring & palatability) and 1 treatment of mixed seeds, the groups of which were composed of half and half of each colour being used.

Treatment	Unpalatable	Palatable	Total
1	5	5	10
2	5	0	5
3	0	5	5
4	10	0	10
5	0	10	10

Table 5–1. Number and type of seed in each treatment Group.

To ensure that the seeds from each group were spread evenly over the desired area, a circular cardboard 'stencil' was cut to 10 cm in diameter and used to ensure the grouped seeds remained within the set area. The pre-prepared seed groups were then sprinkled from a height of at least 10cm to ensure an even but random distribution. Treatments were placed out in a random order with a minimum of 10 metres between each group. To aid in collection of groups following experiments, a coloured golf tee was placed three paces to the north of each group. As an extra precaution, a brief note of the golf tee's location was also taken.

The sites were revisited at 4pm each day and the remaining seeds were counted and recorded. To ensure that missing seeds could be considered to be eaten and not just displaced; searching continued for 5 minutes after the last seed had been found. Seeds recovered were also examined to see if they had been 'discreetly' eaten (where the kernel had been removed via a small hole in the husk which on casual inspection may look intact).

There were three parts to this experiment. In part one we used unpalatable red seeds and palatable green seeds. This was carried out over 1 month from 28th October 2010 at Site 1 with one replicate of each treatment set out per day (a total of 5 groups of seeds per day over 20 days). In part 2, using the same methodology, we carried out the reciprocal experiment from 15th January 2011 to 6th February at Site 2, with unpalatable green seeds and palatable red seeds. As we expected the incongruity of the colour and palatability to reduce the effect of the conspicuous colouring and unpalatability on the predation rate we carried out an extra 10 replicates (30 in total). We carried out the final part from 7th February to 1st March, at site 1, with 20 replicates using palatable green and red seeds. In all cases, it was assumed that the green seeds were cryptic against the green background and that the red seeds were more conspicuous. However, our design was counterbalanced for colour and, thus, our interpretation was not contingent on the validity of these assumptions.

5.1.1.1 Site selection

The sites were selected for a combination of high bird activity and reduced human activity levels (human activity could not be avoided all together due to the inner city location). In particular, areas known to have regular bird feeding activity by humans were avoided, as these were likely to have many human visitors. While the treatment groups were not watched throughout the day a number of different bird species were observed close to or in the immediate area including blackbirds (*Turdus merula*), bluetits (*Cyanistes caeruleus*), bullfinch (*Pyrrhula pyrrhula*), carrion crow (*Corvus corone*), house sparrows (*Passer domesticus*), magpie (*Pica pica*), robin (*Erithacus rubecula*), rock pigeon (*Columba livia*), starling (*Sturnus vulgaris*) and wood pigeon (*Columba palumbus*). From observations carried out in previous unpublished work we knew that these species have also been seen to take interest in and feed from seed groups left out and that consumption of experimental seeds by mammals, such as grey squirrels (S*ciurus carolinensis*), is extremely rare.

The two sites selected, Site 1 (55°52'24.92"N 4°16'49.50"W) and Site 2 (55°52'54.47"N 4°17'27.62"W), were within 1 mile of each other. Both sites were of a sufficiently large area to prevent the need for repeated trials in the same space and overlap of area usage was minimised. In the weeks prior to the

experiment, wild bird seed was scattered to encourage birds to return to the sites throughout the winter months. Following the first experiment, a new site was chosen in which to conduct the second experiment in an attempt to ensure that different birds would be exposed to the new treatments and, thus, the problems with learning and conditioning would be greatly reduced. To maximise undisturbed foraging time seed groups were placed on open areas of grass, away from footpaths and other areas of high traffic. Trials were not conducted during periods of heavy rainfall where muddy conditions would make differentiating seed colour difficult and would reduce feeding activity in the bird population. Treatments were also not conducted during the extended snowfall in late November and December 2010.

5.1.1.2 Seed Treatments

Four types of bait were prepared, 1) palatable green seeds; 2) palatable red seeds; 3) unpalatable green seeds; and 4) unpalatable red seeds. In addition to their colouring, the 'unpalatable' seeds were treated with quinine hydrochloride, a chemical substance with a pronounced bitter taste known to be aversive to both domestic and wild birds (Halpin et al., 2008; Rowland et al., 2010; Skelhorn et al., 2008; Speed et al., 2000).

Standard striped sunflower seeds were dyed using Sugar Flairs food dye Holly Green and Red Extra. The dye concentrates were diluted using 200ml water with 75ml dye gel. 165g of striped sunflower seeds were mixed with 20ml of the dye solution and 200ml of water. This was brought to a simmer and left for 15mins, stirring occasionally. To make the unpalatable seeds 2 tablespoons (~34g) of quinine were added to the water and dye before simmering. The liquid was reduced as much as possible and, after simmering, the seeds and any remaining liquid were spread out on a baking tray to ensure that little dye or quinine was lost. To dry the seeds out completely a domestic oven was set to its lowest setting (50°C) and the seeds were spread on a baking tray. The seeds were checked and turned regularly. Once all traces of surface water had evaporated and the seeds were dry they were removed from the oven. The seeds retained their striped pattern after this treatment.

5.1.1.3 Pilot Study

A pilot study was carried out to test the palatability of the treated sunflower seeds, as we wanted to ensure that the dying process had not made all the seeds unpalatable and that the quinine treated seeds were sufficiently unpalatable to deter predation. To check this 5 groups of 10 red seeds (unpalatable) and 5 groups of 10 green seeds (palatable) were put out at the 2 sites (over 2 miles from the sites used in our subsequent main experiments) and left for the same timescale used in the full scale experiments (9am-4pm). At 4pm, the remaining reds and greens were counted.

From the results of the pilot study (Supplementary Table i) we were confident that the red unpalatable seeds were sufficiently less palatable than the green palatable seeds ($p = 0.056$) to be used in a larger scale test. Another small scale test was carried out to check that taste and colour were not confounded. We compared survival of 4 seed treatments, unpalatable green and red seeds and palatable green and red seeds. We found that when both red and green seeds were equally palatable no significant difference was found in their survival ($p = 0.803$; see Supplementary Table ii). Thus the birds tested did not display a strong preference for either colour.

5.1.1.4 Assessment of collection accuracy.

In order to independently assess the accuracy of the seed counting, random groupings of seeds were placed out (including both red and green seeds) at different sites. A second experimenter (the same one responsible for collection in the main study) then immediately collected as many seeds as he/she could find using the same technique as in the main study. In each case the collector did not know the total number of seeds or the split between red and green seeds. Of the 15 trials, only one inaccurate reading was recorded. This result allows us to be confident in the analysis of the main study that missing seeds are not an artefact caused by the accuracy of the human collection method.

5.1.1.5 Statistical Analysis

Our sampling unit is all the prey of a given colour within a group of 5 or 10 seeds. The percentage of seeds surviving at the end of the test period was

calculated for each treatment group and compared against survival in other treatment groups i.e. we did pairwise comparisons of every seed-group type against every other seed-group type. We also carried out a further set of pairwise tests using the pooled data from all 3 experiments. The data were then analysed using the statistical package SPSS. A Mann Whitney U Test, a non-parametric technique was used to compare the difference in survival within and between experiments. It was applicable here as the samples were unmatched and, in some cases, the sample sizes unequal. We did not use Bonferroni or any other correction to control experiment-wide type 1 errors because (1) we were making a small number of comparisons to test hypotheses derived a priori (Ruxton and Beauchamp, 2008) and (2) because of current concerns about the logical basis and powers costs of such corrections(Nakagawa, 2004).

5.1.2 Results

The first part of our experiment used unpalatable (unP) red and palatable (P) green prey (Table 5–2). We found a significant difference between the percentage of palatable green and unpalatable red seeds that survived to the end of the trials in both groups of 5 and 10 seeds. We found that the (unP) red seeds survived better in both cases ((P) Green, 5 seeds: Mean = 29, SE = 7.2; 10 seeds: Mean = 32.5, SE = 6.9; (unP) Red, 5 seeds: Mean = 89, SE = 4; 10 seeds: = Mean = 82, SE = 5.3, for full table of average % seeds surviving for each group type, per treatment showing S.E. and S.D. see Supplementary Table iii).

Palatable Green & Unpalatable Red									
5g	10g	5r	10r	5g Mixed	5r Mixed	Sample size	U-value	p-value	Highest Rank
X		X				20/20	28	<0.001*	Red (unP)
	X		X			20/20	38	<0.001*	Red (unP)
				X	X	20/20	121.5	0.022*	Red (unP)
X				X		40/20	67.5	<0.001*	Green (P)Mixed
X				X		20/20	59	<0.001*	Green (P)Mixed
		X			X	40/20	296	0.089	N/A
		X			X	20/20	197.5	0.936	N/A
X	X					20/20	185	0.684	N/A
		X	X			20/20	137	0.072	N/A

Table 5–2. Mann-Whitney U Test, P Green & UnP Red seeds.

% seed survival palatable green & unpalatable red seeds (unP = Unpalatable, P = Palatable). Where X straddles two boxes this is where 5 and 10 seed groups have been combined. (NA = no statistically significant result was found and so ranking is not applicable)
*** indicates significance**

A significant difference was also found between the green and red seeds of the mixed seed group, again, with more red (unP) seeds surviving than green (P) seeds in the same group (Table 5–2) (Red mixed: Mean = 88, SE = 5.3; Green mixed: Mean = 74, SE = 6). When we compared the green (P) seeds from single colour groups with those in the mixed group we also found a significant difference, with those palatable seeds in the mixed group surviving significantly better than those in a single colour group (Green only, 5 seeds: Mean = 29, SE = 7.2; Green only, 10 seeds: Mean = 32.5, SE: 6.9; Green mixed: Mean = 74, SE = 6). This was not the case for the red seeds (Table 5–2) where there were no significant differences between the survival of red seeds from single and mixed colour groups (Red only, 5 seeds: Mean = 89, SE = 4; Red only, 10 seeds: Mean = 82, SE = 3.9; Red mixed: Mean = 88, SE = 5.3). When seed survival was compared between homogenous groups of 5 or 10 seeds no significant differences were found for either red or green seeds.

The second part of our experiment used red palatable and green unpalatable prey (Table 5–3). Here we found a significant difference between green (unP) and red (P) seed survival in single colour groups, with green seeds suffering significantly less predation in each case, but no difference in survival was found between differing group sizes for each colour (Red 5 seeds: Mean = 44.7, SE = 6.6; Red 10 seeds: Mean = 49.3, SE = 6.7; Green 5 seeds: Mean = 64, SE = 6.5; Green 10 seeds: Mean = 66.3, SE = 6.4, see Supplementary Table iii). Further, no significant difference was found between survival rates of the red and green seeds in the mixed groups (Red mixed: Mean = 71.3, SE = 6.2; Green mixed: Mean = 73.3, SE = 6) or between the green seeds from single colour and mixed groups. A statistically significant difference was found between red seed from mixed groups and red seed from single colour groups, with lower predation in mixed groups (Table 5–3) (Red mixed: Mean = 71.3, SE = 6.2; Red only, 5 seeds: Mean = 44.7, SE = 6.6; Red only, 10 seeds: Mean = 49.3, SE = 6.7). No difference in survival rate was found between different group sizes in homogeneous groups of 5 or 10 for neither the green or red treatments.

Unpalatable Green & Palatable Red									
5g	10g	5r	10r	5g Mixed	5r Mixed	Sample size	U-value	p-value	Highest Rank
X		X				30/30	323	0.055	Green (unP)
	X		X			30/30	319	0.051	Green (unP)
				X	X	30/30	441.5	0.894	N/A
X				X		60/30	767	0.242	N/A
X				X		30/30	365	0.19	N/A
		X			X	60/30	705	0.085	N/A
		X			X	30/30	266.5	0.005*	Red (P) Mixed
X	X					30/30	435	0.819	N/A
		X	X			30/30	406	0.508	N/A

Table 5–3. Mann-Whitney U Test, UnP Green & P Red seeds.

% seed survival unpalatable green & palatable red seeds (unP = Unpalatable, P = Palatable). Where X straddles two boxes this is where 5 and 10 seed groups have been combined. (NA = no statistically significant result was found and so ranking is not applicable)
*** indicates significance**

For the third part of our experiment both the red and the green seeds were palatable (Table 5–4). The only significant difference in survival rate was found between the green (P) seeds in single colour groups and the green (P) seeds in mixed colour groups, with those in the mixed groups surviving better than their counterparts in single colour groups (Green only, 5 seeds: Mean = 30, SE = 7.8; 10 seeds: Mean = 22, SE = 5.7; Green Mixed: Mean = 51, SE = 10; Supplementary Table iii).

Palatable Green & Palatable Red									
5g	10g	5r	10r	5g Mixed	5r Mixed	Sample size	U-value	p-value	Highest Rank
X		X				20/20	158.5	0.245	N/A
	X		X			20/20	136	0.076	N/A
				X	X	20/20	193.5	0.854	N/A
X				X		40/20	277.5	0.045*	Green(P) Mixed
X				X		20/20	129	0.046*	Green(P) Mixed
		X			X	40/20	368.5	0.613	N/A
		X			X	20/20	185.5	0.686	N/A
X	X					20/20	184	0.649	N/A
		X	X			20/20	198.5	0.967	N/A

Table 5–4. Mann-Whitney U Test, P Green & Red seeds.

% seed survival both green & red palatable seeds (unP = Unpalatable, P = Palatable). Where X straddles two boxes this is where 5 and 10 seed groups have been combined. (NA = no statistically significant result was found and so ranking is not applicable)
*** indicates significance**

Finally, we combined results from all three parts of our experiment to look at the overall effect of palatability and colour (Table 5–5). From this we discovered that, across all group sizes, there was a highly significant difference between the survival rate of palatable seed and unpalatable seed with, on average, more

unpalatable seeds surviving to the end of the trials (Palatable, Green 5 seeds: Mean = 46, SE = 4.4, Green 10 seeds: Mean = 27.3, SE = 4.5; Red 5 seeds: Mean = 53, SE = 3.8; Red 10 seeds: Mean = 46.2, SE = 5; Unpalatable, Green 5 seeds: Mean = 68.7, SE = 4.4; Green 10 seeds: Mean = 66.3, SE = 6.4; Red 5 seeds: Mean = 88.5, SE = 3.3 ; Red 10 seeds: Mean = 82, SE = 3.9).

Comparisons	Sample size	U-value	p-value	Highest Rank
5 Green Seeds (P vs UnP)	80 (P) & 60 (unP)	1625.5	<0.001*	unP
10 Green Seeds (P vs UnP)	40 (P) & 30 (unP)	237.5	<0.0001*	unP
5 Red Seeds (P vs UnP)	100 (P) & 40 (unP)	966	<0.0001*	unP
10 Red Seeds (P vs UnP)	50 (P) & 20 (unP)	216	<0.0001*	unP
5 Unpalatable (R vs G)	40 (R) & 60 (G)	745	<0.001*	Red
10 Unpalatable (R vs G)	20 (R) & 30 (G)	232	0.175	N/A
5 Palatable (R vs G)	100 (R) & 80 (G)	3573	0.209	N/A
10 Palatable (R vs G)	50 (R) & 40 (G)	700.5	0.013*	Red

Table 5–5. Mann-Whitney U Test, across all experiments.

% seed survival across all 3 experiments by colour and palatability (unP = Unpalatable, P = Palatable, R = Red, G = Green). (NA = no statistically significant result was found and so ranking is not applicable)
*** indicates significance**

When looking at differences in predation between red and green unpalatable seeds across all parts of the experiment (Table 5–5), the only significant difference found was between red and green seeds in groups of 5 seeds (this includes seeds from mixed colour groups), where red seeds were found to survive significantly better (Red, Mean: 88.5, SE: 3.3 & Green seeds = Mean 68.7, SE: 4.4). When we then compared survival rates of palatable seeds we found a significant difference between red and green palatable seeds in groups of 10, with red seeds suffering less predation (Red, Mean: 46.2, SE: 5 & Green seeds = Mean 27.3, SE: 4.5). However, unlike the unpalatable seeds we found no difference in survival between red and green palatable seeds in groups of 5. It should be noted that this result is at odds with the results found in the small scale pilot studies carried out prior to the full scale experiments. This suggests that the effects of colour are small and only with the larger sample sizes of the full scale trial can they be picked up.

5.1.3 Discussion

From the analysis of the first part of our experiment with green palatable seeds and red unpalatable seeds we found that the red seeds survived significantly better than green seeds in all conditions, as expected based on the

unpalatability of the quinine. Moreover, we found that there was a significant difference in green seed survival between groups, with those in mixed groups alongside red seeds having a significantly better survival rate than those in green-only groups. This result supports the “aposematic commensalism” hypothesis, with the palatable cryptic prey suffering significantly less predation when grouped together with aposematic neighbours. In comparison, no difference was found between red seeds in mixed groups and those in red only groups. This also supports the “aposematic commensalism” hypothesis, as the added protection the palatable seeds gain apparently does not impose any corresponding costs on the aposematic seeds they are grouped with.

For the second part of our experiment we investigated how much of the effect was generated by colour and how much by palatability and, thus, we reversed the treatment by using green unpalatable seeds and red palatable seeds. Here, as expected due to the uncoupling of palatability and conspicuous colouring, we found that the treatment effects were much weaker. Nevertheless, our results were close to significant ($p = 0.055$ & 0.051) and might suggest a non-significant trend in single colour groups with the unpalatable seeds (green) surviving in greater numbers than the palatable (red) seeds. In this part of the experiment, no significant difference was found between red and green seed survival in mixed colour groups, but when red seeds from the single colour groups of 5 seeds were compared with red seeds from mixed groups, it was found that those grouped with the unpalatable green seeds survived significantly better. This again supports “aposematic commensalism” and, as in the first part of our experiment, this demonstrates that there is a benefit for undefended prey to group with defended prey. However, here, the effect may be weaker when the unpalatability and aposematic colouring are not tied. In other words, by combining unpalatability with the classically aposematic colour red(Roper and Marples, 1997), it is likely there will be a stronger combined aversion than when combined with the colour green, since many birds have an innate aversion to red(Mastrota and Mench, 1995).

For the third and final part of our experiment we used groups in which both the red and green seeds were palatable (). We found that there were no significant differences in survival for the majority of the comparisons. However, we did find that the green palatable seed survived significantly better when it was grouped

with red palatable seed. This suggested that, even in the absence of chemical defences, when in a mixed group of red and green seeds, the green seeds still appeared to benefit from their proximity to the conspicuous red seeds.

This may be relevant when considering selection pressures on potential Batesian mimics, with the conspicuous colouring dishonestly signalling unpalatability. In this case we presented predators with prey groups with no honest signals. We know that as the ratio of dishonest to honest signals is increased the effectiveness of the warning signal is reduced and predation rates for both groups increase(Ruxton et al., 2004). For this experiment we did our best to minimise learning effects, but we would expect that had we continued presenting the same predators with groups of all palatable red seeds they would eventually learn to ignore the red colouration. However, without the time to learn this, red colouration is still effective in improving the survival rate of nearby prey, but to a lesser extent than when the conspicuous signal is supported by chemical defences. Furthermore, these results confirm our hypothesis that palatable prey can improve their survival rate by grouping with neighbours that predators may be pre-adapted to interpret as defended.

Finally, we wanted to analyse the effect of colour and unpalatability across all three parts of the experiment (Table 5–5). We should note that as this analysis pools the data from the 3 separate experiments conducted between early autumn and mid-winter, they may be confounded by the time of year. Here we found that, in all cases, whether coloured red or green, unpalatable seeds had a greater survival rate than palatable seeds. We found that in groups of palatable seeds of homogenous colour, red seeds in the larger groups (10 seeds vs. 5) survived better than green seeds but that there was no difference in the smaller groups. This might suggest that, where the aposematic signal is dishonest, it takes the greater stimulus of a larger group and a greater area of warning colouration to illicit a reaction from predators, which confirms an effect found in previous studies(Gamberale and Tullberg, 1998).

We had expected that, when looking at the unpalatable seeds, in those cases where seeds were both red (warning coloured) and unpalatable, these seeds would survive better than unpalatable green seeds. What we found, however, was that only in the groups of 5 seeds did red seeds survive significantly better

than green. We are unsure as to why this was not the case for the groups of 10 seeds but it may be that this is a product of the smaller sample size obtained for the 10 seed groups. In summary, our results show that (1) undefended non-aposematic prey can benefit by grouping with aposematic and defended prey and that (2) there is no evidence that the aposematic prey suffered greater predation by their association with the palatable prey.

It is important to take protection by association or 'aposematic commensalism' in to account when talking about the evolution of aposematic signals. Is this an effect of proximity? Perhaps predators avoid locations where they have had noxious experiences, with all prey within a certain distance lumped together and avoided? Or perhaps, rather than proximity in space, should we consider proximity in time, with unpalatable prey or an aversive experience causing a period of disinterest in food, of any kind? This could be tested by providing distasteful prey within a test area and observing whether there is any latency to the selection of further prey items and if this changed by moving to a new test area. We might also want to investigate what cues are being used; are they purely visual or might odour be playing some part? We know that odour can be used in conjunction with colour to advertise unprofitability(Kelly and Marples, 2004), and, with the effects of wind, dispersal odour cues are likely to have a much less defined effective area than that of colour. This may mean that neighbouring undefended prey can benefit from aversive odour cues emitted by others by grouping with them. The prey items we used were very different in colour. In all other ways, such as size and shape, they were very similar. Had we had used palatable prey that were different in those aspects, would they still have benefited from their aposematic neighbours?

We speculate that it would be of particular relevance to the evolution of aposematic colouring if we were to find that these benefits could cross kingdoms, with palatable animals able to benefit by grouping with unpalatable plants or vice versa. It is thought that some defended plants use aposematic signals similar to animals(Inbar et al., ; Lev-Yadun, 2009; Rubino and McCarthy). If animals were able to benefit by association with these plant species and vice versa, it might help to explain the convergence of signals between these very different groups. We might also consider that warning signals might have first

evolved within plants, with animals, later, co-opting an already established system by close association with defended plant species.

A similar phenomenon to the one we describe here has been also been found in plants with the discovery of ‘magnet species’(Molina-Montenegro et al., 2008). It was found that species with flowers that were less attractive or profitable to pollinators were able to increase pollination and their seed production by associating with more attractive species. This, along with our results, leads us to speculate that perhaps this type of commensal relationship may be comparatively common within the natural world.

Our results may also point to potential consequences for the evolution of Batesian mimicry. One of the biggest questions on mimicry that remains to be answered is how a species can survive through the intermediate stages of evolving a conspicuous display. Assuming an undefended species initially begins with cryptic patterning, there is presumably a point at which they are neither fully cryptic nor conspicuous and therefore at very high risk of predation (being both easily visible and undefended). We speculate that if such a species was able to reduce their predation rate by associating closely with their defended model, they may be able to survive long enough to evolve a better mimicry. Eventually, increasingly more accurate mimicry could free them to associate less closely with their models. However, the mechanism explored here may also act to reduce the likelihood of Batesian mimicry evolving, because the anti-predatory advantage of close association without mimicry may reduce the fitness advantage of changing appearance towards mimicry, particularly where the model and mimic closely share habitat requirements. To properly examine this we will need to carry out further experiments to examine how differing levels of mimicry affect fitness.

The results shown here suggest that a full understanding of selection pressures associated with mimicry requires consideration of the benefits of close association without mimicry. Although mimicry has been closely studied because of its evolutionary significance most prey species do not evolve to be Batesian mimics and the aposematic commensalism highlighted here may be an important but hitherto neglected factor in explaining this phenomenon.

General Discussion

Using both field and lab experiments I have examined adaptations that reduce predation rate and in particular how colouration and patterning reduce risk of predation, both as a single organism or as part of a group when against the natural environment. While each of the strategies I have examined are important in their own right, it is however important to realise that they do not occur in isolation and they are often encountered simultaneously, either as part of a community or within the same animal and so their effects are likely to be modified. I also did not consider the genetic basis of the traits I studied and any constraints here may have considerable effects on the evolution of anti-predator strategies. Gene studies are also extremely useful in examining the origins of an adaptation and our understanding of how they spread throughout a population. For example some recent work examining the mimetic history of the *Heliconius* butterflies has redefined our understanding of their colour pattern evolution. Previous studies using neutral markers (not linked to the colour pattern loci) suggested that similar colour patterns had arisen independently, multiple times, in each species with populations partitioned by geographic region. However, this most recent study used markers linked to the colour pattern loci and a gene which controls red colour pattern variation. The results of this approach suggested a single origin of the pattern element within each species and demonstrated the importance of using markers from the phenotypic-determining genomic region to understand the evolutionary history of an adaptive trait(Hines et al.).

Symmetry of body plan is something common to most complex organisms and may be to some extent a constraint on the types of patterning an organism can evolve. In chapters 1 and 2 I examined aspects of cryptic and aposematic patterning, both of which have been studied in respect to the effect of symmetry on their anti-predator effects. In chapter 1 I found that asymmetry has no effect on predation rate, a result that is in agreement with other recent studies. However, we must still find a way to marry these findings with symmetry studies that have found symmetrical patterns are more easily detected, learnt and reproduced. These two set of results seem to be

completely at odds with each other. However, a possible solution to this problem may be found by looking at the resting positions of cryptic Lepidoptera. Many cryptic species do not lie perfectly flat against the substrate and, while these more rounded profiles do mean that they may stand out slightly proud of the substrate, it means that predators are likely to only see part of their wing patterning when approaching (Figure 6–1). In these cases despite having a symmetrical wing pattern when both wings are fully visible, the majority of predators are likely to only ever be presented with a highly asymmetrical pattern. Comparatively, the asymmetries used in most studies are considerably smaller in magnitude.

Figure 6–1. Cryptic patterning and resting position.
1) Oak Lutestring (Cymatophorima diluta), a) curved wing resting position b) flattened wing resting position; 2) Yellow Horned (Achlya flavicornis) a) curved wing resting position b) flattened wing resting position; 3) Peach Blossom (Thyatira batis) a) curved wing resting position b) flattened wing resting position. (Image © http://ukmoths.org.uk)

Another way of looking at this question may be to examine why the majority of butterfly species that have both aposematic and cryptic patterning have the aposematic on the dorsal surface of the wings and the cryptic designs on the ventral surface (Figure 6–2). Why do we not find species with the aposematic design on the ventral surface and the cryptic patterning on the dorsal wing surface? With this arrangement butterflies would still be able use the startling technique of quickly revealing the aposematic pattern by resting with their wings open and flicking their wings closed quickly to show the aposematic ventral wing surface.

Figure 6–2. Butterflies that are both cryptic and aposematic.
1.) Peacock (*Inachis io*) 2.) Indian Leaf Butterfly (*Kallima paralekta*) (Image © Wikimedia Commons)

However, we might be able to explain why this is not more commonly seen if asymmetry does aid crypsis or is at least not detrimental. With the cryptic patterning on the ventral surface approaching predators are only able to see one wing surface making the visible pattern highly asymmetrical. Alternatively, if asymmetry has no effect on crypsis this arrangement may still be explained by benefits of symmetry in aposematic patterns. In this case, despite crypsis not benefiting from asymmetry, the symmetry and increase in size of signalling area gained by using the upper wing surface for the aposematic patterning is enough to ensure that this is the preferred surface to display aposematic signals.

One very important factor that needs to be taken in to consideration in any study examining visual signals and crypsis is that animals might perceive colours differently from us. To control for this many contemporary studies have used methods such as spectrometry and digital photography in an attempt to objectively measure colour (Stevens et al., 2007; Stevens and Merilaita, 2009). Nevertheless, there how a colour is perceived and its true spectral value are often very different, as the light that enters the eyes is processed by the visual system and in term those signals interpreted by the brain (Endler, 1990). Even within our own species it has been found that culture and language have direct consequences for the way we interpret and assess colour (Roberson et al., 2005) and it is likely that different species will have even greater differences in higher processing functions (like generalisation, recognition, categorisation). However, it has only been in relatively rare instances that studies have used measures that

relate directly to the visual processing of the receiver (Cassey et al., 2008; Hastad et al., 2005; Langmore et al., 2009; Lovell et al., 2005; Stevens and Cuthill, 2006; Tanaka et al., 2011)

Taking all this into account, I hope I have demonstrated that there still are many fascinating questions left to explore in the field of visual anti-predator adaptations. However, future work should (i) take predator physiology and psychology into account, (ii) look at trade-offs between functions, (iii) look at developmental (iv) and genetic constraints

Appendix i. Colour and Shadow

Colour vision is for many predators an important hunting and foraging tool. Humans and some primates have trichromatic vision and can see in the range of ~400 and ~650 nm(Rowe, 2002). Many avian predators are well equipped with sensitive tetrachromatic colour vision with a range of 300–700nm (Cassey et al., 2008; Finger and Burkhardt, 1994). Their colour vision enables them to pick out shape, colour and patterns, which they can then match to search images to increase their foraging efficiency (Pietrewicz and Kamil, 1979). While there is a considerable amount of research investigating colour vision in animals, it is significantly easier in many ways to investigate it in humans. Research using human volunteers has found that colour vision is used to provide information on the shape, texture, depth, object segmentation and even motion (Shevell and Kingdom, 2008a). Much of this is due to how colour is used to facilitate shadow identification(Kingdom et al., 2004). If we look at any natural scene shadows are to be found everywhere and being able to identify shadows from changes in the amount or quality of light being reflected from a surface is central to 'edge classification'(Gilchrist et al., 1983) and object identification (Cavanagh and Leclerc, 1989).

An object's colour is determined by the wavelengths of light that object reflects and which it absorbs. A green leaf for example and other green plants use Chlorophyll to change light into energy. Chlorophyll absorbs the blue and red light from the spectrum and reflects the green. The green is reflected back out to the viewer making the grass and leaves appear green. However, if the light illuminating a green leaf does not contain any green light to reflect it will appear black (Figure 6–3).

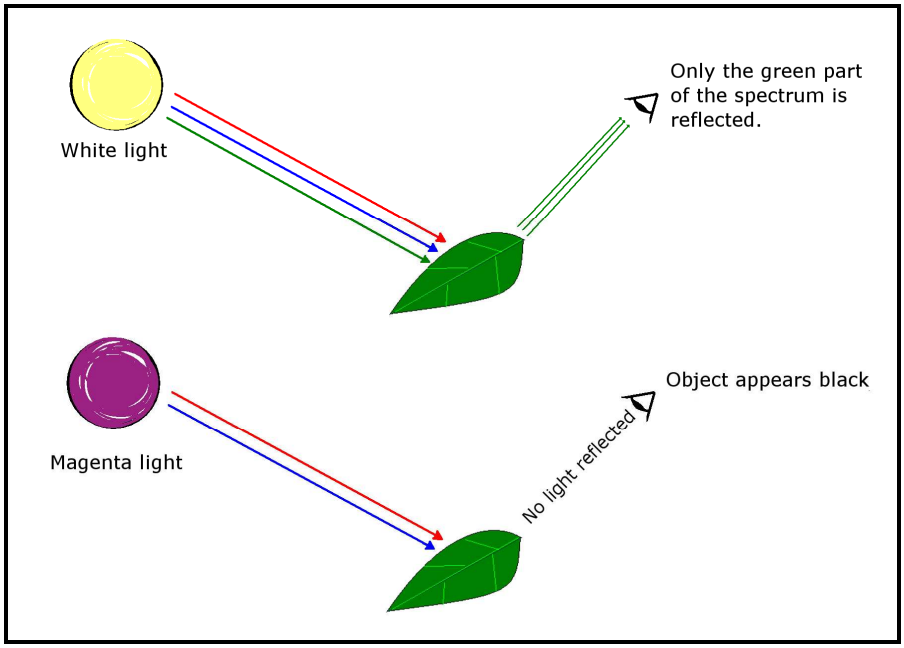


Figure 6–3. Reflectance and absorption.
- How the reflectance and absorption properties of an object allow us to see colour. (Image author's own)

When we travel between different rooms, from indoors to outdoors and between different light sources, the quality of light illuminating everything we see can change dramatically. If we were to carry the same object with us as we moved between different lighting conditions that same object would in fact be reflecting slightly different wavelengths of light depending on the ambient light. If we were to actually perceive the colour differences the world would be a very confusing place with objects constantly shifting in colour throughout the day as light conditions changed. However, our brain interprets the incoming signals and allows us to see a green apple as green at midday, when the main illumination is white sunlight, and also at sunset, when the main illumination is red. This helps us identify objects.

In natural scenes, many potential processes interact to achieve colour constancy. However, these processes are not perfect and we will use information such as illumination, context and prior knowledge to influence our determination of an object's colour (Hansen et al., 2007; Shevell and Kingdom, 2008b). While we must be extremely cautious in extending the findings of human studies to other species, there has been some recent evidence to show that human colour vision can be used as a valid proxy for avian perception of colour (Seddon et al., 2010). Colour constancy for example is something that appears to be common throughout the animal kingdom and has been tested in guppies,

pigeons, goldfish, bees, cichlid fish, tree shrews and monkeys (Brenner and Cornelissen, 2005; Intskirveli et al., 2002).

A change in the way colour is perceived has obvious implications for organisms that depend on colour for camouflage or visual warning signals to reduce predation. In forest environments the light quality can be changed significantly by the leaves filtering out certain wavelengths of light. Chlorophyll predominately removes light from the red and blue ends of the spectrum, with another smaller peak in the orange spectrum (Figure 6–4).

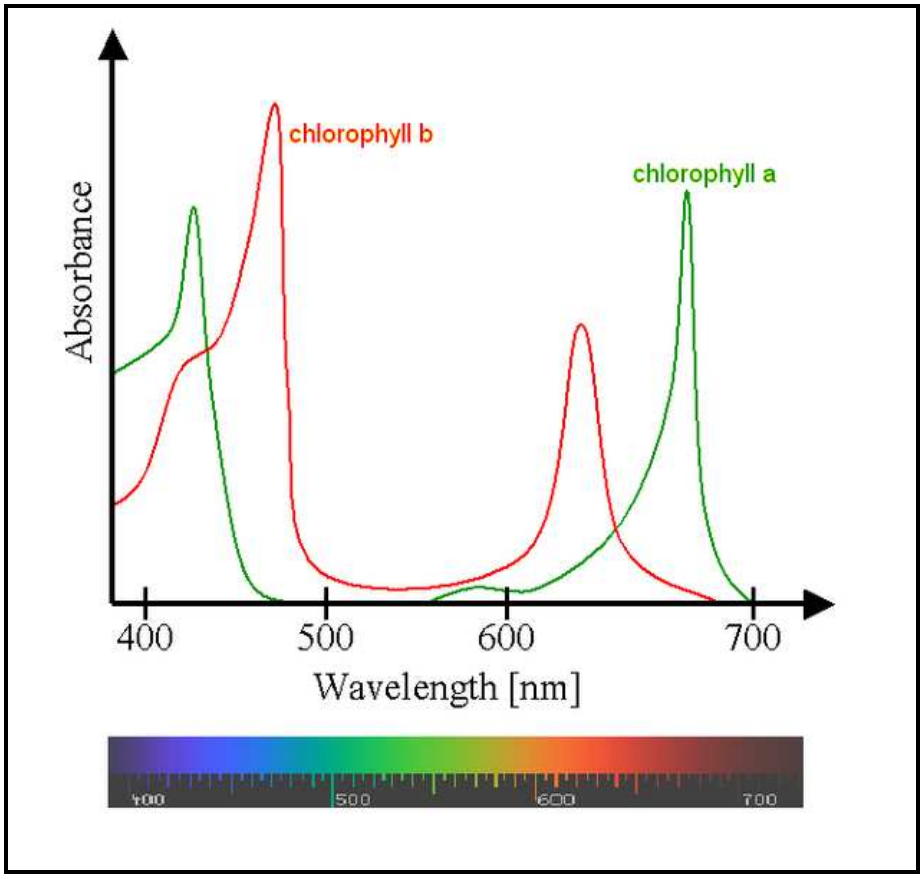


Figure 6–4. Absorbance spectra of free chlorophyll.
Chlorophyll a (green) and b (red) in a solvent. (Image © Wikimedia commons)

John Endler's (1993) paper on the colour of light in forests looked at the effects of cloud cover, time of day, forest type and amount of cover on the chromatic quality of light and its implications on conspicuous signals. He found that forest shade and thin shade (closed canopy or very small gaps) could be characterised as greenish to yellow-green light, woodland shade (open canopy but no direct sunlight) was blue to bluish-grey and small canopy gaps would result in reddish light. In the majority of cases, and particularly in sunny conditions, the amount of red and orange light drops considerably, with red light showing the greatest

drop (Figure 6-5). He also stated that for effective signalling in a forest shade (green light) environment, although green and yellow colours would be the brightest, they are likely to be cryptic against the predominately green background. Therefore, he suggested that patterns incorporating orange or red would be the most effective signalling colours.

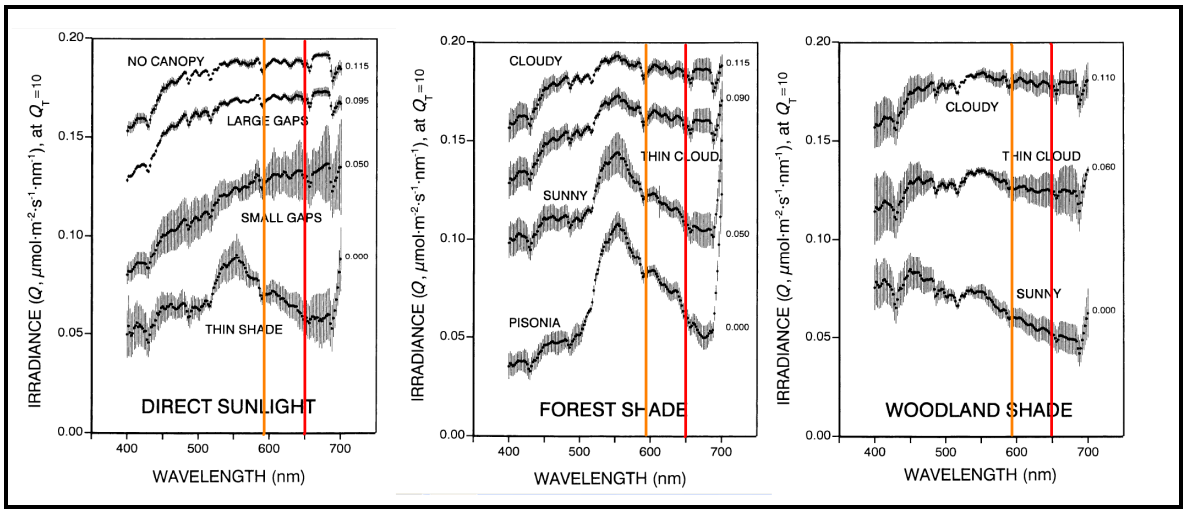


Figure 6–5. Light Spectra.
From various habitats and under varying weather conditions. The orange and red bars mark the approximate position of orange (~590nm) and red (~650nm) light on the spectrum. (Taken and modified from(Endler, 1993).

We can find a similar affect in deep sea environments. In the deep oceans most if not all of the red part of the light spectrum is removed by phytoplankton in the surface waters. In these deep waters you can find animals that under full spectrum light are coloured a bright red, but without any red light to reflect this colouration is an effective camouflage. Therefore, while Endler (1993) suggested that orange and red might be the best options for a conspicuous display. In a more recent study (Gomez and Thery, 2007) it was found that light orange-red colors had a greater brightness contrast than saturated orange/red in understory conditions, but that this was reversed in the full sunlight conditions of the canopy. They also found that yellow and orange are more conspicuous in understory than in the canopy due to a high brightness contrast, but have a moderate chromatic contrast in both environments. Their survey of avain plumage colouration found that the colours, and patch sizes of those colours, differed depending on whether a species was found in the canopy or understory. These results help us to understand how organisms living in contrasted light environments might evolve patterns that are both simultaneously conspicuous and cryptic. For example a pattern with a bright and conspicuous red in full

sunlight might become a muddy, dull brown in forest shade; and a bright yellow while conspicuous in the understory might become cryptic in the predominately green and yellow environment of the canopy.

In a recent paper by Lindstedt et al(2011), the predation rate of two colour morphs of the aposematic wood tiger moth (*Parasemia plantaginis*) was compared using both laboratory and field studies. The laboratory studies used great tits (*Parus major*) as the model predator and while the birds found both the red and orange morphs aversive, red morphs were attacked less than orange morphs. When measured, the contrast of red and orange in full spectrum lighting was found to be similar, however, orange morphs were found to have a higher luminosity. A higher luminosity makes objects easier to see from a greater distance and it was suggested that the difference might be due to the orange morphs being more easily detected against the green background used. These results would lead us to expect that in the field orange morphs would be predated to a greater extent and be less numerous in wild populations. Nevertheless, when a field study was conducted no difference in predation rates was found between the two colour morphs. I propose that the missing component in the lab study might be that the lighting used did not take into account the effect of the forest habitat light conditions and the reduced red/orange spectrum as would be likely to occur in the field. This may change the way in which the two morphs are seen by predators and cause the differences in predation rates seen between lab and field studies.

The colour of light not only changes the colour of an object but changes the colour of the shadow it casts. Objects that are illuminated in colored light will cast a shadow of the complementary colour, an effect called the Helson-Judd effect (Pridmore, 2011). An example most of us will have seen will be the effect of the redder tint of sunlight at sunset and how this gives may give shadows a green tint. This can be replicated by shining a torch shining through a coloured acetate sheet projecting on to a white wall. In the example of the predominately green light of forest shade objects would cast a pink shadow. As this is thought to be caused by the light exciting different cones within the eye to differing extents (Houde-Walter and Pierce, 1992), it would be necessary to carry out tests to see if other animals also perceive this effect. However, if we find that they do this may have implications for camouflage in different micro

habitats. For example if you live predominately in the canopy with a large amount of reflected green light you might want to use pink shades in counter shading or camouflage patterning. A possible example of this might be found in the larval stage of the Small Copper butterfly (*Lycaena phlaeas*) which feeds on the underside of leaves after hatching.

Figure 6–6. Small Copper (*Lycaena phlaeas*).

Finally, I believe we need to examine how colour and shadow are used in disruptive patterning. As previously mentioned, colour and shadow play an important function in providing visual clues to depth, shape and texture. These factors are all key to object recognition and manipulation of the perception of them is a possible way to enhance crypsis, and in particular, disruptive colouration. Disruptive patterning is a form of crypsis in which the patterning breaks up the distinctive outline of an organism hindering detection (Stevens and Merilaita, 2011). As described by Martin Stevens & Sami Merilaita (2009) disruptive colouration can be broken down in to five categories: 1) differential blending, where some colour patches stand out from the background while others blend in ; 2) maximum disruptive contrast, where high luminance or colour contrast break up the surface or outline continuity; 3) disruption of surface through false edges; 4) disruptive marginal patterns (disruptive markings specifically found along the outer edges); and 5) coincident disruptive colouration, which uses highly conspicuous markings to draw ‘attention’ away from the body outline (Stevens and Merilaita, 2009).

Disruption of surface as a mechanism for crypsis was first mentioned by H.B Cott in his book Adaptive Colouration in Animals (Cott, 1940), in which he suggested that breaking up the continuity of the surface through patterning that creates ‘holes’ and false outlines on the surface of the animal. Since then there has been little attention paid to exactly how these false edges and markings are perceived by the receiver. I would argue that one of the ways in which a false edge could be created is with the use of coloured shading and markings that create the illusion of depth and texture. We know from studies carried out on

human vision that by combining patterns of differing luminance and colour contrast we can create the impression of depth on a 2D surface (Figure 6–7)(Kingdom, 2003; Kingdom et al., 2005).

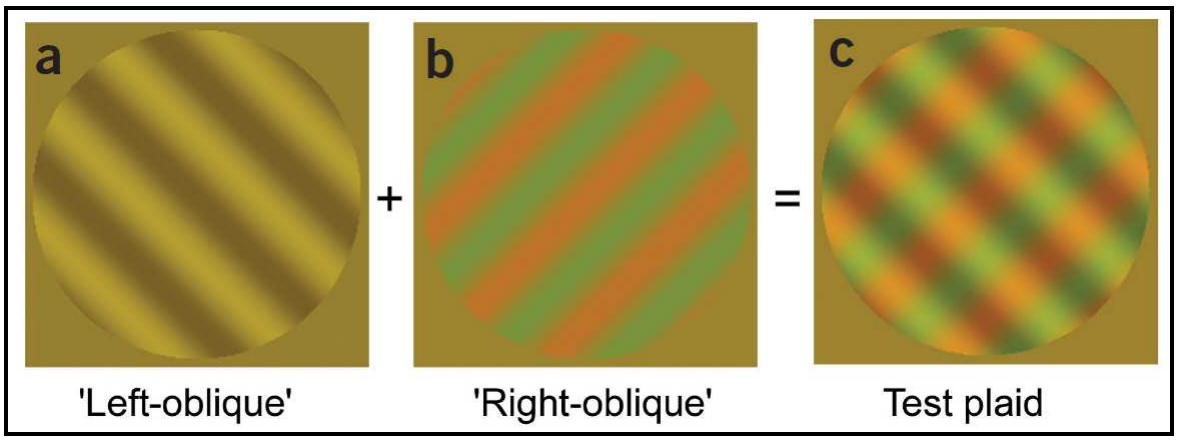


Figure 6–7. Shape from Shading.
(Image taken and adapted from(Kingdom, 2003). The illusion of depth seen in (c) is created by combining the luminance grating (a) with the colour contrast grating (b).)

Therefore, we might expect that evolution might take advantage of this and use patterns that create a false impression of depth to disguise the true contours or outline of an organism (it is also likely that we will find pattern elements that work to flatten a contoured surface). A potential example of shading used to create a false impression of depth might be found in the peach blossom moth (*Thyatira batis*). The moth is fairly common in woodland habitats throughout the UK and has a pattern which incorporates pink and brown spots on a brown background. The adult moths fly in June and July when their woodland habitat is likely to have a mostly green light. Therefore, I speculate that the pink colouration might create a false shadow and the spots interpreted as literal ‘holes’ in the wing surface of the moth. In Figure 6–8 you can see that even when some of the spots on the peach blossom moth are replaced with a photograph of a hole bored in wood the image looks surprisingly similar to the original. Using varying amounts of dark or lighter shading the angle at which the ‘hole’ appears to go down may be changed from straight down or off to an angle.

Figure 6–8. Peach blossom, (*Thyatira batis*) spots viewed as 'holes'.
The image on the left is the original image of the moth and the image on the right is where the spots have been replaced with a photograph of a drilled hole in wood. The only changes made to the original image of the hole were orientating and stretching to fit the spot sizes and the addition of a similar pink tinge to the lighter areas.

It is my opinion that the interplay of colour, shadow and light has been over looked for the most part in animal camouflage. It seems to me to be fertile ground for evolution to work on and for organisms to develop patterns that take advantage of the assumptions and shortcuts visual systems make in the way they process the world.

Appendix ii. Comparison of Eyespot experimental techniques and methodologies

Jones, 1980 & Stevens et al, 2007, 2008 a, 2009a.

Both Jones and Stevens et al carried out similar experiments with the aim of examining predator avoidance of eye-spots and in particular what attributes cause these reactions i.e. is the reaction elicited due to their similarity to eyes or due to their conspicuous colouration? Despite many similarities in their approach these experiments produced two opposing conclusions; with Stevens et al concluding that conspicuousness is the determining factor and Jones that eye mimicry is integral. Here I examine both experiments in an attempt to find possible explanations for such differing opinions.

The basic experimental setup for both Stevens and Jones was similar, with both using avian predators and cardboard stimuli bearing shapes designed to be more or less eye-like in their appearance. However, while Jones chose a lab based system with naïve male domesticated chicks, Stevens used a field setup and an assemblage of wild birds. It is this choice of field over lab setup which introduces many of the differences between the two sets of experiments. In particular the use of captive chicks greatly reduced the number of replicates that Jones could carry out.

Jones produced their stimuli using plain white card with the test patterns marked in indelible black ink bordered by a basic representation of a head and beak. The stimuli were introduced to the chicks from a maximum distance of 680mm (the length of the box in which they were housed). Stevens used printed card with a background colour intended to be half-way between black and white, with the stimuli pinned to trees at a height of 1-3m. The average distance at which they were first encountered is likely to have varied considerably, but we can assume that on average the stimuli were likely to be seen from some distance before being approached. This may have affected the way in which the stimuli were perceived particularly, as pointed out by Stevens, the diamond (or square?) stimuli may look similar to the circular stimuli from a distance.

The criteria to determine what effect the stimuli have had differs considerably between the experiments, with Jones using behavioural cues such as freezing, avoidance, distress and number of steps or jumps of the test chick. Conversely Stevens et al used the removal of a bait attached to the stimulus as an indication of how effective the stimulus was at inducing predator avoidance (with baits removed by non-avian predators discounted).

Overall, both approaches have their strengths and weaknesses with the lab experiments providing greater control and observational opportunities, but lacking in realism and the field studies limiting our ability to observe the way the stimuli are approached and treated by predators, but allowing the stimuli to be selected by the predator community rather than one individual.

A compromise perhaps between the two that might enable us to use the best aspects of both techniques may be an aviary study using wild birds. This would allow greater opportunities to observe predation as it occurs, but in a more natural setting.

	Stimulus	Result		Stimulus	Result
Jones, 1980	Exp1: No eyes	No sig. result (not aversive)	Stevens et al, 2008a	Exp1: No spots (Control)	Least surviving
	Exp1: 1 eye	Sig. avoidance	2008a	Exp1: 1 large spot	Survived sig. better than other stimuli (barr 3 spot)
			2008a	Exp1: 1 small spot	No diff. between small spot stimuli
	Exp1: 2 eyes	Sig. avoidance + greater than 1 eye	2008a	Exp1: 2 large spots	Survived sig. better than other stimuli (barr 3 spot)
	Exp2: Diamond shape + cross pupils	No avoidance - taken as proof that eyes aren't avoid just as conspicuous objects			
	Exp2: Vertical eyes	No avoidance	2009a	Exp1: Vertical bars	No diff. compared to horizontal or circles
				Exp1: Vertical circles	No diff. compared to horizontal or bars

Exp2: 3 eyes	No avoidance	2008a	Exp1: 3 small spots	Best survival (but 1 large & 2 large survived qualitatively better)
Exp3: Circular surround alone	No sig. result (not aversive) - compared to No eyes			
Exp3: Pupils alone	No sig. result (not aversive) - compared to No eyes	2008	Exp5: Paired black circles	Did not survive as well as concentric circles
Exp3: Rectangular + pupil	Sig. increase in 'passive' behaviour & decrease in activity, vocalisations & time in stimulus area.	2009a	Exp1: Horizontal bar	Survived sig. better than no treatment
Exp3: Rectangular surround alone	No sig. result (not aversive) - compared to No eyes			
Exp4: 2 large eyes	Sig. avoidance (not sig. but perhaps slightly more aversive than small eyes)	2008a	Exp1: 2 large eyes	Survived sig. better than smaller treatments
Exp4: 2 small eyes	Sig. avoidance		Exp1: 2 small eyes	Survived sig. less than larger eyes

Appendix iii. British Caterpillar Database

This database was intended to catalogue the physical characteristics of approximately 864 British caterpillars. The main sources of data used were The colour identification guide to: Caterpillars of the British Isles by Jim Porter (Porter, 1997) and A field guide to caterpillars of Britain and Europe by David J. Carter(Carter and Hargreaves, 1986).

The purpose of compiling this database was to use the information to investigate the relationship between aposematic colouration, physical characteristics and life history traits.

To do this I collated data on these physical attributes:

1. The instar being described
2. Colour: Background colour, Head colour, Colour patterns (stripes, spots, etc) Countershading, Cryptic, Aposematic or Masquerade
3. Hair
4. Body shape & texture
5. Body size

And these life cycle attributes:

1. Number of broods e.g. Univoltine/Bivoltine
2. Month of pupation, emergence and laying
3. Are eggs laid eggs laid singly, in groups or scattered
4. Life span
5. Pupation site
6. Over-wintering stage e.g. adult, pupa, etc
7. If larvae are known to be diurnal/nocturnal/corpuscular
8. Gregariousness

The data collected was used in the paper:

Higginson AD, de Wert L, Rowland HM, Ruxton GD, Speed MP (in press) Masquerade is associated with polyphagy and larval overwintering in the Lepidoptera. *Biological Journal of the Linnean Society*

Supplementary Materials

Green (P)Groups	Before	After	Red (UnP) Groups	Before	After
1	10	0	1	10	8
2	10	8	2	10	10
3	10	0	3	10	10
4	10	8	4	10	9
5	10	0	5	10	6
Total	50	16	Total	50	43

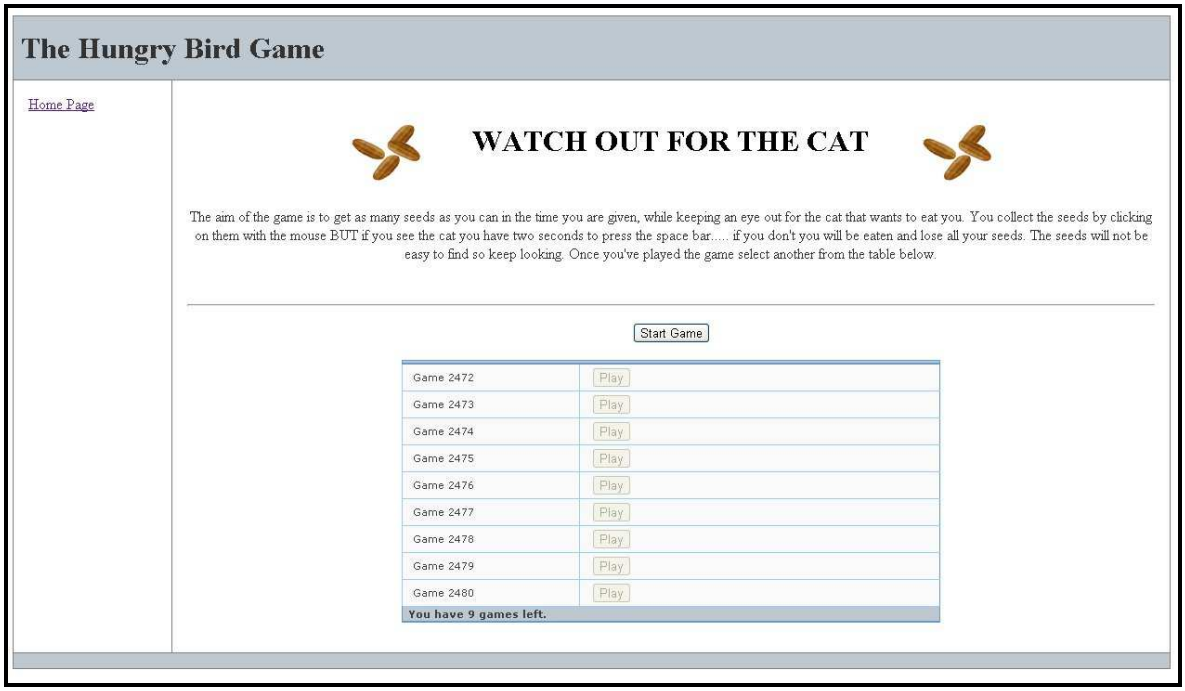
Supplementary Table i. Pilot Results
***Mann-Whitney U test: U-value = 3.000, P-value = 0.056**

	Groups	Average Surviving
Day 1	6x 10 Palatable Green seeds	3.2
	6x 10 Palatable Red seeds	3.3
Day 2	5x 10 Unpalatable Green seeds	6.4
	6x 10 Unpalatable Red seeds	7.2

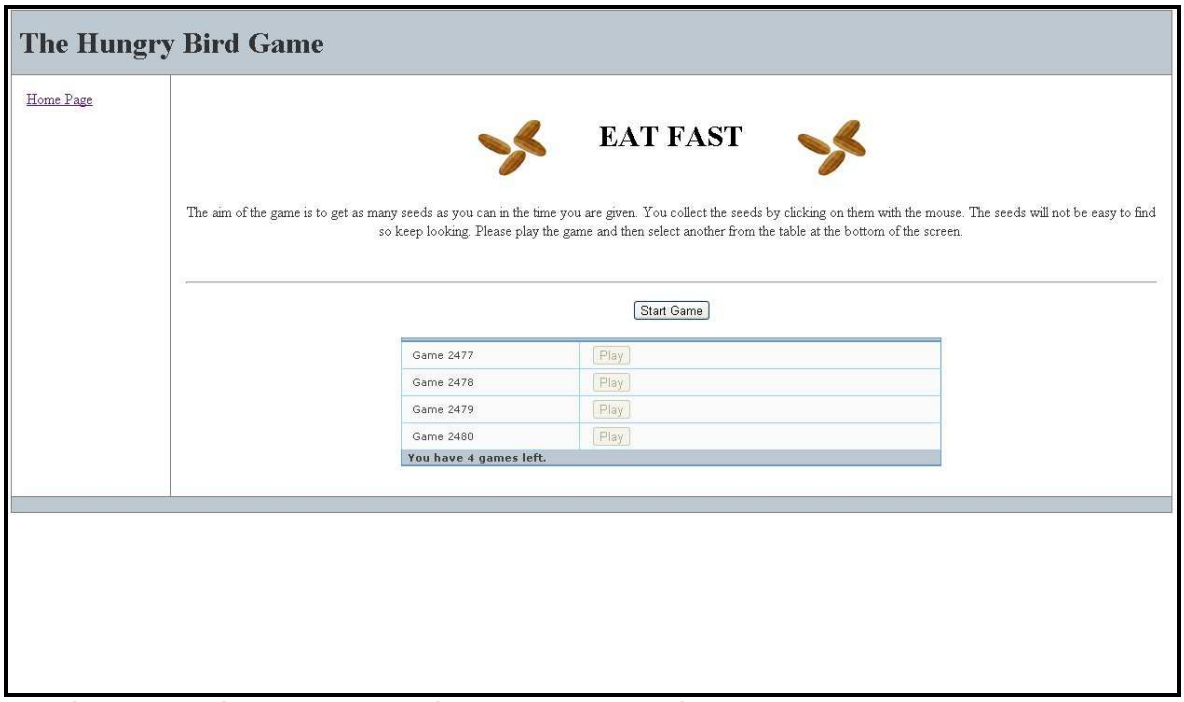
Supplementary Table ii. Small scale test results.
***Anova test of colour: p-value = 0.803**

	Group type	5 seeds	10 seeds	Mixed 5&5
Experiment 1	Average % Red Survival (UnP)	89.0	82.0	88.0
	SD	17.7	17.4	23.8
	SE	4.0	3.9	5.3
	Average % Green Survival (P)	29.0	32.5	74.0
	SD	32.1	30.9	26.8
	SE	7.2	6.9	6.0
Experiment 2	Average % Red Survival (P)	44.7	49.3	71.3
	SD	35.9	36.7	33.9
	SE	6.6	6.7	6.2
	Average % Green Survival (UnP)	64.0	66.3	73.3
	SD	35.8	34.8	32.9
	SE	6.5	6.4	6.0
Experiment 3	Average % Red Survival (P)	42.0	41.5	49.0
	SD	34.9	34.4	44.7
	SE	7.7	7.7	10.0
	Average % Green Survival (P)	30.0	22.0	51.0
	SD	34.6	25.7	44.7
	SE	7.8	5.7	10.0

Supplementary Table iii. Average % of seeds surviving per Group, per treatment (unP = Unpalatable, P = Palatable.)



Supplementary Figure 1. Predator Game Instructions



Supplementary Figure 2. Non-predator Game Instructions

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