Predator Cognition and the Evolution of Deimatic Displays

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Abstract

Deimatic displays are a unique form of prey defence where prey perform a complex display including any combination of movements, postures and visual, auditory, vibratory and/or olfactory stimuli. There is some evidence supporting the idea that displays deter predators, however, it is unclear why. The evolutionary route via which these complex displays evolve is not well understood. Using a novel experimental paradigm, naïve domestic chicks (*Gallus gallus domesticus*) were presented with computer-generated moth-like deimatic prey to investigate how deimatic displays may have evolved, factors influencing their success, and when prey should produce them. Prey that flicked their forewings to reveal cryptic hindwings deterred predators (when forewing movement was fast), but predators were deterred even more when the hindwings revealed were conspicuously coloured. These results demonstrate that deimatism could evolve if fast movement evolved first, followed by the evolution of conspicuous colouration. Since deimatic displays are widely considered to startle predators, I tested whether factors known to influence the magnitude of startle responses also influence the efficacy of deimatic displays (specifically, hunger, background noise, and affective state). I did not find any effect of hunger on the responses of predators to deimatic displays. Background noise was found to influence the distance at which predators were positioned during the time interval when they decided to attack prey. Although unaltered by an anxious-like state, predators in a depressive-like state were found to attack deimatic prey much faster than conspecifics in a neutral affective state. Finally, I investigated whether temperature influenced the likelihood that live Peacock butterflies (*Aglais io*) would perform deimatic displays. They displayed more often and for longer durations at cooler temperatures. Thus, in this thesis I provide the first evidence of a viable evolutionary route to deimatism, and establish the factors likely to influence the efficacy and production of deimatic displays. In doing so, I have increased the understanding of the conditions under which deimatism is likely to evolve.

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Abbreviations

% = Percentage
± = Plus or minus
° = Degrees
ASAB = The Association for the Study of Animal Behaviour
BORIS = Behavioural Observation Research Interactive Software
cm = Centimetre
M = Mean
m = Metre
mm = Millimetre
ms = Millisecond
N = Sample size
s = Second
SE = Standard error
fps = Flicks per second

Chapter 1. Introduction: Predator Cognition and the Evolution of Deimatic Displays

1.1 What are deimatic displays?

Antipredator defences are classic examples of natural selection at work and studying them has been instrumental in developing and testing evolutionary theory (Guilford, 1988; Brodie and Brodie, 1999). These adaptations also influence the outcomes of predator-prey interactions, which can have important effects on both population dynamics and community structure (Naeem, 1988; Lima, 1998; Preisser, Bolnick and Benard, 2005). Whilst many forms of defence are well studied, particularly camouflage (Stevens and Merilaita, 2009; Skelhorn, Rowland and Ruxton, 2010; Skelhorn and Rowe, 2016), aposematism (Mappes, Marples and Endler, 2005; Skelhorn, Halpin and Rowe, 2016) and mimicry (Speed and Turner, 1999; Kikuchi and Pfennig, 2013), others have received much less attention. A prime example are the deimatic displays used by animals from a wide range of taxa, including reptiles (Whiting, Noble and Somaweera, 2015), amphibians (Lenzi-Mattos, M. M. Antoniazzi, *et al.*, 2005), cephalopods (Mather and Mather, 2004; Langridge, 2009), and crustaceans (Staaterman, Claverie and Patek, 2010), and are particularly commonly reported among insects (Maldonado, 1970; Vallin *et al.*, 2005; Olofsson *et al.*, 2012a, 2013; Umbers, Lehtonen and Mappes, 2015; Dookie *et al.*, 2017). The form of these displays differs among species, but they often involve animals assuming characteristic postures and presenting striking auditory, visual and/or olfactory stimuli to would-be predators. For example, when disturbed, underwing moths (*Catocala* sp.) reveal brightly patterned hindwings that contrast with their cryptic forewings (Sargent 1978; Figure 1); peacock butterflies (*Aglais io*) flick their wings rapidly and rotate their body in the direction of predators, exposing two pairs of previously unseen eyespots (Blest, 1957); and elephant hawk moth larvae (*Deilephila elpenor*) inflate and sway their anterior eye-spotted body segments (Edmunds, 1974). Deimatic displays therefore incorporate a variety of components and can vary greatly between different deimatic species.

Figure 1. Deimatic displays (from left to right): Red underwing moth (*Catocala nupta*), photo: John B; Peacock butterfly (*Aglais io*), photo: G. Holmes; Larva of elephant hawkmoth (*Deilephila elpenor*), photo: Lynk media.

The complexity of deimatic displays, and the fact that their form differs so much among species, has meant that defining them has proved somewhat difficult. Several definitions of the term ‘deimatic display’ have been suggested, but most are somewhat subjective and are based on descriptions of the display itself (see Table 1 for examples). Whilst these are perhaps sufficient for identifying putative deimatic displays, they make it difficult to distinguish between deimatism and other forms of defence and tell us nothing about the function of these displays. In recent years, greater attention has been placed on the need to clarify and refine the definition of deimatic displays and the discussion surrounding this has at times been somewhat contentious (see Skelhorn et al. 2016; Umbers and Mappes 2016). Whilst Umbers and Mappes (2016) initially supported the use of definitions based (at least in part) on the physical form of the display, Skelhorn *et al*. (2016) stated that the defining feature of deimatic displays should be the mechanism(s) through which they promote avoidance in predators. The benefit of the latter suggestion is that it tells us about the selective pressures leading to the evolution of these displays, and this in turn provides a meaningful way to differentiate between deimatic displays and other forms of prey defence. In a recent paper, Umbers et al. (2017) updated their position in order to focus more on the mechanism through which deimatic displays deter predators. They now define deimatism as “a behaviour by a sender that gives rise to a sudden transition in sensory input, causing the receiver to recoil reflexively”. Although not perfect (even when predators are deterred by deimatic displays they often do not ‘recoil’ from them), this definition explicitly states the mechanism via which deimatic displays deter predators: they elicit reflexive avoidance responses. This definition is therefore testable and allows deimatic displays to be distinguished from other forms of defence. Consequently, although it is likely that this definition of deimatism will be refined further once we have established exactly how deimatic displays deter predators, it is currently sufficient to underpin research in this area.

Table 1. Definitions of deimatic displays.

Source	Definition
Edmunds, 1974	The adoption of a characteristic display, posture and/or frightening noise, which appears to be designed to intimidate the predator, often involving a variety of different movements and includes bright colours, stridulation and sometimes glandular secretions.
Ruxton et al. 2004	Signals (in the form of the sudden appearance of a bright display or loud noise) induced by the proximity of a predator, which are designed to make a predator hesitate in its attack by inducing an element of fear or confusion in the predator giving the prey individual an increased chance of fleeing before being attacked.
Umbers et al. 2015	“Behaviour in which, when under attack, prey suddenly unleash unexpected defences to frighten their predators and stop the attack.”
Umbers and Mappes, 2016	“A momentary, transient, conspicuous signal that induces a startle response or overloads the senses of an attacking predator, such that the predator pauses, slows or stops the attack.”
Skelhorn et al. 2016	“Any defensive display that causes a predator to misclassify a prey as a potential threat to its immediate safety.”
Umbers et al. 2017	“Deimatism involves a behaviour by a sender that gives rise to a sudden transition in sensory input, causing the receiver to recoil reflexively.”

This most recent definition from Umbers *et al*. (2017) is useful in that it allows those researching deimatism to distinguish between it and other forms of prey defence. For example, unlike crypsis, deimatism is a form of secondary defence, whose advantage is provided after detection by predators, whereas crypsis protects the prey that possess it by their avoidance of detection and interaction with predators. On the other hand, whilst possessing some similarities with aposematism, deimatic displays differ from that form of defence as no learning is required by predators but rather deimatic displays elicit reflexive unlearned responses in the predators that encounter them (Umbers *et al*. 2017). Further, unlike aposematic prey many of whom possess a conspicuous which signal is constantly on display, deimatic prey possess the ability to turn their displays ‘on’ and ‘off’. Although parallels may also be drawn between certain components of deimatism and those of mimicry, deimatic displays differ in that they exploit unlearned responses to dangerous prey in their predators. Therefore, although at first glance deimatism may appear to include components that are seen in other forms of defence, it is a distinctly unique prey defence.

1.2 Do deimatic displays deter predators?

Despite the widespread occurrence of deimatic displays, and the ecological and evolutionary importance of studying prey defences, relatively few empirical studies have attempted to test whether or not these displays actually deter predators, and none have directly addressed the issue of why predators are deterred by them. Although many deimatic prey are seemingly not chemically defended (e.g. Maldonado, 1970; Vallin *et al.*, 2005; Olofsson *et al.*, 2012a; but see Umbers & Mappes, 2015 for a putative example of a deimatic display in a chemically defended species), and may pose no real risk to predators, it has been shown that their displays can cause predators to pause or abandon their attacks (Vallin *et al.*, 2005; Olofsson *et al.*, 2012a, 2013). A small number of studies have monitored predators' responses to deimatic prey, and in some cases, compared these to their responses in control conditions in which they were presented with either dead prey or prey whose displays had been manipulated in a manner likely to reduce the magnitude of the display (see Table 2 for an overview of such studies published between 1970 and the present). Whilst deimatic displays were shown to be ineffective against some predators (Edmunds, 1972), this was not uniformly the case, and there is now good (although not abundant) evidence that least some deimatic displays serve an anti-predator function. However, these observations do not tell us why predators are deterred by deimatic displays, and it is vital that we understand this if we want to determine why these displays have evolved, under what ecological conditions, why they take the physical forms they do, and how they differ from other forms of defence (Skelhorn, Holmes and Rowe, 2016).

Table 2. Research investigating the anti-predator function of deimatic displays. Following a systematic literature search using terms used to refer to deimatic displays, I identified experimental research articles focused on the investigation of the anti-predator function of deimatism and compiled them into the following table. Search terms: 1. Deimatic Behaviour, 2. Deimatic Display, 3. Startle Signal, 4. Deimatic Reaction, 5. Startle Display, 6. Defensive Display, 7. Frightening Display, 8. Frightening Attitude. See Appendix A for further details of the methodology used to create this table.

Prey	**Predator**	**Deimatic Display**	**Methodological Approach**	**Predator Responses**	**Anti-Predator Evidence**	**Authors' suggested mechanisms**
			Observational Studies			
Grandcolas and Desutter-Grandcolas 1998[2]						
Madagascan marbled mantis (*Polyspilota aeruginosa*)	Yellow-vented Bulbul (*Pycnonotus barbatus*)	The mantis stands on its middle and hind legs, its four wings apart, with the black and red stripes visible on its hind wings. Its forelegs are outstretched, their bluish inner sides with black ocelli turned forward. This posture is maintained for several seconds and is emphasised several times by leg stretching and slight wing movements.	An interaction between predator and prey was observed in the field.	The bird attempted to attack the mantis during the display, however it eventually retreated and left the area.	Although the bird charged and attacked the mantid several times during a two-minute period, it eventually withdrew and flew away.	None
Roonwal 1983[8]						
Desert mantis (*Eremiaphila braueri*)	Predator attack simulated by presence of experimenter	Unfolds its wings and stands up on its hind-legs “like a performing bear.”	The experimenter observed prey’s reaction to encountering themselves in the field.	Not recorded	None	None
			Live Predator – Live Prey			
Dias et al. 2014[1*]						
Neotropical harvestman spider	Large wandering spider *Ctenus ornatus*	Intense dorso-ventral movements performed with the legs toward predators that are	Prey were placed into an arena with a predator present and the resulting	Whether the predator attacked or moved and, what direction it moved in.	Half of the predators did not move in relation to prey, 5 (of 12)	None

(*Mischonyx cuspidatus*)		sensitive to air displacements and vibrations.	interactions were recorded.		moved away from prey and 1 predator attacked prey.	
Langridge 2009[1-3,5,7]						
European Common Cuttlefish (*Sepia officinalis*)	Sea bass (*Dicentraarchus labrax*); Smooth hound fish (*Mustelus mustelus*)	A conspicuous black and white pattern is suddenly exposed, involving dark eyes and/or eyespots and a dark contour, the body is flattened and the fin extended to create the illusion of large size. They hover above the substrate and orient the display towards a predator (Hanlon and Messenger, 1996).	Prey were enclosed in a transparent plastic box which was located within a tank containing various predatory fish species, with the aim of the presence of a predator eliciting a display.	Not recorded	None	None
Maldonado 1970[4]						
Praying Mantis (*Stagmatoptera biocellata*)	Venezuelan troupials (*Icterus icterus*); Shiny cow-birds (*Molothrus bonariensis*); Java sparrows (*Padda oryzivora*); Atlantic canaries (*Serinus canaria*)	Antennae are positioned backwards, mouth parts open wide showing the coloured mandibles, prothorax raised, forelegs extended laterally, flexed, and showing black spots; the tegima are held straight up, the shiny wings are simultaneously elevated; the abdomen is twisted to the side and tilted, and colour bands, which are not otherwise visible, are exposed; the insect stridulates, making a noise by rubbing the abdomen up and down between the hindwings;	Prey were placed into a cage which contained a predator.	Non-insectivorous bird species maintained a distance from the mantis, and all of the insectivorous bird species started attacks but did not complete them.	Predators avoided the mantids, and attempted to fly away from them.	Deimatic displays work by frightening predators.

		and the entire insect sways from side to side.				
Olofsson et al. 2012a[1-3,5]						
Swallowtail butterfly (*Papilio machaon*)	Great tits (*Parus major*)	Exposes their brightly coloured dorsal wing surface upon disturbance and, if the disturbance continues, intermittently protracts and relaxes its wing muscles generating a jerky motion of the wings.	Individual predators were presented with a pair of prey on a wooden log upon which they had been trained to forage for mealworms. One of butterflies was alive (able to display), and the other was dead and pinned such that its wings were open. A mealworm was pinned below each butterfly.	i) Which butterfly was visited first, ii) The number of visits to each butterfly, until the mealworm/butterfly was attacked, iii) Which butterfly (/mealworm) was attacked first, iv) The time until the first and the second mealworm/butterfly was attacked, v) Startle scores were assigned using the following scale: 0 = No reaction or approaching the butterfly, 1 = Flinching and/or retreating by hopping away from the butterfly, 2 = Retreating by flying away.	Most birds flew away instantly, or flinched and/or retreated by hopping away from the live butterfly when it performed its display.	That the responses of the blue tits are indicative of the types of responses birds demonstrate towards real danger, and therefore the displays provide a bluff of a genuine threat to the birds.
Olofsson, Jakobsson and Wiklund 2012b[3,5]						
Peacock butterfly (*Aglais io*)	Wood mice (*Apodemus sylvaticus*); Yellow-necked mice (*Apodemus flavicollis*)	Suddenly produces sonic and ultrasonic sounds and displays four large eyespots when attacked by flicking its wings.	Predators were presented with butterflies with their sound producing parts either intact or removed.	The distance to which the mice fled during their first encounter with the display.	Predators fled further from butterflies that produced the auditory component of their display.	The responses of the mice suggest that the auditory components simulate sounds produced by

						rodent prdators.
Staudinger et al. 2011[1,4]						
Longfin squid (*Doryteuthis pealeii*)	Summer flounder (*Paralichthys dentatus*); Bluefish (*Pomatomus saltatrix*)	One or more of the following: Squid face the direction of approaching predator while maintaining position in water column near the surface; Arms flared upwards, exposing beak; Squid hangs vertically in water column near the surface, arms and sometimes tentacles droop downwards; Both tentacles are extended as squid is swimming; Amber and pink coloration with brown banding; used to camouflage against substrate while dropping to the bottom, resting on the bottom and while swimming in the water column; Rapid change in body colour to deep brown or red.	Prey were introduced to a tank containing predators.	The number of predator-prey interactions and their outcome; mortality, escape or abandoned attack	Deimatic displays were successful in deterring bluefish but were not as important in relation to flounder	They act to make the squid appear larger which may be a deterrent to gape-limited predators.
Staudinger et al. 2013[1,2,5,6]						
European Common Cuttlefish (*Sepia officinalis*)	Bluefish; Black seabass (*Centropristis striata*); Summer flounder	Dark mottle body pattern often with dark fin line; entire body uniform dark brown; head and arms white in contrast to mantle, which may be light or dark mottle; head and arms uniform dark in	Prey were enclosed in a transparent cylinder within a tank which contained one of the three predator species.	i) Where in the water column predator-prey interactions occurred for each of the predators. ii) Predator behaviour towards deimatic display (observed from video recordings): Flounder –	Displays disrupted seabass attacks, causing them to hesitate or turn away. They also caused bluefish to abandon their	None

		contrast to mantle, which may be light or dark mottle; cark circle around eyes, often shown with raised head; eyespots on mantle, no movement; eyespots displayed on mantle, cuttlefish rotates body toward approaching predator with drooping arms and hangs vertically with mantle pointing downward in water column; hovering in water column with splayed arms; sitting on substrate, flattened body, with splayed arms		did not purse or attack cuttlefish; Seabass – Raised dorsal fins, spread pectoral fins, slowed/halted swimming, turned away from cuttlefish; Bluefish – Abandoned approach and turned away from cuttlefish.	approach and turn away.	
Vallin et al. 2005[1]						
Peacock butterfly (*Aglais io*)	Blue tits (*Cyanistes caeruleus*)	Performs a repeated sequence of movements exposing major eyespots on the wings accompanied by a hissing noise.	Predators were presented with live prey that were either i) unchanged, ii) eyespots painted black, iii) sections of their wings similar in size to their eyespots painted black, iv) sound producing wing sections removed, v) sections of wing removed but were able to produce sound, vi) sound-producing sections removed and	i) Duration of time the birds spent within 10cm from the butterfly, ii) Whether the butterfly survived, iii) No. of bird-butterfly interactions, iv) The time between interactions.	The butterflies' eyespots were the most pertinent component of the display in deterring predatory attacks.	None

			eyespots painted black.			
Vallin et al. 2007[1]						
Peacock Butterfly (*Aglais io*); Eyed hawkmoth (*Smerinthus ocellatus*)	Great tits; Blue tits	Peacock butterfly: “flicks its wings open, suddenly exposing its eyespots, and continues to close and flick open its wings and also seems to track and follow the bird predator as long as it is under attack (Blest 1957).” Eyed hawkmoth: “exposes its eyespots on the hindwings by protracting its forewings and performs a rocking movement to and fro by bending its legs while keeping the eyespots fully exposed (Blest 1957; Tinbergen 1958; personal observations).”	Both predator species were presented with live individuals of both prey species.	Whether predators retreated in response to their first exposure to the display of both prey species.	No - This study focused on the comparison of the deterrent effect of peacocks and eyed hawkmoths and found that both bird species killed more hawkmoths than peacocks.	None
Watanabe and Yano 2010[1,2,4,6]						
Japanese Giant Mantis (*Tenodera aridifolia*); Giant Asian Mantis (*Hierodula patellifera*); Asian jumping mantis (*Statilia maculata*)	The Japanese skink (*Takydromus tachydromoides*)	Turns toward the predator with the prothoracic legs held close to the thorax at an angle of 180˚ to each other; the antenna are directed toward the predator, and the wings are partially raised	Predators were released into cages containing an individual prey.	Not recorded	No	None

Live Predator – Artificial Prey						
Dookie et al. 2017[2,3,5]						
Defensive sounds of Walnut sphinx caterpillar (*Amorpha juglandis*)	Red-winged blackbird (*Agelaius phoeniceus*)	“When attacked, generates whistles from its spiracles."	The predator was presented with recordings of defensive sounds of prey while feeding.	1. Startle: Based on the scoring system; i) No reaction, ii) Flinching one or both wings without flying away from platform, iii) Full extension of one or both wings without flying away from platform, iv) Shaking/trembling feathers raised up from skin, v) body flinch - a whole body sudden short movement, vi) a sudden upward hop where both feet go up in the air and back down to the ground, vii) flying off platform, flapping both wings 2. Latency to return to the feeding dish	Startle scores were higher in response to caterpillar whistles in comparison to control conditions (no sound).	Caterpillar whistles elicit an innate startle response, causing predators to abandon the prey.
Kang et al. 2017[1-3,5,6]						
Robotic moth based on the Underwing moth genus (*Catocala* sp.)	Black-capped chickadees (*Poecile atricapillus*)	Contrasting hindwings are displayed under an imminent predatory threat to frighten predators.	A robotic moth which differed in size and perform a deimatic display was presented to groups of predators in the field at a feeding station.	Startle response with the following scoring system: 0 = Stayed in the arena and took the mealworm before the display was over; 1 = Flew off to a nearby branch, but landed back on the arena and took the mealworm before the display was over; 2 = Flew off from the arena and did not take	Survival benefits were seen in the artificial deimatic prey regardless of their size.	None

				the mealworm until the display was over.		
Simulated Predator – Live Prey						
Kowalski et al. 2014[1]						
Ornate Bush-cricket (*Poecilimon ornatus*)	Simulated by experimenter	Both sexes rely on secondary defence by stridulating and regurgitating after an attack, at differing frequencies.	A predatory attack was simulated by an experimenter grasping i) the pronotum and ii) the hind legs at the joint between femur and tibia.	Not recorded	None	None
Lenzi-Mattos et al. 2005[1]						
Cuyaba dwarf frog (*Physalaemus nattereri*)	Simulated by experimenter	Puffs up body laterally by inflation of lungs, turns back to predator and elevates hindparts. Black inguinal glands are exhibited with the coccyx. When intensely harassed, the black skin covering the macroglands turns whitish as a consequence of a milky secretion.	Disturbance of the prey's terrarium by the predator was determined to simulate a threatening situation.	Not recorded	None	None
Martins 1989[1]						
Colombian four-eyed frog (*Pleurodema brachyops*)	Simulated by experimenter	Inflating the body and elevating the hindparts, thus displaying the lumbar glands, the orange spots of the inguinal region and posterior surfaces of the thighs. The head is lowered a little and the eyes are open. When re-stimulated, the posture is enhanced and when not,	The deimatic display was stimulated by experimenters preventing prey individuals' ability to escape and tapping them on the back.	Not recorded	None	None

		the posture is abandoned after a few seconds.				
Whiting et al. 2015[2,3]						
Leaf-nosed Lizard (*Ceratophora tennentii*)	Simulated by experimenter	Mouth gapes revealing brightly coloured tongue.	Experimenters waving hands 10cm away in the field and being captured by hand were used as simulations of predatory encounters	Not recorded	None	None

1.3 Why are predators deterred by deimatic displays?

There have been a number of suggestions as to why predators may be deterred by deimatic displays. However, as highlighted by Umbers et al. (2016), these all rely on the assumption that deimatism exploits reflexive responses in predators. Indeed, Umbers et al. (2017) recently identified four ways in which deimatic displays have been proposed to do this: (i) by releasing the ‘startle reflex’ in predators (Crane, 1952; Ruxton, Sherratt and Speed, 2004); (ii) by exploiting predators’ fear responses (Edmunds, 1974; Ruxton, Sherratt and Speed, 2004; Skelhorn, Holmes and Rowe, 2016); (iii) by causing predators to misclassify potential prey as a threat (Skelhorn, Holmes and Rowe, 2016); and, (iv) by overwhelming predators’ senses (Umbers and Mappes, 2016b). I would add one further suggestion: deimatic displays could work by: (v) exploiting reflexive responses to looming stimuli (Kane, Fulton and Rosenthal, 2015). Below, I will explore each of the suggested mechanisms in turn, assess the limited indirect evidence in support of them, and explain how better establishing the effects of displays on predators could inform our understanding of the evolution, ecology and behaviour of deimatic prey. My aim is to provide a thought-provoking discussion of why deimatism works and promote the integration of the experimental psychology and deimatism literatures. Whilst deimatism may (or may not) have functions above and beyond antipredator defence (e.g. communicating with conspecifics; Ruiz-Rodríguez, Martín-Vivaldi and Avilés, 2017), I will focus on understanding why these displays deter predators since the limited work in this area suggests that predation is a key selective pressure driving the evolution of deimatism.

1.3.1 Do deimatic displays release predators’ startle reflexes?

The first, and most commonly proposed, mechanism suggested to explain how deimatic displays deter predators is that they do so by eliciting a startle response. Deimatic displays are often informally referred to as ‘startle displays’, in that predators can often appear startled by a displaying prey (e.g. Olofsson *et al.*, 2012a). However, looking startled is different from being startled. Being startled involves triggering the ‘startle reflex’, a reflexive response that has been carefully defined and extensively studied (Davis, 1984; Vrana, Spence and Lang, 1988; Koch, 1999). The startle response appears to be an adaption that interrupts what an animal is currently doing and produces physiological and behavioural changes (e.g. freezing, increased heart rate, tension of facial muscles) that help it evade an immediate danger (e.g. Eaton, Bombardieri and Meyer, 1977; Skelhorn *et al.*, 2016). What is interesting about the startle reflex is that it occurs in response to a very specific set of stimuli; sensory stimuli, whether auditory or visual, need to have a high intensity and a sudden onset to trigger the response (Koch and Schnitzler, 1997; Koch, 1999; Deuter *et al.*, 2012). For example, in mammals, sounds typically need to be above 80-90dB, with rise times (the time taken for the stimulus to reach its maximum amplitude) of less than 12ms, to elicit a startle response (Davis, 1984), but sounds of 60dB can also be effective if they have close to instantaneous rise times (Åsli and Flaten, 2012). Some deimatic displays do possess the characteristics required to elicit startle responses. For example, Dookie et al. (2017) found that the deimatic sounds produced by the Walnut sphinx caterpillar (*Amorpha juglandis*) elicited behavioural responses in birds typical of those observed in relation to startling stimuli.

Although some studies have found evidence indicative of predators being startled by deimatic displays (e.g. Vallin *et al.*, 2005; Kang, Zahiri and Sherratt, 2017), these findings are based on somewhat subjective scales of predators' behavioural responses. In contrast, within the field of experimental psychology, more objective measures such as the use of heart rate monitors to measure differences in heart rate (Richter *et al.*, 2011), or electrodes placed on the skin to measure facial muscle tension (Lang, Bradley and Cuthbert, 1990). To be certain that predators are startled by deimatic displays, discrete measures of physiological changes that concur with the findings of previous research (e.g. that startling stimuli cause an increase in heart rate (Richter *et al.*, 2011)) are required. However, it is important to remember, that it may not be possible for all deimatic species to produce startling stimuli. Some putative deimatic displays do not appear to have intense and rapidly produced displays (e.g. the mountain katydid (*Acripeza reticulata*), and the larva of the elephant hawkmoth (*Deilephila elpenor*)). Therefore, although the startle response is taxonomically widespread, and exploiting it could be a potentially valuable way for deimatic displays to work, this may not be the case for all deimatic species.

1.3.2 Do deimatic displays elicit predators' fear responses?

Deimatic displays have also been suggested to deter predators by exploiting their innate fear responses (Umbers *et al.*, 2017). Fear responses can be described as either "phasic" or "sustained". Phasic responses occur rapidly (within 100ms of stimulus onset; Pomeroy, 1973) in response to imminent and perceived danger, and their effects dissipate quickly. On the other hand, sustained fear responses (or anxiety) have a slower onset, are aimed toward unspecified threats, and can last for days at a time (Sato and Yamawaki, 2014; Tovote *et al.*, 2016). Deimatism would elicit phasic fear responses, which would cause predators to halt their attacks, and perhaps even distance themselves from deimatic prey. It can prove difficult conceptually to distinguish between startle and fear responses as they can both involve abrupt and rapid changes to physiology and behaviour, including an increase in heart rate, freezing or a rapid evasive movement away from the threatening stimulus (Pomeroy, 1973; Eaton, Bombardieri and Meyer, 1977; Åsli and Flaten, 2012; Yilmaz and Meister, 2013; Sato and Yamawaki, 2014). These defensive reactions need to be rapid in order to allow animals to escape potential harm (Tovote *et al.*, 2016). However, although startle and fear responses may appear quite similar in terms of the behavioural and physiological reactions that they trigger, the kinds of stimuli that elicit them can be quite different. To elicit a phasic fear response, a deimatic display would need to share elements with real or perceived threats in a predator's environment, or with threats that occurred in its recent evolutionary history and still elicit fear responses (Blumstein, 2006; Åsli and Flaten, 2012). For example, mice show fear responses, such as rapid fleeing or freezing, to rapidly looming discs, but only when they approach from above at a speed sufficient to resemble an incoming aerial predator (Yilmaz and Meister, 2013). Therefore, as long as deimatic displays sufficiently resemble perceived dangers, they are likely to trigger fear responses in their predators, even when predators repeatedly encounter deimatic prey. In order to distinguish between startle and fear responses, establishing that deimatic displays elicit the appropriate physiological and behavioural responses expected is first required. It is then necessary to assess the deimatic display to determine whether it shares features with a known threat to predators. Alternatively, one could use more invasive techniques. Lesions of the amygdala are known to diminish typical fear responses, whilst having no effect on startle responses (Gewirtz, McNish and Davis, 1998). Lesioning the same area in the brain of predators and observing their responses to deimatic prey could therefore help determine whether a deimatic display elicits fear or startle responses. Further, using gene knockout technology, it is possible to generate mice that are deficient in α-Calcium-Calmodulin Kinase II (Chen *et al.*, 1994). The heterozygote α-CaMKII mutant mice display characteristic differences in behaviour in that they exhibit reduced fear responses but no change in their startle responses. Therefore, presenting them with deimatic prey and comparing their responses to those of wild-type (not subjected to any gene alterations) mice, would allow us to test whether deimatic displays do elicit fear responses in predators.

1.3.3 Do predators misclassify deimatic prey as a threat?

Misclassification of deimatic prey as a potential threat is the third mechanism by which deimatic prey are suggested to deter predators (Skelhorn, Holmes and Rowe, 2016; Umbers *et al.*, 2017). It is closely linked to the fear mechanism in that predators are responding to a perceived threat. However, they differ in that fear responses are elicited by prey sharing certain elements with threats within predators' environment. On the other hand, misclassification involves predators mistakenly perceiving displaying deimatic prey as a threatening object in itself. Previous work on the misclassification of eyespot markings as eyes of predators altered certain features of the markings, making them more or less similar to real eyes, and monitored predators responses in order to determine any effect on prey survival (Blut *et al.*, 2012). In order to establish whether predators are misclassifying deimatic prey, manipulating predators' experience with the 'model' (i.e. the thing/object that deimatic prey resemble) would be necessary. If predators are misclassifying deimatic displays, then only those predators with experience of the 'model' should avoid deimatic prey.

1.3.4 Do deimatic displays overwhelm predators' senses?

It has also been proposed that deimatic displays cause their deterrent effect by "overwhelming the predator's senses" (Umbers *et al.*, 2017). This is typically referred to as 'sensory overload' (Hebets and Papaj, 2005; Bro-Jørgensen, 2009), and centres around the idea that a signaller can overwhelm the receivers' reception and/or processing system(s) by presenting the receiver with more information than they are able to process at that point in time (Hebets and Papaj, 2005). Is not made clear in the literature exactly how sensory overload could lead to the enhanced survival of deimatic prey. It is possible that, while attempting to process the abundance of sensory information, predators cannot determine what deimatic prey are, and so they pause their attacks in order to fully process the information. On the other hand, it may be the case that predators find this inability to process the information presented by prey aversive in some way and that is where the protective advantage lies for deimatic prey. Although deimatism and other prey defences, such as eyespots in lepidopteran prey, have been suggested to work via sensory overload (Stevens and Ruxton, 2014; De Bona *et al.*, 2015), no empirical evidence in support of this theory has been provided. Therefore, there is currently no evidence of sensory overload being an effective mechanism via which deimatism, or any other form of prey defence, could function.

In order to determine whether sensory overload is the mechanism by which deimatic prey deter predators, first which areas of the brain, or specific neurons, activated by the different components of deimatic displays would need to be identified. Then, live neurophysiological recordings of such areas or neurons in the brains of predators while viewing the deimatic display would be necessary in order to establish whether the multiple components act synergistically to inhibit the processing of one another at a neural level. At a behavioural level, the sensory overload hypothesis would expect predators to readily decide whether or not to attack prey that possessed one component of these displays. However, it would be expected that predators would pause for a greater length of time before deciding about prey when they present them with multiple components at the same time (i.e. the displays of deimatic prey). Although research focusing on this is required, the results of previous research do not suggest that this would be the case. Birds were found to be deterred more by the visual aspects of the deimatic displays of peacock butterflies (their survival was higher), whereas they were not by the auditory aspects (Vallin *et al.*, 2005). Indeed, the visual aspects alone were as deterring as the deimatic display as a whole (Vallin *et al.*, 2005). In combination with the evidence that the auditory component of this display deters rodent predators (Olofsson, Jakobsson and Wiklund, 2012b), and in contrast to the concept of sensory overload, the idea that the multiple components of deimatic displays provide a defence that is simultaneously effective against numerous different predators is supported.

I would not discount the idea that the multiple sensory components of deimatic displays are aversive to predators. However, rather than overwhelming predators' senses, I would suggest that they invoke sensory discomfort or confusion. Previous research has been established that, in humans, certain visual patterns (most notably, but not exclusively, striped patterns with a spatial frequency of close to 3 cycles/degree) are known to cause visual discomfort (visual illusions, sensation of 'tired eyes', headaches) (Wilkins *et al.*, 1984; Fernandez and Wilkins, 2008). Visual discomfort occurs when the subject finds an image uncomfortable to view, whether because it causes distortions of the image (i.e. visual illusions), provokes a sensation of tiredness in the viewer's eyes, or can stimulate headaches (Wilkins *et al.*, 1984; Fernandez and Wilkins, 2008). Greater deviation from the spatial, luminance and chromatic characteristics of natural images is found to induce aversive effects of greater magnitude in observers (Juricevic *et al.*, 2010). Deimatic displays could have evolved to cause visual (or auditory) discomfort, and so predators could avoid them because they find them aversive to look at. Visual discomfort has not been studied in any non-human animals. A first step would be to use the discomfort scale to establish whether deimatic displays cause visual discomfort in human subjects similar to established discomforting images (Juricevic *et al.*, 2010). It may also be possible to establish whether deimatic displays stimulate the same neural areas that visually discomforting images activate in the human brain (Wilkins *et al.*, 1984; Huang *et al.*, 2003).

1.3.5 Are deimatic displays perceived by predators as looming stimuli?

The final potential mechanism that I would suggest is that deimatic displays may be perceived by predators as looming stimuli. Looming objects are known to elicit reflexive recoil and/or escape responses in birds (Schiff, 1965; Lee and Reddish, 1981; Evans, Macedonia and Marler, 1993), crabs (Oliva, Medan and Tomsic, 2007), insects (Tammero and Dickinson, 2002; Santer, 2006), fish (Dill, 1974), mice (Yilmaz and Meister, 2013) and humans (Regan and Vincent, 1995). The detection of these objects are thought to stimulate collision-avoidance neurons in the brain (Wang and Frost, 1992; Sun and Frost, 1998; Gray, Blincow and Robertson, 2010). It has been suggested that the often rapid increase in perceived size during deimatic displays could take advantage of these innate responses (Kane, Fulton and Rosenthal, 2015). Parameters thought to influence the elicitation of these collision-avoidance responses include, the apparent size of the stimulus (Glantz, 1974), and the speed detected on the retina of the borders of the approaching stimulus (Land and Layne, 1995; Hemmi, 2005). Thus, the stimulation of looming neurons by deimatic displays may be influenced by their actual size and, the distance at which predators view the display (the closer the predator the larger the perceived increase in size on their retina). It may be that deimatic displays are therefore more likely to elicit looming responses when predators are in close proximity to them. Further, the speed of the moving aspects of the displays could be critical as previous research has found that responses to looming stimuli increase with increasing velocity of the approaching looming stimulus (Yamawaki, 2011).

Research determining whether deimatic displays possess the characteristics known to stimulate looming neurons is required. In addition, recording the activity of looming neurons in the brains of predators, in response to deimatic prey, is needed to establish whether deimatic displays do stimulate these neurons. Praying mantids would be a good candidate predator for these as their looming neurons have been identified and there are established methods for taking recordings from them (Sato and Yamawaki, 2014). Although evidence supporting this mechanism in relation to deimatism is currently lacking, other species have been known to take advantage of this existing reflex. For example, the painted redstart (*Myioborus pictus*) flushes out hemipteran prey by performing a distinctive display which is thought to stimulate the prey’s looming responses causing them to flee and exposing them to predation (Jablonski and Strausfeld, 2000). Thus, if deimatic displays are perceived as looming stimuli by predators the factors that influence the magnitude of these defensive responses may play a role in the success of deimatism as a form of prey defence.

Five suggested mechanisms of deimatism have been proposed, however conclusive empirical evidence for all of them is still lacking. It will be important to bear in mind two factors when investigating the merits of these mechanisms. The first is that there is not likely to be one mechanism by which all deimatic prey deter their predators, and so, differences in display form and ecology between species must be accounted for when investing how deimatic displays deter predators. The second factor is that the examination of the responses of the predators of deimatic prey themselves is likely to be crucial. It is impossible to conclude whether a predator has been startled, scared, or deterred by a looming stimulus without first ascertaining whether a startle response, fear response or response typical to the presentation of a looming stimulus has been elicited. While I do not deny that measuring some of these responses may prove difficult, it is not however impossible.

I have briefly outlined how establishing the neurological effects of deimatic displays on predators could help us to distinguish between the different mechanisms through which it is proposed to work. For example, the amygdala is involved in fear but not startle responses (Gewirtz, McNish and Davis, 1998), and specific collision-avoidance neurons are known to be activated by looming stimuli (Wang and Frost, 1992; Sun and Frost, 1998; Gray, Blincow and Robertson, 2010). I have also explained how understanding how deimatic displays deter predators could allow us to predict what aspects of deimatic signals are likely to be most effective at eliciting avoidance responses. For example, we know what types of stimuli elicit startle responses and trigger collision avoidance neurons. However, there are other ways in which a better understanding of the neurological effects of predators could help us to understand the form taken by deimatic displays. For example, understanding the early processing of visual signals may help us to determine what aspects of visual stimuli are likely to be most salient, and consequently draw most attention. Previous research has successfully modelled salience maps of complex visual scenes based on primate neural circuity (Itti, Koch and Niebur, 1998). These models allow the identification of specific areas whose features are such that they are more likely to attract the attention of the observer (Itti and Koch, 2001). Analysing the visual components of deimatic displays using similar methods may allow the identification of areas on deimatic prey or within their visual displays that are most salient in drawing predators attention. This could aid their misperception as a threatening or frightening object hence supporting their success in avoiding predation. Research investigating the mechanisms driving the success of deimatic displays and that exploring their effect on neural systems in predator brain, are likely to inform each other as behavioural results can provide direction for focused neurological studies. Although the wealth of literature on visual processing would undoubtedly be a source of useful knowledge regarding predators' perception of deimatic displays, the lack of any research in relation to deimatism in this area means that currently discussion of this topic would currently be little more than speculation. As such it lies outside the scope of my thesis as I have chosen to focus on understanding the mechanisms of deimatic displays and the identification of factors that may influence their evolution.

1.4 What factors affect the efficacy of deimatic displays?

Understanding the mechanisms through which deimatism works is also important because it allows us to make predictions about the factors that are likely to influence the efficacy, and consequently the evolution, of deimatism. For example, many factors are known to influence the magnitude of startle responses, including (to highlight a few): age (Acri *et al.*, 1995; Ludewig *et al.*, 2003), hunger (Drobes *et al.*, 2001; Rejeski *et al.*, 2010; Blechert *et al.*, 2014; Ferreira de Sa *et al.*, 2014), rearing environment (Varty *et al.*, 2000; Sánchez *et al.*, 2005), affective state (Davis, 1979; Swerdlow *et al.*, 1986; Liang *et al.*, 1992; Allen, Trinder and Brennan, 1999; Kaviani *et al.*, 2004; Grillon *et al.*, 2005; Cornwell *et al.*, 2008; Schulz, Alpers and Hofmann, 2008), light levels (Grillon *et al.*, 1997; Walker and Davis, 1997, 2002), and background noise (Hoffman and Fleshler, 1963; Hoffman and Searle, 1965; Davis, 1974; Cory and Ison, 1979; Gerrard and Ison, 1990; Flaten, Nordmark and Elden, 2005; Blumenthal *et al.*, 2006). If deimatic displays startle predators, then it follows that they would be expected to evolve under conditions that enhance startle magnitude and not under those that reduce it. For example, chemically-induced prenatal stress is known to increase the magnitude of startle responses in rats (*Rattus norvegicus*; Kjær *et al.*, 2011). Thus, if deimatic displays do startle predators, then they would be more effective against those predators whose mothers experienced a stressful environment during gestation. It follows that we would expect deimatism to evolve in environments that are stressful to predators during the build up to the birth of their young, whether that be due to lack of food sources, high risk of predation, or lack of sites to birth and raise their young. This is only one example of the many factors known to influence startle responses and, it is clear that understanding how they do so can help us understand the conditions under which deimatic displays are most likely to be successful in deterring predators. This would also help us predict the environments in which we would expect deimatism to evolve.

As startle and fear responses are expressed behaviourally in similar ways, it would not be surprising if the factors outlined in relation to startle above also effect fear responses. In addition, it is known that the presence of other conspecifics can reduce the fear responses of rats (Davitz and Mason, 1955; Morrison and Hill, 1967). This would suggest that, if deimatic displays elicit fear responses in predators, they would be less effective against predators that forage in groups. Thus, evidence supporting the idea that social buffering of fear reduced the deterrent effect of deimatism, would lead us to expect deimatic displays to evolve in environments where they predators are not likely to forage for prey in groups. Conversely, experiencing a fear-inducing stimulus in the presence of a predator can increase the fear response in Carolina anole lizards (*Anolis carolinensis*; Edson and Gallup, 1972) and chickens (*Gallus gallus domesticus*; Gallup, Cummings and Nash, 1972). This would suggest that deimatic displays would be more effective in environments where their predators are in close proximity to predators of their own.

With regard to the three other suggested mechanisms of deimatism, we do not possess a comprehensive understanding of the factors influencing their elicitation. However, it is possible to hypothesise as to what may influence deimatic displays ability to deter predators through them. First, misclassification of prey is very likely to be influenced by experience. In order for deimatic displays to be mistaken for a potential threat, predators would have to have had experience of that threat. Consequently, deimatism is unlikely to work against completely naïve predators. If deimatic displays avoid predation because they are misclassified by predators, I would therefore expect them to have evolved in areas where naïve predators are not common.

Predator experience, however, is not something I would expect to alter deimatic prey’s ability to overwhelm predator senses. I would expect predator physiology and psychology to alter the efficacy of deimatic displays that deter predators by overloading predators’ senses. I can imagine that the ability to process multiple sources of sensory information is likely to be more difficult in individuals that are experiencing poor physical health. In addition, the deficit in selective attention (the ability to attend to one stimulus when a number of stimuli are being simultaneously presented) demonstrated in individuals experiencing depression

(Purcell *et al.*, 1997; Gualtieri, Johnson and Benedict, 2006), would suggest that predators in a depression-like state would be more susceptible to their sensory system being overwhelmed by deimatic prey. Thus, I would expect deimatic prey to be more successful (and therefore more likely to evolve) in environments that produce a depression-like state in predators.

Regarding the successful emulation of looming stimuli by deimatic displays, a number of factors are likely to play a role. It is known that human subjects perceive looming stimuli as approaching faster when they are known threatening objects in comparison to non-threatening objects (Vagnoni, Lourenco and Longo, 2012). Thus, responses to threatening looming stimuli are produced faster than in response no non-threatening looming stimuli. Therefore, whether predators perceive deimatic prey as threatening could have an important influence on the speed at which they respond to deimatic displays. In addition, a recent study has found that stress accelerates the innate defensive responses of mice towards looming stimuli (Li *et al.*, 2018). If deimatic displays are perceived by predators as looming stimuli, then it is likely that they would be more effective against predators experiencing higher levels of stress. High levels of predation risk could increase the likelihood that deimatic prey are perceived as looming objects. Similar to the phenomenon of fear-potentiated startle, whereby a cue of a fearful event very soon before experiencing a startling stimulus will increase the magnitude of the startle response (Davis *et al.*, 1993), the increased likelihood of encountering a predator may increase the likelihood of predators perceiving objects in their environment as their own predators looming toward them to attempt an attack.

Clearly the ability of a deimatic display to effectively defend prey from a predatory attack will be influenced by numerous factors affecting predator behaviour, some intrinsic to the predator, and others dependent upon current environmental conditions or those experienced in the past. Understanding what these factors are, and how they can influence predators’ responses will help us to understand not only what contributes to the success (or failure) of deimatic displays, but also what types of ecological conditions might facilitate their evolution.

1.5 What influences display production?

Understanding how deimatic displays deter predators is however only one aspect of deimatic research. Another area which is greatly lacking attention is research examining the behaviour of actual deimatic species themselves. Unlike many other forms of defence, the magnitude, and to some extent the form, of the display is often under the control of the prey. Therefore, we also need to understand the factors that might influence the behaviour of deimatic prey. Although sparse, some research has been carried out on the differences in the behaviour of deimatic prey, and on understanding the stimuli that elicit deimatic display production. For example, mountain katydids (*Acripeza reticulata*) were found to perform their deimatic display only after experiencing tactile stimulation by an experimenter (Umbers and Mappes, 2015). In addition, looming stimuli of various shapes were found to elicit deimatic displays in cuttlefish (*Sepia officinalis*), however, star- and fish-shaped stimuli produced the most intense displays (Cartron *et al.*, 2013). Whilst others have considered how the perceived risk posed by predators might influence different aspects of display production. For example, Vallin et al. (2005) found that the distance from the predator at which peacock butterflies (*Aglais io*) would commence their deimatic display was greater for the second approach of a predator in comparison to the first. In other words, prey started displaying when predators were further away upon their second interaction with them. Interestingly, in cuttlefish, it is the type of predator that determines the production of deimatic displays, with display being produced toward sea bass (*Dicentrarchus labrax*) but not in response to dogfish (*Scyliorhinus canicula*) or velvet crabs (*Necora puber*; Langridge, Broom and Osorio, 2007).It is thought that cuttlefish present their deimatic display to the visually capable teleost predators (sea bass), but avoid drawing extra attention to themselves by not presenting them to predators that are less visually capable (dogfish and velvet crabs).

However, there are many outstanding questions with regard to display production. We do not know what drives prey to initiate display production. Although there is some evidence that prey do not commence their display until physical contact has been made with a predator (e.g. Umbers and Mappes, 2015), this is not always the case. It has been shown that some deimatic species do perform their displays without tactile stimulation having occurred (e.g. Vallin *et al.*, 2005; Olofsson *et al.*, 2013). Deciding when to begin displaying is critical as making a mistake and displaying before a predator has detected your presence could be fatal. Research investigating the factors influencing display initiation could help us understand this further. For example, we know that the distance at which prey commence their display increases between the first and second interaction between prey (Vallin *et al.*, 2005). However, a study focused solely on predator approach and display initiation across a range of species could help us understand the role of distance even further. In addition, it is possible that deimatic prey are more likely to initiate their displays in the presence of a greater number of predators as the chance of their having been detected is increased. Thus, comparing the behaviour of deimatic prey in the presence of a single predator, in comparison to multiple predators, could demonstrate whether this is the case.

I discussed earlier how predator psychology could influence their responses to deimatic displays. However, we do not know if, or how, prey psychology could influence display production. Experiencing stress due to a lack of food, high predation risk, or lack of available mates could influence many aspects of display production such as duration or intensity, as display performance may be costly in terms of the time and/or energy it requires. Carrying out studies where these factors are manipulated and observing the effects on prey behaviour would be an effective way to test whether they play a role in display production. One example of such a study would be to alter the amount food available to prey and simulate an encounter with a predator. Indeed, understanding the role of physiological stress on display production is important. If displays are so energetically costly that prey do not perform them when their physical resources are diminished, not only would we not expect them to evolve in areas where prey food sources are sparse, but there is also a possibility that selection could favour the loss of deimatic displays in areas that experience a deterioration in the quality of the environment.

Finally, we do not know how changes in the environmental conditions of deimatic prey influence display production. Environmental factors, such as light levels, are known to alter the behaviour of prey at different times of day. For example, guppies (*Poecilia reticulata*) are known to avoid the performance of courtship and other visually conspicuous behaviours during times of day when light levels are high and they are more likely to be detected by predators (Endler, 1987). It is possible that deimatic prey may be influenced by the light environment in a similar way as the performance of deimatic displays is a very conspicuous behaviour. Studies investigating how changes in factor such as light level, temperature, and background noise influence the responses of deimatic prey toward predators would help us understand how display production is influenced by the environment in which prey find themselves at a given point in time. This would also help expand our understanding of the evolution of deimatic displays, as it would clarify the conditions under which prey are most, and least, likely to perform their displays.

1.6 Aims of the thesis

The field of deimatic research is still in its infancy, and as such, there are currently more questions to be addressed than there are answers. In my thesis, I aim to provide answers to the questions of how deimatism evolved, which factors influence predators' responses to deimatic displays, and, which factors influence display production and duration. Chapter 3 addresses the question – how do deimatic displays evolve? These displays often have several components that are unlikely to have evolved simultaneously. I therefore test the feasibility of an hypothesis proposed by Umbers *et al.* (2017): that the movement component of displays evolved first, and was followed by the evolution of conspicuously coloured body parts revealed by the existing movement. To do this, I test the two key predictions underlying this hypothesis: that movement alone is sufficient to deter predators, and that a combination of movement and conspicuous coloration deters predators more than movement alone.

In the following three chapters, I test whether three factors known to influence the magnitude of startle responses also influence the efficacy of deimatic displays (Chapter 4: Predator hunger level; Chapter 5: Background noise within the predator's environment; Chapter 6: Predator affective state). The results of these studies will inform our knowledge of the conditions under which deimatism is most effective, and shed light on whether deimatic displays startle predators.

Finally, in Chapter 7, I turn my attention to understanding what factors might influence the production of deimatic displays. I investigate the influence of environmental temperature on the display behaviour of a lepidopteran deimatic species. The factors influencing the behaviour of deimatic species is an area that is currently greatly lacking in empirical evidence. By addressing these three overarching questions I hope to shed light on some of the areas of deimatic research within which we are currently lacking any empirical evidence. Thus, the findings presented in this thesis will provide invaluable information to the field of deimatic research and will I hope provide stimulation for further questioning and discussion.

Chapter 2. General Methods: Experiments using domestic chickens (*Gallus gallus domesticus*)

2.1 Introduction

The aim of my thesis is to investigate factors which may have played a role in the evolution of deimatic displays. I focus mainly on factors that could influence predators' behavioural responses to deimatic prey (Chapters 4-6), although I also consider factors influencing the production of displays by prey (Chapter 7). The idea that predator psychology can play an important role in the evolution of prey defence is not a new one (Guilford and Dawkins, 1991; Rowe and Skelhorn, 2004), but it's role in the evolution of deimatism is not well appreciated (Skelhorn, Holmes and Rowe, 2016). This is an important oversight because understanding how and why predators are deterred by deimatic displays could shed light on the evolutionary pathway(s) via which these displays evolve, and the conditions under which they are most likely to evolve. For example, if deimatic displays are more successful against naïve predators, it follows that they would be more likely to evolve in habitats where naïve predators are more abundant and deimatic prey are scarce.

Four of my chapters (Chapters 3-6) use the same experimental paradigm to investigate how deimatic displays might have evolved (Chapter 3), and how both environmental conditions and predator state might affect their efficacy (Chapters 4-6). In each experiment, I presented naïve domestic chicks (*Gallus gallus domesticus*) with artificial prey projected onto the floor of an experimental arena. In this Chapter, I outline the procedure and details of this experimental system and justify my approach. Further details of the experimental manipulations specific to each study will be presented in the individual study chapters. Chapter 7 investigates how environmental conditions influence the likelihood that prey perform deimatic displays. For this reason, it used an entirely different methodology, which is discussed in full in Chapter 7.

2.2 Predators

I chose to use juvenile domestic chicks (*Gallus gallus domesticus*) as model avian predators in the experiments examining the responses of avian predators to deimatic displays. There were a number of reasons for this choice. I had complete control over chicks' experiences of prey from the beginning of their life. The chicks entered the lab on the day they hatched and had not yet consumed food. Therefore, I was able to guarantee that their responses to prey would not be influenced by any previous experiences that I was unaware of. For example, if I had used wild birds they may have had learned aversions to particular colours, or they may have learned about naturally-occurring deimatic prey. If this was the case, their responses to experimental prey may have reflected how they generalised about the live prey they had experienced and may not reflect how birds without such experience responded to the experimental prey. It could be argued that the same experiential control could be obtained by using hand-reared wild birds. However, hand-rearing wild birds is extremely time intensive, and, once reared it is only possible to test them once in a naïve state (after one test they are no longer naive). Therefore, the time and resources required to hand-rear birds, and the ethical issues associated with the removal of large numbers of juvenile birds from their natural habitat for use in a single experiment was not justifiable.

Although domestic chickens have undergone years of inbreeding they still retain behavioural responses that are similar to those of their wild counterparts. For example, it is known that chicks have an innate aversion to colours typically used by aposematic prey (Roper and Wistow, 1986; Roper, 1990; Marples and Roper, 1996; Roper and Marples, 1997; Aronsson and Gamberale-Stille, 2008). These colours biases are similar to those seen in other species of avian predator (Kelly and Marples, 2004; Svádová *et al.*, 2009) .In addition, both domestic chicks and other birds appear to be similar in that they learn to avoid defended prey more quickly when they are conspicuous (Roper and Redston, 1987; Alatalo and Mappes, 1996). Their responses are therefore comparable to those of wilds birds, and I can make generalisations from their behaviour about that of other bird species.

In fact, many previous studies investigating the evolution of prey defences have used domestic chicks as model predators when investigating prey defences (e.g. Roper and Wistow, 1986; Marples and Roper, 1996; Roper and Marples, 1997;

Skelhorn and Rowe, 2005; Halpin, Skelhorn and Rowe, 2008). They have the advantage of being very amenable to reward-based training and, as such, are excellent at learning to attack artificial prey. Because of this, the numbers of chicks that did not reach the training criterion for inclusion in testing was low. Moreover, it was also possible to train chicks to attack artificial prey within a short period of time, meaning that a cohort of 30 chicks could be trained and tested in 14 days. This low drop-out rate and fast learning rate allowed me to use fewer individuals and keep them in the laboratory for shorter periods, both of which are desirable from an ethical standpoint and in order to maximise data collection. Therefore, the use of juvenile domestic chicks as my model predator allowed me to have complete control over their life experience up until they participated in my experiments, while also using animals that are easily trained and can be used to collect data efficiently whilst minimising ethical concerns.

2.2.1 Housing of chicks

Newly-hatched domestic chicks of mixed sex (Ross strain) were purchased for use in my experiments from P.D. Hook Hatcheries Ltd., Thirsk, UK. The chicks were housed in the laboratory in a floor pen measuring 56 x 85.5 x 208 cm (the approved size for 42 chicks, which was the typical cohort size). The floor of the pen was covered with wood chips and the pen contained a food hopper, a water hopper, a bale of hay, and a plastic shelter measuring 41 x 37 x 54.5 cm. The birds were maintained on a 12:12 hour light/dark cycle beneath fluorescent lighting and the room temperature was kept at 25-29˚C by three electric heaters placed next to their pen. Water and chick starter crumb HPS were made available *ad libitum*, except prior to training and experimentation when brief periods of food (but not water) deprivation were required to ensure the chicks' motivation to forage.

Upon arrival, the chicks were marked with non-toxic coloured marker pens to allow individual identification. Chicks were visually inspected and weighed daily in order to monitor their health and welfare. All of the chicks gained weight during the course of the experiments. At the end of the experiments, where possible, the chicks were rehomed to a free-range farm. In the case that this was not possible, they were euthanised by an animal technician trained to do so using Home Office approved techniques. Each of my experimental protocols received ethical approval from the

Newcastle University Animal Welfare and Ethical Review Body (see Chapters 4-7 for individual project approval codes), and followed the UK Home Office Guidelines and the Association for the Study of Animal Behaviour's Guidelines for the Treatment of Animals in Research and Teaching.

2.3 Prey

Deimatic displays are highly complex and dynamic, often including a combination of movements, postures, and visual, olfactory, auditory and/or vibratory stimuli (e.g. Maldonado 1970; Edmunds 1974; Lenzi-Mattos et al. 2005; Langridge 2009). Controlling the multiple components of the displays and ensuring that deimatic prey perform their display consistently can prove difficult when using live individuals. Consequently, I chose to use computer-generated artificial deimatic prey. This gave me full control of where, when and how the deimatic prey displayed. The prey were computer-generated moth-like images projected onto the floor of the experimental arena (additional detail on the arena, images and projection can be found in the following section). The use of this experimental apparatus also allowed me to control the background against which prey were presented, ensuring that prey and background images had equal luminance prior to display. Thus, I could ensure that the only factors producing differential responses in the chicks were those that I manipulated in a given experiment. This approach allowed me to ask questions that I would have been unable to address had I used live prey. For example, the ability to alter hindwing colouration and the speed of forewing movement allowed me to investigate a possible pathway by which deimatic displays may have evolved (Chapter 3). The ability to keep display initiation and the rate of display constant also allowed me to ensure that each individual chick received the same experience of the deimatic display, something which would have been much more difficult (if not impossible) had I been working with live prey. I was therefore able to manipulate both environmental factors and predator state while ensuring that all experimental subjects had a uniform experience of the deimatic display itself (Chapters 4-6).

It could be argued that this ability to remove a great deal of noise from the predator-prey interactions within my experiments does not result in responses that are generalisable to those in natural settings. However, such controlled experimental designs have been used many times in previous research in order to identify important characteristics of prey defences which are then possible to test with live subjects in more natural settings. For example, artificial prey have been used previously to investigate camouflage (Troscianko *et al.*, 2013), eyespot markings (Stevens, Stubbins and Hardman, 2008), and deimatic displays (Skelhorn *et al.*, 2014; Kang, Zahiri and Sherratt, 2017). Indeed, the experimental system I used was adapted from one initially used to investigate the evolution of camouflage (Troscianko, Skelhorn and Stevens, 2017). Thus, not only is the use of an experimental system presenting artificial prey justified, it allowed me to ask questions about deimatic displays that otherwise would have been impossible to test.

2.4 Experimental Paradigm

The experimental paradigm involved presenting computer-generated artificial moth-like prey images to domestic chicks. The prey images were presented to the chicks by projecting them onto the floor of a raised experimental arena. Prey were presented against background images of tree bark which were also projected onto the arena floor. Consequently, this unique apparatus allowed me to present chicks with prey in a manner similar to that in which they would encounter insect prey in a natural setting, but with greater control over the form of the preys' deimatic display. In doing so, I was able to present deimatic prey to predators with complete control not only over the characteristics of the display itself, but also over the experience of the predator, which has not been previously possible within this field.

2.4.1 Experimental Arena

It should be noted that one of my collaborators, Dr J Troscianko, created the experimental arena and all of the backgrounds and artificial prey used in these studies. The apparatus consisted of a raised rectangular experimental arena (32 × 71 × 103.5 cm) with opaque white walls (Figure 2). An Optoma ML1500 LED DLP projector positioned beneath the arena projected background and prey images onto the floor of the arena. The arena floor was made of PMMA (Poly(methyl methacrylate)) a substance also known as acrylic glass. On top of this was placed a 0.5 mm thick sheet of white PTFE (Polytetrafluoroethylene). This structure of the arena floor allowed me to ensure that the display looked the same to the chicks regardless of their viewing angle, an important consideration seeing as they would be walking across it and viewing it from various different viewpoints.

On either side of the arena were two areas (hereafter “buddy arenas”) measuring 32 x 18 x 70 cm served to house 2 buddy chicks. Two chicks were present in each buddy arena at all times, i.e. a total of four buddy chicks were present during all training and experimental sessions. Buddy chicks did not participate as experimental subjects, and only served as companion chicks to reduce any stress associated with social isolation in the experimental chicks. The buddy arenas were separated from the experimental arena using wire mesh screens, allowing experimental chicks constant visual and auditory contact with buddy chicks without any physical contact being possible. Water was available *ad libitum* to buddy chicks while present in the buddy arenas.

2.4.2 Background

Background images were created from 57 monochromatic photographs of tree bark (oak, beech, birch, holly and ash) which were taken using a Canon 5D MKII with a Nikkor EL 80mm lens at F/22 to ensure the depth of field. These photographs were taken under diffuse light conditions and were manipulated to ensure an overall similarity in mean luminance and contrast (variance in luminance). These images were then cropped and scaled to a 1:1 aspect ratio to the monitor’s resolution of 1280px by 800px using bilinear interpolation. The pixel values were converted to 32-bit greyscale and log-transformed, which resulted in an approximately normal distribution of pixel luminance values. For each image, a histogram of pixel values with 10,000 bins was analysed. The 1st, 50th and 99th percentile luminance values were calculated, and their bins were modelled using a quadratic function against the desired values for these percentiles to ensure that the median (50th percentile) was halfway between the luminance at the upper and lower limits, based on a log scale. The background images produced all had approximately equal mean and median luminance, similar luminance distributions (i.e. contrast in luminance), and, equal numbers of pixels at their upper and lower extremes.

Figure 2. Experimental Arena. Images of arena showing the projector (A) and the layout of the experimental arena in the centre and buddy areas on either side, which would each contain two buddy chicks (B).

2.4.3 Artificial Prey

Artificial prey were loosely based on deimatic lepidoptera that repeatedly open and close their cryptic forewings when disturbed to flash conspicuous hindwings (e.g. Underwing moths; *Catocala* sp. and Peacock butterflies; *Aglais io*). Some chicks received prey in the test trial that flashed conspicuous hindwings, some received prey that flashed background-matching hindwings, and others received background-matching prey that did not move at all. They were not, however, intended to represent any particular species. Each prey image was triangular in shape and was generated from the background against which it was presented using code written specifically for that purpose (similar to that used by Troscianko et al. 2013 and Troscianko et al. 2017). The chicks were initially trained to attack stationary cryptic prey during training and they then received a single artificial prey in the test trial.

Training Prey

I initially trained chicks to find stationary, cryptic, triangular prey for food rewards. Chicks were trained to find two cryptic prey types, background-matching prey and prey with distractive markings (small conspicuous markings thought to distract attention from salient prey outlines (Dimitrova *et al.*, 2009; see Fig. 3). The method used created unique two-tone prey that match the general pattern and luminance of the background, with patterning that did not reach the prey edge in order that they did not possess disruptive colouration.

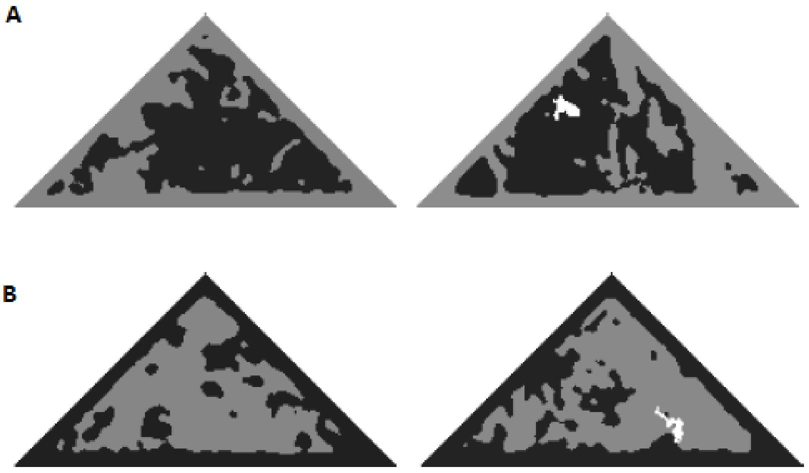

Figure 3. Examples of training prey. (A) Pattern with dark pixels on light, (B) Pattern with light pixels on dark. Prey on the left are background-matching and prey on the right have distractive markings.

To create the background-matching training prey, a triangular section of the background image was selected from a random location for each prey, measuring 200px wide by 100px high. Prior to threshold modelling a Gaussian gradient was applied to the edges of the prey image which made it less likely that underlying patterns would appear nearer the edge of the prey. This step allowed the avoidance of the creation of salient internal lines in the background-matching prey parallel with the prey's outline, while also ensuring that no patterns touch the very edges. The prey image was then spilt into 50% pattern and 50% background using thresholding. If the thresholded proportion did was not within 1% of the target limits the thresholding process was repeated. Prey images were generated with one of two patterns; dark-on-light or light-on-dark, and each chick received only one of these treatments. The light value of the pattern was equal to the 95th percentile of the background's luminance, and the dark value was equal to the 5th percentile.

In order to produce the distractive training prey, the procedure for the production of background-matching prey was followed, but a single distractive marking was added to each prey. The distractive markings were created by repeatedly sampling the background image using a thresholding selection tool until a selection area of 50-60 pixels was formed with maximal width and height dimensions not in excess of 20 pixels. This outline of the marking was then filled in with white and randomly placed on the prey image while ensuring that it was not placed within 2 pixels of the prey edge. The position of each of the prey images was randomly allocated by the image software.

The use of two different prey types in my training sessions, and their type of cryptic colouration, stemmed from the fact that this system was originally conceived for use in studies investigating the features of different camouflage patterns. In fact, the chicks generating data in my first study (Chapter 3) were used in an initial experiment investigating the difference in camouflage between the patterns of the training prey prior to participation in the experiment relating to deimatism (for further details see Chapter 3). This system of training with prey that differed in the characteristics of their background-matching colouration had the added advantage of providing the chicks with a broader range of what constituted prey items. Thus, chicks showed no signs of neophobia during test sessions when presented with test prey that differed somewhat in appearance to training prey. I went on to use these same cryptic training prey for all 4 studies using this experimental set up. This was due to the fact that I would be presenting the chicks with experimental prey that had background-matching forewings during the test trial. Therefore, training chicks with prey that had another type of colouration or patterning would have proved problematic.

Experimental Prey

Chicks experienced either Deimatic, Background-matching or Control prey. The background-matching pattern used for experimental prey were adjusted to be symmetrical by constructing one half of the prey and mirroring it to create the second half. They were also slightly darker than the training prey (with and 80:20 distribution of dark and light pixels). This made the prey easier to find than the training prey; chicks finding and interacting with prey being essential for experimental participation. Deimatic prey images consisted of a background-matching triangle, with similar patterning to training prey. The hindwings were conspicuously coloured a uniform bright red colour (Figure 4B, 4C). Background-matching prey were the same as Deimatic prey, except that when their forewings opened they revealed hindwings that were coloured with the same background-matching pattern as their forewings (Figure 4D, 4E). Control prey looked exactly the same as the experimental Deimatic and Background-matching prey, but they remained stationary throughout the trial (Figure 4F, 4G). Prey were presented in one of two positions, either on the right- or the left-hand side of the arena (Figure 4A), and the position of prey was counterbalanced across experimental groups.

When activated, the moving prey’s ‘forewings’ opened, ‘hinged’ at the peak of the triangular prey, to reveal its ‘hindwings’, before returning to the original position (Figure 4). The movement mimicked deimatic moth species such as the underwing moths. This motion of opening and closing was defined as a single wing flick and prey continuously wing flicked from activation to the end of the trial. The forewings flicked at one of three different speeds which fall within the range observed in live lepidoptera (Olofsson, Jakobsson and Wiklund, 2012b; Olofsson *et al.*, 2013): Slow, 1 flick per second (fps); Moderate, 1.8 fps; and Fast: 2.22 fps.

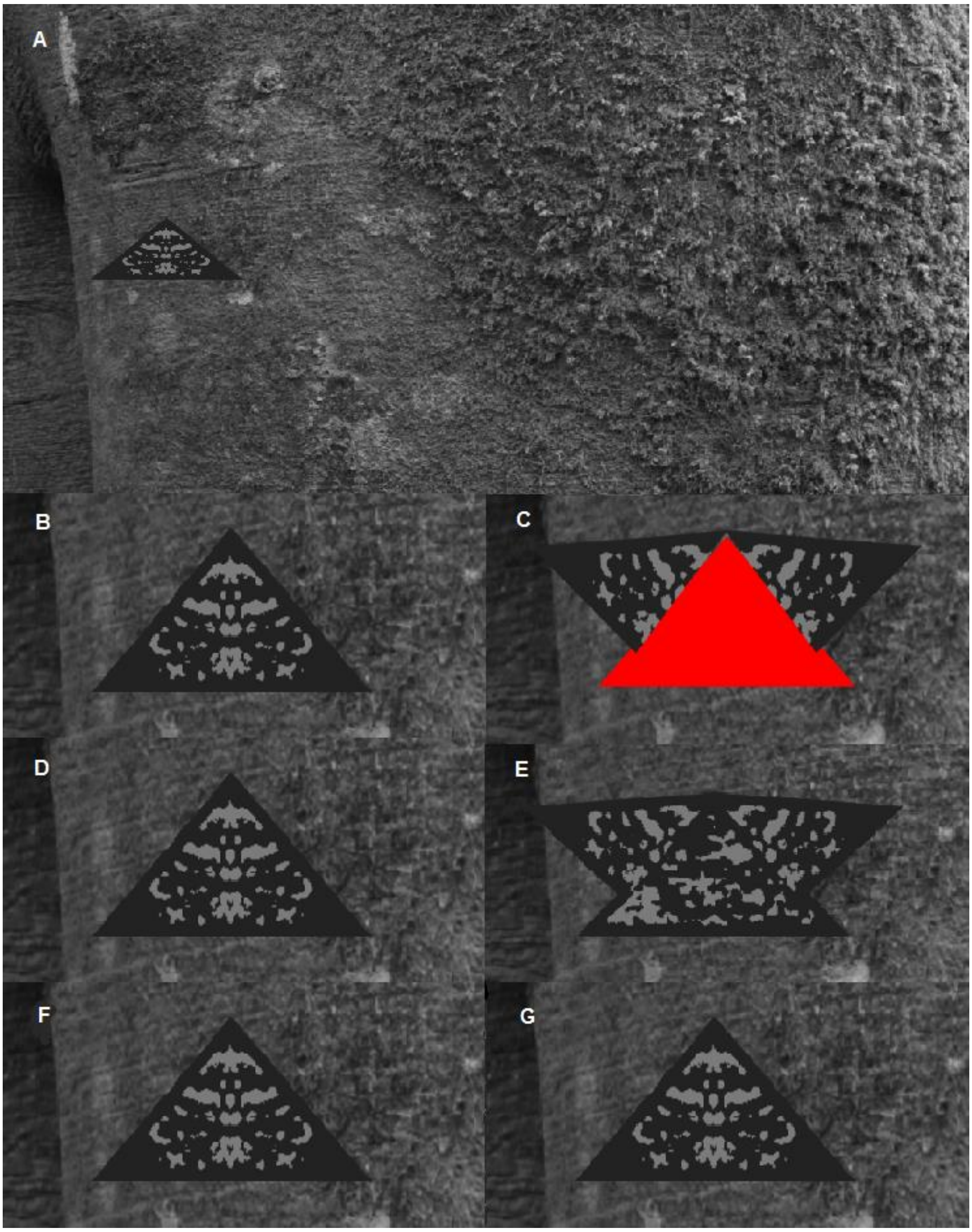

Figure 4. Background image and each of the prey types Deimatic, Background-matching and Control. (A) Example of a prey image on the natural bark background image. Conspicuous deimatic prey with forewings closed (B) and open (C), revealing bright red underwing colouration. Background-matching prey with forewing closed (D) and open (E), revealing background-matching underwing colouration. Control prey (F) with forewing always closed (G).

2.5 Experimental Protocol

2.5.1 Training Trials

On the first day after hatching the chicks were all placed into the experimental arena with mealworms (*Tenebrio molitor*) scattered across the floor for 30 minutes. This habituated chicks to both foraging in the experimental arena and eating mealworms. After this, they were returned to their home pen and left to acclimatise to the laboratory. The following day, experimental chicks were placed singly in the arena to learn the task. Throughout all training and experimental sessions, experimental chicks were accompanied by two buddy chicks were placed in each of the two buddy arenas (Figure 2). For each cohort, twelve individuals were used as buddy chicks. They were separated into 3 groups of 4 individuals and were rotated such that each group spent no longer than an hour in the buddy area at a time and had at least a two-hour interval before returning.

All of the experimental chicks received one training session per day for 6 consecutive days. Prior to each session, chicks were moved to an experimental holding pen (56 × 67 × 126 cm) for approximately one hour where water, but not food, was available *ad libitum*, in order to ensure they were motivated to participate. Prey images were initially presented against a uniformly grey background and chicks were rewarded with live mealworms for pecking or scratching on the prey image. Mealworms are a food that chicks find highly rewarding and provided a strong incentive for participation in the task. As the number of presentations experienced increased over the course of the training trials, so too did the number of mealworms the chicks received. Thus, the chicks received on average approximately 30 mealworms per day.

Once the chicks were accurately pecking or scratching the prey item for four consecutive presentations, the visibility of the background image was increased to become more visible. There were ten levels of background image opacity ranging from solid grey with no background image visible (0) to the fully visible background image (9; Figure 5). Chicks searched for prey at each background image opacity level until they were pecking consistently and independently. Chicks were initially rewarded with a mealworm for every peck and scratch carried out on a prey image. However, once they reached background image level 5, they only received a mealworm for their first interaction with the prey image before the next presentation began. There were 20 possible presentations within each training session. However, as training trials had a maximum length of 12 minutes, during training days 1-3, when chicks were at their slowest, it was common for them to receive less. From training day 4 onwards, chicks received all 20 presentations in every training session. Only chicks that successfully attacked (pecked or scratched) the prey image in 80% of prey presentations at opacity level 7 or above on the final training day were included in the test trial. On average, two chicks from each cohort were excluded.

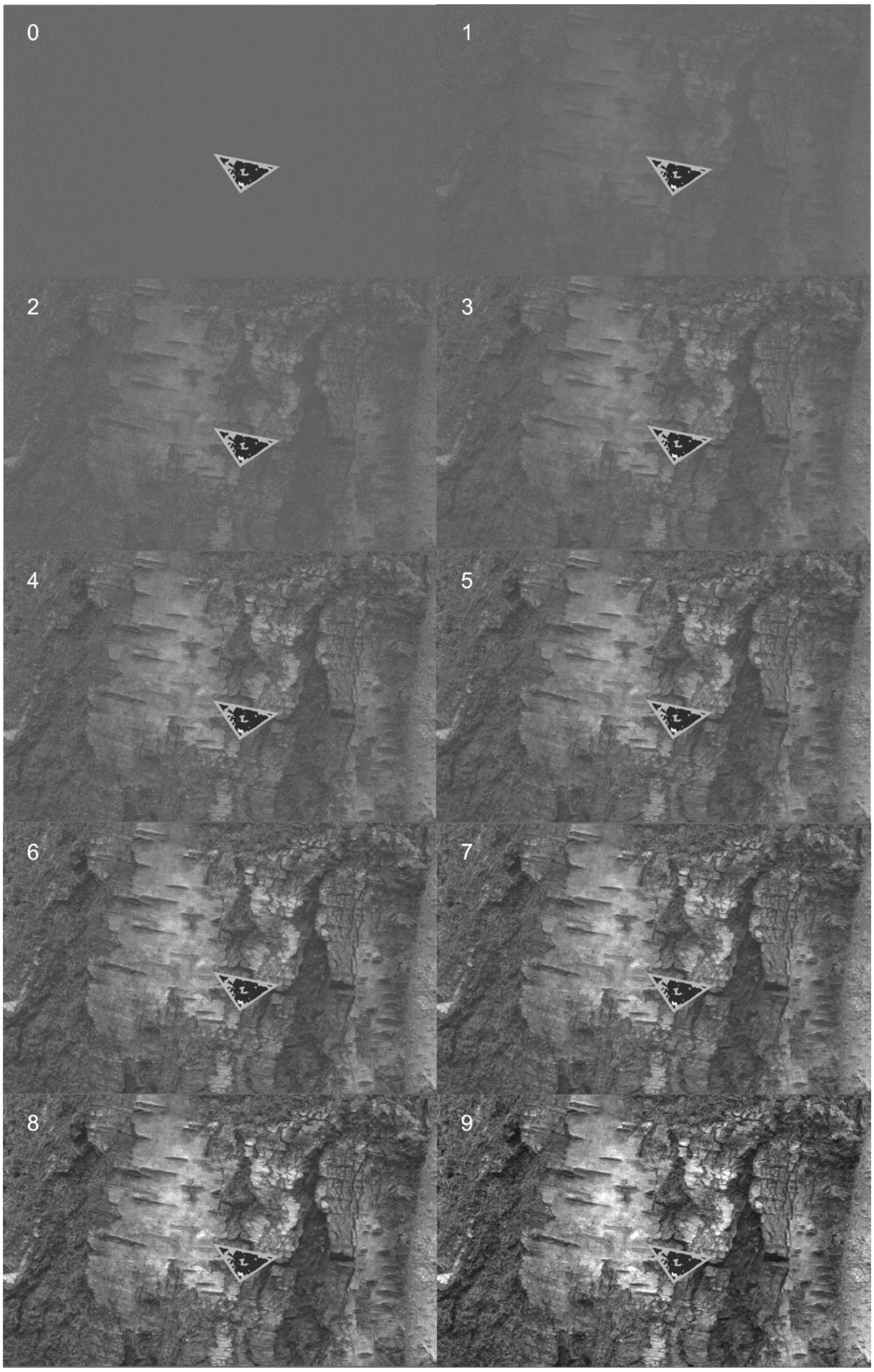

Figure 5. Background image levels used during training trials. Background images ranged from fully transparent (0) to fully opaque (9).

2.5.2 Test Trials

During the test trial, chicks were placed into the experimental arena on the opposite side to the prey image, facing away from it. The midpoint of the arena was marked on either side of the arena in order that the experimenter could see it, but the chicks could not. When the head of the chick crossed the midpoint of the arena, the experimenter activated the prey image using the control keypad. The first peck or scratch of the prey image, and all subsequent contact events were recorded live during the trial using the control keypad. Trials lasted for a total of 10 minutes after activation of the prey, when the trial was automatically ended. All trials were video recorded using a JVC Everio GZ-315DEK camcorder. From the video files, I measured the length of time chicks spent in close proximity to the prey by separating the screen image of the experimental arena into a grid and using the video analysis software BORIS (Friard and Gamba, 2016) to measure the length of time spent in the half of the arena containing the prey image (Near), and the half of the arena without it (Far). The live coding of pecks and scratches allowed me to measure the latency for chicks to attack prey after exposure to their display.

2.6 Concluding Remarks

The training and testing procedures outlined here were used in all four of the chick experiments presented in this thesis (Chapters 3-6). Specific experimental manipulations and any alterations to said procedures are outlined as necessary in the methods sections of individual chapters.

Chapter 3. Testing the feasibility of the startle-first route to deimatism

This chapter is based on: Holmes GG, Delferriere E, Rowe C, Troscianko J & Skelhorn J. (2018) ‘Testing the feasibility of the startle-first route to deimatism,’ *Scientific Reports*, **8**: 10737.

3.1 Abstract

Many prey species perform deimatic displays that are thought to scare or startle would-be predators, or, elicit other reflexive responses that lead to attacks being delayed or abandoned. The form of these displays differs among species, but often includes prey revealing previously-hidden conspicuous visual components. The evolutionary route(s) to deimatism are poorly understood, but it has recently been suggested that the behavioural component of the displays evolves first followed by a conspicuous visual component. This is known as the “startle-first hypothesis”. Here I test the two key predictions of this hypothesis: 1) that movement can deter predators in the absence of conspicuously coloured display components; and, 2) that the combination of movement and conspicuously coloured display components is more effective than movement alone. I show that both these predictions hold, but only when the movement is fast. This provides the first evidence for the feasibility of ‘the startle-first hypothesis’ of the evolution of deimatism.

3.2 Introduction

Deimatic displays occur across a wide range of different animal groups, and often involve prey adopting characteristic movements and postures while revealing conspicuous visual display components to an attacking predator. For example, cuttlefish (*Sepia officinalis*) flatten their bodies, extend their peripheral fin and suddenly change colour to present a conspicuous dark eyespot pattern on the rear of the mantle and a dark contour that runs parallel to the body's edge (Langridge, 2009); and underwing moths (*Catocala* spp.) open their cryptic forewings to reveal striking, conspicuously patterned hindwings (Sargent, 1978; Schlenoff, 1985). There is some evidence that this kind of display can deter predators (Vallin *et al.*, 2005; Olofsson *et al.*, 2012a, 2013; Olofsson, Jakobsson and Wiklund, 2012b), however, we know very little about the evolutionary pathways via which these displays evolve (Umbers, Lehtonen and Mappes, 2015).

It is unlikely that visual and behavioural components of deimatic displays evolved simultaneously. This raises the question of whether the conspicuous colours and patterns often associated with deimatic displays evolve before or after the behavioural component of the displays (i.e. the characteristic movements and/or postures; Umbers et al. 2017). Since hidden visual display components are likely to be useless without the movement that reveals them, Umbers et al. (2017) recently suggested that in undefended prey at least, the behavioural component of the display evolves first followed by the conspicuous colours and patterns (also see Umbers *et al.* (2017) for other potential routes to deimatism). They refer to this as 'the startle-first hypothesis'(Umbers, Bona, *et al.*, 2017), and it relies on two key assumptions: that the behavioural components of deimatic displays have a deterrent effect on predators, and that this is enhanced by the evolution of conspicuous visual display components. However, these assumptions have not been tested.

Here, I critically test the predictions that: (1) movement alone in the absence of a conspicuous visual component is sufficient to deter predators; and, (2) the combined effect of movement and a conspicuous visual component is more effective than movement alone. I used the experimental system outlined in Chapter 2 in order to test these predictions. After learning to attack artificial prey, each chick received one test trial in which it was presented with a single prey item. The type of prey chicks received differed among our seven experimental groups. Chicks received either Control (stationary background-matching prey), Deimatic (opened their forewings to reveal conspicuous red hindwings), or Background-matching prey (opened their forewings to reveal background-matching hindwings). Deimatic and Background-matching prey produced their displays at one of three different speeds (Slow, Moderate, or Fast). This allowed us to determine whether or not movement speed could influence the benefits to displaying, and consequently, the likelihood that deimatic displays would be selected for.

3.3 Methods

3.3.1 Subjects

Eighty newly-hatched domestic chicks (*Gallus gallus domesticus*) of the ‘Ross’ strain were used in this experiment: 56 served as experimental chicks and 24 acted as buddy chicks. The chicks were of mixed sex and were purchased in two cohorts (39 in the first cohort and 41 in the second) from a commercial hatchery in Yorkshire (U.K.) as day-old hatchlings. Details of the chicks housing during the course of the study, the training protocol and experimental procedure on the test day are outlined in Chapter 2.

3.3.2 Experiment

The chicks in this study were trained to attack artificial prey as detailed in Chapter 2. After completion of the training trials, on days 10 and 11 post-hatch, they participated in an experiment investigating how camouflage patterns influence detection (Troscianko, Skelhorn and Stevens, 2017). This experiment consisted of two test trials carried out over consecutive days. These trials took the same format as the training trials, but the type and order of prey presented differed among the six experimental groups. The experiment had a 3 x 2 design, whereby in the first 10 presentations in each of the two trials, chicks encountered either all background matching, all disruptive, or five of each prey type in a random order; and in the following 10 presentations they encountered either all background matching, or all distractive prey. I ensured that chicks from each of the six groups used in the camouflage experiment were counterbalanced across the seven groups used in my deimatism experiment in order to minimise any possible effect of the experimental treatments in the camouflage experiment.

On day 12 post-hatch, chicks were assigned to one of seven experimental groups (N = 8 for each group), and then received a single test trial in which they were presented with a single artificial prey of one treatment type. There were three types of prey; Control, Background-matching and Deimatic. Control prey were stationary and background-matching. Background-matching and Deimatic prey moved their forewings either at Fast, Moderate, or Slow speeds. Forewing speed was measured using number of flicks per second (FPS), with one wing flick encompassing the forewing moved from closed to open, and back to closed again. Fast prey moved at 2.2 FPS, Moderate at 1.8 FPS and Slow at 1.2 FPS. Background-matching prey revealed background-matching hindwings and Deimatic prey revealed conspicuous red hindwings (a colour known to be innately aversive to domestic chicks; Roper & Marples 1997). Thus, chicks received either: i) Control, ii) Background-matching/Fast, iii) Background-matching/Moderate, iv) Background-matching/Slow, v) Deimatic/Fast, vi) Deimatic/Moderate, or vii) Deimatic/Slow prey.

Ethical approval for this study was received from the Newcastle University Animal Welfare Ethical Review Body (Project ID No. 500).

3.3.3 Data Analysis

Latency to Attack Prey

I recorded the latency to first contact the prey (the length of time from when the chick crossed the midpoint of the experimental arena until it first attacked (pecked or scratched) the prey), and used this as a measure of how willing birds were to attack displaying prey. A number of potential outliers were identified, however taking into account my sample size and the range of behaviours observed across all of my experimental groups, I deemed them to be realistic chick responses and so included them for analysis. The design of my experimental system was such that it was impossible for me to be blind to the experimental aims and manipulations while live scoring the occurrence of attacking behaviour. Because the behaviours were so specific, I did not believe that this would influence the data collected. However, in order to ensure that this was indeed the case, I enlisted an independent observer outside of our research group to score the video recordings of test trials blind to the aims of my experiment. This observer scored: i) the point at which the chick's head crossed the midpoint of the arena (the cue used by the experimenter to activate prey during test trials); and, ii) the first time a chick pecked or scratched the prey item. Using the statistical software package IBM SPSS Statistics 23, I compared the latencies scored live by the experimenter and those scored by the independent observer using the Intraclass Correlation Coefficient (ICC). ICCs are measured on a scale of 0 to 1, where 1 represented perfect reliability between observers and 0 represents no reliability. I found that the ICC = 0.896, with 95% CI (0.829, 0.938). This score provides evidence for the reliability of measurements between the two observers. This result supports the validity of the data presented here.

Since these data did not meet the assumptions of parametric tests, I performed a series of planned contrasts using Kruskal-Wallis tests. With six degrees of freedom among the seven experimental groups, I tested the following *a priori* predictions based on the 'behaviour first hypothesis'. First, I predicted that birds in the (i) Background-matching/Fast group, (ii) Background-matching/Moderate group, and (iii) Background-matching/Slow group would take longer to attack prey than those in the Control group. That is, movement alone would be sufficient to deter predators. Second, (iv) birds in the Deimatic/Fast group would take significantly longer to attack prey than those in the Background-matching/Fast group; (v) birds in the Deimatic/Moderate group would take significantly longer to attack prey than those in the Background-matching/Moderate group; and (vi) birds in the Deimatic/Slow group would take significantly longer to attack prey than those in the Background-matching/Slow group. That is, that movement combined with conspicuous colouration would be more effective than movement alone or no movement at all.

It was not necessary to use Bonferroni or other corrections to control for type I error rate as I carried out a small number of planned comparisons testing quite distinct *a priori* predictions (Ruxton and Beauchamp, 2008). These analyses were carried out using the statistical software package R.

Proportion of time in proximity to prey during pre-attack interval

In the videos of the test trials, I subdivided the experimental arena into two sections; the half containing the prey image (Near), and the half without (Far). Using the behavioural video scoring software BORIS (Friard and Gamba, 2016), I then measured the length of time each chick spent in the Near half of the arena during the interval between prey activation (in the case of Control prey this was the point at which the chick's head crossed the midpoint of the arena and the timer started), and prey attack (the pre-attack interval). I then calculated the proportion of time spent Near during the pre-attack interval. This provides a measure of the birds' willingness to spend time in proximity to prey during the time that they decide whether to attack the prey image. Since these were proportion data, I carried out an arcsine square root transformation. The residuals of the transformed data were normally distributed and the transformed data were homoscedastic meaning that it was appropriate to use a one-way ANOVA to compare the difference among groups. I then carried out a series of orthogonal planned contrasts testing the six predictions outlined for the previous measure.

3.4 Results

3.4.1 Latency to attack prey

I found that the time that chicks took to attack prey differed among my experimental groups (Kruskal-Wallis test: $\chi^2 = 16.57$, $p = 0.011$, df = 6; Figure 6). As predicted (see methods and materials section), chicks in the Background-matching/Fast group took significantly longer to attack prey than chicks in the Control group (Kruskal-Wallis test: $\chi^2 = 6.10$, $p = 0.014$, df = 1). Contrary to my predictions, there was no significant difference in attack latency between the Background-matching/Moderate group and the Control group (Kruskal-Wallis test: $\chi^2 = 0.798$, $p = 0.372$, df = 1), or between the Background-matching/Slow group and the Control group (Kruskal-Wallis test: $\chi^2 = 3.780$, $p = 0.052$, df = 1). However, the latter approached significance and so should be interpreted with caution. These results indicate that fast movement can be an effective deterrent in the absence of other conspicuous visual display components.

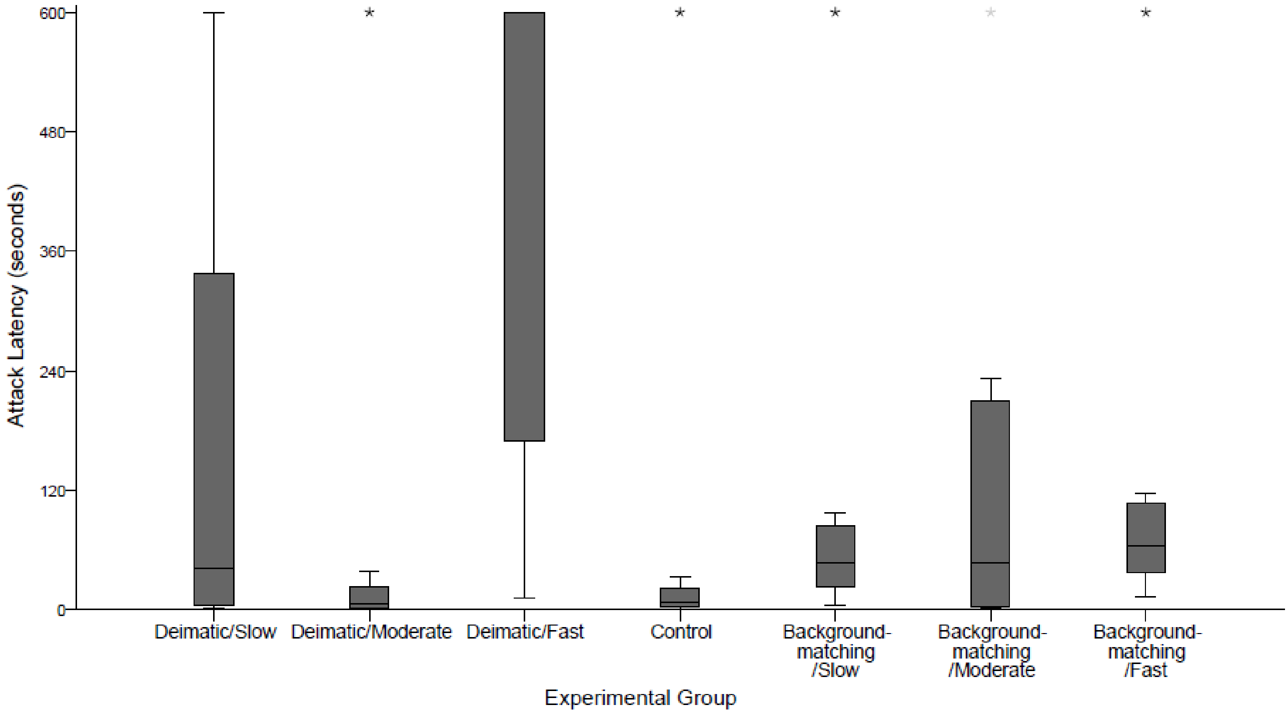

Figure 6. Latency to attack prey during the test trial. Each box represents the middle 50% of scores for each experimental group. The line dividing each box into two represent the median of those data. The upper and lower whiskers extend to include the data outside the middle 50%. Asterisks (*) represent possible outliers, each of which has been examined and is valid for inclusion within the analysis.

As predicted, chicks in the Deimatic/Fast group took significantly longer to attack prey than chicks in the Background-matching/Fast group (Kruskal-Wallis test: χ^2 =4.422, *p* = 0.033, df = 1), attacking the Deimatic/Fast (M = 418.69s, SE = 90.14s) prey over three times faster than Background-matching/Fast (M = 128.68s, SE = 68.47s) prey. However, I found no significant difference in the attack latencies between chicks in the Deimatic/Moderate group and chicks in the Background-matching/Moderate group (Kruskal-Wallis test: χ^2 = 1.461, *p* = 0.246, df = 1), or between chicks in the Deimatic/Slow group and chicks in the Background-matching/Slow group (Kruskal-Wallis test: χ^2 = 0.100, *p* = 0.805, df = 1). This indicates that conspicuous colouration enhanced the deterrent effect of movement, but only when prey moved at the fast speed.

3.4.2 Proportion of time in proximity to prey during pre-attack interval

I found a significant difference among the experimental groups (ANOVA: $F_{(6)}$ = 2.795, p = 0.02; Figure 7). However, in contrast to my predictions, there was no difference between the Background-matching/Fast and Control groups (t = -1.428, df = 49, p = 0.16), the Background-matching/Moderate and Control groups (t = -1.037, df = 49, p = 0.305) and the Background-matching/Slow and Control groups (t = -1.479, df = 49, p = 0.146). Nor did I find any difference between the Deimatic/Fast and Background-matching/Fast groups (t = 1.736, df = 49, p = 0.089), the Deimatic/Medium Background-matching/Moderate groups (t = -1.561, df = 49, p = 0.125), or the Deimatic/Slow and Background-matching/Slow groups (t = -0.552, df = 49, p = 0.583). These results suggest that the amount of time spent in proximity to prey during the pre-attack interval did not differ among the different prey types presented in this experiment.

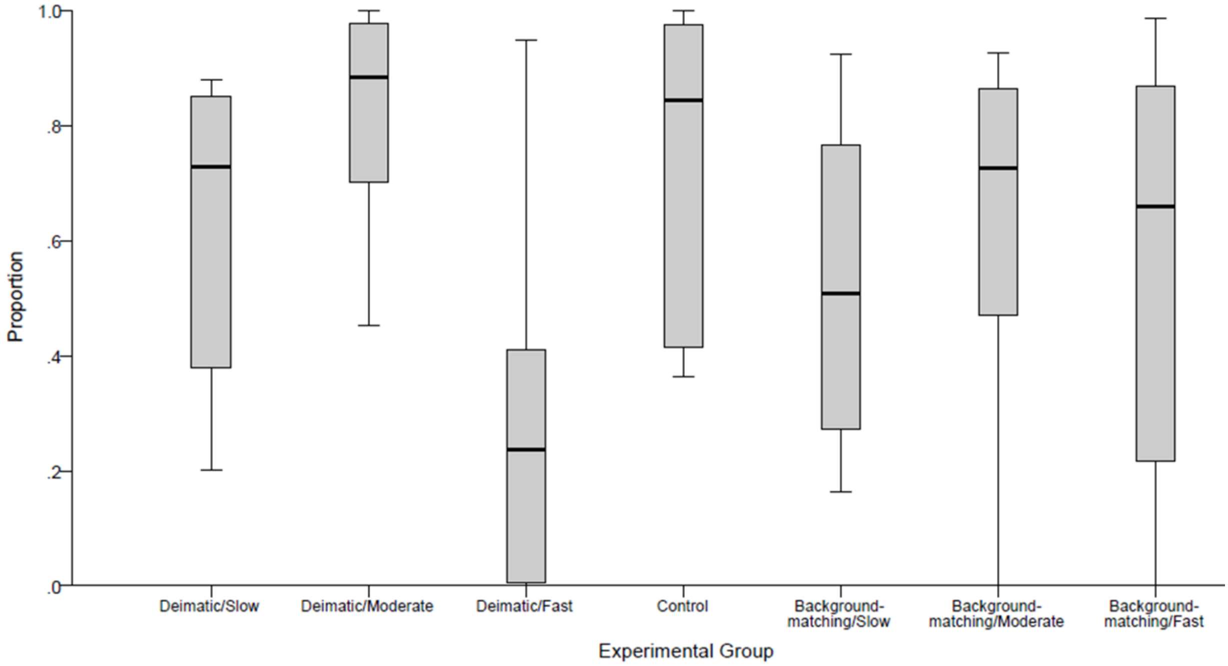

Figure 7. Proportion of time in proximity to prey during pre-attack interval. Each box represents the middle 50% of scores for each experimental group. The line dividing each box into two represent the median of those data. The upper and lower whiskers extend to include the data outside the middle 50%.

3.5 Discussion

Many deimatic displays involve prey revealing previously-hidden conspicuous visual components when approached or attacked by a predator (Blest, 1957; Maldonado, 1970; Sargent, 1978; Vallin *et al.*, 2005) . Understanding how these displays have evolved has proved challenging because the conspicuous visual components are unlikely to be effective without the movement that reveals them, and it is unlikely that the visual components and the behaviour evolved simultaneously. None of the prey types influenced the likelihood of predators spending time in proximity to them during the pre-attack interval. My results provide clear support for the two key predictions underlying the startle-first hypothesis, whereby movement is thought to evolve before conspicuous display components. I found that fast movement by prey was sufficient to cause birds to delay their attacks, and the combined effect of fast movement and a novel conspicuous visual component was more effective than movement alone. It is therefore feasible that natural selection could initially favour displays in which prey quickly move body parts, and could subsequently favour the evolution of conspicuous visual components that augment these behavioural displays (Umbers *et al.*, 2017).

My findings also suggest that deimatic displays need not necessarily include conspicuous components, other than fast movement, in order to be effective. This suggests that deimatism could evolve even in situations where there are factors constraining the evolution of other conspicuous display components (Edmunds, 1974). For example, where the cost of producing bright pigmentation or loud sounds is prohibitively high or restricted by an animal’s physiology (Hoback and Wagner, 1997; Bennet-Clark, 1998; Hooper, Tsubaki and Silva-Jothy, 1999; Hill, 2000; Talloen, van Dyck and Lens, 2004). Indeed, most studies investigating the antipredator benefits of deimatism focus on species with displays containing highly conspicuous visual and/or auditory components (e.g. Maldonado, 1970; Schlenoff, 1985; Vallin *et al.*, 2005; Olofsson *et al.*, 2012a, 2013; Olofsson, Jakobsson and Wiklund, 2012b; Umbers and Mappes, 2015; Kang *et al.*, 2016; Dookie *et al.*, 2017; Kang, Zahiri and Sherratt, 2017; although see Dias et al. 2014). This may be because most deimatic displays have them, but an alternative explanation is that we haven’t fully considered the possibility that species could benefit from deimatism without them. Deimatism could be more widespread than previously thought, and the form of these displays could be more diverse than we currently appreciate. For example, many cryptic species of moth (e.g. the Early Thorn moth, *Selenia dentaria* and the Hebrew Character, *Orthosia gothica*) occasionally flick their wings when threatened (Skelhorn, *pers. Obs.*), a movement which may be involved with preparation for flight. However, it is not difficult to imagine such a movement proving the rudimentary basis for the wing movements seen in lepidopteran displays (Blest 1957; Cott 1940).

Intriguingly, I only found a significant deterrent effect of movement when the prey moved at the fastest of the three speeds used. This suggests that the speed or number of displays (since here, as in natural systems, the two are correlated) needs to exceed a particular threshold in order to have a deterrent effect. This would be consistent with (although not a critical test of) the idea that deimatic displays work by startling predators, as rapid onset (or rise time) of a startling stimulus is required in order to successfully elicit a startle response (Turpin, Schaefer and Boucsein, 1999). However, it is worth noting that the difference in reaction times between chicks in the Control group and chicks in the Background-matching/Slow group approached significance. I would therefore not rule out the possibility that a wider range of movement speeds could have deterrent effects on predators, and would recommend further research in this area.

It is also worth noting that my experiment used naïve predators searching for artificial deimatic prey. This was crucial to control movement speed and ensure that predators' responses were not influenced by their previous experience with deimatic prey. However, this experiment should be considered as an initial step toward testing the order in which the various components of deimatic displays evolve. Here I have shown that movement alone can be effective in deterring predators. The next logical step is to determine whether the movements used in real deimatic displays are effective in deterring the natural predators of deimatic species and enhancing prey survival, in the absence of other display components. The only way to truly examine such movements would be to present predators with live prey, ideally within a natural setting.

Although I have shown that this evolutionary pathway is feasible, it doesn't preclude the existence of other pathways. Conspicuous visual patterns have evolved in some lepidopteran species to aid in mate recognition (Jiggins *et al.*, 2001) and as markers of individual quality in relation to sexual selection and mate choice

(Robertson and Monteiro, 2005). It is possible that these existing visual patterns were incorporated into deimatic displays after the evolution of the moving and/or behavioural components. In addition, selection may favour the addition of other display components (e.g. producing a loud auditory stimulus, incorporating a movement which appears to lead to a sudden increase in size; Umbers et al. 2017), if they confer additional benefits in terms of enhancing the deterrent effect of the display or preventing habituation. It is therefore possible that there are multiple pathways to the evolution of deimatic displays.

In conclusion, I have provided evidence that the startle-first hypothesis for the evolution of deimatism is feasible, i.e. that selection could favour the evolution of the behavioural components of deimatic display followed by the evolution of other conspicuous visual features. This is the first evidence of any evolutionary pathway in relation to deimatism, and helps us to understand the how the numerous and often conspicuous components came to form a cohesive defensive mechanism. It should be noted that the route outlined here relates only to those species that use movement to reveal previous hidden visual components. Some deimatic species appear to perform displays that rely on other sensory modalities (Dias *et al.*, 2014; Dookie *et al.*, 2017), or utilise visual components that are on constant display (Janzen, Hallwachs and Burns, 2010), and further work is needed to determine how such displays evolve. As is becoming apparent with all aspects of deimatism, it is important that we do not take a “one-size-fits-all” approach, but remain aware that just as displays of different species differ, so too will their evolutionary route.

Chapter 4. The role of predator hunger in the evolution of deimatic displays

4.1 Abstract

Deimatic displays are often considered to deter predators by eliciting predators' reflexive startle responses. If this is the case, then any factor that influences the magnitude of startle displays should also increase the antipredator efficacy of deimatic displays. In the experimental psychology literature, hunger has been consistently shown to increase startle responses when startling stimuli are presented in the presence of food, or images of food. If deimatic displays do indeed elicit predators' startle responses, and hunger increases the magnitude of startle responses, then deimatic displays should be more effective at deterring predators when they are in a state of increased hunger. In this study, I manipulated predator hunger levels in order to establish whether deimatic displays do elicit the startle reflex in predators. Domestic chicks were presented with artificial deimatic, background-matching and control prey at two different hunger levels. I found no effect of hunger on either the latency to attack deimatic prey or the amount of time spent in proximity to them prior to deciding whether to attack. This could have been due to the hunger manipulation not being successful but could also be evidence that deimatic displays do not deter predators by startling them.

4.2 Introduction

In Chapter 3, I provided evidence in support of one potential evolutionary route of deimatic displays. In this chapter, I turn my attention to why deimatic displays deter predators, and whether this could inform our understanding of the factors that might influence their efficacy, and consequently their evolution. As outlined in Chapter 1, deimatic displays are thought to cause predators to pause or abort their attacks by eliciting reflexive fear or startle responses (Maldonado, 1970; Edmunds, 1974; Schlenoff, 1985; Ruxton, Sherratt and Speed, 2004): rapid involuntary physiological responses (e.g. increased heart rate, changes in skin conductance, tension of the facial muscles) to sudden and intense sensory stimuli (Koch, 1999). However, there is currently no empirical evidence to either support or refute this idea. Perhaps one reason for this is that measuring startle responses in predators appears challenging. In humans, non-human primates and rodents, a number of methods of measuring startle have been established and validated. These include measuring changes in heart rate, skin conductance and muscle tension (Cook *et al.*, 1991; Koch, 1999; Dreissen *et al.*, 2012). Whilst it is possible to measure some of these variables in non-mammalian taxa (e.g. birds), this often requires invasive techniques, and they have yet to be validated as accurate measures of startle in these taxa.

Another possible way of establishing whether deimatism startles predators is to determine whether the efficacy of deimatic displays is influenced by any of the factors established by experimental psychologists to influence the magnitude of startle displays (see Chapter 1 for examples). One of these factors is hunger, which has been found to influence the magnitude of startle responses in humans (Drobes *et al.*, 2001; Hawk *et al.*, 2004; Rejeski *et al.*, 2010; Blechert *et al.*, 2014; Ferreira de Sa *et al.*, 2014) and rodents (Fechter and Ison, 1972; Schneider and Spanagel, 2008; Toufexis *et al.*, 2016). Research into the effect of hunger on the magnitude of startle responses has had conflicting results. Some studies have shown that hunger increases the magnitude of startle responses (Drobes *et al.*, 2001; Rejeski *et al.*, 2010; Blechert *et al.*, 2014; Ferreira de Sa *et al.*, 2014), whilst others have shown that it decreases the magnitude of startle responses (Fechter and Ison, 1972; Hawk *et al.*, 2004; Schneider and Spanagel, 2008; Toufexis *et al.*, 2016). Crucially, however, hunger consistently increases the magnitude of startle responses when subjects are presented with startling stimuli in the presence of food (Rejeski *et al.*, 2010), or images of food (Drobes *et al.*, 2001; Blechert *et al.*, 2014; Ferreira de Sa *et al.*, 2014). It has been suggested that in the presence of food, startling stimuli frustrate the expectation of a food reward (Wagner, 1963). This then leads to a state of increased stress, which in turn enhances the magnitude of the startle response (Wagner, 1963). In addition, it has been established that increased hunger stimulates increased attention to food cues (Piech, Pastorino and Zald, 2010), and increased attention towards startling stimuli is known to increase the magnitude of the startle response (Blumenthal, 2001). As deimatic displays are often initiated upon tactile contact from predators (e.g. Lenzi-Mattos et al. 2005; Umbers & Mappes 2015), one could argue that, prior to prey displaying, predators are approaching what they perceive to be a food item causing them to be more attentive which could increase their startle responses. Therefore, being in the presence of food, predators with increased hunger would be expected to show an increased magnitude of startle response by either of these mechanisms.

Here, I tested whether hunger influenced the responses of naïve domestic chicks (*Gallus gallus domesticus*) to deimatic prey. I manipulated hunger levels by restricting access to food for different amounts of time, thus creating groups of chicks with high, and low hunger levels. I then presented chicks with either stationary background-matching prey (Control), or prey that revealed either background-matching (Background-matching) or, conspicuously coloured hindwings (Deimatic), using the experimental paradigm outlined in Chapter 2. Since the chicks were initially trained to attack artificial prey in return for a food reward, I expected chicks to view artificial prey as a food source. Consequently, in this food-related context, I predicted that hunger would enhance startle responses and increase the deterrent effect of deimatic displays (assuming that deimatic displays do in fact startle predators).

4.3 Methods

4.3.1 Subjects

In total, 96 one day old domestic chicks of the ‘Ross’ strain participated in this experiment; 60 experimental chicks and 36 buddy chicks. The chicks were purchased from a commercial hatchery in Yorkshire (U.K.) in three cohorts and were of mixed sex. The housing arrangements and experimental paradigm used in this study are described in detail in Chapter 2. I received ethical approval for this study from the Newcastle University Animal Welfare Ethical Review Body (Project ID No.: ID 500).

4.3.2 Experiment

Training trials

Training trials were carried out as outlined in Chapter 2, in that chicks received one training trial per day over consecutive days until they reached the training criterion. This is with the exception of the manner in which the chicks were food deprived. In all training trials, each bird was moved into the food deprivation pen 100 minutes prior to the start of its trial. This ensured that all chicks spent equal time outside of their home cage prior to training/testing. During training trials, chicks were fed to satiation at 50 minutes, with all chicks having the same 50 minutes of food deprivation prior to the start of their training trial. Feeding the chicks to satiation involved moving them briefly (20-30 sec) to the experimental arena with buddy chicks present in the buddy areas where they were given (5-20) mealworms to consume. The number of mealworms consumed during the pre-trial feeding session increased to 20 over the course of the training days as the chicks themselves increased in size and capacity to eat. All chicks consumed 20 mealworms prior to participation in the test trial. Chicks in the home and food deprivation pens were in constant audio and visual contact with each other, to avoid any possible stressful effects as a result of being placed in the deprivation pen.

Test trial

After completion of the training protocol (see Chapter 2), the chicks participated in a single test trial. They were randomly assigned to one of six experimental groups, with each group experiencing one of the three prey types (Control, Background-matching, Deimatic) at one of the two hunger levels (Low, High; see Table 3). The computer-generated prey used here were the same as those outlined in Chapter 2, however the Background-matching and Deimatic prey moved only at the Fast speed (2.2 flicks per second), as this was found to be the most effective in deterring predators in an earlier study (see Chapter 3). Hunger levels were manipulated by feeding chicks to satiation at some point during the 100 minutes spent in the food deprivation pen. For the test trial, feeding to satiation occurred either after 10 minutes (High hunger treatment), or after 90 minutes (Low hunger treatment). This allowed me to alter the chicks' length of food deprivation while ensuring that each experimental treatment spent the same length of time in the food deprivation pen on the test day (as well as during training trials).

Table 3. Experimental groups.

Group	Prey Type	Hunger Level	Group Name
1	Control	Low	Control/Low
2		High	Control/High
3	Background-matching	Low	Background-matching/Low
4		High	Background-matching/High
5	Deimatic	Low	Deimatic/Low
6		High	Deimatic/High

During the test trial, each chick received a single prey presentation where moving prey were activated upon their crossing the midpoint of the arena (see Chapter 2 for further detail). The type of prey presented was determined by the experimental group membership of each individual (Table 3). All trials were video recorded using a JVC Everio GZ-315DEK camcorder. The latency to first peck or scratch prey was taken as a measure of the chicks' willingness to attack the prey images. From the videos of test trials, the arena was divided into two halves, one containing the prey image (Near) and the other half not containing the prey image (Far). Using the behavioural video scoring software BORIS (Behavioural Observation Research Interactive Software; Friard and Gamba, 2016), I calculated the proportion of time that chicks spent in the Near sections of the arena during the time interval between prey activation (when the chick's head crossed the midpoint in the case of Control prey), and prey attack (referred to from this point as the pre-attack interval).

4.3.3 Data Analysis

Data were analysed using the statistical software IBM SPSS (v23).

Latency to attack

The time taken for chicks to first peck or scratch prey after prey activation was live coded by the experimenter during the test trial using a keypad specifically coded for this purpose. It should be noted that prey activation in stationary control prey was taken as the point at which an individual chick's head crossed the midpoint of the arena. A number of potential outliers were identified. However, taking into account my sample size, and the range of behaviours observed across all of my experimental groups, I deemed them to be realistic chick responses and so included them for analysis. The data were found to violate the assumptions of normality, as the residuals were not normally distributed and the data were heteroscedastic. I carried out a log10-transformation which produced data that had non-normally distributed residuals but were homoscedastic. A series of Kruskal-Wallis tests was, therefore, used to investigate a series of planned comparisons.

I predicted that i) birds in the Deimatic/Low group would attack prey faster than those in the Deimatic/High group, ii) birds in the Background-matching/Low group would attack prey faster than those in the Background-matching/High group, and, iii) birds in the Control/High group would attack prey faster than those in the Control/Low group. In other words, hunger would enhance the deterrent effect of displays (irrespective of whether the hindwings were background matching or conspicuous) but would make birds more motivated to attack innocuous Control prey. In line with my findings from Chapter 3, I also predicted that Background-matching prey would be more deterrent than Control prey, and that Deimatic prey would be more deterrent than Background-matching prey. Further, I expected this to hold across the experimental manipulation. This would result in iv) chicks in the Background-matching/High and Background-matching/Low groups taking longer to attack prey than chicks in the Control/High and Control/Low groups, and v) chicks in the Deimatic/High and Deimatic/Low groups taking longer to attack prey than chicks in the Background-matching/High and Background-matching/Low prey.

As I used a small number of specific planned comparisons, it was not necessary to carry out Bonferroni corrections to control for Type I error rate (Ruxton and Beauchamp, 2008).

Proportion of time spent in proximity to prey during pre-attack interval

Using the behavioural video scoring software, BORIS (Friard and Gamba, 2016), I calculated the proportion of time that each chick spent in the Near section of the arena (the half of the arena containing the prey image) during the pre-attack interval. Since these data were proportional rather than continuous, they were arcsine square root transformed to make their distribution more evenly spread. The residuals of the transformed data were normally distributed, and the data were homoscedastic. Therefore, I used a one-way ANOVA to examine the differences among the experimental groups followed by a series of orthogonal planned contrasts. Those planned contrasts tested the following predictions: i) that chicks in the Deimatic/High group would spend less time in the Near section of the arena than those in the Deimatic/Low group, ii) that the time spent in the Near section would be less for those in the Background-matching/High group than those in the Background-matching/Low group and iii) that chicks in the Control/High group would spend a greater amount of time in the Near section of the arena than those in the Control/Low group. In light of the results of my first experiment (Chapter 3), I predicted that chicks would not differ in the proportion of time spent in proximity to iv) Deimatic prey compared to Background-matching prey and v) Background-matching and Control prey, for both Low and High hunger chicks.

4.4 Results

4.4.1 Latency to attack prey

In contrast to my predictions, I found no evidence that hunger influenced the speed at which birds attacked prey. There was no significant difference in the birds' latencies to attack prey between the Deimatic/High and Deimatic/Low groups (Kruskal-Wallis test: $\chi^2 = 0.706$, df = 1, p = 0.413), Background-matching/High and

Background-matching/Low groups (χ^2 = 0.368, df = 1, p = 0.559), or Control/High and Control/Low groups (χ^2 = 0.348, df = 1, p = 0.579; Figure 8).

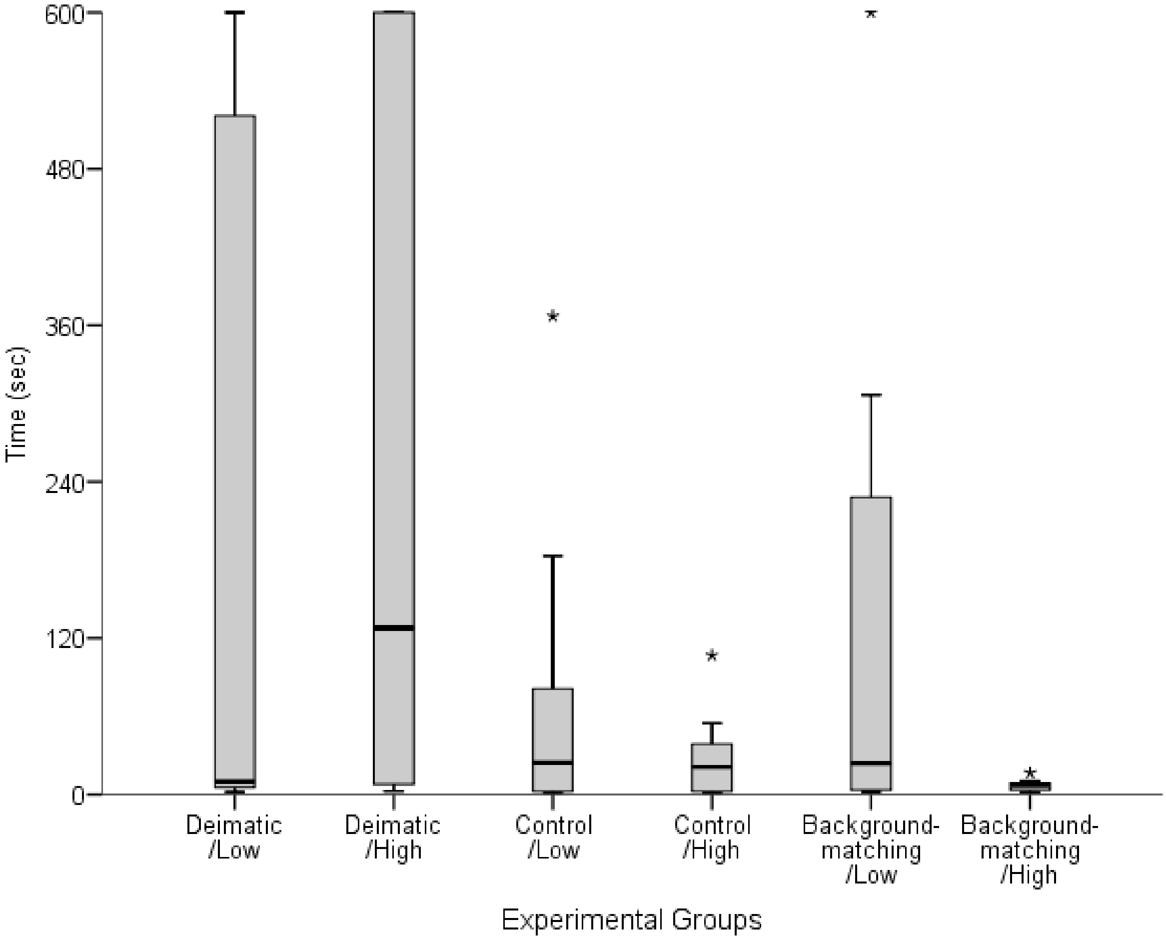

Figure 8. Latency (in seconds) to attack prey during the test trial. Each box represents the middle 50% of scores for each experimental group. The line dividing each box into two represent the median of those data. The upper and lower whiskers extend to include the data outside the middle 50%. Asterisks (*) represent possible outliers, each of which has been examined and is valid for inclusion within the analysis.

Further, I found no significant difference in birds' latencies to attack prey between either the Deimatic and Background-matching groups (Kruskal-Wallis test: χ^2 = 2.05, df = 1, p = 0.152), or the Background-matching and Control groups (Kruskal-Wallis test: χ^2 =0.320, df = 1, p = 0.859). This suggests that, in contrast to my previous study (see Chapter 3), prey's displays had no detectable deterrent effect on predators.

4.4.2 Proportion of time in proximity to prey during pre-attack interval

No effect of hunger was found on the proportion of time chicks spent in proximity to prey in the interval between prey activation and attack (one-way ANOVA: $F_{(5)}$ = 1.157, p = 0.339; Figure 9). In contrast to my predictions, I found no difference in the proportion of time birds spent in proximity to prey between the Deimatic/High and Deimatic/Low groups (t = 1.719, df = 74, p = 0.09), the Background-matching/High and Background-matching/Low groups (t = 0.819, df = 74, p = 0.415), and the Control/High and Control/Low groups (t = -0.986, df = 74, p = 0.327).

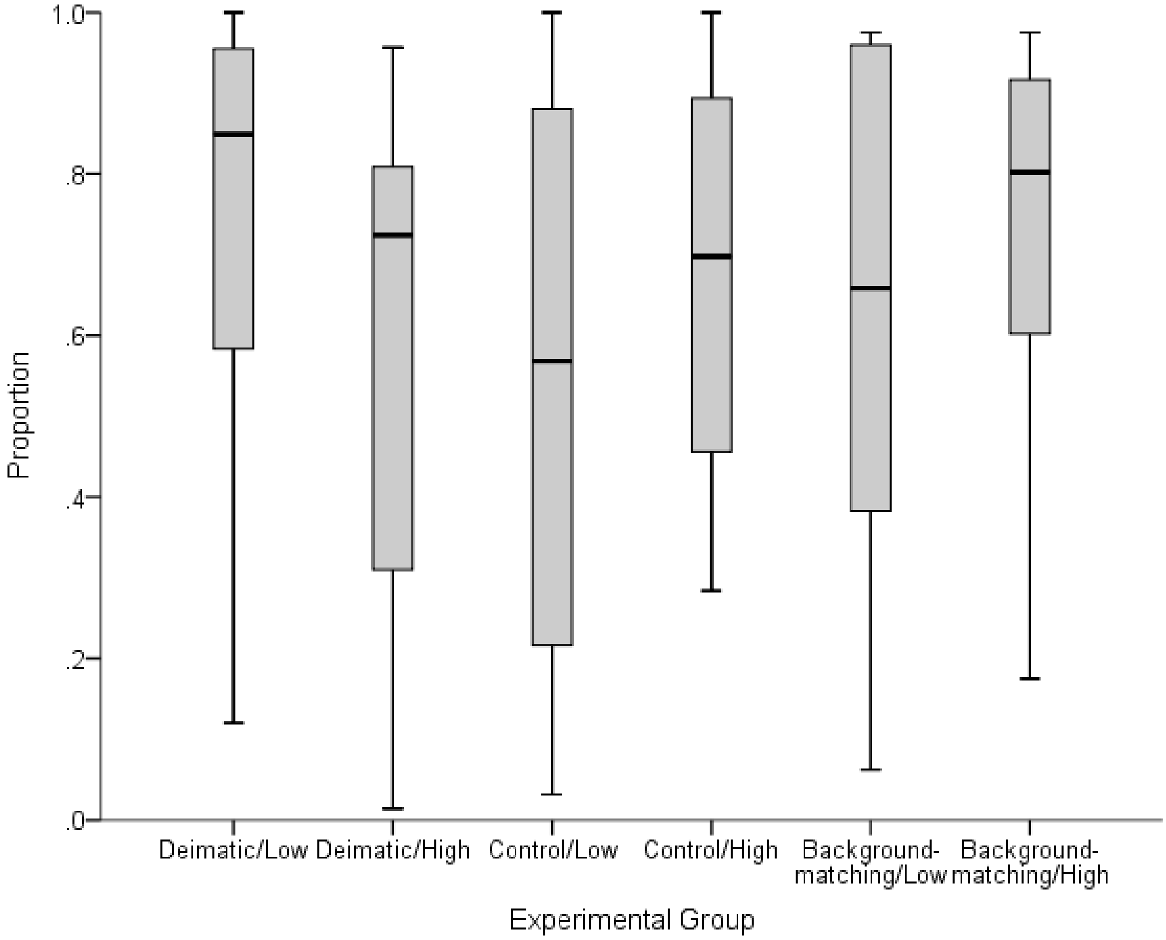

Figure 9. Proportion of time spent in proximity to prey during the pre-attack interval. Each box represents the middle 50% of scores for each experimental group. The line dividing each box into two represent the median of those data. The upper and lower whiskers extend to include the data outside the middle 50%.

Nor was there any difference in the proportion of time spent in proximity to prey between the Deimatic and Background-matching groups (t = 0.246, df = 74, p =

0.806), or between the Background-matching and Control groups (t = 1.008, df = 74, p = 0.317). These results suggest that hunger does not influence the proportion of time predators spent in proximity to prey while deciding whether to attack them.

4.5 Discussion

Contrary to expectation, hunger level did not have any effect on predators' responses to any of the prey presented in this experiment. There was no difference in the latency to attack, or the time spent near prey during the pre-attack interval for any of my prey types between the High and Low hunger manipulations. In addition, in contrast to the experiment outlined in Chapter 3, I found no evidence that prey with background-matching or deimatic displays had a deterrent effect on predators. Predators took a similar amount of time to attack both prey types, and they did not differ in the time they spent in proximity to prey during the pre-attack interval. Therefore, these results do not suggest that predator hunger level influences their responses to deimatic displays.

In my previous study (see Chapter 3), I found that predators were deterred by both Background-matching and Deimatic prey. Thus, it was surprising that I did not find a similar effect in this study. It is possible that the Deimatic and Background-matching prey did deter predators, but this effect was masked by the large amount of variance between the groups. Alternatively, the food deprivation manipulation may have affected the results. In Chapter 3, chicks were food deprived for 60 minutes prior to participation in the test trial. Perhaps shorter periods of deprivation (i.e. 10 minutes) reduce any deterrent effect as the birds are under less energetic stress. On the other hand, longer periods of deprivation (i.e. 90 minutes) may lead to such a high level of energetic stress that it overrides any deterrence provided by the displays. Further research examining the influence of a broader range of food deprivation periods would be required in order to clarify whether food deprivation removes the deterrent effect seen in Chapter 1.

The lack of any effect of hunger on the birds' responses to prey may have occurred for a number of reasons. As outlined above, no difference was found in the behaviour of the birds toward the three prey types presented to them. This lack of an establishment of an initial difference between the three prey groups may have made it difficult to then detect any difference produced by the hunger manipulation. It is therefore difficult to determine what amount of variation in the data is due to the birds' responses solely to the prey, and what is because of the effect of their hunger level. Thus, within the experimental protocol used here, it proves difficult to state for certain whether we are seeing results representative of solely the effects of different levels of hunger. Another possibility is that the sample size in this study was not large enough to detect significant differences among my experimental groups. However, in my previous study where I detected differences (Chapter 3), I had a smaller sample size (n=8 per group) compared to the current study (n=12-15 per group), suggesting that my sample size should have been sufficient to detect any effect of hunger were it present. Therefore, I think my failure to find an effect of hunger on birds' responses to deimatic prey is more likely due to the fact that the relationship between prey defence and energetic state is more complex than could be accounted for within my experimental paradigm.

The lack of any effect of increased hunger may be due to the failure of my manipulation to induce a change of state in the chicks. My experimental protocol was based on the concept that hunger level should increase with increasing time without access to food. However, an alternative manipulation of energetic state (e.g. manipulation of body mass) may have produced more definitive differences between experimental groups. The manipulation of body mass has been carried out in other behavioural research using avian subjects. For example, Barnett et al. (2012) manipulated the energetic state of the starlings (*Sturnus vulgaris*) that participated in their experiment. They initially used food restriction over consecutive days to reduce birds' body mass to a known percentage of their free-feeding mass, and subsequently increased their body mass by providing food ad lib. This allowed birds' responses to prey to be tested at different masses, but crucially at similar hunger levels, as birds were tested after the same degree of food deprivation throughout. This approach would also have the added advantage of controlling for individual differences in factors such as metabolic rate. However, it is also worth noting that energetic state and nutrient requirements can influence the likelihood that predators will consume toxic aposematic prey (Sherratt, 2003; Barnett, Bateson and Rowe, 2007; Barnett *et al.*, 2012; Halpin, Skelhorn and Rowe, 2014b). Therefore, increased need to consume food may in fact increase the likelihood of predators consuming prey they would be deterred by when satiated. Thus, even if hunger does enhance the startling effect of deimatism, it may also motivate birds to recover from their initial response more quickly, meaning that there may be no net effect of hunger on attack latencies.

Finally, it may be that hunger did not influence predators' responses to deimatic prey because deimatic displays do not startle predators, or because predators did not perceive the artificial prey as food or images of food. As discussed earlier, hunger-induced enhancements of startle response magnitude are only consistently seen in humans and rodents when they are in the presence of food (Drobes *et al.*, 2001; Blechert *et al.*, 2014; Ferreira de Sa *et al.*, 2014) or images of food (Rejeski *et al.*, 2010). It may be the case that birds don't perceive the test trial as a food-related contest as, although they are likely to associate their experiences in the arena with receiving a food reward, there is no food present when they view the display. Another possible explanation is that we see no effect because in fact deimatic displays don't function by startling predators. Although commonly proposed to be the mechanism behind the deterrent effect of these displays (Maldonado, 1970; Edmunds, 1974; Schlenoff, 1985; Ruxton, Sherratt and Speed, 2004), this may simply not be the case. These results could provide potential evidence of deimatic displays not acting to startle predators. In the next chapter, I investigate this further by testing whether background noise (an environmental factor known to enhance startle responses) enhances the antipredator efficacy of deimatic displays.

Chapter 5. The role of background noise in the evolution of deimatic displays

5.1 Abstract

It is important to establish what factors influence the efficacy of deimatic displays in order to gain a better understanding of the ecological conditions under which deimatism is likely to evolve, and to shed light on the mechanisms through which deimatism deters predators. Whilst I did not find any evidence to support the idea that hunger influenced the efficacy of deimatic displays (Chapter 4), it is possible that other factors known to enhance or diminish the magnitude of startle displays may alter the efficacy of deimatism. There is good evidence that background noise enhances startle responses in humans and rodents. Here, I tested the prediction that background noise would enhance the deterrent effect of deimatic displays on predators by comparing chicks' responses to control and deimatic prey at two levels of noise (ambient noise levels, and enhanced background noise where white noise was played), using the experimental system outlined in Chapter 2. In line with my previous study (see Chapter 3), I found that chicks took longer to attack deimatic prey than control prey. However, the effect of background noise on the efficacy of deimatism was more complex. Whilst I found no evidence that background noise affected the speed of predatory attacks, chicks in the background noise condition spent significantly more of the time prior to attack in the half of the arena furthest away from the deimatic prey compared to chicks in the ambient noise condition. This was not true of chicks exposed to control prey. These results suggest that background noise may improve the chances of survival for deimatic prey by providing them an opportunity to escape while predators decide whether or not to attack.

5.2 Introduction

In the previous chapter, I found no evidence that hunger influenced the efficacy of deimatic displays, despite the fact that hunger is known to enhance the magnitude of startle responses under conditions of frustrative non-reward (i.e. when food, or images of food, are present during the administration of the startling stimulus; Wagner, 1963). This may have been because deimatic displays do not startle predators, or it may be because my experimental manipulation did not successfully produce a difference in predator hunger. However, other factors also influence the magnitude of startle responses and potentially influence the efficacy of deimatic displays, including a number of environmental factors. For example, light levels affect the startle responses of both human and rodent subjects (Grillon *et al.*, 1997; Walker and Davis, 1997, 2002), but in different ways: high levels of illumination increases the startle response in rodents (Walker and Davis, 1997, 2002), whilst humans show stronger startle responses in darkness (Grillon *et al.*, 1997). These differences can potentially be explained by the time period when these species are least active, since in both species, startle responses are increased under the lighting conditions experienced during the times when they are likely to be sleeping and therefore more vulnerable (Grillon *et al.*, 1997; Walker and Davis, 1997). The nutrient content of the available diet has also been shown to affect the magnitude of startle responses. Rats (*Rattus norvegicus*) fed on an nutrient deficient diet were found to have reduced startle magnitudes (Burhans *et al.*, 2006; Unger *et al.*, 2006). Thus, it is possible that environments with nutrient-poor prey may produce predators with reduced startle response magnitudes. Finally, the cold pressor test (where subjects submerge their hands in water at 0-4°C and are presented with startling stimuli before, during and after submersion) has been found to reduce startle responses in human subjects (Tavernor *et al.*, 2000), suggesting that ambient temperature may be important in determining the magnitude of startle responses, and consequently the efficacy of deimatic displays.

In this chapter, I manipulated background noise, because its effects on startle responses are well established (Hoffman and Fleshler, 1963; Hoffman and Searle, 1965; Davis, 1974; Cory and Ison, 1979; Gerrard and Ison, 1990; Flaten, Nordmark and Elden, 2005; Blumenthal *et al.*, 2006).Background noise is known to increase the magnitude of the startle responses of both humans and rodents (Hoffman and

Fleshler, 1963; Hoffman and Searle, 1965; Davis, 1974; Cory and Ison, 1979; Gerrard and Ison, 1990; Flaten, Nordmark and Elden, 2005; Blumenthal *et al.*, 2006). In rats, presentation of an auditory startling stimulus in the presence of background noise (white noise ranging from 50-90dB) increases the acoustic startle response, or ASR (Hoffman and Fleshler, 1963; Hoffman and Searle, 1965; Davis, 1974; Cory and Ison, 1979; Gerrard and Ison, 1990). In these studies, the ASR was measured by testing subjects in a specialised cage with a spring-loaded floor linked to either a stabilimeter or accelerometer, such that, when the rat was startled and jumped it was possible to objectively measure the magnitude of that response. Similarly in human studies, background noise (white noise at 40-70 dB) produced increased eye-blink magnitudes in response to auditory startling stimuli (Flaten, Nordmark and Elden, 2005; Blumenthal *et al.*, 2006). It is thought that these amplified startle responses are due to background noise causing anxiety in experimental subjects, as anxiolytic drugs have been found to prevent this enhancing effect (Kellogg *et al.*, 1991). This makes sense as it has been established that startle response magnitudes are increased in anxious individuals (Kaviani *et al.*, 2004; Grillon *et al.*, 2005). An important consideration for me was the fact that previous research primarily presented auditory startling stimuli, whereas in the case of my experimental system, the chicks would be receiving visual stimulation only. However, studies have shown the same influence of background noise in relation to tactile startling stimuli (Ison and Russo, 1990), suggesting that this effect is not isolated to auditory stimuli. There is also some evidence that background noise can enhance the magnitude of startle responses elicited by visual stimuli in birds (Stitt *et al.*, 1976). The investigation of the influence of background noise in this context is therefore appropriate.

Background noise is also a factor that deimatic prey and their predators are likely to experience at varying frequencies and volumes within their environment. Understanding the effect, if any, of background noise is important as it will help us to understand the environmental conditions under which deimatic displays are likely to evolve and, the effect that anthropogenic changes in noise levels could have on the efficacy of deimatic displays, and consequently prey survival. In this study, I presented naïve domestic chicks with Deimatic and Control prey, using the experimental system outlined in Chapter 2, at either ambient sound levels (Ambient Noise) or with white noise playing at a level of 80db (Background Noise). The background noise stimulus was selected on the basis that it had the greatest effect on the magnitude of birds' startle responses elicited by visual stimuli (Stitt *et al.*, 1976). I predicted that Deimatic prey would produce a greater deterrent response in chicks subject to the Background Noise treatment in comparison to those in the Ambient Noise treatment. I did not expect there to be a difference in the responses of chicks to Control prey between the two sound treatments.

5.3 Methods

5.3.1 Subjects

Two cohorts of 42 one-day-old domestic chicks (*Gallus gallus domesticus*) of mixed sex (Ross strain), were purchased from a commercial hatchery in Yorkshire (U.K.) for participation in this study. Of the 84 chicks, 24 acted as buddy chicks and 60 participated as experimental subjects. Details of chick housing, as well as training and experimental paradigms used in this study, are described in detail in Chapter 2. Two identical laboratories were used for the duration of this study: one for housing chicks and one for training and testing chicks. I received ethical approval for this study from the Newcastle University Animal Welfare and Ethical Review Body (Project ID Number: 611).

5.3.2 Experiment

Training trials

On days 1-7, I exposed chicks to silence for 6 of the 12 daylight hours, and background noise for the remaining 6 daylight hours in their home laboratory. This was to ensure that that all chicks were equally familiar with background noise and ambient noise, in addition to being familiar with the background noise stimulus itself. The background noise consisted of white noise at 80dB played from a pair of Logitech Z200 speakers. One of the speakers was positioned on the side of the home pen, and the other was positioned on the side of the food deprivation pen. (Note: the food deprivation pen and home pen consisted of a divided rectangular pen, such that 25% of the area constituted the food deprivation area and the other 75% the home pen.) The speakers were attached to the top of the pen side facing inward and downward at a 30° angle. This positioning ensured that the sound level was uniform throughout the home and food deprivation pens regardless of chick position. I confirmed this by using a sound level meter (Max Measure MM-SMB01) to take daily measurements of the background noise stimulus in the centre and all four corners of the home and food deprivation pens. I found that all measurements fell within an 80 ± 0.5 dB range. Chicks were exposed to ambient and white noise in a randomised block design, in such a way that out of every two hours, chicks received one hour of ambient noise and one hour of white noise. The specific order in which chicks received ambient noise and white noise was different every day.

Training trials were carried out in the same manner as described in Chapter 2, aside from the chicks' housing and food deprivation occurring in the home laboratory, and only the training trials themselves occurring in the experimental laboratory. In half of the training trials chicks were exposed to the Background Noise treatment (white noise at 80 dB), and in the other half they were exposed to the Ambient Noise treatment (ambient noise level of the testing laboratory; 54.5 ± 2 dB). The Background Noise stimulus was played over a pair of Logitech Z200 speakers. Each speaker was attached to the top of the division (in the centre) separating the buddy chick arenas from the experimental arena. They were positioned facing inward and downward (at a 30˚ angle). Using a sound level meter (Max Measure MM-SMB01), I took daily measurements of the Background Noise stimulus in the centre and four corners of the experimental arena to ensure that the sound level remained within an 80 ± 0.5 dB range throughout. Trials were presented in a randomised block design, such that, out of every two trials, one was performed with white noise (Background Noise) and one was performed without (Ambient Noise). This resulted in 8 different orders of sound presentation, and each chick was randomly assigned to one of those. I ensured that an equal number of chicks were assigned to each presentation order. In addition, the order in which chicks were tested was randomised such that each chick participated in their training trials at a different time each day.

Test trial

On day 8, the test trial was carried out. Chicks were presented with one of two possible prey types, Deimatic or Control (see Chapter 2 for detailed descriptions). It should be noted that Deimatic prey were only presented at the Fast speed (2.2 fps) as the results of earlier experiments (Chapter 3) indicate that these are most effective in deterring predators. The 60 experimental chicks were randomly assigned to one of four groups, such that each chick experienced one prey type and one sound condition; i) Control/Background Noise, ii) Control/Ambient Noise, iii) Deimatic/Background Noise, iv) Deimatic/Ambient Noise. The Ambient Noise and Background Noise treatments were identical to those used for the training trials. The speaker positioning in relation to the experimental arena was also the same. The order of use of buddy chick groups (buddy chicks were assigned to groups of four individuals which were used for one hour at a time and the groups were rotated so that each had a two hour break between use) ensured that all buddy chicks experienced equal amounts of exposure to both the Ambient noise treatment and the Background noise treatment to avoid any potential influence of buddy chick experience.

Test trials proceeded as outlined in Chapter 2. Trials lasted for a total of 10 minutes after activation of prey display (i.e. after the chicks crossed the mid-point of the arena). All trials were video recorded using a JVC Everio GZ-315DEK camcorder. The latency to first peck or scratch prey was taken as a measure of the chicks' willingness to attack the prey images. The proportion of time spent in proximity to prey (i.e. in the half of the arena containing the prey item) in the time period between prey activation and a chick's first contact was taken as a measure of the level of the chick's aversion to the prey image.

5.3.3 Data Analysis

All data were analysed using the scientific statistical analysis software IBM Statistics SPSS (v23).

Latency to attack

The latency to attack data were homoscedastic however, the residuals of the data were not normally distributed, and as such, it was necessary to transform the data using a Log10 transformation. The transformed data had normally distributed residuals and remained homoscedastic. I then carried out a two-way ANOVA to determine whether prey type and sound condition influenced the latency to attack prey. I first predicted that there would be a main effect of prey type, with chicks attacking deimatic prey slower than control prey. This is because, irrespective of the noise environment, I would expect the chicks to be deterred by deimatic displays. Second, I predicted that there would not be a main effect of the noise condition, because I would only expect differences in the noise environment to alter the chicks' responses toward deimatic prey. Finally, I predicted that there would be an interaction between prey type and noise type such that the Background Noise treatment would increase the time taken to attack deimatic prey, but this would not be true for control prey. This is due to the fact that I would expect chicks' responses to deimatic prey to mirror those of startling stimuli, but I would not expect the stationary control prey to possess any similarity to a startling stimulus.

Proportion of time in proximity to prey during pre-attack interval

Using the behavioural video scoring software, BORIS (Friard and Gamba, 2016), I calculated the proportion of time that chicks spent in the half of the experimental arena containing the prey image (Near) during the interval between prey activation and predator attack (the pre-attack interval). For Control prey that were stationary, the activation point was taken as the point at which the chicks crossed the midpoint of the arena.

These data were proportional, and so I transformed them using an arcsine square root transformation. As proportional data tend to lie outside of the 0.3-0.7 range of the data, arcsine square root transformation extends both ends (between 0.0-0.3 and 0.7-1.0) of the distribution to give the data a more normal distribution. The transformed data had non-normally distributed residuals, however, the experimental groups displayed equal variances. Therefore, I used Kruskal-Wallis tests to investigate a series of *a priori* predictions using a series of planned comparisons. I predicted that: i) chicks would spend more time in the Near section of the arena when presented with Deimatic/Ambient Noise prey than when presented with Deimatic/Background Noise prey, ii) chicks would not differ in the amount of time they spent Near prey in the Control/Ambient Noise and Control/Background Noise groups, and iii) chicks would spend less time Near Deimatic prey than Control prey regardless of the sound treatment.

5.4 Results

5.4.1 Latency to attack prey

In line with my predictions, I found a main effect of prey type in that chicks took significantly longer to attack Deimatic prey than Control prey ($F_{(1,51)} = 18.112$, $p < 0.001$; Figure 10), indicating that they found Deimatic prey more aversive. In addition, there was no main effect of sound condition as predicted ($F_{(1,51)} = 0.452$, $p = 0.504$). However, I did not find any interaction between prey type and sound condition ($F_{(1,51)} = 0.301$, $p = 0.585$). This suggests that, in contrast to my predictions, Background Noise did not alter the level to which birds found Deimatic prey aversive.

5.4.2 Proportion of time in proximity to prey during pre-attack interval

In contrast to my predictions, there was no significant difference in the amount of time chicks spend in close proximity to Deimatic prey compared to Control prey (Kruskal-Wallis: $\chi^2 = 2.311$, df = 1, p = 0.128; Figure 11). However, as predicted, there was no significant effect of background noise on the proportion of time chicks spent in proximity to control prey during the pre-attack interval (Kruskal-Wallis: $\chi^2 = 0.003$, df = 1, p = 0.977), whereas chicks exposed to background noise spent significantly less time in close proximity to deimatic prey during the pre-attack interval than those exposed to ambient noise (Kruskal-Wallis: $\chi^2 = 4.154$, df = 1, p = 0.043). Taken together, these results suggest that background noise alters birds' willingness to remain in proximity to Deimatic prey, but not control prey, during the time when they are deciding whether or not to attack.

Finally, I ran a one-sample Wilcoxon test for each of the experimental groups to determine whether they showed a significant preference for either side of the arena (i.e. whether the median proportion of time spent in close proximity to the prey was significantly different from chance levels of 0.5). The Control/Ambient Noise, Control/Background Noise, and Deimatic/Ambient Noise groups showed no significant preference for either side of the arena (One-Sample Wilcoxon: T = 39, p = 1.000; T = 67, p = 0.363; and T = 47, p = 0.917, respectively). However, chicks in the Deimatic/Background Noise group spent significantly more time in the half of the arena furthest from the prey than in the half containing the prey (one-sample Wilcoxon: T = 13, p = 0.013).

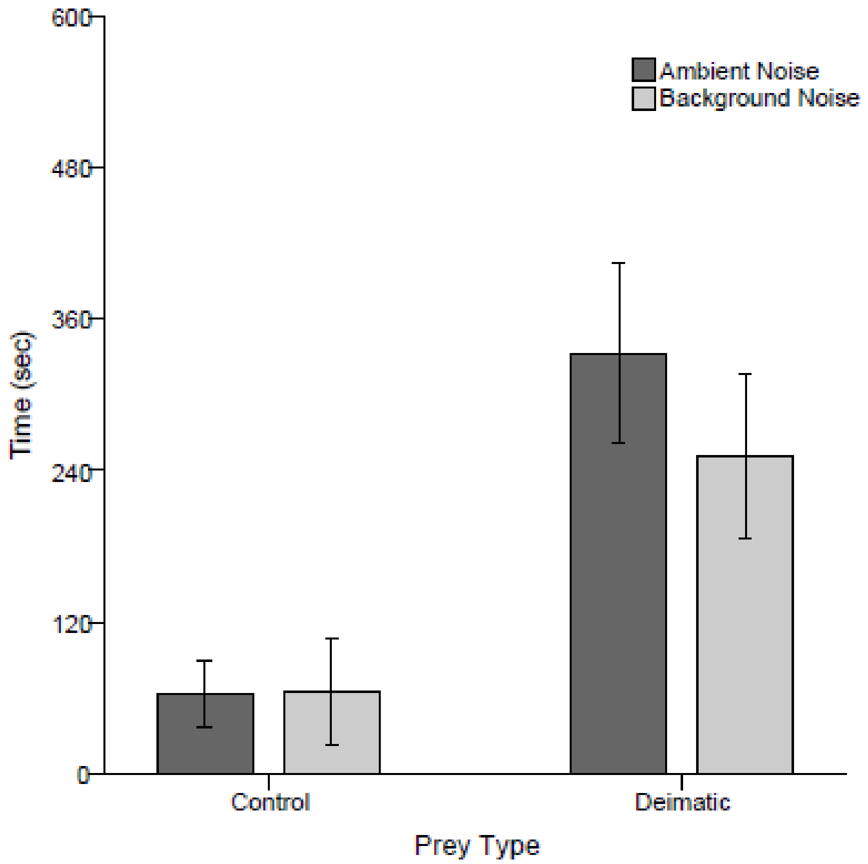

Figure 10. Latency (in seconds) to attack (M±SE) prey during the test trial according to prey type and sound condition.

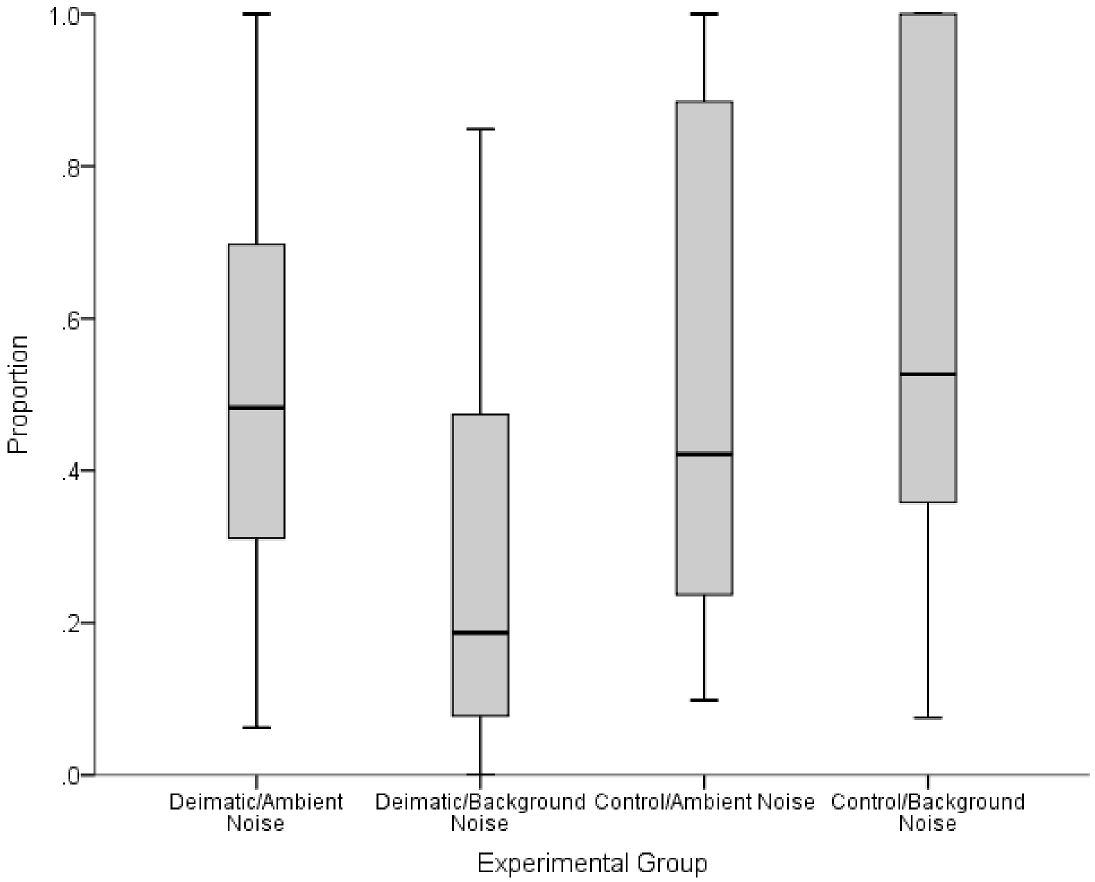

Figure 11. Proportion of time spent in the Near half of the arena during the pre-attack interval. Each box represents the middle 50% of scores for each experimental group. The line dividing each box into two represent the median of those data. The upper and lower whiskers extend to include the data outside the middle 50%.

5.5 Discussion

I found that birds were much slower to attack Deimatic prey than Control prey. This is consistent with the findings presented in Chapter 3 and, indicates that birds were deterred by deimatic displays. As expected, background noise had no effect on birds' responses to control prey. However, the effects of background noise on birds' responses to deimatic prey were more complex. I found no effect of background noise on the time taken to attack deimatic prey, but birds exposed to background noise spent significantly more time in the half of the arena furthest from deimatic prey than those exposed to ambient noise. It is difficult to determine how the observed behavioural changes in response to background noise might influence the survival of deimatic prey in natural settings. However, it seems reasonable to assume that if predators position themselves further away from prey while deciding whether to attack them, prey may have a greater opportunity to escape. My results, therefore, provide the first evidence that environmental factors could potentially influence the efficacy of deimatism, and consequently the likelihood that it will evolve.

My findings should be viewed as the first step towards understanding the effect of background noise on the efficacy of deimatism. Further research using live deimatic prey, and ecologically relevant noise types and levels, is required in order to establish whether background noise enhances the survival of deimatic prey in natural settings. If background noise does increase the likelihood of deimatic prey surviving encounters with predators, then it may affect the likelihood of deimatism evolving and both the behaviour and ecology of deimatic prey. All else being equal, I would expect deimatism to evolve more readily in noisy environments, since the antipredator benefits of deimatism would be greater there. I would also expect deimatic prey to show preferences for habitats/microhabitats with higher levels of background noise, or to show increased activity at times of the day when background noise is most prevalent. For example, just after dawn when many passerines are known to spend a higher proportion of time singing in comparison to other times of day (Hinde, 1952; Morton, 1975). Moreover, it is not only natural sources of noise that could potentially influence the efficacy of deimatism, anthropogenic noise could have similar effects, which could be one reason why deimatic species like the Peacock butterfly (*Aglais io*) appear to do well in urban environments (Bergerot *et al.*, 2011). Currently, these predictions are speculative. Whilst there is good evidence that prey often select environments that maximise the efficacy of their antipredator defences (Herrebout, Kuyten and De Ruiter, 1963; Greene, 1989; Skelhorn and Ruxton, 2013), microhabitat selection in deimatic prey has not yet been investigated. Similarly, we do not know what, if any, environmental conditions might promote the evolution of deimatism, but comparative analyses (like those used for other forms of defence; Sword, 1999; Michie *et al.*, 2011; Nokelainen, Lindstedt and Mappes, 2013; Galarza *et al.*, 2014) could help to address this issue. It is also worth noting that the predictions above are in relation to deimatic displays with visual components. It is difficult to predict what would happen if the display had an auditory component. Whilst background noise is known to enhance auditory startle, this is only true if the noise is not sufficiently loud to interfere with the detection of the deimatic sounds (Ison and Russo, 1990).

The results of this study highlight the importance of considering deimatic displays within the bigger picture of their varying environment. Previous research has tended to investigate the efficacy of deimatic displays within a narrow range of tightly-controlled experimental settings (temperature, lighting and noise level). I would suggest that future research should aim to investigate how a range of environmental factors, such as those outlined in the introduction, influence the efficacy of deimatism. This would enable us to build a more complete picture, not only of the environments in which deimatic prey are likely to survive best, but also the environments in which they are likely to evolve. In addition, finding an effect of background noise on a predator behaviour (proximity to prey during the time when deciding to attack) that was not found to be important in previous studies (Chapters 3 and 4), highlights the importance of collecting data on a wide range of predator behaviours rather than just whether predators attack prey or not. The factors improving the likelihood of deimatic prey survival may provide their advantage in more subtle ways than stopping a predatory attack completely.

If predators perceive the visual components of a deimatic display in the presence of background noise in the same manner by which they treat a multimodal signal, then previous research investigating the integration of multimodal stimuli, and in particular those focusing on visual and auditory stimuli, can provide further insight into the effect of background noise on the responses of predators to deimatic displays. It has been found that multimodal stimuli can excite cells in the superior colliculus (SC) such that their response in comparison to unimodal stimuli is enhanced (Meredith and Stein, 1986), and those SC neurons connect to areas of the brain stem and spinal cord that are involved in attention and orientation behaviours. Therefore, multimodal signals may have higher salience and consequently receivers may direct more of their attention toward them. Thus, the processing of the auditory background noise in combination with the visual deimatic display may have led to increased neural activity which could increase the saliency of the visual components causing predators to retreat to a position of ‘safety’ in order to observe the deimatic prey and make an informed decision whether to attack or not. Research measuring the activity of these neurons while predators experience the conditions of my experimental test trial would be necessary in order to confirm this for certain. Background noise may also influence birds’ abilities to categorise visual signals. Human subjects were found to discriminate visual targets faster on trials where the auditory stimulus they were simultaneously exposed to was “matching” in its features (Marks, 1987). This concept of matching relates to the phenomena of various aspects of sound and vision being perceived as complimentary in humans. For example, high-pitched sounds are known to evoke jagged, sharp visual images, whereas smooth, rounded images are evoked by low-pitched sounds (Karwoski and Odbert, 1938). In addition, high-pitched sounds are known to provoke the perception of bright images (Marks, 1975). Therefore, if predators found the background noise and the visual signals incongruent, they may have found it difficult to identify/categorize the prey and this may have led them to distance themselves from it as they couldn’t determine whether or not it posed a significant risk. Research investigating the characteristics of the background noise sound and the visual deimatic display could shed further light on this interaction. Similarly, these pitch/image phenomena could be important in relation to deimatic displays containing both auditory and visual components.

Finally, it is also worth noting that my findings are consistent with the idea that deimatic displays deter predators by eliciting reflexive startle responses. Background noise is known to increase the magnitude of startle responses (Hoffman and Fleshler, 1963; Hoffman and Searle, 1965; Flaten, Nordmark and Elden, 2005; Blumenthal *et al.*, 2006), and this study provides some evidence that background noise also increases the deterrent effect of deimatic displays. However, whilst this finding could be explained by assuming that predators are more startled by the deimatic prey in the presence of background noise, this study does not test this directly. Further, the effect of background noise on birds’ responses to deimatic prey did not extend to all measures of predator behaviour, meaning that any speculation about the mechanisms through which birds are deterred by deimatic prey should be treated with caution. In light of the suggestion that the effect of background noise on the magnitude of startle responses is mediated by its inducing states of anxiety (Kellogg *et al.*, 1991), further investigation of the influence of different affective states on predator responses to deimatic displays would perhaps provide more clarity on this matter.

In conclusion, background noise does influence the responses of birds to deimatic prey, not by altering their speed of attack, but by causing them to maintain a distance from prey while deciding whether to attack. This effect could have important implications for prey survival, although research testing this idea with live prey is required. In the next chapter, I will investigate whether predator affective state influences the efficacy of deimatism. It is likely that many of the factors know to influence the magnitude of startle responses (and consequently the efficacy of deimatism) are mediated by changes in affective state (Kellogg *et al.*, 1991; Walker and Davis, 1997; Grillon *et al.*, 1999). Determining how affective state influences predators' responses to affective state may therefore help us to understand the role, if any, of startle in the success of deimatic displays.

Chapter 6. The role of predator affective state in the evolution of deimatic displays

6.1 Abstract

In the previous chapter, I found some evidence that background noise influenced birds' reactions to deimatic prey. One explanation as to why the efficacy of deimatic displays is increased in the presence of background noise is that it may evoke anxiety-like states in predators. Anxiety-like states are known to enhance the magnitude of startle responses in a number of species. In contrast, depressive-like states are known to reduce startle response magnitudes, although this relationship can depend on the severity of the depressive-like state. In this chapter, I tested whether predators' affective states influence their responses to deimatic prey. I used an established experimental protocol in which chicks were exposed to conspecific vocalisations known to elicit either: i) Neutral, ii) Anxious-like, or iii) Depressive-like states. The following day, they were presented with either stationary Control prey or Deimatic prey, using the experimental paradigm outlined in Chapter 2. I found that significantly fewer chicks attacked deimatic compared to control prey across the three affective state treatments. For those prey that were attacked, I found no evidence that the latency to attack deimatic prey differed from the time taken to attack control prey for either birds in an anxious-like state or birds in a neutral state. However, chicks in a depressive-like state attacked deimatic prey significantly faster than control prey. These results suggest that the evolution of deimatism is more likely to occur in environments that do not cultivate depressed predators.

6.2 Introduction

In Chapter 5, I found that background noise decreased the amount of time that predators spent in proximity to deimatic prey when deciding whether or not to attack, which could be advantageous for deimatic prey if they take this opportunity to escape. One possible explanation for background noise increasing the efficacy of deimatic displays is that it may elicit anxious-like states in predators (Kellogg *et al.*, 1991), which can enhance the magnitude of startle responses (Davis, 1979; Swerdlow *et al.*, 1986; Liang *et al.*, 1992; Grillon *et al.*, 2005; Cornwell *et al.*, 2008; Schulz, Alpers and Hofmann, 2008). In contrast, depressive-like states are known to reduce the magnitude of startle responses, although this relationship can vary with the intensity of the depressive state experienced (Allen, Trinder and Brennan, 1999; Kaviani *et al.*, 2004; Forbes *et al.*, 2005).

Anxiety or anxious-like states are known to cause an increase in the magnitude of startle responses in both humans (Grillon *et al.*, 2005; Cornwell *et al.*, 2008; Schulz, Alpers and Hofmann, 2008) and rodents (Davis, 1979; Swerdlow *et al.*, 1986; Liang *et al.*, 1992). Human subjects with anxiety disorders, and those currently experiencing social anxiety, have been shown to display enhanced startle responses to both auditory and tactile stimuli (Grillon *et al.*, 2005; Cornwell *et al.*, 2008; Schulz, Alpers and Hofmann, 2008). It could be suggested that this increase is due to the subjects being fearful rather than anxious. However, lesions of the brain region associated with anxiety (the bed nucleus of the stria terminalis) block the increase in startle magnitudes in rats, while lesions in regions associated with fear (the amygdala) do not (Gewirtz, McNish and Davis, 1998). Similarly, the administration of drugs that are known to manipulated anxiety levels also influence the magnitude of auditory-induced startle responses: anxiety-inducing compounds enhance startle responses in rats (Davis, 1979; Swerdlow *et al.*, 1986; Liang *et al.*, 1992), whereas anxiolytic (anxiety-reducing) drugs reduce startle responses (Davis, 1979). If deimatic displays startle predators, it would be reasonable to expect that deimatic displays would be more effective when predators are in an anxious state.

However, not all affective states are thought to enhance the magnitude of startle responses, depression or depressive-like states are known in general to reduce startle response magnitudes: patients suffering from a major depressive episode have reduced startle responses to auditory stimuli in comparison to healthy subjects (Allen, Trinder and Brennan, 1999). However, the effect of depression on the magnitude of auditory startle responses is complex, and may depend on the degree to which people are depressed (Kaviani *et al.*, 2004; Forbes *et al.*, 2005). This results in a general pattern of low levels of depression leading to enhanced startle, whereas subjects with higher levels of depression have greatly reduced startle response magnitudes (Kaviani *et al.*, 2004). Therefore, predator responses may depend upon the level of depression that they are experiencing. Mild levels of depression should enhance the startle response and thus lead to higher survival of deimatic prey. On the other hand, reduced startle responses would be seen in those with high levels of depression leading to lower survival of deimatic prey.

Understanding any influence of affective state on the interactions between deimatic prey and their predators is important because it will allow us to better predict the environments in which deimatic displays are likely to be most effective. Negative affective states could be induced by negative stimuli or experiences within predators' environment such as, encounters with their own predators, lack of food, lack of available mates, or negative social experiences. If so, these factors could have knock-on effects on predators' responses to deimatic prey. By establishing how predator affective state influences the success of deimatic displays, we can infer the type of environments in which they could be more or less successful, and thus can make predictions about the conditions under which they may evolve. Intriguingly, we know very little about how predator affect influences the efficacy of any form of defence. The exception to this being a study by Brilot et al. (2009) that found that an anxious-like affective state did not influence the responses of starlings (*Sturnus vulgaris*) to eyespot markings. Thus, investigating the influence of affective state on predatory responses to deimatic prey could further our understanding of the conditions altering the efficacy of deimatic displays, and, the conditions likely to be conducive to their evolution.

In this chapter, I manipulated predator affective state by exposing domestic chicks (*Gallus gallus domesticus*) to repeated playback of different conspecific vocalisations to induce either a neutral, anxious-like or depressive-like state. This manipulation centres around the concept that chick distress vocalisations reflect their affective state. When held in isolation, chicks initially enter an anxious-like state and vocalise at a high rate. After more extensive isolation, they enter a more depressive-like state, and vocalising is reduced to approximately 40-50% of the rate at which anxious chicks vocalise (Sufka *et al.*, 2006). The playback protocol used in this study was designed by Drs Herborn, Asher and Wilson. They have used this protocol in a number of studies in order to manipulate affective state. In those studies, the results of cognitive bias testing and the thermal imaging data both consistently supported the induction of different affective state (*pers. comms*.). Thermal imaging has been used previously to assess stress level in domestic chicks (Herborn *et al.*, 2015), and chicks that had heard vocalisations from conspecifics in an anxious-like state showed a significant and acute increase in surface temperature. In contrast, chicks that had heard vocalisations from conspecifics in a depressive-like state had a significantly elevated baseline surface temperature suggestive of exposure to chronic stress. Thus, I felt confident that this protocol would successfully alter affective state in the chicks I used in this study. Using this protocol, I aimed to manipulate affective state in the chicks whilst avoiding the use of invasive procedures such as drug administration or isolation (Sufka and Weed, 1994). Chicks experienced one day of playback of chick vocalisations intended to induce (i) Neutral, ii) Anxious-like, or iii) Depressive-like states, before being presented with either Control or Deimatic prey. In this way, I aimed to establish whether or not predator affective state influenced the efficacy of deimatic displays.

6.3 Methods

6.3.1 Subjects

A total of 113 domestic chicks (Ross strain), tested in 3 cohorts, of mixed sex participated in this study. They were purchased on the day that they hatched from a commercial hatchery in Yorkshire (U.K.). 77 chicks participated in the experiment (Cohort 1 = 28; Cohort 2 = 25; Cohort 3 = 24) and 36 (12 in each cohort) were used as buddy chicks. Details of food and housing are outlined in Chapter 2. Each cohort was divided into three groups of experimental chicks and one group of buddy chicks which were each housed in one of four identical laboratories. This allowed me to play chicks in each of the experimental laboratories different vocalisations: those intended to incite neutral, anxious-like and depressive-like states respectively (see below). The three home laboratories for the experimental chicks were alternated between the three cohorts of chicks, such that each room housed each affective state condition once. All training and testing trials were performed in the fourth laboratory, which also housed the buddy chicks. They were housed in the testing laboratory in conditions identical to those encountered by the three experimental groups (minus the playback of vocalisations). This avoided buddy chicks experiencing any of the playback manipulations whilst maintaining them under the same conditions as the experimental chicks so that their behaviour would not influence experimental chicks during training and test trials. Ethical approval for this study was received from the Newcastle University Animal Welfare and Ethical Review Body (Project ID No.: ID 635).

6.3.2 Experiment

Training trials

Chicks received one training trial per day on days 1-6 of life. These trials were carried out as outlined in Chapter 2, with the exception that chicks were moved from their home laboratory to the experimental laboratory immediately after being food deprived and prior to participation in the training trial.

Affective State Manipulation

I used recordings of vocalisations provided by Drs Herborn, Asher and Wilson. 42 commercially sourced (P.D. Hook Hatchery Ltd., Thirsk, Yorkshire, UK) domestic chicks (Ross Strain) were housed in the laboratory in order to record their distress and contact vocalisations. The chicks were randomly assigned to groups of 6 individuals. Between 2 and 7 days of age, five individuals from one group per day were captured and individually transported to a separate room in a cardboard box. They were then left in isolation in a 1x1 m pen for 10 minutes. Distress vocalisations during this time were recorded using an Arbimon Acoustic^{TM} recorder, and these were used to construct the Anxious-like and Depressive-like stimuli. If a trial failed, or a chick did not vocalise, the sixth chick was tested. After all of the birds were isolated, the chicks from the five successful trials per day were placed together into the pen used for the isolation recordings and were allowed to settle for 30 minutes before they were recorded for 10 minutes in order to capture contact vocalisations which were used as Neutral stimuli.

From the 10 minute isolation recordings, vocalisations were counted, extracted and saved as waveform audio files using the mark_pauses code in the speech analysis software Praat (Boersma, 2002). Vocalisation rates in minutes 1-3 and 7-10 were used to generate “anxious-like” and “depressive-like” stimuli respectively, where average vocalisation rates were 99 and 66 vocalisations per minute respectively. A highpass filter was applied at 1200 Hz, to remove low frequency fan noise from the extracted audio files without altering vocalisation attributes (>2kHz). A pool of 10 vocalisations was produced with sufficient silences to give the proportion of silence observed on average in anxious-like and depressive-like phases. To generate sequences, vocalisations were then selected at random without replacement from the pool. This process was repeated and the resulting periods were linked with a brief (100 ms) inter-stimulus interval until 3 minutes of audio were produced. This generated sequences of real vocalisations with realistic distress vocalisation rates and timings. Six 3-minute stimuli were constructed per chick per day, and the stimuli from the 5 chicks per day were randomly assembled to produce 6 unique 15-minute playback stimuli. For contact vocalisations, 50 vocalisations were extracted per group per recording, i.e. approximately 10 vocalisations per individual. Contact vocalisation rate was defined as the total vocalisation rate divided by five individuals. 15-minute playback stimuli were produced in the same manner as above, but instead drawing from the pool of 50 vocalisations and corresponding silences of the same duration. Six different 15-minute contact vocalisation stimuli were produced. The same 10 vocalisations were used to produce anxious-like and depressive-like stimuli, and contact vocalisations were drawn from the same individuals. Playback stimuli were constructed as 15 minutes of vocalisation playback plus 45 minutes of silence.

On day seven, chicks were exposed to 15 minutes of playback, every hour for their 12 waking hours, of either i) chick contact vocalisations (Neutral), ii) chick distress vocalisations at a fast rate (Anxious), or iii) chick distress vocalisations at a slow rate (Depressed). The 6 playback stimuli were played on a loop throughout the day at natural vocalisation volume (60-65 dB for contact vocalisations, 70-75 dB for anxious-like and depressive-like vocalisations). Thus, the chicks experienced the 6 playback stimuli twice over the course of the day. As the playback sessions began from the moment when the laboratory lights switched on and chicks woke from their nocturnal fast (chicks do not eat in the dark and as such we can assume that they wake hungry), the timing of the start of the first playback sessions was arranged such that the chicks experienced the 45 minutes of silence first to avoid any impact of the playback on their first feeding session of the day.

Test trial

The day after the affective state manipulation, the chicks participated in the test trial. They were randomly assigned to receive either deimatic or control prey, thus creating six experimental groups, with each group experiencing one prey type and one affective state manipulation; i) Deimatic/Neutral, ii) Deimatic/Anxious-Like, iii) Deimatic/Depressive-Like, iv) Control/Neutral, v) Control/Anxious-Like, and vi) Control/Depressive-Like. The computer-generated prey used here were the same as those outlined in Chapter 2. The Deimatic prey moved only at the Fast speed (2.2 flicks per second), as this was found to be the most effective in deterring predators in Chapter 3.

During the test trial, each chick received one prey presentation (the type of which was determined by their experimental group) in the experimental arena and their behaviour was recorded for ten minutes after presentation with the prey image (for further detail see Chapter 2). All trials were video recorded using a JVC Everio GZ-315DEK camcorder, and the videos were analysed using the behavioural video scoring software BORIS (Behavioural Observation Research Interactive Software; Friard and Gamba, 2016). I recorded the number of chicks that attacked the prey item, and for those chicks that did attack a prey item, I measured the latency to first peck or scratch prey as a measure of the chicks’ willingness to attack the prey images. For those chicks that attacked prey, I measured the amount of time chicks spent in each half of the arena after prey activation (measured as the time when the chicks’ head crossed the midpoint of the arena in the case of Control prey) and prior to attacking prey (the pre-attack interval) using the video scoring software BORIS (Friard and Gamba, 2016). I used these data to calculate the proportion of time chicks spent in the half of the arena containing the prey item prior to attacking it.

6.3.3 Data Analysis

All of the data collected throughout this study were analysed using the statistical software package IBM SPSS Statistics v23.

The Number of Chicks That Attacked Prey

The latency to attack data were bimodally distributed and as such proved difficult to analyse with the same approach that I have used in previous chapters. Therefore, I investigated whether or not there was a difference in the number of chicks that attacked prey among the experimental groups, i.e. whether prey type (Deimatic/Control), and/or affective state (Neutral/Anxious-Like/Depressive-Like) influenced whether prey were attacked or not. I used a binomial general linear model (GLM) with the number of chicks that attacked prey as the outcome variable, and prey type and affective state as fixed factors. I tested for a main effect of prey type, a main effect of affective state, and the interaction between those two factors on the number of chicks that attacked prey. As previous studies have shown deimatic prey to be more deterring than control prey (see Chapters 3&5), I expected a greater number of control prey to be attacked in comparison to deimatic prey. I also expected that chicks in a neutral state would attack more prey than those in an anxious-like state, and that chicks in a depressive-like state would differ from those in a neutral state (however, whether they would attack fewer or more would depend on the level of depressive-like state that the chicks are experiencing). Finally, I expected an interaction between prey type and affective state, as affective state should have an effect on deimatic prey to a greater extent than controls.

Latency to Attack

I extracted the latency to attack data for only those chicks who attacked prey during the test trial. These data were heteroscedastic and their residuals were not normally distributed. I carried out a Log10 transformation on the data and this provided data that were still heteroscedastic, but the residuals were normally distributed. This allowed me to test the data using Welch’s ANOVA and I then used t-tests (which accounted for the heteroscedasticity of the data) to test a series of planned contrasts examining a series of planned contrasts which did not assume equal variances among groups. I predicted that chicks in the affective state conditions would take longer to attack deimatic compared to control prey, i.e. chicks in all of the affective state groups would be more deterred by deimatic displays. However, I expected chicks in the anxious-like groups to take longer to attack deimatic than those in the neutral groups if an anxious-like state enhances the deterrent effect of deimatic displays. Finally, I predicted that chicks in the depressive-like groups would attack deimatic prey either slower or faster than chicks in the neutral groups, depending on whether they were experiencing mild or severe depressive-like symptoms.

Proportion of time in proximity to prey during pre-attack interval

Again, I looked only at the data relating to those chicks that attacked prey during the test trial. As these data were proportional, I carried out an arcsine square root transformation in order to extend the higher and lower ends of the distribution giving the data a distribution more similar to normality. The transformed data were heteroscedastic and the residuals were not normally distributed. As ANOVA are quite robust to violations of normality, I decided to use a Welch's ANOVA which would also take into account the unequal group variances. I then used t-tests (which accounted for the heteroscedasticity of the data) to test a series of planned contrasts examining the same predictions as outlined above in relation to the latency to attack.

6.4 Results

6.4.1 The number of chicks that attacked prey

I found a significant difference in the number of chicks that attacked prey among my experimental groups (Binomial GLM: $\chi^2 = 49.371$, df = 6, $p < 0.001$; Figure 12). There was a main effect of prey type ($\chi^2 = 15.897$, df = 1, $p < 0.001$), with significantly more chicks attacking control than deimatic prey. However, I found no effect of affective state ($\chi^2 = 2.969$, df = 2, $p = 0.227$), nor any interaction between state and prey type ($\chi^2 = 1.527$, df = 2, $p = 0.466$). These results suggest that deimatic prey survive better than control prey, however, this protective effect is not altered by predator affective state.

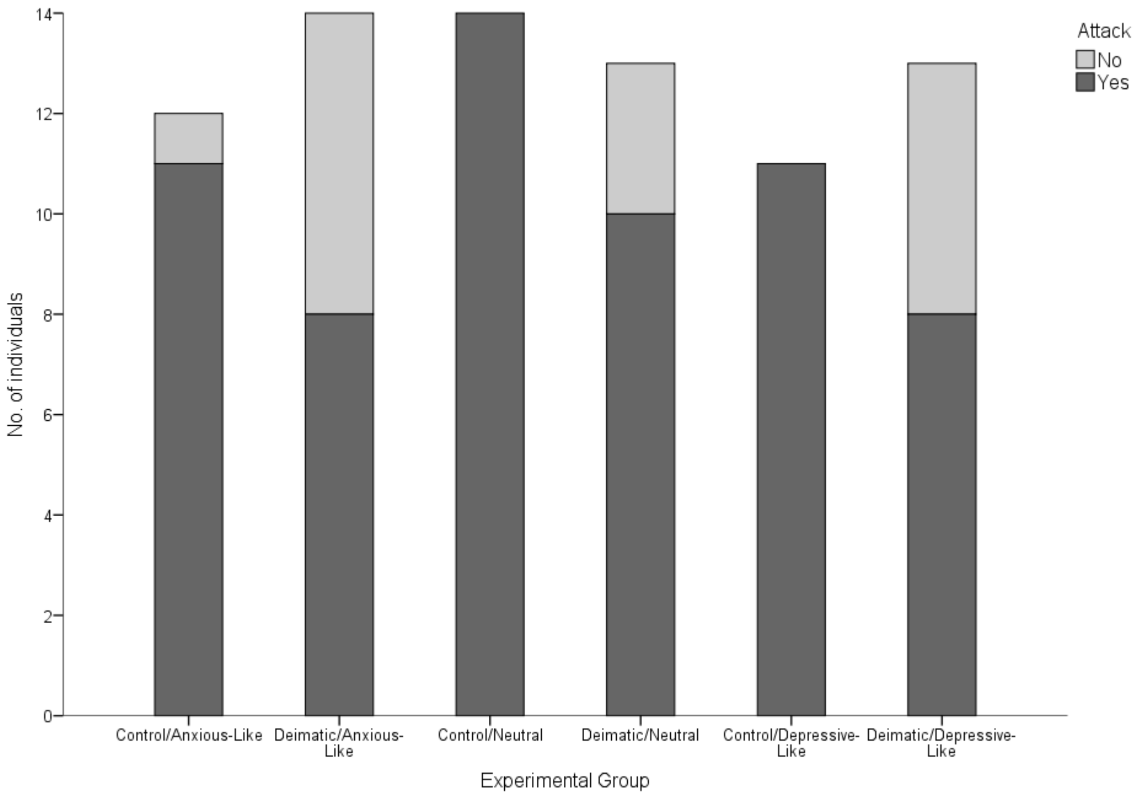

Figure 12. The number of chicks that attacked prey during the test trial for each of the experimental groups.

6.4.2 Latency to attack prey

When considering only those chicks that attacked prey during the test trial, I found a significant difference in attack latency among the six experimental groups (Welch's ANOVA: $F_{(5,23.838)}$ = 4.313, p = 0.006; Figure 13). In contrast to the results of previous studies (Chapters 3-5), the latency to attack control and deimatic prey did not differ in chicks in a neutral state (t = -1.528, df = 14.639, p = 0.148), although this could be because I only included those chicks that attacked prey in this analysis whereas in previous chapters I included data from all chicks. Furthermore, I found no evidence that chicks in an anxious-like state differed in the speed at which they attacked deimatic and control prey (t = -1.344, df = 8.998, p = 0.212). In contrast to my predictions, chicks in a depressive-like state attacked deimatic prey (M = 21.67s, SE = 13.27s) significantly faster than control prey (M = 78.59s, SE = 41.42s; t = 2.285, df = 13.682, p = 0.039).

Contrary to my predictions, there was no difference in the latency to attack deimatic prey between chicks in an anxious-like and a neutral state (t = -0.958, df =

13.9, p = 0.354). Nor did chicks in depressive-like and neutral state differ in their speed of attacking deimatic prey (t = 1.827, df = 15.6, p = 0.087). This result is approaching significance and so interpretation should be made with caution. However, these results would suggest that deimatic prey are attacked at equal speeds regardless of predator affective state.

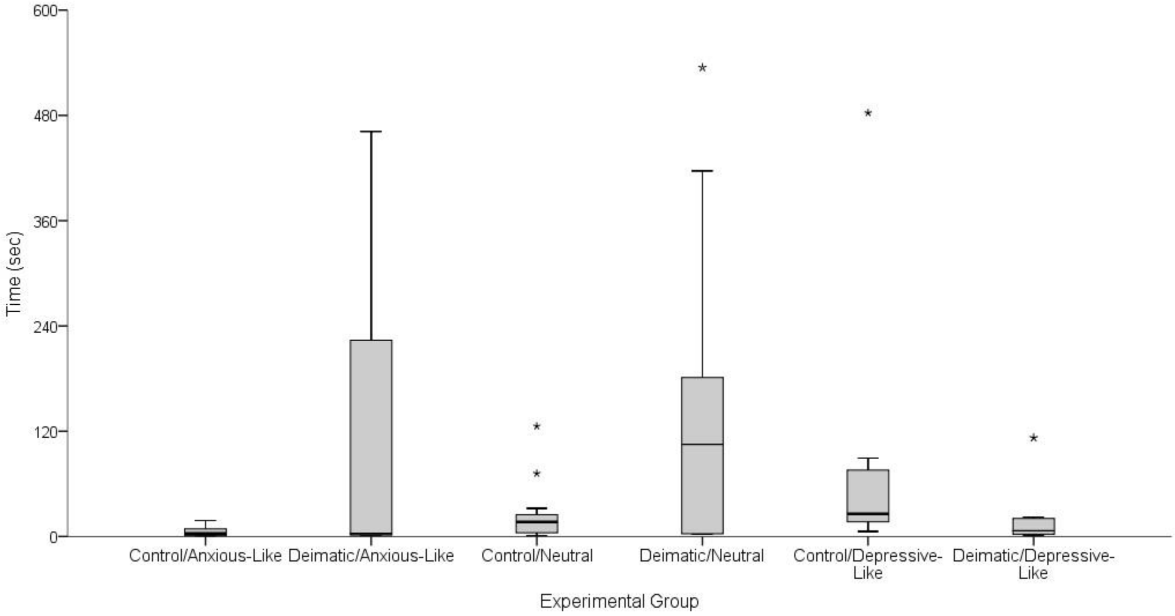

Figure 13. Latency to attack prey during test trial. Each box represents the middle 50% of scores for each experimental group. The line dividing each box into two represent the median of those data. The upper and lower whiskers extend to include the data outside the middle 50%. Asterisks (*) represent possible outliers, each of which has been examined and is valid for inclusion within the analysis.

6.4.3 Proportion of time in proximity to prey during pre-attack interval

Again, when considering only those chicks that attacked prey during the test trial, I found a significant difference among the experimental groups in the proportion of time chicks spent in proximity to prey during the pre-attack interval (Welch’s ANOVA: $F_{(5,24.142)}$ = 8.565, p < 0.001; Figure 14). Consistent with previous results (Chapters 3 and 4), there was no difference in the time spent in proximity to control and deimatic prey during the pre-attack interval in chicks in a neutral state (t = 0.797, df = 17.099, p = 0.436). In contrast to my predictions, chicks in an anxious-like state did not differ in the time they spent in proximity to control and deimatic prey prior to attacking them (t = 0.455, df = 12.483, p = 0.657). In addition, chicks in a depressive-like state spent significantly more time in proximity to deimatic prey in comparison to control prey during the pre-attack interval (t = -6.317, df = 14.703, p < 0.001).

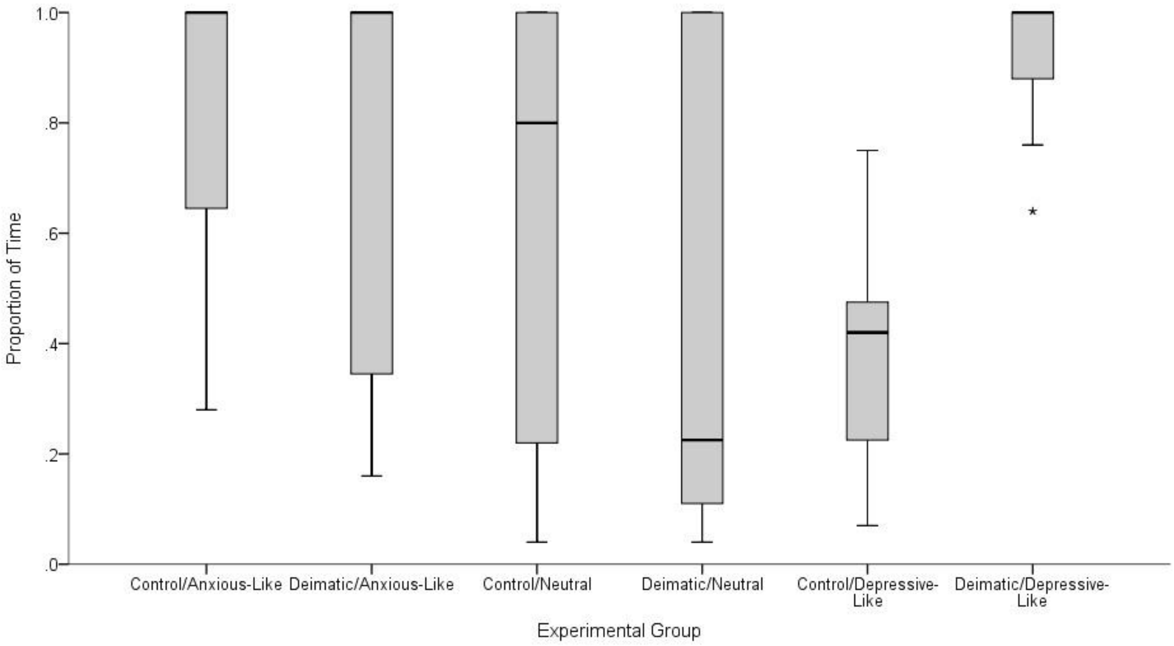

Figure 14. Proportion of time spent in proximity to prey during the pre-attack interval. Each box represents the middle 50% of scores for each experimental group. The line dividing each box into two represent the median of those data. The upper and lower whiskers extend to include the data outside the middle 50%. Asterisks (*) represent possible outliers, each of which has been examined and is valid for inclusion within the analysis.

There was no difference in the time spent in proximity to deimatic prey between chicks in an anxious-like state and those in a neutral state (t = 1.260, df = 15.928, p = 0.226). However, chicks in a depressive-like state spent significantly more time in proximity to deimatic prey prior to attacking them in comparison to those in a neutral state (t = -2.634, df = 12.683, p = 0.021). These results suggest that in chicks that do attack prey, those in a depressive-like state may be less deterred by deimatic prey than those in a neutral or an anxious-like state.

6.5 Discussion

I found that significantly fewer chicks attacked deimatic prey in comparison to control prey across the three affective state treatments: deimatism was effective at enhancing prey survival in each of the affective state groups examined here. This finding is in line with the results of my previous studies (Chapters 3-5), and supports the idea that deimatism has an antipredator function. I found no evidence that affective state influenced the likelihood of chicks attacking prey, suggesting that affective state may not influence the number of chicks that attack deimatic prey or control prey. However, there may be other explanations for not finding a significant effect of affective state in terms of the number of chicks that attacked prey. I may not have been successful in altering the chicks affective state, and so, no difference was seen between them. On the other hand, it could be because affective state does not influence the likelihood of predators attacking prey. The influence of affective state may relate to another aspect of predator behaviour.

When considering only those chicks that attacked prey, I did not find any evidence that chicks subjected to playback of stimuli intended to induce an anxious-like state responded to deimatic prey any differently than those exposed to playback stimuli intended to induce a neutral state. They did not differ in their latency to attack prey, nor did they differ in the proportion of time spent in the half of the arena containing the prey in the interval between prey activation and attack. This lack of difference could be explained in several ways. It may be because anxious-like states do not influence predators’ responses to deimatic prey. This would suggest that deimatic displays do not deter predators by startling them, since anxious-like states have consistently been shown to enhance startle responses (Liang *et al.*, 1992; Grillon *et al.*, 2005; Schulz, Alpers and Hofmann, 2008). Alternatively, my sample size may not have been sufficient to detect any differences that do exist. The sample size in this study (N= 11-14 per experimental group) was larger than those in previous studies that did detect differences (e.g. N = 8 per experimental group in Chapter 3). However, it is possible that the effect size was smaller here than in other studies. Another possibility is that the manipulation that I used did not successfully induce an anxious-like state in the chicks. Although I attempted to confirm that my manipulations had been successful by measuring the number of distress vocalisations that chicks produced in their home pen on the stimulus playback day, the low quality of the audio files made this impossible.

The effect of a depressive-like state on predators’ responses to deimatic prey is less clear-cut. Some of the chicks in the groups exposed to playback stimuli that were intended to induce a depressive-like state were deterred by deimatic displays, as chicks in a depressive-like state were more likely to attack control prey than deimatic prey. However, those chicks in a depression-like state that did attack prey, attacked deimatic prey more quickly than control prey and spent more time in close proximity to them while deciding whether to attack. This seems to suggest that deimatism elicited a bimodal response (seen in all of the affective state groups, but most pronounced in the depressive-like groups), with some chicks being deterred (those that did not attack them) and others not being deterred (those that did attack them). In fact, those that did attack deimatic prey appeared to be to some degree attracted rather than deterred by them. This bimodality of response is not surprising given that bimodality of symptomology in human patients of depression is not uncommon (Everitt, 1981). This could perhaps be explained by assuming that some of the chicks were in a severe depressive state whilst others were only mildly depressed. Mild depressive states enhance startle (Kaviani *et al.*, 2004), which would explain those chicks that did not attack deimatic prey. On the other hand more severe depressive states reduce startle response magnitudes (Kaviani *et al.*, 2004), which may explain why they are not deterred by them. However, even if startle magnitude was reduced in severely depressed chicks, it still is not clear why chicks should attack deimatic prey faster than control prey, as opposed to attacking them at similar rates. One potential explanation for this, is that these chicks were not startled by the deimatic displays and attacked deimatic prey more quickly than control prey because the displays drew their attention. Irrespective of the underlying mechanism, it is clear that deimatic display are less beneficial to prey (and may even be costly) when predators are in a depressive-like state.

That predators experiencing a depressive-like state altered both their attack speed and the time spent in proximity to prey while deciding whether to attack has important implications for the efficacy and evolution of deimatic displays. This is the first evidence of any factor known to significantly reduce the efficacy of deimatic displays in deterring predators. Depressive-like states are characterised in humans by experiencing anhedonia, fatigue and pessimism (Nettle and Bateson, 2012), and are often associated with rumination on past negative events (Eysenck, Payne and Santos, 2006). Such a state could therefore occur in predators living in an environment where they have regular negative experiences over a prolonged period of time. For example, those inhabiting environments where food is scarce throughout entire seasons. Indeed, food scarcity in humans is known to increase anxiety and depression (Hadley and Patil, 2006) and unhealthy behaviours, such as drug abuse and binge consumption of high calorie foods (Carr, 2011). In addition, those predators whose habitat has a high abundance of their own predators meaning they are constantly confronted with a high risk of predation could also experience depressive-like states. If this is the case, then such environments that are consistently threatening or stressful to predators, are also those in which deimatic species should avoid in order to maximise their survival. Further, with deimatic displays being significantly less effective in deterring predators in such environments, deimatism may be less likely to evolve in these areas.

It should be noted that, in contrast to previous findings (see Chapters 3&5), I did not find a significant difference in the latency to attack deimatic and control prey in chicks experiencing a neutral state. One reason for this may be that the analysis in this study focused on birds that attacked prey, whereas previous studies account for both attacking, and non-attacking birds. However, in Chapter 4 (where the latency to attack analysis included both chicks that did, and chicks that did not, attack prey), I also failed to establish a significant difference between control and deimatic prey. Therefore, it is possible that an unidentified factor differed between my experiments, and this influenced how deterred predators were by deimatic prey. Alternatively, the playback of the neutral stimuli may have had an unexpected effect on chicks, which could have influenced their responses to deimatic prey. Taken together with the fact that I do not know the affective state of my experimental subjects in previous studies (Chapters 3-5), it is difficult to make direct comparisons between this and other studies. Consequently, the findings of this study should be interpreted with caution and should act as a starting point for future research assessing the influence of predator affective state on the efficacy of deimatism.

In conclusion, this study provides some evidence that predator affective state could influence the responses of predators to deimatic displays in those chicks that attack prey. However, future research is required in order to ascertain whether those responses are caused by the effects of affective state on the magnitude of predators' startle responses. In Chapter 7, I will examine another aspect of deimatism that until this point has not been addressed at all – the factors that may influence whether deimatic prey display and the duration of their display. I will present an investigation of the role that temperature plays on the displaying behaviour of the deimatic species the peacock butterfly (*Aglais io*).

Chapter 7. The effect of temperature on the displaying behaviour of peacock butterflies (*Aglais io*)

7. 1 Abstract

My previous chapters have concentrated on understanding what factors influence predators’ responses to deimatic displays. However, this is only part of the story. Prey choose when to display, and may even control the form and magnitude of the display. Since these factors could have a knock-on effect on the efficacy of the displays, it is important to understand what factors influence the production of them. Little is known about the factors that may influence the frequency or duration of deimatic displays, however, it is possible that variation in environmental conditions could influence their efficacy. Deimatic species often encounter variation in temperature across space and time within their environment. There is reason to believe that variation in temperature could affect deimatic behaviour, although it may do so in two ways. For example, deimatic displays are energetically expensive, and the cost is likely to be greater at lower temperatures, which could result in prey reducing the magnitude or duration of displays. Conversely, although deimatic displays are energetically costly, the cost of display production may be less than the costs associated with fleeing. Therefore, deimatic displays may only be used as a last resort defence in adverse conditions when fleeing is not an option, e.g. at low temperatures. Here, I performed an experiment in which I compared the displaying and flying behaviour of peacock butterflies (*Aglais io*) housed at two different ecologically-relevant temperatures (8 and 18°C). I did this under both control conditions where butterflies where left undisturbed, and, following a simulated predatory attack. I found that at 8°C deimatic displays were common, but very few butterflies performed flying behaviour. In contrast, at 18°C flying behaviour was common, but very few butterflies performed deimatic displays. In addition, at 8°C, butterflies displayed for longer after simulated predation than in the control condition, whereas at 18°C, the duration of flying behaviour was similar in the control and simulated predation conditions. These results suggest that deimatic displays in peacock butterflies are likely to play a defensive role, but their use may be restricted to the lower end of the range of temperatures that prey encounter in natural settings.

7.2 Introduction

Previous chapters have investigated factors that may influence predators’ responses to deimatic displays. However, this is only part of the story as we know very little about what factors affect the behaviour of deimatic prey. There is some evidence that prey control both when they display, and the form/magnitude of the display. For example, peacock butterflies (*Aglais io*) can either leave their wings open and unmoving when displaying or flick their wings throughout the displays; and deimatic butterflies that flick their wings are known to deter predators more effectively than those with static displays (Olofsson *et al.*, 2012a). In addition, cuttlefish (*Sepia officinalis*) are known to vary the intensity of their displays according to both the type of predator present and whether the predator continues to pursue an attack (Langridge, 2006; 2009). This suggests that cuttlefish adapt their displays to best counter the particular threat they face, although experiments determining predators’ responses to different display types are needed to confirm this. These studies, along with the results from Chapter 2 (Holmes *et al.*, 2018), suggest that the deterrent effect of displays increases with display magnitude and that (at least some) deimatic prey can control display magnitude in what appears to be an adaptive manner. Consequently, in order to gain a full understanding of what factors influence the evolution of deimatic displays, we need to know not only what factors influence predators’ responses to these displays, but also what factors influence the display behaviour of deimatic prey.

One factor that is likely to influence display production is temperature. Temperature can vary over space and time, and many deimatic species encounter a range of temperatures throughout their lifetime. For example, peacock butterflies (*Aglais io*) overwinter as adults, and in Britain, encounter average summer temperatures of 14.7°C, and average winter temperatures of 5.0°C (Met Office, UK). Indeed, this temperature variation may be even greater in other parts of their range. However, predicting exactly how temperature influences the production of deimatic displays is difficult, and there are two competing predictions. First, deimatic displays could be suppressed at low temperatures due to the potential energetic costs of performing them. Since deimatic displays often involve the movement of body parts into key postures and/or specific whole-body movements, the energetic cost of these displays is likely to increase with decreasing temperature. Furthermore, this may be particularly evident in ectothermic species that need to heat their muscles before movement can be performed (Kammer, 1970; Esch, 1988). Alternatively, it has been suggested that the magnitude and/or likelihood of deimatic prey displaying could be greater at cooler temperatures. This is because it may be more difficult to deter predators at lower temperatures due to their increased energetic needs. For example, lower temperatures cause avian predators to consume more aposematic prey (Chatelain, Halpin and Rowe, 2013). Therefore, displays of greater magnitude may be needed at low temperatures in order to ensure similar levels of antipredator efficacy seen at higher temperatures. In addition, although the cost of producing deimatic displays is likely higher at low temperatures, these displays may be the only option that prey have. Escaping is likely to be even more energetically expensive than displaying, and may be particularly difficult for prey that overwinter in enclosed spaces (Wiklund *et al.*, 2008). Thus, prey may use deimatism as a last defensive resort at lower temperature when fleeing is not possible and invest in fleeing at higher temperatures.

Unfortunately, we know very little about the effect of temperature on either the production or efficacy of deimatic displays. Most of the research investigating the success of displays in deterring predators has been carried out under a narrow range of environmental conditions, which are often tightly controlled. For instance, peacock butterflies used in deimatic research are usually maintained and tested within a narrow range of temperatures (6-10°C) (Vallin *et al.*, 2005; Vallin, Jakobsson and Wiklund, 2007; Olofsson, Jakobsson and Wiklund, 2012b; Olofsson *et al.*, 2013). Maintaining and testing butterflies at the lower end of the temperature range they typically encounter in natural settings is not exclusive to peacock butterflies but is also found in relation to other lepidopteran species (e.g. swallowtail butterflies (*Papilio machaon*; Olofsson et al., 2012a); eyed hawk-moths (*Smerinthus ocellatus*; Vallin et al., 2007)). There has been one study that monitored the survival of deimatic prey in natural settings including the temperature range (Wiklund *et al.* 2008), but this focused solely on overwintering butterflies and did not directly study the effect of temperature on either display production or survival. Thus, although the temperature ranges used in the studies mentioned were ecologically relevant, they represent a small amount of the variety of temperatures that deimatic prey encounter during their lifetimes. This is an important consideration as carrying out research under specific narrow environmental settings means we cannot assess whether deimatic displays are a defence that is important over a broad range of conditions, or how variations in environmental conditions may influence their efficacy. Such knowledge could be vital in understanding how deimatic displays came to evolve.

Figure 15. The dorsal wing colouration of the peacock butterfly (*Aglais io*; photo: G Holmes)

Here, I investigated the effect of two different temperatures (8 and 18°C) on the deimatic display of peacock butterflies. The ventral side of the wings of peacock butterflies are cryptically coloured and appear to mimic dead leaves (Brakefield, Shreeve and Thomas1992). In contrast, the dorsal side of their wings are brightly coloured with two pairs of eyespot markings (Blest, 1957; see Figure 15). Their deimatic display consists of opening their wings rapidly to simultaneously reveal their conspicuously coloured dorsal wings and to produce a characteristic hissing sound caused by the anal veins of the forewings rubbing against the costal veins of the hindwings (Blest, 1957). Wings can either remain open for the duration of the display, or butterflies can continue to open and close the wings rapidly (referred to as wing-flicking; Blest, 1957) throughout the display. In this species, the adult imago hibernates over the winter months in sheltered enclosed spaces such as rock formations and hollow trees (Wiklund *et al.*, 2008). The following spring, they become active again, mate, and lay eggs on their host plant the stinging nettle

(*Urtica dioica*; C. Wiklund, Gotthard, & Nylin, 2003). Thus, during their lifetime individuals experience a wide range of temperatures, and their level of activity and behaviour also changes over that period. peacock butterflies were also of interest to us as they have been the subject of a number of previous studies on deimatic displays run under specific temperature settings (e.g. Olofsson, Jakobsson and Wiklund 2012b; Olofsson et al. 2013; Vallin et al. 2005, 2007). The aim of this study was to provide the first empirical test of the effect of temperature on the production of deimatic displays using ecologically valid temperatures. I observed the duration of both deimatic displays and flying behaviour of butterflies maintained at 8°C and 18°C, after either no stimulation (Control), or after a simulated predatory attack (Attack). These temperatures were selected because they represent the minimum and maximum temperatures for the UK during the butterflies' most active time of year (June-August; Met Office, 2015).

7. 3 Methods

7.3.1 Subjects

A total of 24 peacock butterflies were used in this study. peacock butterfly pupae were purchased from the commercial lepidopteran breeding company (*Devon Butterflies*, Devon, UK) and placed in a plastic greenhouse (65 x 49 x 125 cm) where they were left to eclose. Upon eclosing, the butterflies were allowed to dry before being carefully moved into a clear plastic vivarium (7.2 x 8.6 x 9.1 cm). The floor and roof of the vivarium was lined with tissue paper and contained a plastic tray (17.5 x 11.5 x 3.5 cm) containing cotton wool soaked in a 20% w/v solution of sucrose and water. The sucrose solution served to supply both their food and water requirements, and the cotton wool was changed regularly to avoid the growth of any mould or bacteria. The sucrose solution was available *ad libitum,* and the butterflies were permitted two weeks to feed on the sucrose solution. The vivarium was housed under a 12:12 hour light/dark schedule under uncovered fluorescent bulbs in a laboratory maintained at 18-23˚C. This lighting schedule and environmental temperature reflected the conditions that these butterflies would experience at this point in their life cycle in the U.K.

On the day prior to participation in an experiment, each butterfly was moved to a 300ml plastic container with a piece of sponge containing the same 20% sucrose solution. A section of black mesh netting (approx. 12 x 12 cm) held in place with an elastic band acted as a lid, while still allowing air to enter. The butterflies where housed in these conditions for the duration of the experiment. This allowed for individual identification without the need to physically mark the butterflies. It also prevented individuals from interacting, meaning that their behaviour was independent of that of conspecifics. At the end of the experiment the butterflies were returned to the communal vivarium for use in future studies. All procedures adhered to the Association for the Study of Animal Behaviour's Guidelines for the Treatment of Animals in Research and Teaching, 2012.

7.3.2 Test Trial

This experiment involved the recording and measurement of butterflies' behaviour at 8˚C and 18˚C, with (Attack) and without (Control) a simulated predatory attack. Through this approach I was able to assess the extent to which temperature affected the occurrence of butterflies' deimatic displays, whilst controlling for any general effects of temperature on behaviour in the absence of a predatory attack. I monitored the butterflies' behaviour under both control conditions, and following a simulated attack, at each of the two temperatures. The experimental period occurred over two consecutive days, with two trials each day (morning and afternoon). Half of the butterflies were tested at 18˚C on day 1 and at 8˚C on day 2, and for the other half the reverse was true. At each temperature, control and attack conditions were performed on the same day: one in the morning session and one in the afternoon session. Half of the butterflies first received the control trial followed by the test trial, and the other half received the test trial followed by the control trial.

The experiments were performed in climate-controlled chambers designed to allow full control of the temperature. Twelve hours prior to the test trial, the butterflies were placed, in their individual containers, into the chamber which was maintained at the temperature at which they would be tested the following day. This allowed butterflies to acclimatise to the temperature prior to their participation in the experiment. For both experiments, each test trial involved individual butterflies being placed into a transparent plastic experimental arena (7.2 × 8.6 × 9.1 cm) and allowed to acclimatise for 2 minutes. A video camera was positioned directly above the arena, such that the entire arena was recorded throughout the experiment. A sheet of transparent cellophane was placed over the top of the arena to ensure that the butterfly did not leave the arena during the experiment, and cardboard screens were placed around the arena to ensure that test subjects could not view the rest of the room. Except when simulating a predatory attack, the experimenters were absent from the chambers for the duration of the experiment, in order to ensure that they did not influence the butterflies' behaviour. In the Attack condition, an experimenter blind to the experimental design pinched the butterfly's abdomen with metal forceps for 1 second. A rigid section of foam was placed between the forceps to ensure that they always pinched the butterflies with the same pressure. In the Control condition, the butterfly received no tactile stimulation (the experimenter entered the room and approached the butterflies but did not perform the attack simulation). In all trials the butterfly was video recorded for 10 minutes using a JVC Everio GZ-315DEK camcorder and the videos were stored for analysis.

7.3.3 Video Scoring

Initial observation of approximately 10% of the videos recorded from both experiments made it possible for me to identify the behaviours performed by the butterflies. These included sitting with wings closed; walking with wings closed; flying and displaying. I decided to focus on flying and displaying as these could reflect anti-predator responses, and it was clear from my preliminary observations that flying was more common at warmer temperatures and displaying at cooler temperatures. I defined flying as moving through the air with wings moving and none of the butterflies' legs in contact with any of the experimental arena walls, floor, or with the cellophane cover. Displaying was defined as butterflies having either their wings open and stationary, or, with wings being opened and closed (referred to as wing-flicking), while standing or walking with all four legs in contact with either the experimental arena floor, walls, or the cellophane cover. I used the behavioural video coding software BORIS (Behavioural Observation Research Interactive Software; Friard & Gamba, 2016) to record the duration of time the butterflies spent i) flying and ii) performing their display over the course of the ten-minute trial. For both flying and displaying, bouts of behaviour were measured from start to finish, and the total length of time of the behaviour was calculated by adding all of these together. When multiple bouts of the same type of behaviour happened in close succession, individual bouts were distinguished by a lack of behaviour occurring for more than one second. If no activity occurred for less than one second, I considered that to be one bout.

7.4 Statistical Analyses and Results

I used the statistical analysis software IBM SPSS Statistics v23 to run the following analyses.

Initial inspection of the data revealed that butterflies behaved differently at 8˚C and 18˚C. At 8 ˚C, only one individual displayed flying behaviour (across both treatments), whereas 23 butterflies displayed when attacked and 9 displayed under control conditions. In contrast, at 18 ˚C, nearly all individuals demonstrated flying behaviour, whereas only 4 individuals (across both treatments) performed deimatic displays (see Table 4). Given the scarcity of flying behaviour at 8˚C and display behaviour at 18˚C, I chose to compare (i) how the duration of display behaviour differed between the control condition and the simulated attack condition at 8˚C; and (ii) how the duration of flying behaviour differed between the control condition and the simulated attack condition at 18˚C. If these behaviours serve an antipredator function, I would expect butterflies to perform them for longer following simulated attack than in the control condition in which they were left undisturbed.

Table 4. Frequency of behaviours. The number of individuals that displayed or flew for each of the experimental conditions. N = 24 for all conditions.

Behaviour	8°C Attack	8°C Control	18°C Attack	18°C Control
Displaying	23	9	3	1
Flying	1	0	23	20

The residuals of the duration of displaying data were not normally distributed, and the data were heteroscedastic. The same was found to be the case for the duration of flying data. Consequently, I used Wilcoxon Signed-Rank Tests to determine whether these behaviours differed between experimental groups. Butterflies housed at 8˚C displayed for significantly longer after experiencing a simulated predatory attack than after experiencing the control conditions (Wilcoxon

Signed-Rank Test: *z* = -3.457, *p* < 0.001; Figure 16A). However, I found no significant difference in the duration of flying between the two conditions (Wilcoxon Signed-Rank Test: *z* = -0.335, *p* = 0.754; Figure 16B). Taken together, these findings suggest that butterflies tend to use deimatic displays at lower temperatures, and that these displays are performed in response to a perceived threat. In contrast, butterflies tended to fly more at higher temperatures. However, there is no evidence that this is an antipredator response because simulated predator attacks had no detectable effect on flight duration.

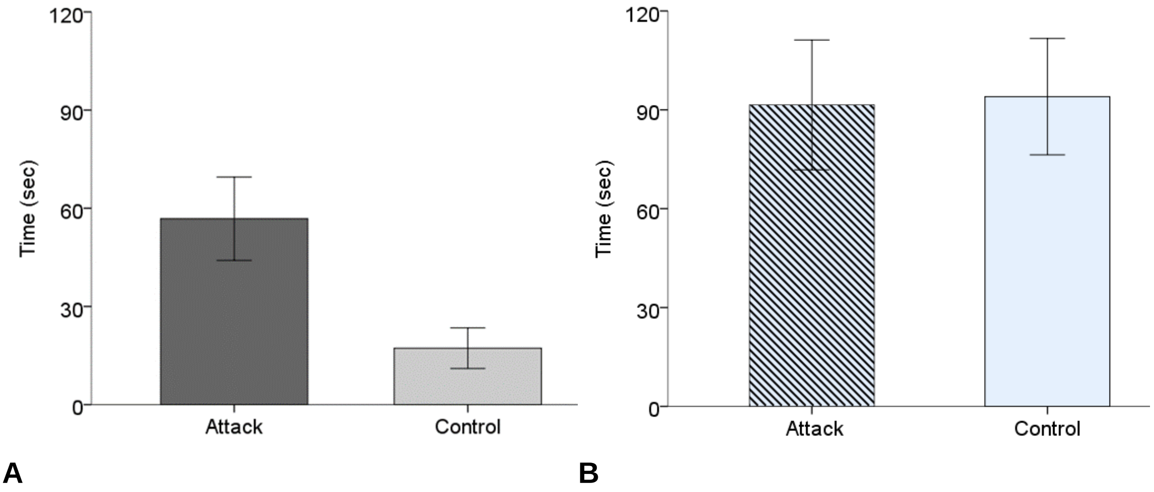

Figure 16. Behaviour durations in at 8°C and 18°C. A) The length of time (M ± SE) the butterflies spent performing their deimatic display at 8˚C after experiencing either no interaction (Control), or a simulated predatory attack (Attack). B**)** The length of time (M ± SE) the butterflies spent flying at 18˚C in response to the Control and Attack conditions.

7.5 Discussion

These results provide the first evidence that temperature influences the defensive behaviour of peacock butterflies. I found that most individuals performed their deimatic display at the lower temperature, and very few individuals performed displays at the higher temperature. Furthermore, at lower temperatures, the duration of deimatic displays was significantly longer when butterflies had experienced a simulated predatory attack compared to when they had not. In contrast, few individuals flew at the lower temperature whereas most of them flew at the higher temperature. Moreover, at the higher temperature, I found no evidence that the duration of flying behaviour was influenced by whether or not the butterflies had experienced a simulated attack. These results suggest that peacock butterflies perform deimatic displays in response to simulated predatory attacks at low temperatures, but at higher temperatures, they tend to invest in flying behaviour. However, it is unclear whether flying should be considered as an antipredator defence *per se* since flying duration was not influenced by simulated predatory attack.

Taking into consideration the ecology of peacock butterflies, it seems likely that the use of deimatism is restricted to times when temperatures are reasonably low. For example, the colder months of the year when butterflies are overwintering, or colder times of day (spring mornings). It is difficult to predict exactly how low the temperature is required to be before butterflies will begin to use deimatic displays. What we can say at the moment is that the threshold lies somewhere between 8 and 18°C. Neither is it certain how display duration varies with temperature across that range. In other words, does display duration gradually decrease as temperature increases? Or, are displays all-or-nothing, in that display duration is unaffected by temperature until a cut-off point at which butterflies stop displaying? Furthermore, we do not know what happens to displaying behaviour at temperatures higher or lower than those tested here. Answering all of these questions is crucial if we are to understand (i) at what point in the lifecycle of butterflies deimatism is an important form of defence, and (ii) under what conditions deimatism is likely to be favoured by selection. In order to answer these outstanding queries, this experiment would need to be repeated at regular temperature intervals starting at the minimum temperature that butterflies encounter in nature and continuing to the maximum temperature they encounter.

It is currently unclear why displaying behaviour seems to be restricted to colder temperatures, but there are two potential explanations that are not necessarily mutually exclusive. First, this may be an adaptive response to enhanced predation risk, i.e. predators may be hungrier and therefore harder to deter at lower temperatures. There is some evidence that this is the case for other forms of prey defence. For example, aposematism is less effective against predators maintained at lower temperatures (Chatelain, Halpin and Rowe, 2013). A second explanation for the observed difference in display duration is that deimatic displays may be used as a last resort when other more energetically-costly forms of defence (e.g. fleeing) are not possible. This is supported, to some extent, by the fact that flying behaviour was rarely observed at low temperatures. It may be possible to disentangle these two explanations experimentally. For example, to determine whether predators are more difficult to deter at low temperatures, one could house predators at a range of different temperatures and monitor their responses to deimatic prey. It would be crucial, however, that predators experience similar deimatic displays at each temperature in order to avoid any confounds of temperature-mediated differences in prey display behaviour. The system I used in Chapters 3-6 where the displays of artificial deimatic prey can be entirely controlled would be ideal for this purpose. One could then test whether deimatic displays act as a form of ‘last resort’ defence by establishing whether restricting fleeing in other ways (e.g. by tethering butterflies or restricting food consumption) also leads to increased investment in deimatic displays.

Given our limited knowledge of the factors influencing display production, it is difficult to predict to what extend my findings may apply to other species. I would expect to find a similar effect of temperature on the display production of other lepidopteran species with similar ecologies (e.g. the European swallowtail; *Papilio machaon*). However, my findings are not likely to hold across all deimatic species. For example, deimatism is seen in many tropical species that live at temperatures above those at which peacock butterflies appear to stop performing deimatic displays. This raises the question as to why these species use deimatism when presumably the temperature of their environment does not fall low enough to restrict prey escape ability, or enhance predator hunger levels. It may be that in the tropics, deimatism is found in species that have poor escape abilities for reasons other than low ambient temperatures. For example, they may have no where to escape to, or, they may be pursued by very fast or agile predators. Having said this, it remains a possibility that even in the tropics deimatism is restricted to lower temperatures, but for very different reasons. For example, performing deimatic displays at higher ambient temperatures may be costly because they generate unwanted body heat.

Not only do these results highlight the importance of understanding what factors influence the production of deimatic displays, but they also have implications for both our interpretation of existing research and the design of future studies. Previous studies investigating the antipredator efficacy of deimatic displays have tended to house deimatic prey and perform experimental trials at low temperatures (e.g. Olofsson, Jakobsson, & Wiklund, 2012b; Olofsson *et al*., 2013; Vallin *et al*. 2005, 2007). These are typically at the lower end of the temperature range of that which these species would encounter in nature, and lower than the lowest temperature I used in this study. It is therefore important to note that although their findings are valid, they may not hold at higher temperatures, meaning that the benefit of deimatism may have been overestimated. Future studies should take this into account and explore the benefit of deimatism across the range of environmental conditions regularly experienced by deimatic prey.

In conclusion, I have found that the production of deimatic displays appears to be restricted to lower temperatures. This is the first evidence of the influence of any environmental factor on the production of deimatic displays. However, my work should be seen as a small step toward understanding how the production (and thus potentially the efficacy) of deimatic displays can vary across space and time depending on environmental conditions. There are many other factors that could influence display production: prey's physiological and affective state, the level of stress prey experience during larval development, and whether prey are currently in the breeding season. For example, if deimatic prey are in a poor physiological state, performing their deimatic display may prove too energetically costly, and their chances of surviving a predatory encounter may be reduced. If this were the case, I would expect deimatic displays to be more likely to evolve in environments where deimatic prey have sufficient resources to enable the production of displays. Understanding what factors influence display production, and how and why this is the case, can inform us as to the conditions most, or least, conducive to the evolution of deimatic displays and the type of environments in which they are likely to thrive.

Chapter 8. General Discussion

The overarching aim of my thesis was to provide a better understanding of the role of cognition in the evolution of deimatic displays. In this chapter I will briefly recap my findings and explain how, and to what extent, they help us to answer three key questions about deimatic displays: 1) How do deimatic displays evolve? 2) Why are predators deterred by deimatic displays? and 3) What factors influence the production of deimatic displays? I then go on to consider the important gaps in our knowledge of deimatism and what should be done in order to address them.

8.1 How do deimatic displays evolve?

In Chapter 3, I provided the first empirical evidence that an evolutionary pathway to deimatism was feasible. I demonstrated that naïve predators were deterred by movement alone, if that movement was of sufficient speed; and that movement that revealed conspicuously-coloured hindwings deterred predators more effectively than movement that revealed background-matching hindwings. This suggests that the movement component of deimatic displays could evolve first (perhaps by intensifying existing movements to the point that they deter predators), and that this could be followed by the evolution of a conspicuously coloured body part that is revealed by the movement. Thus, the combination of movement and conspicuous colouration could become a cohesive deimatic display. It is worth noting that whilst this study does not account for the fact that many deimatic displays also have auditory components (e.g. the “hissing” sound produced by peacock butterflies (*Aglais io*) while performing their display), it is easy to imagine that such components could evolve because they further enhance the efficacy of the deimatic display. Alternatively, auditory display components could function to deter a different guild of predators. For example, the hissing of peacock butterflies (*Aglais io*) appears to be aimed at mammalian predators (Olofsson, Jakobsson and Wiklund, 2012b).

Whilst my findings clearly suggest that one potential mechanism is feasible, this does not preclude the possibility that other pathways are possible, and further research investigating other potential routes to deimatism is needed. One such evolutionary pathway is the “defence-first evolutionary sequence” proposed by Umbers *et al.* (2017). This hypothesis proposes that cryptic prey first acquire a chemical defence, and subsequently a conspicuously coloured body part (which is constantly on display) in order to advertise this defence to would-be predators. Finally, prey evolve to conceal the conspicuous colouration at rest such that they now only expose it when confronted by a predator (Umbers, *et al.*, 2017). However, the authors do not state how this would be achieved. For example, they do not clarify whether this requires morphological changes, or changes in posture. Moreover, it is difficult to see how adaptations that hide conspicuous colour patches from view could be favoured by selection unless, that is, the movement required to reveal them is already in place. This may be due to that movement having a startling effect on predators. As we know that static conspicuous signals are effective at advertising chemical defences (Mappes, Marples and Endler, 2005), the key predictions necessary to test in relation to this hypothesis would therefore be: 1) that moving conspicuous displays are more effective than static ones, and 2) that the survival of deimatic prey that are cryptic when at rest is higher than those that are conspicuous at rest.

Intriguingly, both of the pathways mentioned above consider only the evolution of displays in which previously-hidden conspicuously coloured body parts are revealed by movement. They do not, however, provide any explanation for those displays that involve visual signals that are constantly on display. For example, the eyespots of many deimatic frogs are always visible to predators (Lenzi-Mattos *et al.*, 2005). It may be that such conspicuous visual markings evolved for other purposes (e.g. sexual selection and mate choice) and that the addition of specific postures allowed their presentation to predators as a deimatic display. In addition, research into the evolution of deimatism in cephalopods is greatly lacking. The common cuttlefish (*Sepia officinalis*) can change its appearance, and the deimatic body colouration is one of a number of different patterns it can adopt for both defensive purposes, and, in order to conceal themselves while hunting prey (Adamo *et al.*, 2006). It is likely that certain visual components evolved because predators found them aversive, e.g. eyespot markings, but that the addition of other visual patterns in combination with specific body postures enhanced their effect in deterring predators thus leading to the evolution of a deimatic display. This is all currently, however, no more than speculation as research testing these ideas is required in order to establish their likelihood.

In addition to testing the feasibility of an evolutionary pathway to deimatism, I also investigated whether a number of factors (predator hunger, environmental background noise, predator affective state) influenced the efficacy of deimatic displays, and therefore, the likelihood that these displays would evolve. I found no evidence that hunger (see Chapter 4) influenced predators' responses to deimatic prey, which could suggest that deimatic displays are equally effective against predators regardless of their level of hunger. Consequently, predator hunger, and any factors that influence this (e.g. abundance of food in the environment), may not alter the likelihood of deimatic displays evolving. However, more research is needed in this area, as an alternative explanation for my findings is that the hunger manipulation I used did not successfully alter the hunger state of the predators in my study.

I did, however, find that background noise (see Chapter 5) enhanced the efficacy of deimatic displays: in its presence, predators spent a greater proportion of time at a greater distance from deimatic prey while they were deciding whether to attack them. This suggests that deimatic displays may be more likely to evolve in areas where background noise is present. I also found some evidence that predators' affective state could influence their responses to deimatic prey. Whilst I found no evidence that anxious-like states influenced predators' responses to deimatic prey, depressive-like states seemed to have complex effects on predator behaviour (Chapter 6). Whilst some individuals in a depressive-like state were deterred by deimatic prey, the individuals that attacked prey, did so more quickly than the chicks that attacked control prey. Thus, environments where predators are more likely to experience depressive-like states could contain predators that do not find deimatic displays as deterring as those in other affective states. Such states could be induced by a variety of experiences including (but not contained to), low food abundance, high levels of predation risk to themselves, high competition for mates, and low number of sites to raise offspring.

These studies have provided the first insights into both the pathways via which deimatic displays could have evolved and the types of environments in which deimatic displays are likely to evolve. However, much more work is needed in this area. Moving forward, research into the evolution of deimatism should follow two paths. The first being the conceptualisation and testing of hypotheses relating to the evolutionary pathways via which a broader range of deimatic displays have evolved, and the second being the continued pursuit of understanding the conditions most, and least, conducive to deimatic display success.

8.2 Why are predators deterred by deimatic displays?

We do not know why predators are deterred by deimatic displays, however, several suggestions have been made (see Chapter 1). Whilst my studies investigating the factors that may influence the efficacy of deimatism (as outlined above) were not intended to directly test why predators are deterred by deimatic displays, they do shed some light on this question. The factors I chose to investigate were all known to influence the magnitude of startle responses. Hunger, in the presence of food (Rejeski *et al.*, 2010), or images of food (Drobes *et al.*, 2001; Blechert *et al.*, 2014; Ferreira de Sa *et al.*, 2014), is known to increase the startle response magnitude, so too is background noise (Hoffman and Fleshler, 1963; Hoffman and Searle, 1965; Davis, 1974; Cory and Ison, 1979; Gerrard and Ison, 1990; Flaten, Nordmark and Elden, 2005; Blumenthal *et al.*, 2006). Animals experiencing either an anxious-like or a mild depressive-like state also show enhanced startle responses (Davis, 1979; Swerdlow *et al.*, 1986; Liang *et al.*, 1992; Cornwell *et al.*, 2008; Schulz, Alpers and Hofmann, 2008), and those experiencing severe depressive-like states show reduced startle responses (Kaviani *et al.*, 2004). While some of my findings are consistent with the idea that deimatic displays startle predators (i.e. the increase in deterrence of displays with background noise, and, for those subjects experiencing a more severe depressive-like state), others are not (the lack of any effect of hunger or an anxiety-like state). Therefore, whilst there is some tentative support for the idea that deimatic displays startle predators, it is difficult to draw robust conclusions from my data. In order to test this more directly, it may be necessary to carry out studies examining measures of predators' physiological and neural responses to deimatic displays in order to establish whether these are similar to those seen in response to startling stimuli. For example, one could use heart monitors to measure any increases in heart rate, or, apply electrodes to detect the subtle muscle tensions associated with the startle reflex. It may also be fruitful to establish the effects of anxiolytic drugs on the efficacy of deimatic displays, since such drugs are known to reduce the magnitude of startle responses (Riba *et al.*, 2001).

As outlined in Chapter 1, startle is only one of five mechanisms through which deimatism has been suggested to deter predators. Research testing the feasibility of the remaining four mechanisms is also needed. It would be difficult to test whether deimatic displays cause sensory overload in predators without live neurophysiological recordings. However, it may be possible to establish whether deimatic displays are likely to cause visual discomfort by presenting them to human subjects and using the discomfort scale to assess any aversive visual impact (Juricevic *et al.*, 2010). In addition, it may be possible to adapt models developed within the areas of human design to predict visual discomfort in order to assess whether the visual signals used by deimatic prey are likely to cause visual discomfort (Oh, Lee and Bovik, 2016). It could also be possible to test whether deimatism elicits fear responses. For example, one could present predators with deimatic prey before and after lesioning of brain areas associated with fear responses (the amygdala; Gewirtz, McNish and Davis, 1998; Walker, Toufexis and Davis, 2003). If fear is driving the deterrent effect of deimatism, then survival of deimatic prey should be lower after lesioning. Similarly, it should be relatively straightforward to establish whether predators misclassify deimatic prey as a threatening object. This could be achieved by manipulating naïve predators' experience with fearful stimuli before exposing them to deimatic prey. If deimatic prey are aversive because they are misclassified as something frightening, then these displays will be more effective if predators have learned to be frightened of the relevant stimuli (i.e. they have learned that it poses a risk to them). Finally, many species are known to demonstrate recoil and/or evasive behavioural responses in relation to looming stimuli (Schiff, 1965; Dill, 1974; Lee and Reddish, 1981; Evans, Macedonia and Marler, 1993; Regan and Vincent, 1995; Tammero and Dickinson, 2002; Santer, 2006; Oliva, Medan and Tomsic, 2007; Yilmaz and Meister, 2013), and it has been suggested that deimatism exploits this. It should be possible to test this by establishing whether deimatic displays share features with stimuli known to provoke collision avoidance responses (e.g. velocity of stimulus approach, direction of approach, stopping distance (Yamamoto, Nakata and Nakagawa, 2003; Yamawaki, 2011)).Therefore, although the results of my research provide some clarification as to the workings of deimatic displays, much further research is required.

8.3 What factors influence the production of deimatic displays?

Very little of the research carried out on deimatism has focused on understanding prey behaviour, and those studies that have tend to investigate what types of stimuli elicit their production (e.g. Maldonado, Benko and Isern, 1970; King and Adamo, 2006; Wood, Pennoyer and Derby, 2008; Cartron *et al.*, 2013; Umbers and Mappes, 2015; Mooney *et al.*, 2016). In Chapter 7, I adopted a novel approach, investigating instead how predators' environments (temperature) influence the likelihood and duration of deimatic displays. I found that most butterflies performed their deimatic displays at 8°C, and that the display duration was greater after experiencing a simulated predator attack. In contrast, very few butterflies performed their displays at 18°C. When maintained at this higher temperature, butterflies tended to fly instead, although the duration of flying behaviour was not influenced by whether they had experienced a simulated predator attack. This is the first evidence that the production of deimatic displays could be confined to certain environmental conditions. Further research investigating the factors that influence display initiation, production and magnitude (e.g. light level, and background noise) are now required if we are to understand the range of conditions under which prey are likely employ deimatic displays. We also need to understand both how environmentally-mediated changes to the form of deimatic displays influence their antipredator efficacy, and whether factors that influence display production have direct effects on predator behaviour. For example, low temperatures caused peacock butterflies to increase display duration. This could enhance the deterrent effect of the displays, but this will depend upon whether temperature also affects startle response magnitude or hunger levels in predators. To tease these effects apart one would need to manipulate display duration and predator temperature independently. This would be difficult to achieve using live predators and prey, as one would need to stage an encounter in which predators and prey were kept at different temperatures. However, this should be possible using artificial prey, or video recordings of live prey.

8.4 Future research directions

The research presented in this thesis provides novel and useful data to the field of deimatic research. However, there are still a lot of unanswered questions that need to be addressed. For example, definitions of deimatism tend to agree that its defining feature is that it exploits unlearned reflexes in predators (Edmunds, 1974; Ruxton, Sherratt and Speed, 2004; Umbers *et al.*, 2017). As a consequence, studies monitoring predators' responses to deimatic prey have tended to focus on their initial responses to them (e.g. Vallin *et al.*, 2005; Olofsson *et al.*, 2013). However, this ignores the fact that predator responses to deimatic prey may change over repeated encounters, and how their responses change will have an important effect on the survival of deimatic prey. There is some evidence that predators responses to deimatic prey can change with experience: jumping spiders (*Phidippus audax*) are more likely to attack deimatic flies (*Anastrepha ludens*) after repeatedly encountering them (Aguilar-Argüello, Díaz-Castelazo and Rao, 2018). However, it is unclear whether this is also the case in other predator-prey systems. Whilst it may be common for predators to learn that deimatic prey are bluffing and habituate to their displays, one could also make alternative predictions. For example, some predators may not have the opportunity to learn that deimatic prey pose no real threat because the cost of investigating potentially threatening animals is too high. This could lead predators to retain their initial responses to deimatic prey even after repeated encounters with them. Alternatively, predators may find deimatic displays aversive, perhaps because they elicit physiological responses that are energetically costly, such as increases in heart rate. If this is the case, predators could learn to avoid deimatic prey and the likelihood of attacking prey could decrease over successive encounters.

Understanding how predators' responses to deimatic prey change across repeated encounters may also help us to better understand the form taken by deimatic displays, and what factors influence their efficacy. It seems reasonable to assume that selection may have favoured display components that either promote avoidance learning or inhibit the speed at which predators learn that deimatic prey are bluffing. Crucially, there are also many factors that could influence the speed of learning, and these could have a knock-on effect on the survival benefit of deimatism to prey. For example, age (Ludewig *et al.*, 2003), longer and more regular intervals between exposure to startling stimuli (Davis, 1970), and, various aspects of (human) personality (e.g. extraversion, impulsiveness, risk-taking, neuroticism; (LaRowe *et al.*, 2006; Blanch, Balada and Aluja, 2014)), are all known to increase the rate of habituation to startling stimuli, and could therefore decrease the antipredator benefit of deimatism. On the other hand, anxiety in humans is known to decrease the rate of habituation to startling stimuli (Campbell *et al.*, 2014). Thus, it will also be important to understand whether any of these factors influence predators behaviour toward deimatic prey over repeated encounters.

Another important unanswered question in this field of research is whether the evolution of deimatism has led to the evolution of other behavioural adaptations in prey, and if so, whether these adaptations influence the ecology of deimatic species. This certainly seems to be the case in other forms of prey defence. For example, both masquerading prey and cryptic prey have been shown to possess adaptive microhabitat selection strategies that optimise the efficacy of their defences (Shine *et al.*, 2000; Skelhorn and Ruxton, 2013). Thus, the evolution of these defences may have influenced where prey are found. It may also have influenced when prey are active, as it has been suggested that the evolution of camouflage could cause some prey to forage at night (Skelhorn *et al.*, 2011). We might therefore expect deimatic prey to show similar adaptations. This hypothesis could be tested by using comparative analyses to establish whether deimatism is associated with particular environmental traits. One could then stage predatory encounters to determine whether deimatism is most effective under those environmental conditions, and finally test whether deimatic prey favour the microhabitats in which they survive best.

8.5 Concluding Remarks

Throughout this thesis I have provided novel insight into a number of aspects of deimatism. In doing so, I have increased our understanding of the evolution of deimatic displays, the factors that may influence their success in deterring predators, and, a factor that may influence the length of their production. My research was informed by the accumulated knowledge within the field of experimental psychology, and our knowledge of the ecology of deimatic prey themselves. I did this with the hope that my approach, in addition to my findings themselves, may provide stimulation for the generation of more hypotheses. Deimatic displays are complex and varied, but with careful consideration and the application of novel methodologies, we can understand their production and success.

Appendix A. Chapter 1. Introduction: Predator Cognition and the Evolution of Deimatic Displays

A.1 Table 2 Search Methods

A list of terms commonly used to refer deimatic displays was compiled. It contained the following terms: deimatic display, startle display, deimatic behaviour, responsive defence, frightening attitude, defensive display, deimatic reaction, dymantic display, dymantic colouration, frightening display, startle signal and bluff display. I entered each of these as a search term in the Web of Science online literature database on the following dates: 13/06/2017, 18/06/2017, 02/07/2017, 09/07/2017, and 14/07/2017. The results of these searches were then ordered by publication date, and I read each paper to establish whether there was a statement in the paper relating to the search term. Any papers without any use of the search term within the text were excluded at that point.

I gathered a total of 294 papers, of those papers, 66% were related to research focused on predator-prey interactions and 33% were not. A total of 35 papers (11%) were directly related to deimatic displays and of those 35 papers 29 were empirical articles and 6 were review articles. Of the 35 papers relating to deimatic research, 18 particularly focused on researching the anti-predator function of deimatic displays, and thus were included in Table 2 as seen in Chapter 1.

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