Modelling the ecology and co-evolution of animal warning signals

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By

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ABSTRACT

The study of defensive colouration in animals, specifically aposematism whereby prey advertise their unpalatability with a bright warning signal, has served for over a century as an important case study in evolutionary adaptation. Though well studied, to date there are a number of questions and paradoxes which remain unresolved. This thesis presents a series of theoretical models aiming to re-appraise our understanding of warning signal evolution and optimality based on new theory, concepts and published empirical data.

The first chapter of the thesis shows, contrary to existing theory, that the initial evolution of warning signals may have been facilitated by novel food wariness in predators and proceeds to explore a series of ecological scenarios that can help evaluate the generality of this argument. The second chapter develops and applies models from the first chapter to a spatially distributed metapopulation, finding that population structure, clustering, isolation and migration levels are key factors influencing the viable spread of aposematism as an anti-predator trait. The simulations show that the most favourable conditions for the spread of a novel mutant in a metapopulation are where aposematism first reaches fixation in one or more sub-habitats followed by low levels of outward migration.

Chapter 3 focuses on determining the optimal defence strategies of prey given that aposematism is already established within a prey population, and specifically examines how conspicuousness may have become a reliable indicator of prey toxicity as suggested from recent empirical studies. Previous models show that positive correlations can arise when defence and aposematic display compete for a common resource. Chapter 3 presents new results to suggest that positive correlations between conspicuousness and toxicity can arise when toxicity and display deplete a common resource, irrespective of whether bright colouration is used as a warning signal or not, given that it provides some additional fecundity advantage e.g. from thermoregulation or sexual signalling. Finally, the second part of the chapter looks more closely at when prey should adopt aposematism over pure crypsis and finds that resource availability can influence this decision.

Chapter 4 presents the first theoretical model to examine the optimality of combined warning signals with crypsis and provides a series of predictions for what ecological conditions may influence the combination of those key traits. A range of prey distributions were tested in a simulated two dimensional habitat. The results show that where prey are distributed in close spatial proximity to predators or where they are commonly viewed from a short distance, that the optimal strategy is to adopt pure aposematism with no combined display. For all other tested prey distributions some combination of warning display, cryptic colouration and defence proved optimal. Further testing showed that the combination of cryptic displays with conspicuous warning signals could extend the phenotype space over which a positive correlation is observed between defence level and warning signal strength, suggesting that combined displays may have important implications for models of signal honesty in aposematic prey.

Finally chapter 5 investigates mimicry between prey species. This chapter presents the first model to explicitly consider the coevolution of mimetic appearance alongside evolution in defence levels in prey and in turn makes exciting new predictions about the dynamics of mimetic evolution. Three novel predictions are that 1. Müllerian co-mimics may gain an additional advantage from mimicry in that they can reduce their toxin investment in their post-mimetic state. 2. That increased toxicity of the model, rather than shifting warning patterns may be a common outcome of Batesian mimicry. 3. That the post-mimetic evolution and optimization of toxin levels in both species can change the dynamics of the relationship from mutualistic to parasitic. These results could influence a paradigm shift in the understanding of mimetic systems and shape future theoretical and empirical studies on mimetic evolution.

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General Introduction

Aposematism is a well known anti-predator mechanism in which a prey advertises a secondary defence, such as a toxin, with a distinctive and usually bright warning signal (Cott 1940; Edmunds 1974a; Poulton 1890; Wallace 1867). Animal warning colouration and mimicry between aposematic species have for over a century represented key case studies in evolutionary adaptation and co-evolution. The complex and spectacular patterns of warning colouration observed widely across taxa provide a mere snapshot in time of continual (co)evolutionary processes which have operated over millions of years. Given the long timescales involved, observing evolution is impossible in all but the simplest and short lived of life forms (Paterson et al. 2010). Despite being limited in most cases to observing only the products of evolution, this is enough for us to hypothesize the purpose of such adaptations and to empirically test their function. It seems Wallace was correct in his hypothesis on the purpose of (what we now know are in fact) warning signals in his letter to Darwin dated 24th February, 1867 in which he asserts *"Any gaudy & conspicuous colour therefore, that would plainly distinguish them from the brown & green eatable catterpillars, would enable birds to recognise them easily as a kind not fit for food, & thus they would escape seizure which is as bad as being eaten."*, the validity of such wariness of conspicuous warning signals by predators has since been repeatedly confirmed in empirical experiments (Gamberale & Tullberg 1998; Kelly & Marples 2004; Sillén-Tullberg 1985). What remains unresolved for the most part is understanding how warning signals may have evolved in the first place (Franks 2009; Ruxton et al. 2004; Sherratt 2002; Speed 2001), especially given that in even in the most simple case it involves potentially conflicting interests between the receiver aiming to discriminate between palatable and unpalatable prey (predators) and the signaller wishing to escape attack (prey) but also perhaps avoid costs of detection. As such, warning signal evolution may have been influenced heavily by ecological factors such as costs of displays and toxins, size and structure of populations, detection and encounter rates as well as predator psychology and foraging patterns. Further complexity arises when warning signals are shared across species boundaries in mimetic systems as will be explored in chapter 5. This co-

evolutionary conflict and sensitivity to ecological conditions ensure that warning signal evolution is still a topic of active theoretical debate (Ruxton et al. 2004).

Our inability to observe the evolution of complex traits such as warning signals and mimetic resemblance places these subjects firmly in the realm of theoretical biology. Empirical experiments and subsequent data explaining the function of these traits allow us to theorise the processes and evolutionary events that may have occurred to give rise to such adaptations. In the last 50 years with huge advances year on year in the accessibility and processing power of computers, computational modelling has increasingly played a crucial role in expanding our knowledge and understanding of the pathways and processes of evolution. Modelling gives us the ability to replicate thousands of years of evolution within hours, even minutes; though inevitably only modelling a simplified subset of key conditions and assumptions. This ability to simulate evolutionary processes enables us to build numerically validated models based on empirical observations, allowing us to test theories of how and why selection has acted to give rise to the adaptations that we see in modern organisms. Although theoretical models do not provide empirical proof, they put forward numerically validated stories of how evolution may have acted to shape life as we observe it today.

This thesis presents a series of theoretical models designed to build on, extend and in some cases question existing theory of warning signal evolution and optimality, covering two main themes; 1. Identifying the conditions which may have favoured the initial evolution of aposematic signalling and 2. Exploring honesty and cheating in warning signals. The following subheadings outline these concepts in more detail.

Identifying the conditions which may have favoured warning signal evolution

The evolution of warning signals in prey presents an interesting and paradoxical example of adaptation. A trait which, when sufficiently abundant can yield reduced mortality for its adopters by enhancing predator avoidance learning, yet when rare causes raised mortality due to increased conspicuousness and hence

attacks from naive predators. A number of theories exist to explain how warning signals may have evolved, including prey grouping and kin selection (Brodie & Agrawal 2001; Guilford 1985), dietary wariness in predators (Marples et al. 2005; Speed 2001; Thomas et al. 2003), instantaneous avoidance learning (Puurtinen & Kaitala 2006; Speed 2001) and finally in an attempt to escape from Batesian mimics (Franks 2009), for a more exhaustive list see summary in Ruxton et al. (2004). The primary evolution of aposematism whereby it first emerged as an anti-predator adaptation is even more difficult to comprehend given that predators may not have possessed any receiver biases favouring bright colouration in prey (Sherratt 2002).

In the first two chapters of the thesis, the focus is on identifying the conditions which may have promoted the evolution of warning signals in prey. Chapter 1 utilizes agent based stochastic models to re-examine the effect that novel food wariness may have had on the primary evolution of aposematism in light of empirical evidence suggesting that levels of wariness in predators may be higher than previously thought (Marples et al. 1998). The use of agent based, stochastic models allow for a more realistic representation of the number dependent nature of aposematism (Puurtinen & Kaitala 2006) and allows specific ecological conditions which may have been crucial to the initial evolution of aposematisn to be easily and explicitly tested e.g. the effects of multiple predators, increased predator longevity, season length, palatable and unpalatable prey abundance, predator numbers and variation in dietary wariness, all of which are shown to be crucial to the survival of the aposematic form.

Chapter 2 again utilizes agent based stochastic models, presenting the first application of metapopulation theory to the evolution of warning signals. This model extends the single habitat model of the first chapter to explore the conditions which may have favoured the spread of aposematism over spatially distributed prey populations. The use of agent based stochastic models again allows specific spatial ecological parameters to be explicitly tested such as metapopulation size, prey migration levels, sub-habitat clustering, predator movement and patch occupancy. The results give an interesting and novel insight into the conditions which may have allowed aposematism to spread in such populations.

Honesty and cheating in warning signals

The early chapters in the thesis examine how warning signals may have first evolved, however, this only represents part of the bigger picture of warning signal evolution. Natural selection is a continual process and over evolutionary time acts to select, optimize and hone the most beneficial traits. Warning signals are no exception to this rule. The effectiveness and optimality of warning signal strength, structure and underlying prey defences are under constant selective pressure. How natural selection has acted to produce the spectrum of warning signal forms observed in the natural world is every bit as interesting as how aposematism came to exist.

The second part of the thesis considers the optimality of warning signals given that they have already evolved. These chapters address pertinent issues such as in chapter 3 examining the conditions under which conspicuousness can act as an honest signal of prey toxicity and how resource availability may dictate when aposematism proves optimal over crypsis. Chapter 4 explores the conditions which may favour the combination of warning signals with cryptic camouflage in prey, looking at how prey and predator proximity and distribution might affect the optimality of combined displays. In the final chapter, the dynamics of mimicry are examined in a co-evolutionary framework in which two prey species can evolve in appearance in addition to toxicity. In this model we see that cheating and defection both within and between species can be commonplace and shape the nature and dynamics of the mimetic relationships that form.

Each chapter is presented as individual manuscripts in journal format. Preceding each chapter is a brief overview of the rationale and findings presented along with reference to the overall theme of the thesis and a statement of how the work was distributed between co-authors (Thomas Lee was primary author on all manuscripts).

Published papers

Chapter 1

LEE, T. J., MARPLES, N. M. & SPEED, M. P. 2010. Can dietary conservatism explain theprimary evolution of aposematism? *Animal Behaviour,* 79, 63-74.

Chapter 2

LEE, T. J. & SPEED, M. P. 2010. The effect of metapopulation dynamics on the survival and spread of a novel, conspicuous prey. *Journal of Theoretical Biology*, 267, 319-329.

Chapter 3

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Chapter 1 - Can dietary conservatism explain the primary evolution of aposematism?

1.1 Overview

The primary evolution of aposematism presents an evolutionary paradox. A trait which, when sufficiently abundant can yield reduced mortality to prey which adopt a common, bright signal to advertise their unpalatability, yet when rare causes raised mortality due to increased attacks from naive predators. The literature currently proposes two seemingly opposing theories. Firstly, that aposematic signalling came to fruition by a co-evolutionary process whereby variability in a prey's conspicuousness levels and defences co-evolved with predators preferences to create a world in which conspicuousness became a reliable indicator of prey defences (Sherratt 2002). Secondly, that a mutation which increases the conspicuousness of an individual in a cryptic, defended prey population could invoke pre-existing receiver biases in predators that favoured its survival and fixation (Marples et al. 2005).

This chapter describes a series of individual based stochastic models devised to investigate the role of predatory receiver biases (namely dietary conservatism (abbreviated to DC in this and subsequent chapters) and avoidance learning) on the initial evolution of warning signals. A number of previous theoretical studies have expressed some scepticism as to the importance of dietary conservatism and neophobia in promoting the survival and spread of a novel aposematic morph *i.e.* promoting the initial evolution of aposematism in otherwise cryptic populations (Mallet & Singer 1987; Speed 2001). In light of new empirical data showing that the length of avoidance shown by predators can be significantly higher than previously thought (Marples et al. 1998), a re-appraisal of the effects of dietary conservatism on the initial evolution of aposematism is timely.

The same empirical data also shows that there can be high levels of variance in DC tendency between individual predators. The second part of the chapter therefore considers how the application of more realistic ecological factors may affect the viability of dietary conservatism as a driver of aposematic evolution. This

includes considering cases whereby multiple predators, each with different DC tendencies may attack the focal prey population simultaneously or individually but with temporal spacing. Another important ecological factor tested is predator longevity. Previous models have ignored the fact that predators often outlive their prey, hence dietary conservatism may only last a short proportion of the predator's lifetime and therefore may be much less effective at facilitating the evolution of aposematism (Mallet & Singer 1987). Finally, the effects of adding palatable prey to the model system are considered. By utilizing individual based stochastic models, these ecologically realistic scenarios could be tested explicitly with random predator-prey selection and interaction during simulated seasons.

The simulations presented in this chapter show that dietary conservatism, at the levels found empirically and without increased avoidance learning rates over that of the cryptic form can, in a limited range of scenarios explain the coexistence and even fixation of a novel aposematic prey. Although fixation of the aposematic morph could be demonstrated with very high levels of predator dietary conservatism, lower levels of dietary conservatism promoted a stable abundance of aposematic prey to exist over long periods of evolutionary time. A novel prediction of the model is therefore that dietary conservatism can facilitate the long term, stable persistence of the aposematic morph in a state of dynamic equilibrium. This result persists because predator dietary conservatism can prevent extinction of the aposematic morph, yet due to the number dependent nature of selection for aposematism (Puurtinen & Kaitala 2006), in some cases the critical abundance of aposematic prey required for fixation is never met and hence over time, the abundance of the aposematic morph fluctuates yet never fixates or becomes extinct.

Further simulations show, however, that for more ecologically realistic scenarios e.g. where prey suffer predation from more than one predator or where predators significantly outlive their prey, the benefits of dietary conservatism are less pronounced in that dynamic equilibrium between the competing morphs could no longer be demonstrated. In these less favourable conditions, fixation of the aposematic morph required an additional receiver bias to be exhibited by the predators, that being accelerated avoidance learning of the aposematic prey.

Overall, this chapter suggests that dietary conservatism and the receiver bias hypothesis remains a valid explanation for the initial evolution of aposematism. It is suggested that there may be reason to expect that such wariness of novel prey could have evolved before aposematism as a general foraging mechanism. Under a range of limited scenarios, such wariness may have promoted the fixation of a novel, aposematic mutant within a localized population. An equally important and less restrictive prediction is that dietary conservatism may promote the long term, stable persistence of a novel, conspicuous prey form, a result which has so far been unreported in the literature and may have implications to the coevolutionary hypothesis of warning signal evolution proposed by Sherratt (2002).

This work was devised from discussion between Thomas Lee, Mike Speed and Nicola Marples. Model design was undertaken by Thomas Lee with assistance from Mike Speed. Coding, implementation and testing were performed by Thomas Lee. Writing by Thomas Lee with editorial advice from Mike Speed and Nicola Marples.

1.2 Abstract

Predators are often unwilling to eat prey with novel appearances (so called dietary conservatism). It has repeatedly, though controversially been argued that such wariness can contribute to the evolution of bright colouration in prey animals with effective secondary defences such as toxins. In this paper we report the results of novel evolutionary simulations in which bright prey emerge in otherwise cryptic, defended populations, and predators vary in their level of dietary wariness. A novel prediction from our simulations is that rare aposematic prey can evolve to a dynamic equilibrium with their cryptic conspecifics, and persist for long evolutionary timescales without ever reaching fixation in prey populations. Furthermore, we show that when conditions are more beneficial for new aposematic forms, for example because there are many palatable prey in a habitat, then dietary conservatism can indeed explain the evolution of aposematism, but the generality of this result was considerably restricted by variation in levels of dietary conservatism seen within

predator populations and by increased predator longevity. We use the results to consider the case that "receiver biases" could explain aposematism, rather than recently suggested models of signal reliability.

1.3 Introduction

Aposematism is a well known anti-predator mechanism in which a prey advertises a secondary defence, such as a toxin, with a distinctive and usually bright warning signal (Cott 1940; Edmunds 1974a; Poulton 1890). Conspicuous warning signals have been shown to have a number of effects on predators that favour prey survival; for example causing wariness and cautious handling of brightly coloured prey (Gamberale & Tullberg 1998; Kelly & Marples 2004; Sillén-Tullberg 1985), accelerating predator avoidance learning (Gittleman et al. 1980; Lindström et al. 2001; Roper & Wistow 1986) and by reducing predator forgetting (Roper & Redston 1987; Siddall & Marples 2008; Speed 2000; Yachi & Higashi 1998). Furthermore, as A.R. Wallace originally proposed when devising the idea of aposematism, warning signals may be used by educated predators to help them reliably discriminate defended prey from edible, cryptic prey (Wallace 1889; Wallace 1867).

It is widely hypothesized that aposematic warning displays must have initially evolved from defended cryptic prey populations (Harvey et al. 1982; Leimar et al. 1986; Yachi & Higashi 1998). In the literature it is assumed that rare novel mutants emerging from within these populations are especially vulnerable to extinction because, having attracted the attention of ignorant predators (because of their enhanced conspicuousness), they are very likely to be attacked and killed (Mallet & Singer 1987). Their low initial numbers would heighten the risk of extinction, since with low absolute numbers all individuals may be consumed before predators learn to associate their appearance with the defences they experience. Given that aposematism is observed across many species and in many diverse habitats, it appears there is some paradox to resolve: the existence of a trait whose persistence is easy to explain when it is common, but difficult to explain when it is new and rare (Mallet & Singer 1987; Servedio 2000; Speed & Ruxton 2005b, 2007).

We can consider the problem in two ways: first is the "primary" evolution of aposematism, in which aposematism as a class of anti-predator defence is absolutely novel, and did not previously exist. The challenge here is to explain how aposematism evolved from rarity even though predators had no initial reason to treat conspicuous prey with caution. In addition, we could consider "secondary" evolution in which aposematism is relatively novel; turning up for the first time in a particular species, but already existing in other species. The evolution of aposematism is easier to explain in this "secondary" context, because it is often reasonable to expect that evolution has already prepared predators to be wary of aposematic colour patterns (see empirical studies such as Coppinger 1970; Gamberale & Tullberg 1998; Roper & Cook 1989; Schuler & Hesse 1985; Sillén-Tullberg 1985). Hence, when new aposematic prey emerge, predators are already biased to "go slow", to handle them cautiously and to learn about them quickly (Guilford 1994).

The recent literature provides two competing hypotheses to explain the more challenging case of the primary evolution of aposematism. First, it has been argued by Sherratt (2002) that predator biases and bright aposematic colouration originally emerged from a co-evolutionary process. Cautious handling of bright prey by predators emerges in Sherratt's theoretical model because bright, edible prey are increasingly eaten and removed from the prey population while bright defended prey tend to escape and reproduce. If appearance and defence are heritable traits, then brightness consequently becomes a reliable signifier of unprofitability, and at evolutionary stability it pays predators to be wary, and to avoid eating conspicuous prey.

In contrast, Marples et al. (2005) proposed an alternative argument, focusing on the repeated demonstration that predators often avoid contact with new foods (neophobia) and subsequently they may be reluctant to include them in their diet (so called dietary conservatism). There is considerable evidence that both neophobia and dietary conservatism can be invoked in predators by novel prey forms whether these are conspicuous or not (Kelly & Marples 2004; Marples & Brakefield 1995; Marples & Kelly 1999; Marples et al. 2007; Marples et al. 1998; Mastrota & Mench 1994). Marples et al. (2005) recently argued that such wariness of novelty is likely to be a general consequence of optimal foraging decision-making and thus easily preceded the primary evolution of aposematism. For foraging animals, novelty implies some

degree of uncertainty and risk of injury; if valuable familiar foods are already present in a habitat, then the optimal strategy in relation to new food items will often be avoidance for some period. In field and lab experiments, dietary conservatism has been shown to facilitate the evolution of novel prey forms (Thomas et al. 2004; Thomas et al. 2003), and consequently Marples et al. (2005) argued that the primary evolution of aposematism could easily be explained if avoidance of novel prey forms caused by dietary conservatism in predators is sufficiently high.

Though this argument is plausible, some important issues require quantitative evaluation before a well founded judgement can, in our view, be made. For example, field data indicate that (within a population) predators can be very variable in their levels of dietary conservatism; without numerical investigation, it is not clear how likely it is for a novel morph to survive and prosper when, for example a majority of mobile predators are willing to eat novel prey without hesitation (see data in Marples et al. 1998). Furthermore, if predators typically outlive their prey (e.g. with invertebrate prey and vertebrate predators) it is possible that the benefits to rare prey of predatory dietary conservatism apply only to the first prey generation, so that even if novel morphs are initially favoured, they subsequently face a rapid extinction because of long-lived predators that are no longer wary (a similar point is made rather forcefully in Mallet & Singer 1987).

There are, in fact only two published theoretical models that evaluate the importance of predator wariness of novelty on the initial evolution of aposematic warning displays (Puurtinen & Kaitala 2006; Speed 2001) [note different authors use different terms to describe the reluctance of predators to ingest novel prey; for simplicity we will term this dietary conservatism throughout]. Of these papers Puurtinen & Kaitala (2006) is by far the most rigorous and extensive. Whilst both of these papers demonstrate that dietary conservatism could benefit aposematic mutants, neither includes the kind of ecological details, such as variability in predator lifespan and wariness, that are key to evaluating the plausibility of the arguments of Marples et al. (2005). In addition, neither of these papers uses evolutionary modelling as a tool, and as we describe in this paper a stochastic-evolutionary approach yields some important, and so far unreported, findings about the dynamics of aposematic evolution.

If Marples et al. (2005) are correct to assert that wariness of novel prey explains aposematism, then an important evolutionary paradox is easily resolved by a simple and easily generalised argument. If, on the other hand, Mallet & Singer's (1987) view is correct, that the ephemeral nature of dietary conservatism makes it unimportant, the primary evolution of aposematism is more problematic and may require a coevolutionary solution like that proposed by Sherratt (2002). In this paper we therefore present a stochastic evolutionary model in which rare, bright prey forms emerge in populations of cryptic defended prey. We examine whether on its own, dietary conservatism can explain the initial evolution of aposematism and whether additional psychological biases such as accelerated learning of conspicuous signals are necessary. Unlike previous theoretical models we consider the interaction of dietary conservatism with ecologically relevant conditions such as varied duration of predator lifespan, migration of predators into a focal habitat and the number of predators attacking any one time. We show that dietary conservatism can be effective in preventing extinction of novel prey forms, and in causing their fixation in a population; however a number of ecologically relevant factors tend to diminish the effectiveness of predator dietary wariness in facilitating the evolution of aposematism. We use the results to argue for a "receiver bias" explanation of aposematism in our discussion.

1.4 Model description

Using stochastic, evolutionary simulations we consider the evolution of aposematism in a prey population that possesses some kind of effective secondary defence such as a toxin. At the start of the simulations the prey are overwhelmingly cryptic in appearance, and we introduce a single mutant individual that has a more conspicuous appearance than the rest of the population. We simulate interactions between members of the prey population and one or more predators for a specified period (a season) after which the prey species reproduces by asexual reproduction. We iterate this sequence over many prey generations and follow evolutionary change in the abundances of cryptic and aposematic forms of the prey. We make some simplifying assumptions: specifically (i) that there is only one predator and one prey population (which is closed to immigration and emigration), (ii) that the lifespan of

the prey and the predator are equal and (iii) that the habitat contains only the focal defended prey species. As we develop the model some of these assumptions are relaxed. We first present a general framework within which the predator-prey environment is described, and then consider how dietary conservatism is represented computationally, before describing and developing scenarios for simulation.

A MATLAB script was produced to model a finitely sized habitat in which one predator and a number of prey (N) reside. Within the prey population two distinct prey types exist, cryptic (c_c, of number N_c) and aposematic (c_a, of number N_a). We assume that both prey types are equally distasteful to the predator. At the start of the simulation we assume that a single aposematic mutant exists (though the number of aposematic prey within the prey population could be varied). The model is run for a finite number of generations each of finite time limit (T). Each prey type is assigned an arbitrary conspicuousness value, (c_a) for the aposematic prey type and (c_c) for the cryptic type which represents the probability of detection by the predator, given that a predator and prey are within some minimum level of proximity. The two prey types are also each assigned an avoidance learning rate, (α_a) for the aposematic prey type and (α_c) for the cryptic prey, used to determine the rate at which the predator learns to avoid each prey type as a result of their distastefulness.

During each generation, the predator moves through the habitat at random until it comes within striking distance of one individual prey; we assume time taken for this stage is *1/Total number of living prey* (in arbitrary time units). For any plausible predator locomotive pattern and prey distribution, there would be variation between inter-encounter times, but this complexity would not affect our model predictions and so has been ignored here. The idea that time between encounters is inversely proportional to the density of targets is reasonable for a wide range of predator search scenarios and prey distributions (Ruxton & Bailey 2005). The chance of the predator detecting a prey individual is dependent on the prey's conspicuousness value (c_a, c_c) which is compared against a randomly generated number between 0 and 1 inclusive. If for example, we set c_c =0.01 and c_a=0.02 then the aposematic prey has twice the chance of being detected over its cryptic counterpart. If the prey in striking distance is not detected, the predator moves randomly through the habitat and repeats this process until a victim is finally selected.

Once the victim has been selected, the predator then has the option of rejecting it without an attack, based on a variable rejection probability (R_a for aposematic prey, R_c for cryptic prey). Because predators are assumed to learn about their prey, this probability of rejection on a specific prey type increases the more often it has been attacked; for simplicity we use a negative exponential function in accordance with the majority of empirical data on avoidance learning in individual predators. For aposematic prey:

$$R_a = 1 - e^{-\alpha_a \cdot n_a} \qquad (1.1a)$$

And for cryptic prey:

$$R_c = 1 - e^{-\alpha_c \cdot n_c} \qquad (1.1b)$$

Where n_a and n_c refer to the number of attacks that a predator has had with an aposematic or a cryptic prey respectively. When predators are inexperienced then n_a and n_c equal zero and probability of rejection given detection is zero (In appendix, Figure A1.1 we display the avoidance learning curves for a range of learning rates). In the model, if rejection does occur we assume the time taken for this is 0.5 arbitrary time units. If the victim is not rejected by the predator then the victim is killed and the population updated at a time cost of 1 arbitrary time unit. The predation process continues until the current time measure (t) eventually reaches the time limit for the generation (T). The prey are then repopulated stochastically and asexually whereby each new individual in the population is assigned a randomly generated number between 0 and 1 which is compared to the ratio of prey morphs surviving the previous season.

We assume that crypsis is common in the environment and not subject to the effects of dietary conservatism. Aposematism, however, is assumed to be novel and aposematic prey can promote dietary conservatism in the simulations. We make the assumption that in the habitat modelled, the only way for a prey of the same species to be novel enough in appearance to invoke DC is to evolve conspicuousness due to the fact that alternative cryptic appearances can often be limited (Franks 2009). We

omit the possibility that cryptic prey may subsequently invoke predator dietary conservatism as we assume that crypsis is likely to have been overwhelmingly common prior to the primary evolution of aposematism. Although not explicitly considered in our model, alternative cryptic forms of other species in the same habitat would be sufficiently common and similar in appearance enough to prevent the treatment of any cryptic form as novel.

In the main body of this manuscript we describe the simplest method of modelling dietary conservatism, a fixed-number model (Speed 2001), in which the predator has a dietary conservatism "memory" (termed DC_{num}) for which a value is assigned *e.g.* 30. In this case the first 30 aposematic prey encountered (that is, detected) by the predator per generation are rejected (chance of rejection $R=1$). When sufficient aposematic prey have been encountered by the predator *i.e.* when number encountered $=DC_{num}+1$, dietary conservatism is absent and the predator now behaves like an inexperienced animal (so that $R_a=0$); subsequent learning can increase the value of R_a (see Speed 2001). We also used a more complex method which assumes that predators use knowledge about the temporal spacing of prey encounters as well as the number of novel forms avoided. As the results of this method are qualitatively almost identical to the fixed number method, we present these results in our appendix.

Table 1.1 – Standard parameters used for the DC simulations presented in chapter 1 (unless otherwise stated)

Parameter Name	Parameter Description	Fixed Values
T	Arbitrary time limit for each generation	100
N	Total number of prey	400
N_a	Number of aposematic prey	1
N_c	Number of cryptic prey	399
c_a	Conspicuousness of aposematic prey (also representative of the detection probability)	0.02
c_c	Conspicuousness of cryptic prey (also representative of the detection probability)	0.01
α_a	Predator avoidance learning rate for aposematic prey	Variable (see individual model description)
α_c	Predator avoidance learning rate for cryptic prey	Variable (see individual model description)

$Pred_{gen}$	Prey generation intervals at which the predators are replaced	Variable (see individual model description)
DC_{num}	Number of prey rejected due to fixed Dietary Conservatism memory	Variable (see individual model description)
Z	Event weighting for moving average Dietary Conservatism models	Variable (see individual model description)

1.5 Application of the model

In the first instance we evaluate the possibility that aposematism could evolve by chance, by running "null models" without dietary conservatism ($DC_{num}=0$) and with systematic variation in learning rates. Having evaluated null models, we next consider the value of dietary conservatism to aposematic survival and examine how this is affected by variation in key ecological parameters such as population size and season length.

1.5.1 Null Models

We first considered the fate of a novel mutant in a "null model" in which there is a single predator with no dietary conservatism and there are no benefits of accelerated learning for aposematism (i.e. α_a and α_c both take a value of 0.04). For each run of the model, we simulated a total of 4000 generations with a generation time limit of $T = 100$. Pilot runs showed that the probability of fixation without dietary conservatism and without accelerated learning is very low so we repeated the model for a large number of runs (10^4) to get a sound estimate of fixation frequency. In all cases with these conditions ($N_c=399$, $N_a=1$, $T=100$, $generations=4000$, $c_a=0.02$, $c_c=0.01$, $\alpha_a=0.04$, $\alpha_c=0.04$); the outcome was the same: extinction of the novel aposematic form.

The null model was then re-tested with varying levels of predator avoidance learning rate for aposematic prey (α_a). A combination of random drift and accelerated learning about aposematism could for example be sufficient to explain the initial evolution of aposematism (Mallet & Singer 1987). The learning rate for cryptic prey was set to $\alpha_c=0.04$, and we used a range of values for α_a (0.04, and then 0.1 to 0.9 by increments of 0.1, and 0.99). In all runs except one, accelerated learning proved insufficient to enable the novel conspicuous mutant to evolve (in one run of the 10000 with $\alpha_a=0.99$ the aposematic prey survived and reached fixation). In the

circumstances simulated, the bright rare novel prey were very likely to be seen and killed before random drift had any opportunity to cause their numbers to increase to the point that accelerated learning facilitated the evolution of aposematism.

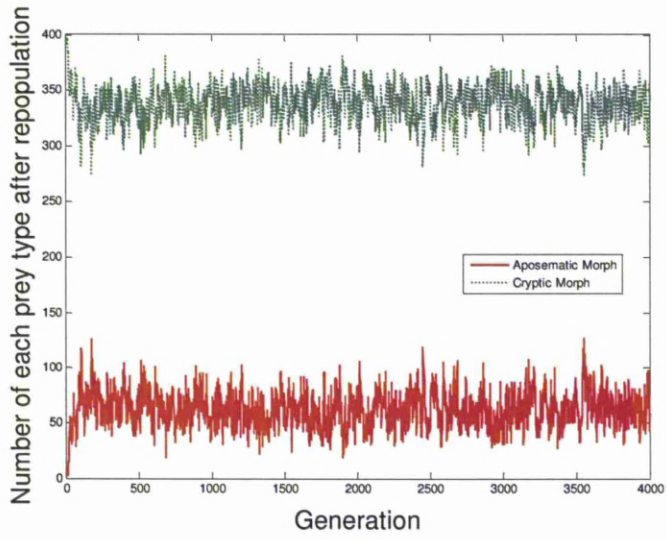
1.5.2 Effects of dietary conservatism: single habitat, single predator

Table 1.2 - Pool of predators and associated fixed D.C values used in subsequent models in chapter 1 (values from Marples et al. 1998).

Predator Number	1	2	3	4	5	6	7	8	9	10	11	12	13
Number Of Novel Prey Avoided	125	49	114	3	15	10	3	10	14	6	3	24	6

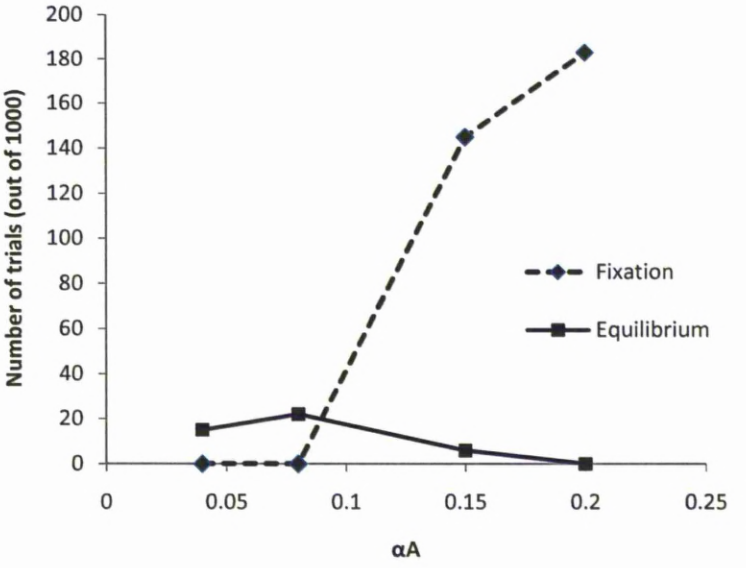
When predator dietary conservatism is implemented we saw improved survival for the aposematic mutant. To the initial null model with equal avoidance learning rates (α_a=0.04 and α_c=0.04) as described above, we introduced dietary conservatism with the "fixed number" model (DC_{num}= 15, so that the first 15 aposematic prey encountered by the predator per generation will be rejected and not killed). Of 1000 runs the aposematic prey became extinct 985 times. In the remaining 15 runs, the aposematic prey did not reach fixation, but instead came to a dynamic equilibrium shown in Figure 1.1.

Figure 1.1 – Example of dynamic equilibrium between the competing morphs in the single habitat, single predator model with fixed dietary conservatism DC_{num}= 15 ($N_{c=399}$, $N_{a=1}$, $T=100$, $generations=4000$, $c_a=0.02$, $c_c=0.01$, $\alpha_a=0.04$, $\alpha_c=0.04$). Here we observe the aposematic morph coexisting with the cryptic morph at low abundance over a period of 4000 generations.



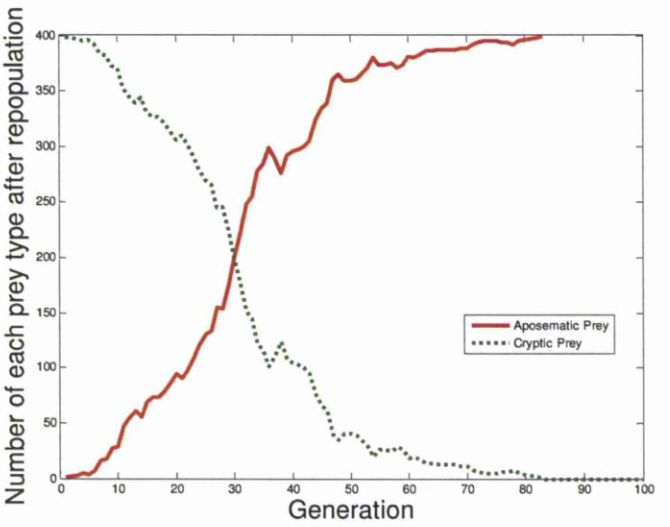
Here we see the coexistence of both prey types sustained over a period of 4000 generations with no overall directional trend visible in either prey population (to check that there is no directional effect, the correlation coefficient of the aposematic prey numbers for the last 3000 generations of one of the runs was calculated, with a result of r=0.005). This dynamic co-existence of aposematic and cryptic prey can also be demonstrated where the predator avoidance learning rate is increased in favour of aposematic prey ($\alpha_a=0.04$-0.15) see Figure 1.2.

Figure 1.2 – Number of trials reaching coexistence and fixation in the single predator model with fixed dietary conservatism, $DC_{num} = 15$ ($N_{c=399}$, $N_{a=1}$, $T=100$, *generations=4000*, $c_a=0.02$, $c_c=0.01$, $\alpha_a = 0.04\text{-}0.2$, $\alpha_c = 0.04$).



The dynamic equilibrium generally pertained for a lower set of dietary conservatism values. For example, if we increased the predator's dietary conservatism level to 80 ($DC_{num} = 80$), which is within the range of observed dietary conservatism levels of wild birds in empirical studies (see Table 1.2) the aposematic morph reached fixation in 193 runs (see example in Figure 1.3) and never stayed in a state of dynamic equilibrium (as in Figure 1.1).

Figure 1.3 – Example of Fixation of the aposematic morph in the single habitat, single predator model with fixed dietary conservatism, DC_{num} = 80 (N_c=399, Na=1, T=100, generations=4000, c_a=0.02, c_c=0.01, α_a =0.04, α_c =0.04).



1.5.3 General effects of key parameters on the survival of novel aposematic prey

We used the fixed dietary conservatism model here to examine the survival of the rare aposematic form. We first varied learning rate for the aposematic form α_a so that it increased from a value of 0.04 where it has equality with cryptic prey (α_c). As learning rate for aposematic prey increased, the nature of the outcome changed in the sense that fixation of the aposematic prey increased and it replaced the dynamic equilibrium as an outcome (Figure 1.4a). Increases in the total initial population size (N, Figure 1.4b) decreased the likelihood that aposematism would evolve.

Figure 1.4a – Number of trials (out of 100) in which the novel aposematic morph reached fixation or equilibrium for varied levels of (α_a) in the Fixed DC model (*DC_{num}= 25,N_c=399, N_a=1, T=100, generations=4000, c_a=0.02, c_c=0.01, α_c =0.04*).

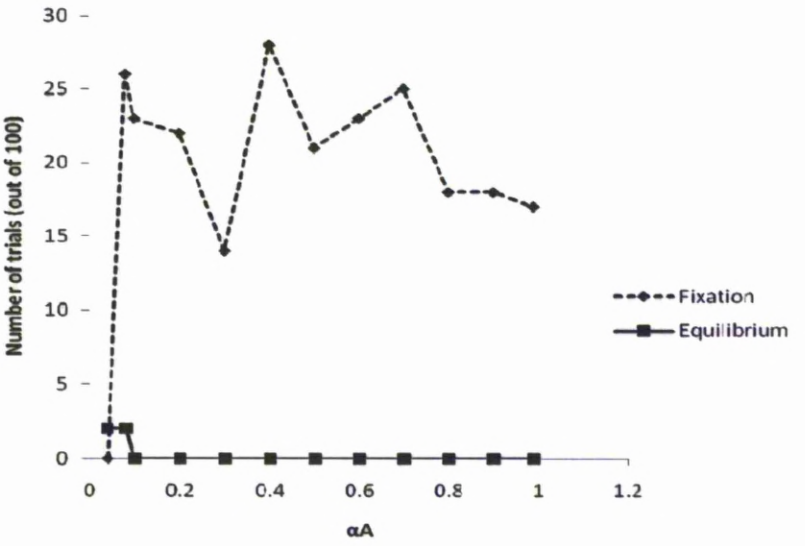
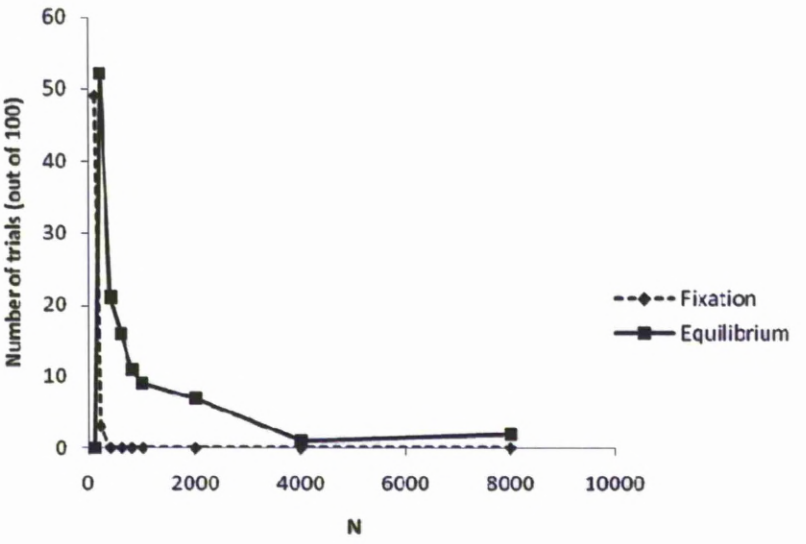


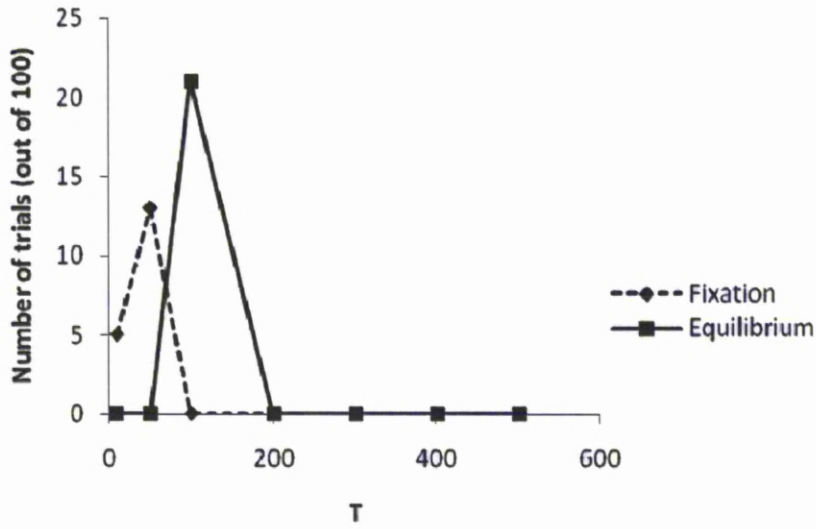
Figure 1.4b – Number of trials (out of 100) in which the novel aposematic morph reached fixation or equilibrium for varied levels of (N) in the Fixed DC model (*DC_{num}= 25, N_a=1, T=100, generations=4000, c_a=0.02, c_c=0.01, α_c =0.04,α_a =0.04*).



This result is in agreement with other models of aposematism (Mallet & Singer 1987; Puurtinen & Kaitala 2006), and follows because the *per capita* risk to the common

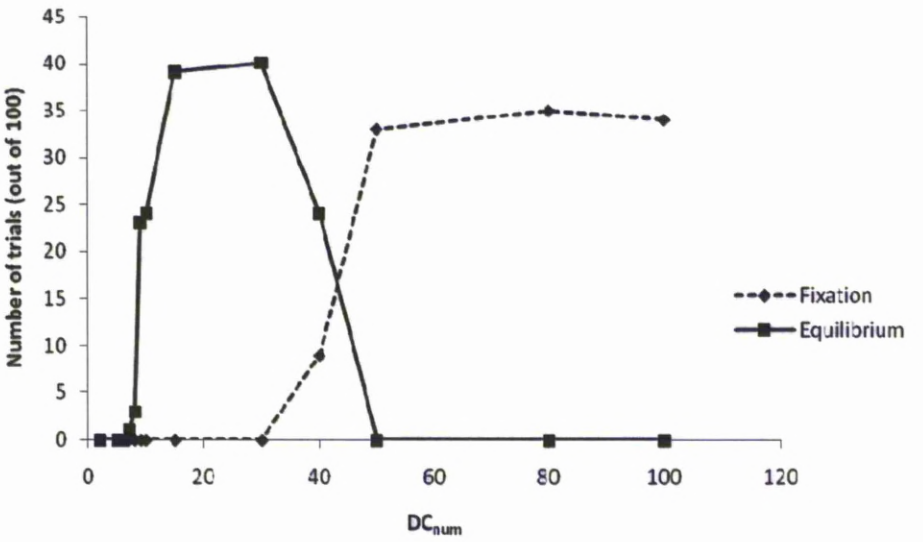
cryptic prey decreases with its abundance, giving the rare aposematic form a lower relative fitness, other things being equal. Increases in season length (*T*, Figure 1.4c) did not favour aposematism either; however the cause here is that the costs of conspicuousness increase with season length, and this disproportionately affects the fitness of the rare, bright forms (Puurtinen & Kaitala 2006).

Figure 1.4c – Number of trials (out of 100) in which the novel aposematic morph reached fixation or equilibrium for varied levels of (T) in the Fixed DC model (DC_{num}= *25*, N_c=*399*,N_a=*1* , *generations=4000*, c_a=*0.02*, c_c=*0.01*, α_c =*0.04*, α_a =*0.04*).



Finally, increasing the level of dietary conservatism decreased the probability of extinction for the aposematic mutant, first increasing the likelihood of persistence through dynamic equilibrium, and subsequently increasing the likelihood of fixation for the aposematic form (Figure 1.4d).

Figure 1.4d – Number of trials (out of 100) in which the novel aposematic morph reached fixation or equilibrium for varied levels of (DC_{num}) in the Fixed DC model (N_c=399,N_a=1 ,**T=100**, *generations=4000*, c_a=0.02, c_c=0.01, α_c **=0.04**, α_a =0.04).



1.5.4 Development of the simple model

We next considered a model in which predators may outlive their prey and in which prey may be subjected to predation by more than one predator. We only used one method for modelling dietary conservatism (the fixed number model) because published data from which we draw our descriptions of dietary conservatism in wild birds is given in terms of number (Marples et al. 1998), and we presently have no information about rate of encounter and loss of dietary conservatism in wild birds. In the results shown below, we set learning rate about cryptic prey to α_c=0.04, and varied learning rate about aposematic prey between values of α_a=**0.04** to 0.99.

We extended the simple model to account for the fact that predators may outlive their prey by introducing a variable, $Pred_{gen}$ (of values 1-5), which specifies predator lifespan in terms of the number of prey generations that a predator lives. When for example $Pred_{gen}$ =4, after every fourth prey generation, the predator dies and is replaced by a new naive predator (all encounter memories are reset to 0). We use this parameter in two scenarios. In the first, a "dead" predator is always replaced by a predator with the same value of dietary conservatism. We take the value of dietary conservatism to be DC_{num}=29, which is the mean dietary conservatism value

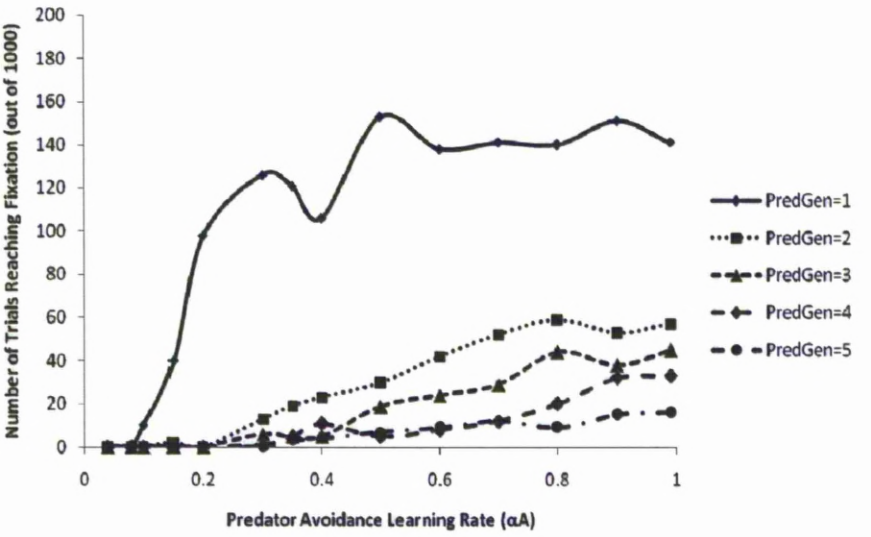
shown by real predators in the study of Marples et al. (1998), see table 1.2. In our second use of $Pred_{gen}$, DC_{num} for each predator is drawn randomly from the dataset in table 1.2; hence the mean DC level in both sets of simulations is the same, but in the latter case there is a wide variance in DC values. We adopt the standard parameters of the previous models ($N_{c=399}$, $N_{a=1}$, T=100, generations=4000, c_a=0.02, c_c=0.01, α_a =0.04, α_c =0.04).

1.5.4.1 Results

As the predator lifespan increased with a fixed DC_{num} value, the benefit of dietary conservatism to the survival of the aposematic morph was removed. For example, where predator – prey lifespan is equal ($Pred_{gen}$=1), we observe fixation of the aposematic morph in 2 of the 1000 trials and dynamic equilibrium in 188 trials. Where predator lifespan was increased ($Pred_{gen}$ =2-5) in all trials the aposematic morph became extinct. Only when we added accelerated learning about aposematism did the novel morph survive. For example with $Pred_{gen}$=5, and avoidance learning rate of α_a =*0.3* the aposematic morph reached fixation in 4 of the 1000 trials (and never stayed in dynamic equilibrium). Lower avoidance learning rates for aposematic prey α_a <*0.3* yielded no fixation or dynamic equilibrium in any trials.

When we repeated the simulations but now drew DC_{num} values randomly from table 1.2, aposematism was even less likely to evolve. Where predator lifespan matches that of the prey, crypsis was now never successfully invaded by aposematism (Figure 1.5), unless predators learnt about the novel morph more rapidly than they learnt about the cryptic morph.

Figure 1.5 – Predator replacement model results. The effect of increasing the avoidance learning rate for aposematic prey (α_a=0.04=0.99) with the predators living for 1-5 prey generations ($pred_{gen}$=1-5) with standard parameters (N_c=399, N_a=1, T=100, generations=4000, c_a=0.02, α_c=0.01, α_c =0.04).



Furthermore, if for example, predators lived for 5 prey generations, as might be reasonable for a vertebrate predator and its prey with two generations per year, the probability of fixation of an aposematic mutant is only 0.019 if learning about the novel morph is virtually instantaneous (α_a=1) and learning about the cryptic morph is much slower (α_c=0.04). Our conclusion is that variation in levels of dietary conservatism and predator lifespan work to prevent dietary conservatism as a cause of the primary evolution of aposematism.

1.5.5 Multiple predators

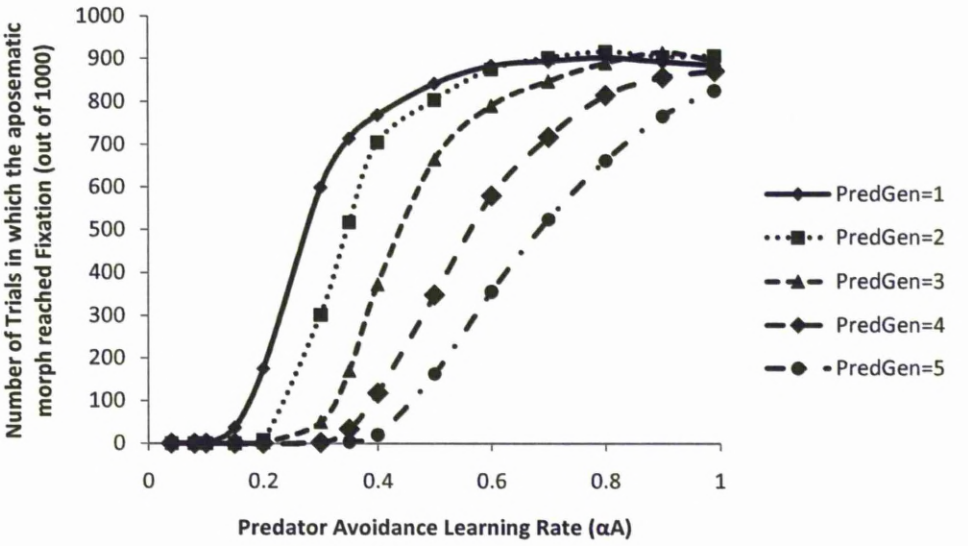
Finally we considered how the results would change if there was more than one predator within the single habitat. We allow 13 predators (with DC levels as in table 2, from Marples et al. 1998) to predate. A single predator is randomly selected from the pool of predators and allowed to browse the prey population until it attacks or declines a neighbouring prey. After this, a new predator will then be randomly selected from the pool and allowed to find a victim and so on until 13 predators have had the opportunity to sample prey. Predation iterates until the time limit for the

generation is reached. However, we scaled the time per generation (T) by multiplying it by the total number of predators in the habitat to account for the increased duration caused by additional predation events. As previously, the model was tested for a series of predator avoidance learning rates (α_a=*0.04-0.99*) and with the predator living for 1-5 prey generations ($Pred_{gen}$=*1-5*) with each combination being tested for a total of 1000 repetitions and the results recorded.

1.5.5.1 Results

In general, adding multiple predators makes it harder for a novel mutant to evolve. This result pertains due to increased predation in the modelled system, given that the generation time is increased substantially to allow all 13 predators (rather than a single predator) to predate. Consider for example the situation in which predators live for one prey generation. With multiple predators aposematism did not ever evolve unless learning was substantially accelerated (α_a=0.15; Figure 1.6), whereas with one predator a value of only α_a=0.1 was required (Figure 1.5).

Figure 1.6 – Multi-predator attack model results. The effect of increasing the avoidance learning rate for aposematic prey (α_a=0.04-0.99) with the predators living for 1-5 prey generations ($Pred_{gen}$=1-5) with standard parameters (N_c=399, N_a=1, T=100, generations=4000, c_a=0.02, c_c=0.01, α_c =0.04).



However, once an abundance threshold is reached at which aposematism can be favoured, the addition of multiple predators makes it much more likely that monomorphic aposematism is the outcome (compare Y axes on Figures 1.5 and 1.6). If aposematism has a survival advantage over crypsis it is because it causes dietary conservatism and accelerates learning; sufficient to increase the number of aposematic prey to a critical level, thus adding more predators to the system increases the fitness difference between the two prey forms. A similar point has been made by Puurtinen & Kaitala (2006); our results show, however, that so long as aposematism can be favoured in terms of its fitness advantage over crypsis, the benefit from multiple predators can in fact offset the detrimental effect of long-lived predators, making the evolution of aposematism easier to account for. Increasing the number of predators that share the same resource effectively increases predation intensity (the fraction of prey that are eaten in any generation). This increase in predation intensity decreases the strength of stochastic variation caused by the random encounters of predators and prey.

1.5.6 Palatable Cryptic Prey

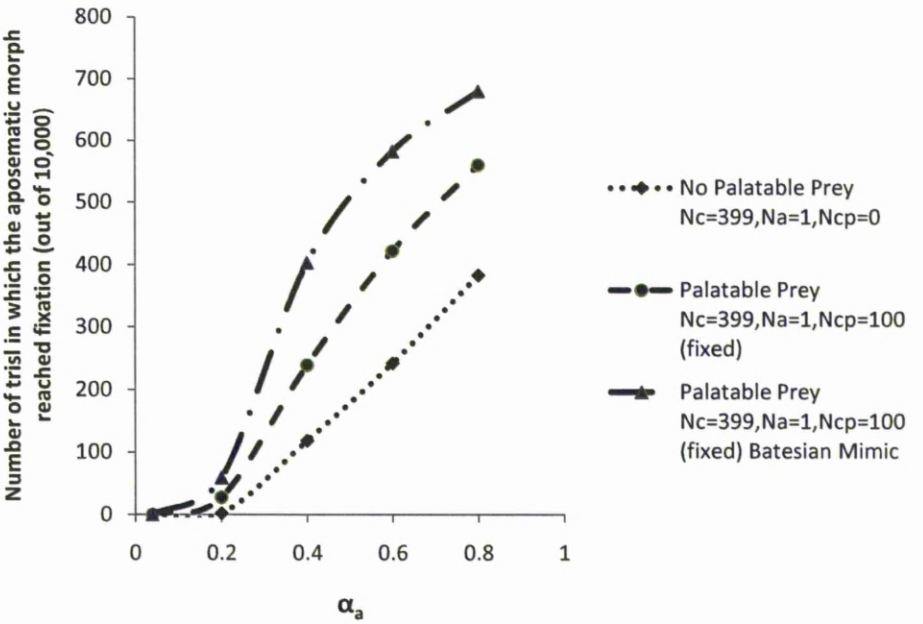
So far we have considered only two prey types, cryptic and aposematic, within one defended species. We now relax this assumption and add a second edible species of fixed number at the start of each generation. Because we are not interested in its evolution, this species is assumed to be uniformly cryptic (and for this prey we assume its conspicuousness is equal to that of the cryptic morph in the defended prey population i.e. (C_{cp}=*0.01*). In the first instance we assume that the additional palatable prey have no visual resemblance to the defended species (i.e. though cryptic they are visually distinct), hence interaction and consumption of the new palatable prey acts only to occupy the predator's time. In a second set of simulations we consider a scenario in which the palatable cryptic morph now resembles the cryptic prey (effectively acting as a parasitic mimic of the defended cryptic form) so that it can reverse predator's avoidance learning about the defended cryptic prey. This was achieved by simply decrementing the defended cryptic prey counter (n_c) for each palatable prey that is attacked thus assuming that learning is reversed by the mimic at the same rate as it occurs about the defended cryptic prey as per equation 1.1b. We tested these scenarios for the most ecologically realistic of our models; first, we consider the model whereby the predator is replaced every 5 prey generations ($pred_{gen}$=5) with a predator of fixed dietary conservatism (DC_{num}=29), this being the mean level found in real predators from table 1.2 (see Figure 1.4). We also applied the addition of palatable prey to the model in which the predators DC_{num} is replaced randomly from table 1.2 (see appendix figure A1.4). Both models were tested for 10,000 repetitions and for a range of avoidance learning rates about aposematic prey (α_a =*0.04*,α_a =*0.2*, α_a =*0.4*,α_a =*0.6*, α_a =*0.8*).

1.5.6.1 Results

In all cases, the addition of palatable prey to the model yields greater probability of fixation for the aposematic morph. In the first case whereby we assume that palatable prey have no effect on predator learning, their existence simply dilutes the predation intensity on the focal prey population, which is analogous to reducing the season length (see Figure 1.7). We show in earlier parameter testing that reduced predation intensity yields greater survival of the rare aposematic morph

(Figure 1.4c). A similar point is made by Puurtinen & Kaitala (2006). Low predation intensity on the focal prey will also promote the increase in abundance of the aposematic morph by random drift.

Figure 1.7 – Addition of palatable prey showing the number of trials (out of 10,000) in which the novel aposematic morph reached fixation where (α_a =0.04, α_a =0.2, α_a =0.4,α_a =0.6, α_a =0.8) in the fixed DC model (DC_{num}=29, N_a=1, T=100, generations=4000, $pred_{gen}$=5, c_a=0.02, c_c=0.01, α_c =0.04).



If, as in our second implementation of palatable prey, we assume that both the palatable and defended cryptic prey are engaged in a parasitic mimetic relationship, the probability of fixation of the aposematic morph is further improved due to reduced avoidance learning about the cryptic defended prey (Franks 2009). We are not aiming to explicitly model the dynamics of mimetic evolution, merely to to show that if constraints were in place to prevent the palatable prey from following the unpalatable prey to evolve conspicuousness; that Batesian mimicry of the ancestral cryptic defended form can yield greater survival for a novel, defended conspicuous mutant.

1.5.7 Palatable Conspicuous Prey

Our previous model developments demonstrate that dietary conservatism alone (without accelerated avoidance learning for aposematic prey) proves insufficient to explain the increase in abundance of a conspicuous mutant in the focal defended prey population in models where we assume that predators vary in their levels of dietary conservatism and where predators live longer than their prey. For completeness we present the results of similar models in which we consider that all prey are edible to determine whether dietary conservatism could facilitate conspicuousness to evolve in palatable prey. We present this model separately to the defended prey models as combining the two would yield identical results yet add unnecessary complexity in terms of controlling predation intensity. We tested two scenarios, the first in which we assume a single predator with the average DC value from the empirical data ($DC_{num}=29$) (see table 1.2) and secondly where predators DC varies across generations. In both presentations we assume that avoidance learning does not occur ($\alpha_a=0$, $\alpha_c=0$) and that predators live for 5 prey generations ($pred_{gen}=5$). All other parameters remain unchanged ($N_{c=399}$, $N_{a=1}$, $T=100$, $generations=4000$, $c_a=0.02$, $c_c=0.01$). Each model was tested for 1000 repetitions.

1.5.7.1 Results

Where we assume that a single predator of fixed DC ($DC_{num}=29$) attacks the prey population, we observed that out of 1000 repetitions, the conspicuous prey did not reach fixation in any trail. However in 4 of the 1000 trials the conspicuous prey did reach a state of dynamic equilibrium as described in earlier models. Where the prey suffer predation from predators of varied DC tendency over time, in all trials the conspicuous prey rapidly became extinct. Our models show therefore, that dietary conservatism, at the levels tested is unlikely to account for the evolution of conspicuousness in palatable prey thus affirming the assumption that only sufficiently defended prey species can afford raised conspicuousness (Sherratt 2002), exposing variation in predator avoidance learning rates as a potential driver for this trend.

1.6 General Discussion

Our simulations present a number of novel and potentially important insights into the role that predator wariness of novelty may play in the evolution of aposematism. We first consider our main predictions before looking at the role of dietary conservatism in the primary evolution of aposematism.

1.6.1 Dynamic equilibria and the persistence of novel aposematic forms

One novel prediction from our evolutionary simulations is that aposematic morphs may remain within a population, reaching a dynamic equilibrium in which they oscillate in abundance around some mean value. This results because the avoidance of novel prey is density dependent. While a prey is very rare it may gain complete protection because predators manifest strong dietary conservatism and avoid the new aposematic morph; but when it becomes too common dietary conservatism wears off, and the aposematic prey is now attacked by predators ignorant of its defence level (on top of being discovered more readily than cryptic prey). A rare morph can increase in abundance until it passes some threshold, beyond which selection causes it to shrink in number, and hence a dynamic equilibrium results (Figure 1.4a). An example of the coexistence of aposematic and cryptic prey forms can be found in (Zrzavy & Nedved 1999). If conditions were to become more favourable for the aposematic form, the population may then switch to monomorphic aposematism (Figures 1.4b,1.4c). These results are important, because previous arguments against the importance of dietary conservatism in aposematism assumed that when wariness wanes the aposematic prey simply becomes extinct, and hence that predator wariness of novelty is only of trivial importance to the evolution of aposematism (Mallet & Singer 1987). Similarly, earlier theoretical models identified that the fitness effects on novel aposematic morphs of dietary conservatism change in a nonmonotonic manner with the abundance of the aposematic morph (Puurtinen & Kaitala 2006; Speed 2001), but suggested that this diminished the importance of

dietary conservatism in the evolution of aposematism. Our evolutionary simulations show that this is not necessarily the case.

1.6.2 Variation in predators

The equilibrium abundance of aposematic prey is defined in part by the level of dietary conservatism in the predator community. If predators die and are sometimes replaced by individuals with very low levels of dietary conservatism, then the population may rapidly move to monomorphic crypsis. In contrast, if dead predators are replaced by very conservative individuals, monomorphic aposematism can result. The empirical dataset we used to simulate variation in dietary conservatism (from Marples et al. 1998) included about 60% of individuals with low levels of dietary conservatism, with the remainder manifesting high levels. With this mixture of predators, the novel aposematic morph tended to become extinct unless we could also assume that predators learn more quickly about aposematism than crypsis. Hence a key ecological question is not merely what the mean level of dietary conservatism is within a population of predators (as in Puurtinen & Kaitala 2006; Speed 2001), but how much variation there is in dietary conservatism within a predator population.

In a further exploration of the model's predictions we found that increasing predator lifespan compared to those of their prey substantially diluted the benefits of dietary conservatism. This prediction follows from the fact that if predators live longer, the period in which their dietary conservatism protects novel prey forms a decreasing proportion of their total existence. Furthermore increasing the number of predators worked to the detriment of rare novel forms (though when there were sufficient aposematic prey, aposematism was itself more often favoured with more predators). Both of these conditions; long lived predators and multiple predators per habitat seem to be ecologically widespread, and we found that dietary conservatism would only help to explain aposematism when bright colouration also caused biases in learning rates.

The addition of palatable prey promoted the evolution of aposematism by reducing predation intensity on the focal prey population and by the promotion of

random drift. Where Batesian mimicry between the defended and palatable cryptic forms exists, aposematism evolved even more readily because learnt avoidance of the cryptic defended prey is relatively degraded. The existence of palatable alternatives is also important because availability of abundant, alternative palatable prey is likely to increase the duration and intensity of dietary conservatism as a foraging strategy, and promote avoidance of novel aposematic prey forms. Hence the available level of alternative palatable prey may have been very important in determining whether novel aposematic prey can evolve from rarity.

Finally our models considering the evolution of conspicuousness in palatable prey affirm the theoretical consensus that conspicuous warning displays are most likely to have initially evolved and established in defended prey populations. Our models show that dietary conservatism, although offering initial protection, is unlikely to account for the sustained increase in abundance of undefended conspicuous prey under realistic ecological conditions.

1.6.3 Receiver biases or reliable signalling?

Sherratt's (2002) coevolutionary hypothesis, described in the introduction, focused on the idea that brightness imposes a special kind of cost on prey, and this cost can only be borne by individuals that are sufficiently well defended to survive attacks (see also a related verbal model in Guilford & Dawkins 1993). Sherratt's idea neatly explains the initial evolution of aposmatism, the coevolution of dietary wariness, and the conspicuousness of warning signals.

In contrast an explanation of aposematism using a receiver-bias explanation of dietary conservatism has a number of problems. First, on the face of it, the argument of Marples et al. (2005) focuses on novelty not conspicuousness *per se*, hence it does not obviously explain why brightness is so strongly associated with aposematic signalling. Second, as we showed in our simulations, although there are conditions which do predict aposematism, there are also several ecological factors which make the dietary conservatism explanation less compelling, specifically: (i) within population variation in dietary conservatism, (ii) relatively long predator lifespans and (iii) the existence of multiple predators within a habitat. In our

simulations each of these conditions reduces the chances of aposematism evolving from rarity via the cause of dietary conservatism in predators. This is not to say, of course, that the specific conditions which favour the dietary conservatism explanation for aposematism cannot exist, merely that they are quite restrictive. We do not however dismiss the explanation of Marples et al. (2005) because it seems to us that plausible modifications to their theory would allow us to present a reasonable alternative view to that proposed by Sherratt (2002).

First, while it is true that dietary conservatism focuses on novelty of prey colouration rather than conspicuousness, it can be argued that if members of the ancestral prey population had an optimally cryptic appearance (Merilaita et al. 1999) almost any change in their colouration that appeared novel to a predator would disrupt their original crypsis, and render the prey more conspicuous to predators. Genuine novelty in appearance, of the sort that evokes dietary wariness, is therefore often inconsistent with an assumption that conspicuousness is not increased. Thus, although it is sometimes argued that aposematic signalling is fundamentally about raised distinctiveness rather than heightened conspicuousness (Franks 2009; Merilaita & Ruxton 2007; Puurtinen & Kaitala 2006), the two often amount to the same thing (Wallace 1889). If this is generally true, the association between conspicuousness and aposematism in the primary evolution of warning signals, in our view, is not problematic.

For dietary conservatism to have a more compelling role in the evolution of aposematism we would need to be able to assume that aposematic colouration speeds up avoidance learning by predators (see Figures 1.5, 1.6, 1.7). There are several reasons why such accelerated learning by predators may indeed have pertained in the primary evolution of aposematism (see discussion in Guilford & Dawkins 1993). First, there may be intrinsic receiver biases that favour rapid learning with conspicuousness, distinctiveness and novelty. By definition, conspicuousness attracts attention, and the evidence in studies of animal psychology is that learning about an event is most rapid when a lot of attention is paid to it (Pearce 1997). Brightness may then be very common in aposematism simply because the visual contrast of a prey against its background is a salient cue, which attracts attention and accelerates predator learning. Similarly, studies of animal cognition show that distinctiveness can also aid in learning (Kraemer 1984; Pearce 1997). As we argued

earlier, a prey that is novel compared to the ancestral conspecific form is likely to be more distinctive because it will be more conspicuous, hence we may expect that learning will be more rapid. It is in our view unlikely that such generally observed biases in predator cognition are specifically an outcome of predator-prey coevolution. Rather they may explain the primary evolution of aposematism; subsequent predator-prey coevolution may then explain the quite specific responses seen to warning colours themselves in modern predators (Roper & Cook 1989; Schuler & Hesse 1985).

A second reason not to dismiss the dietary conservatism explanation of Marples et al. (2005) is that even in the absence of biased learning, our models can predict the long-term persistence of rare aposematic morphs, and thereby allow for evolutionary effects of conspicuousness on predator psychology. Although previous theoretical models recognise that the benefits of aposematic warning signals are significantly density dependent (Mallet & Singer 1987; Puurtinen & Kaitala 2006; Speed 2001), our evolutionary simulations show that the presence of dietary conservatism in predators can cause stable dimorphic prey populations to evolve. This stable coexistence of prey morphs may side-step the initial expectation that a novel aposematic mutant will rapidly become extinct and instead may allow the aposematic morph to co-exist for long periods of time at low abundance. Furthermore, if aposematic forms do persist for long periods because of dietary conservatism, we might well expect predators to undergo selection to optimize their responses to those prey hence increasing wariness and evolving cognitive learning abilities towards the conspicuous signal.

There may be some synthesis between the arguments of Marples et al. (2005) and Sherratt (2002); pre-aposematic wariness in predators could facilitate the increase in abundance of novel coloured, more conspicuous, defended prey and allow them to survive for long periods of time (via the co-existence predicted in our models) or indeed by fixation of the focal population. The prior existence of brightness and toxicity in such prey could trigger the co-evolutionary process described by Sherratt with the predators co-evolving wariness of conspicuousness, forcing the conspicuousness and abundance of aposematic species to increase.

1.7 Conclusions

The literature currently proposes two different scenarios for the primary evolution of aposematism. One is coevolutionary (Sherratt 2002), the other relies on receiver biases in predators. With our numerical-evolutionary simulations we evaluated the "receiver model" recently described by Marples et al. (2005), in which wariness of novel prey favours novel aposematic mutants. There are a number of conditions in which this account is supported, but ecological conditions such as inter-individual variation in wariness and differences in life spans between prey and predators does restrict the generality of the explanation. Nonetheless, it is possible to argue that there were additional receiver biases in early predators which could overcome the limitations shown in our models. The coevolutionary model of Sherratt (2002) is ingenious and persuasive, but we conclude that receiver explanations are none the less also plausible and reasonable.

Chapter 2 - The effect of metapopulation dynamics on the survival and spread of a novel, conspicuous prey.

2.1 Overview

The first chapter showed that the positive effects of dietary conservatism on the survival of a novel aposematic prey can be significantly diluted when predators outlive their prey and where predators of varied D.C. tendency attack the focal prey population, either simultaneously or in sequence. In light of the significant effect that ecological conditions may have on the initial evolution of aposematism as found in chapter 1, this chapter further explores the effects of ecology and population structure on the evolution of warning signals, utilizing an individual based stochastic metapopulation framework. This work presents the first application of metapopulation theory to the evolution and spread of aposematic colouration in prey populations. The metapopulation model developed not only provides a more realistic representation of real population structure but also allows the number dependent effects of prey migration and sub-habitat clustering to be considered in detail.

Many aposematic species reside across spatially distributed sub-habitats (Gill 1978; Marsh & Trenham 2001; Menendez et al. 2002; Shure et al. 1989). The models presented in this chapter allow predictions to be made as to the conditions which are likely to have promoted the spread of aposematism from rarity in such populations, while at the same time investigating whether the dynamics of metapopulation structure itself can facilitate the spread of the aposematic form. Another reason for this approach is that preceding theoretical work, indeed the same work that dismissed the effectiveness of novel food wariness on warning signal evolution (Mallet & Singer 1987), predicted that metapopulation structure could promote the spread of aposematic individuals in otherwise cryptic prey populations simply by the effects of random drift and outward migration. The simulations presented in this chapter consider a series of conditions such as predator migration, variability in predator patch occupancy and variation in predatory biases between patches. Other key parameters are also tested including prey population size, migration rates and geographical isolation and clustering.

The results provide insights about how metapopulation structure may have influenced the evolution of aposematism in prey species and allow us to predict a set of conditions which most likely allowed aposematism to fixate in highly structured populations. The results show that fixation of the aposematic form is most likely when a cluster of sub-habitats begin the simulation at aposeme fixation and where out-migration rates are low. Predators whose territory approximately matches that of a prey subpopulation also promoted fixation of the aposematic form. The results also provide an in-depth look at how source-sink dynamics and migration may influence and affect the balance of viable outmigration of aposematic prey given the number dependent nature of selection for aposematism (Puurtinen & Kaitala 2006) and the shifting balance predictions of Mallet & Singer (1987). Analysis of the dynamics of migration and selection show that for the viable spread of the aposematic form across the metapopulation to occur, a careful balance exists between the number of aposematic prey lost to out-migration from a single source habitat and the numbers gained from selection within the subpopulation, in addition to those replenished from in-migration. If this balance is maintained, aposematism can successfully spread throughout the modelled metapopulation.

This work was devised from discussion between Thomas Lee and Mike Speed with advice from Nicola Marples and Graeme Ruxton. Model designed by Thomas Lee with assistance from Mike Speed. Coding, implementation and testing was undertaken by Thomas Lee. Writing by Thomas Lee with editorial assistance from Mike Speed.

2.2 Abstract

Animals that deploy chemical defences against predators often signal their unprofitability using bright colouration. This pairing of toxicity and conspicuous patterning is known as aposematism. Explaining the evolution and spread of aposematic traits in previously cryptic species has been the focus of much empirical and theoretical work over the last two decades. Existing research concerning the initial evolution of aposematism does not however properly consider that many

aposematic species (such as members of the hymenoptera, the lepidoptera, and amphibia) are highly mobile. We argue in this paper that the evolution of aposematic displays is therefore often best understood within a metapopulation framework, hence in this paper we present the first explicit metapopulation model of the evolution of aposematism. Our most general finding is that migration tends to reduce the probability that an aposematic prey can increase from rarity and spread across a large population. Hence, the best case scenarios for the spread of aposematism required fixation of the aposematic form in one or more isolated sub-habitats prior to some event which subsequently enabled migration. We observed that changes in frequency of new aposematic forms within source habitats are likely to be nonmonotonic. First, aposematic prey tend to decline in frequency as they migrate outwards from the source habitat to neighbouring sink habitats, but subsequently they increase in relative abundance in the source, as the descendents of earlier migrants migrate back from newly converted sub-populations. This pattern of initial loss and subsequent gain between new source and neighbouring sink habitats is then repeated as the aposematic form spreads via a moving cline.

2.3 Introduction

Many prey protect themselves from predation by the possession of a secondary defence, often some form of toxin. To deter predation toxicity is often accompanied by bright colouration and this pairing of defensive traits is known as aposematism. Aposematism is notably common throughout the animal kingdom (Cott 1940; Edmunds 1974a; Poulton 1890), and it is now well established that aposematic displays provoke a number of responses from predators that increases the probability that a prey survives encounters (such as wariness, and accelerated learning; see review in Ruxton 2004).

Though the proximate function of aposematic displays is well understood, the evolutionary processes that bring them into existence are less clear. It is a widely held assumption that aposematic warning displays initially evolved in species already adopting some form of secondary defence combined with crypsis (Harvey et al. 1982; Leimar et al. 1986; Yachi & Higashi 1998). In such populations, a novel, aposematic mutant would face two major evolutionary hurdles. First, the novel

conspicuous prey would attract the attention of naive predators making it likely that it is attacked and killed. Second, even if the novel conspicuous prey survived and reproduced, with low absolute numbers, all individuals may be consumed before predators learn that the prey are unprofitable. Theory therefore predicts a critical abundance level above which aposematic displays are sufficiently common to be selectively favoured and it is generally assumed that this level is much higher than the initial abundance of new aposematic mutants (Mallet & Singer 1987; Puurtinen & Kaitala 2006; Servedio 2000; Speed & Ruxton 2005a; Speed & Ruxton 2007).

There are a growing number of explanations which seek to resolve this evolutionary puzzle (review in Ruxton et al. 2004). A recent evolutionary model presented by Lee et al. (2010) for example, showed that predator wariness of phenotypic novelty in prey (sometimes known as dietary conservatism) may have played an important role in the initial evolution of aposematic warning displays, in contrast to the somewhat sceptical views previously presented in the literature (Mallet & Singer 1987; Speed 2001). Lee *et al* used stochastic evolutionary modelling of prey within a single habitat and found that dynamically stable dimorphisms between aposematic and cryptic prey could be sustained over long periods of time in the presence of a predator showing even quite modest levels of wariness.

A striking feature of existing theoretical models of aposematism, is that researchers have considered only single predator-prey habitats despite strong evidence that many aposematic species reside in patches and generally conform to a metapopulation structure. For example the red-spotted newt (*Notophthalmus viridescens*), believed to be aposematic, is a resident in the fragmented pondscape of eastern America and its red (eft) stage is the vehicle for inter-habitat migration (Gill 1978; Shure et al. 1989). Indeed, the majority of amphibian species are thought to adhere to some level of metapopulation structure and dynamics (Marsh & Trenham 2001) with numerous examples deploying aposematic colouration as an antipredator defence, most pertinently the Dendrobatidae frogs (Saporito et al. 2007). Similarly, many aposematic Lepidoptera adhere to general metapopulation structure, one example being the six-spot burnet moth (*Zygaena filipendulae*),(Menendez et al. 2002).

Despite the omission of spatial structure from formal models, one of the earliest theoretical treatments of the evolution of aposematism by Mallet & Singer (1987) pre-supposes some level of mobility in prey and therefore invites a metapopulation approach. Mallet & Singer (1987) argued that if aposematism evolved within a single habitat the trait could spread outwards destabilising crypsis in neighbouring localities, causing a moving cline of prey colouration which would lead to the entire prey population switching from crypsis to aposematism. A recent paper by (Endler & Rojas 2009) examines the effect of prey dispersion across varied predator (receiver) territories on the viability of frequency dependant traits such as aposematic warning displays with some interesting insights. To date however, there have to our knowledge, been no specific metapopulation treatments of aposematic evolution, and so the major aim of our present paper is to create a metapopulation framework and evaluate how and whether aposematism might evolve and spread over a large, heterogeneous environment. Our population structure-approach is similar to that taken by Sherratt (2006), but where Sherratt focused on explaining diversity in mimicry systems, we are concerned with examining how the dynamics of prey evolution within and between habitats interact to prevent or facilitate a change in prey defence.

In this paper we build on the simple one-habitat model of predation described in Lee et al. (2010) and in the first section we compare aposematic evolution in a self-contained single population (summarised from their paper) with evolution in a metapopulation structure. In the second section of the paper, we consider in detail the evolutionary dynamics in the case suggested by Mallet & Singer (1987), in which aposematism has evolved in one or more isolated sub-habitats which becomes susceptible to the influences of prey migration if an isolating barrier is removed. We show that metapopulation structure and the inter-habitat movement of prey make it more difficult for aposematism to evolve from rarity. Where we assume that aposematism has evolved in isolated source sub-habitats we find that the optimal conditions for its spread across the entire metapopulation are where levels of migration are low, where the number of clustered source habitats is high and finally when a single predators foraging area matches the area inhabited by a single sub-population.

2.3.1 Model Introduction

We consider a habitat with avian predators that are strongly territorial in their foraging area (Smith 1974; Snow 1966), and hence each habitat within our metapopulation contains a single predator who forages within that habitat for its lifetime (however in later models we relax this structural rigidity and allow predators to move between habitats both within and between prey seasons). Our metapopulation model consists of a number of sub-habitats represented in a regular square lattice. Prey are allowed to move between sub-habitats, with a given number allowed to migrate to each of the 8 surrounding sub habitats at the end of each season (Sherratt 2006). Predators, are assumed to die after a defined number of prey seasons after which they are replaced by another predator whose prey choice decisions may be different from its predecessor. We are not modelling the initial evolution of aposematism, rather the survival of conspicuous mutants in a world in which predators have already evolved innate predispositions to avoid novel prey with bright aposematic colouration for some number of exposures (see Ruxton et al. 2004 for a review). In field studies with wild birds, the level of wariness was found to vary considerably between individuals (see table 2.1) and as such we assign wariness levels randomly drawn from this dataset. In addition, it is well known that aposematic signals may accelerate avoidance learning rates in predators, and in our simulations we evaluate the effects of a wide range of learning rates. We first describe a single habitat, and then the metapopulation structure.

2.3.2 Description of a single habitat

Within each cell of the metapopulation we modelled a finitely sized hypothetical habitat in which one predator and a number of prey (N) reside. Within the prey population two distinct prey types exist, cryptic (c_c, of number N_c) and aposematic (c_a, of number N_a, where N_c+ N_a=400). We assume that both prey types are equally distasteful to the predator and we assume that at the start of the simulations prey are cryptic with the exception of rare aposematic mutants that can emerge at the start of a prey generation. Each prey type is assigned a value for conspicuousness, (c_a) for the aposematic prey type and (c_c) for the cryptic type which represents the probability of detection by the predator, given that a predator and prey

are within some minimum level of proximity. The two prey types are also each assigned an avoidance learning rate, (α_a) for the aposematic prey type and (α_c) for the cryptic prey, used to determine the rate at which the predator learns to avoid each prey type as a result of their distastefulness. The model iterates in prey generations (seasons) which have a finite time limit (T).

During each season, the predator moves through the habitat at random until it comes within striking distance of one individual prey; we assume time taken for this stage is *1/Total number of living prey in that habitat* (in arbitrary time units). The chance of the predator detecting a prey individual is dependent on the prey's conspicuousness value (c_a, c_c) which is compared against a randomly generated number between 0 and 1 inclusive. If for example, we set c_c =0.01 and c_a=0.02 then the aposematic prey has twice the chance of being detected over its cryptic counterpart (as is the case in all our models). If the prey in striking distance is not detected, the predator moves randomly through the habitat and repeats this process until a victim is finally selected. Once the victim has been selected, the predator then has the option of rejecting it without attacking, based on a rejection probability (R). This is dependent on two predator behaviours.

2.3.3 Predator wariness of bright, novel prey

The predator has a wariness "memory" (termed DC_{num}) for which a value is assigned *e.g.* 30. In this case the first 30 aposematic prey encountered by the predator are rejected (chance of rejection R_a=*1*). When sufficient aposematic prey have been encountered by the predator *i.e.* when number encountered =DC_{num}+1, wariness is absent and the predator now behaves like an inexperienced animal (so that rejection probability R_a=0, see equation 2.1a); subsequent learning can increase the value of R_a. More complex methods of modelling wariness have been shown to make no material difference to the evolutionary outcomes (Lee et al. 2010). We based all of our predator wariness values (DC_{num}) on the field data for dietary conservatism in wild blackbirds (Marples, 1998).

2.3.4 Avoidance Learning

Here, the predator can increase the chance of rejection of a prey type based on previous experiences (R_a for aposematic prey, R_c for cryptic prey). The probability of rejection of a specific prey type increases the more often it has been attacked; for simplicity we use a negative exponential term to describe this. For aposematic prey:

$$R_a = 1 - e^{-\alpha_a . L_a} \qquad (2.1a)$$

And for cryptic prey:

$$R_c = 1 - e^{-\alpha_c . L_c} \qquad (2.1b)$$

Where L_a and L_c refer to the number of attacks that a predator has had with an aposematic or a cryptic prey respectively. When predators are inexperienced then L_a and L_c equal zero and probability of attack given detection is 1 (In appendix figure A2.1 we display the avoidance learning curves for a range of α values). If rejection does occur we assume the time taken for this is 0.5 arbitrary time units. If the victim is not rejected by the predator then the victim is killed and the population updated at a time cost of 1 arbitrary time unit. The predation process continues until the current time measure (t) eventually reaches the time limit for the generation (T). The prey are then repopulated stochastically and asexually whereby each new individual in the population is assigned a randomly generated number between 0 and 1 which is compared to the ratio of prey morphs surviving the previous season. With an asexual population random drift is caused by the stochastic nature of predator behaviour and the stochastic repopulation of prey between generations.

2.3.5 A metapopulation extension to the single habitat model

We modelled a 50 x 50 grid of sub-habitats arranged in a regular square lattice structure, with each sub habitat containing a prey population and predator as described above. Initially, we allocate each sub-habitat a predator with a DC_{num} sampled randomly from the predator pool (Table 2.1). The predators are assumed to be naive at the start of the simulation i.e. all encounter memories are 0.

In all metapopulation models we consider a mutation rate of 10^{-5}. The initial number of aposematic prey is then calculated by multiplying this mutation rate by the total number of prey across all sub-habitats (10^{-5} x (400 x 50 x 50) = 10) hence each prey generation we spawn 10 new aposematic mutants (c_a=0.02) randomly across the entire metapopulation. We omit the effects of back-mutation in the cryptic form due to all models starting with overwhelming numbers of cryptic prey. Pilot runs were performed to determine the chance that a cryptic morph could invade from rarity (with a mutation rate of 10^{-5}). In all cases the cryptic morph rapidly became extinct (Puurtinen & Kaitala 2006) therefore for simplicity, back-mutation to the cryptic form was omitted from our model.

2.3.6 Migration

Once the starting population and predator locations have been established, the predation process for each sub-habitat is executed in sequence,starting with the upper left sub-habitat. After the predation/repopulation process in each sub-habitat is completed, the starting populations for the next generation are established (as per the single-habitat model description above). Within each sub-habitat, a proportion of the new prey population are then randomly selected for migration (8 x N_{mig}) which are then further randomly divided into 8 subgroups of size (N_{mig}) which represent the migratory population destined for each of the surrounding 8 sub-habitats. Migration occurs after all sub-habitats have completed their predation and re-population sequence, representing a single prey generation. This process is analogous to all sub-habitats being simulated simultaneously, however computational constraints force the process to be executed sequentially.

The overall model iterates for a defined number of prey generations (mig_{gen}). Each sub-habitat will consequently gain and lose (8xN_{mig}) prey individuals each generation. At the borders of the metapopulation, we assume a continuous torus arrangement so that no migratory boundaries exist (Sherratt 2006)

2.3.7 Predator Lifespan & Replacement

We allow predators to outlive their prey by a definable number of generations using a parameter called ($pred_{gen}$) which defines the age at which a predator is replaced. In models where predators live for greater than 1 prey generation, at the beginning of the simulation we assign predators a random age (0 : $pred_{gen}$-1 inclusive) which is incremented each prey generation. When the predator's age reaches the critical value ($pred_{gen}$), the predator is replaced. We assume that when replacement occurs, a new naive predator migrates into each sub-habitat with a DC_{num} drawn randomly from the pool of predator DC values (Table 2.1).

Table 2.1 - Pool of predators and associated fixed D.C values used in subsequent simulation in chapter 2 (values from Marples et al. 1998)

Predator Number	1	2	3	4	5	6	7	8	9	10	11	12	13
Number Of Novel Prey Avoided	125	49	114	3	15	10	3	10	14	6	3	24	6

2.4 Detailed scenarios and results

2.4.1 Part 1: Single Habitat vs. Metapopulation Models

In a recently published paper, Lee et al. (2010) provided a set of predictions for the conditions under which aposematism might have evolved in single habitats. We re-ran these simulations, but now applied the same parameters to the metapopulation model. The resulting dataset is large and hence we present a table comparing the results of single and meta-population models (Table 2.2) here in the main text, but provide a detailed set of results in the Appendix.

Table 2.2 – Comparison between the results of the single habitat models of Lee et al, 2010 and the equivalent metapopulation implementation. In all simulations α_c=0.04.

Modelled Scenario & Parameters	**Single Habitat Model (Lee et al. in press)**	**Metapopulation Model**
No Predator wariness (Null model) variable learning rate for aposematic prey (α_a=0.04-0.99, $pred_{gen}$=1)	No increase in abundance of aposematic prey	No increase in abundance of aposematic prey
Random Predator wariness with equal predator-prey lifespans (α_a=0.04-0.99, $pred_{gen}$=1)	Fixation of the aposematic morph occurred where (α_a=>0.08). No dynamic equilibrium in any trials.	Dynamic equilibrium where (α_a=0.04-0.17). Fixation of the aposematic morph where (α_a>=0.18).
Random Predator wariness with the predator living for 5 prey generations ($pred_{gen}$=5), (α_a=0.04-0.99)	Fixation of the aposematic morph where (α_a=>0.35). No dynamic equilibrium in any trials.	No increase in abundance of aposematic prey.

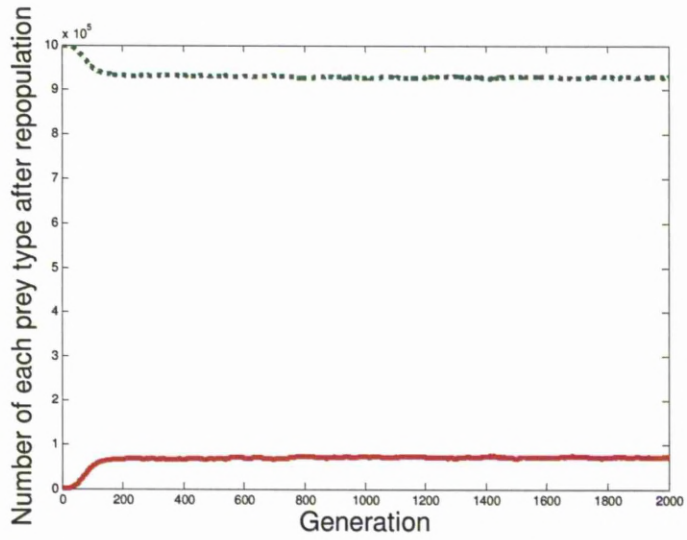
As with Lee et al. we considered the critical value of avoidance learning rate about aposematic displays as an index of how easily aposematism could evolve. If conditions in the model were favourable for aposematism to evolve from rarity and spread, then α_a need not be much greater than α_c to enable this, since aposematism would not need a strong selective benefit from biased avoidance learning rates. In contrast where conditions were unfavourable for the evolution and spread of aposematism, it is likely that the minimum value of α_a would need to be much greater than α_c to compensate and improve selection for the aposematic prey to enable them to evolve and spread. In the metapopulation models presented in this section we allow migration to occur at a fixed rate of (N_{mig}=10) equating to 20% of the prey population and adopt the standard set of parameters (Table 2.3).

Table 1.3 - Standard parameters used in all metapopulation simulations presented in chapter 2 (unless otherwise stated)

Parameter Name	Parameter Description	Fixed Values
T	Arbitrary time limit for each generation	100
N	Total number of prey	400
generations	Number of Generations Simulated	2000
N_a	Number of aposematic prey	10 spawned across the entire metapopulation each
N_c	Number of cryptic prey	399
c_a	Conspicuousness of aposematic prey (also representative of the detection probability)	0.02
c_c	Conspicuousness of cryptic prey (also representative of the detection probability)	0.01
α_a	Predator avoidance learning rate for aposematic prey	Variable (see individual model description)
α_c	Predator avoidance learning rate for cryptic prey	0.04
DC_{num}	Number of prey rejected due to fixed Dietary Conservatism memory	Variable (taken from empirical data in Marples,
mig_{gen}	Prey generation in which we allow prey migration to begin	2
$mutation_{rate}$	Rate of incidence of aposematic mutants	10^{-5}
N_{mig}	Number of prey migrating to each surrounding habitat	Variable (see individual model description)
$pred_{gen}$	Prey generation intervals at which the predators are replaced	Variable (see individual model description)

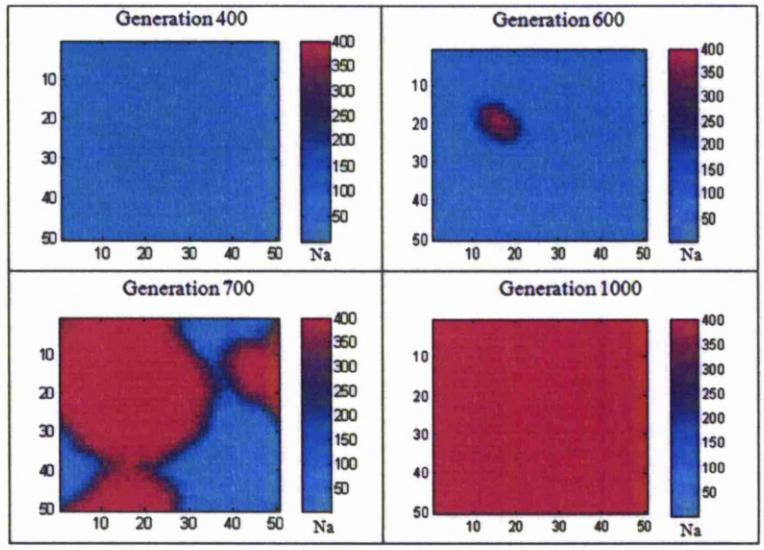
When we simulated predators that have no initial wariness in handling bright prey, both single and meta-population models yielded the same result, in that the aposematic morph became extinct. When predator wariness is added to the model, aposematism evolved in both the single and meta-population models, however metapopulation structure and migration tended to prevent the conversion of crypsis to aposematic colouration. For our tested level of migration (20% of the prey population moving from each habitat, each season), aposematism would only evolve to reach fixation with a higher learning rate (α_a>=0.18) than in the single habitat model (α_a=0.08). However, where migration prevented fixation, it could lead to stable dimorphisms in the prey populations (see Table 2.2 and Figure 2.1) which could not be demonstrated in single habitat models.

Figure 2.1 – Stable coexistence of competing prey morphs demonstrated over 2000 prey generations (generations=2000,, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.17, $pred_{gen}$=1, mig_{gen}=2, $mutation_{rate}$=10-5, DC_{num}=random [see Table 2.1], N_{mig}=10). The solid red line represents the number of aposematic prey and the dotted green line represents the number of cryptic prey.



Where fixation of the aposematic morph occurred in our initial metapopulation models, the spread of the aposematic prey propagated outwards from a single source habitat in all trials (example in Figure 2.2). Finally, when predators live for 5 prey generations and with levels of predator wariness selected randomly from the dataset (table 2.1), the inter-habitat migration of prey (at the level of 20%, N_{mig}=10) acts to prevent any increase in abundance of the aposematic morph even when we assume virtually instantaneous avoidance learning (α_a=0.99).

Figure 2.2 –Metapopulation model plot showing the temporal spread of aposematism from a single zone within the metapopulation (generations=2000, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.18, $pred_{gen}$=1, mig_{gen}=2, $mutation_{rate}$=10-5, DC_{num}=random [see Table 2.1]), N_{mig}=10).



2.4.2 Model development: migration from source habitats

We next consider that aposematism may have initially evolved within one or more habitats that are isolated from the rest of the metapopulation, but at some point, prey migration becomes possible (see Mallet & Singer, 1987). In model terms, at the start of the simulation we simply assign all individuals of these source habitats to be aposematic (c_a=0.02) and begin migration from generation 1 (mig_{gen}=1). Initial pilot runs showed that where we consider that aposematism has evolved in just 1 single sub-habitat, the critical avoidance learning rate required for viable out-migration and spread was very high (α_a=0.9, almost single trial learning) with our migration rate of N_{mig}=10. Further pilot tests revealed that adding four geographically separate source habitats made no difference to the critical avoidance learning rate required for aposematism to spread, however clumping of several source habitats greatly increased the probability of successful export of the aposematic form across the whole habitat.

We next adopted the more favourable case where we assume aposematism has evolved in a localised clump of four source habitats and we evaluated the circumstances under which aposematism could spread into the rest of the

metapopulation from this starting point. All parameters are as per the standard model (generations=2000, T=100, c_a=0.02, c_c=0.01, α_c =0.04, $mutation_{rate}=10^{-5}$, N_{mig}=10). We consider the case in which predators live for 5 prey generations ($pred_{gen}$=5). A series of tests were performed with increasing values of avoidance learning rate for aposematic prey (α_a=*0.04-0.99*) to determine if fixation of the aposematic morph was possible from these starting points.

In previous metapopulation models where aposematic prey arise randomly across the metapopulation and where N_{mig}=10, fixation of the aposematic morph was never demonstrated in models where predators outlive their prey (Table 2.2). Where we assume that migration from a group of 4 clumped source habitats occurs at a rate of N_{mig}=10, we now demonstrate fixation of the aposematic morph across the entire metapopulation when α_a >=*0.55* (Figures 2.3 and 2.4).

Figure 2.3 – Metapopulation abundance plot with 4 grouped source habitats starting at aposematic fixation – (generations=2000, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.55, $pred_{gen}$=5, mig_{gen}=1, $mutation_{rate}$=10-5, N_{mig}=10, DC_{num}=random [see Table 2.1]). The solid red line represents the number of aposematic prey and the dotted green line represents the number of cryptic prey.

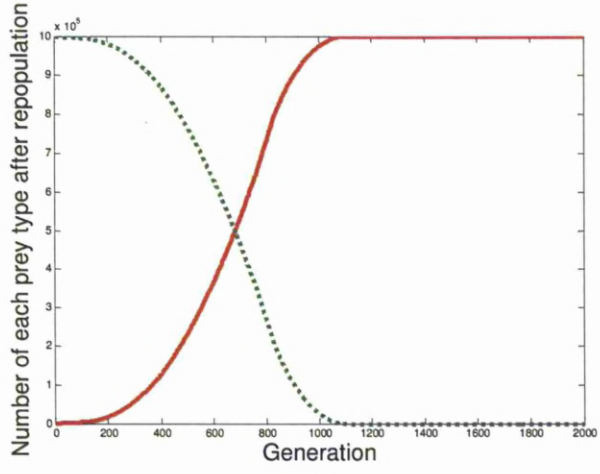
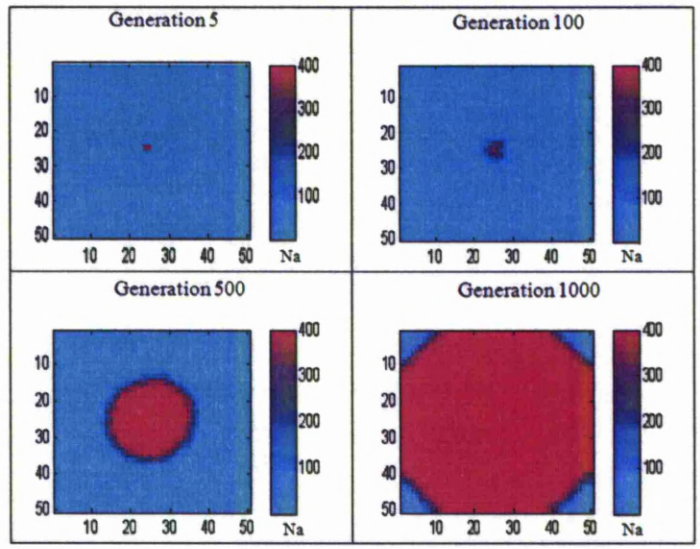


Figure 2.4–Metapopulation model plot showing the temporal spread of aposematism from the centre 4 clustered sub-habitats (generations=2000, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.38, $pred_{gen}$=5, mig_{gen}=1, $mutation_{rate}$=10-5, N_{mig}=10, DC_{num}=random [see Table 2.1]).



2.4.3 Variation in Migration Rate

Next, we considered the effects of migration rate on the viability of the aposematic morph. We adopted the same parameters as the previous source habitat model in which we assume 4 clumped sub-habitats begin at aposeme fixation and the predators live for 5 prey generations ($pred_{gen}$=5). However, we now re-tested the model with varied levels of migration (N_{mig}=2,N_{mig}=5 and N_{mig}=20). For each, we tested a series of avoidance learning rates for aposematic prey (α_a = *0.04-0.99*) to determine the critical level required for aposeme fixation across the entire metapopulation.

Where migration rate was highest (N_{mig}=20) the aposematic morph did not increase in abundance in any of the trials (α_a=*0.04-0.99*) and rapidly became extinct. In contrast when migration was reduced to N_{mig}=5 there was dramatically improved survival for the aposematic morph (with fixation occurring where α_a >=*0.38*. Finally when we reduced migration rates further to N_{mig}=2 we obtained a further reduction in

the critical avoidance learning rate required for total aposeme fixation across the entire metapopulation (α_a=0.18).

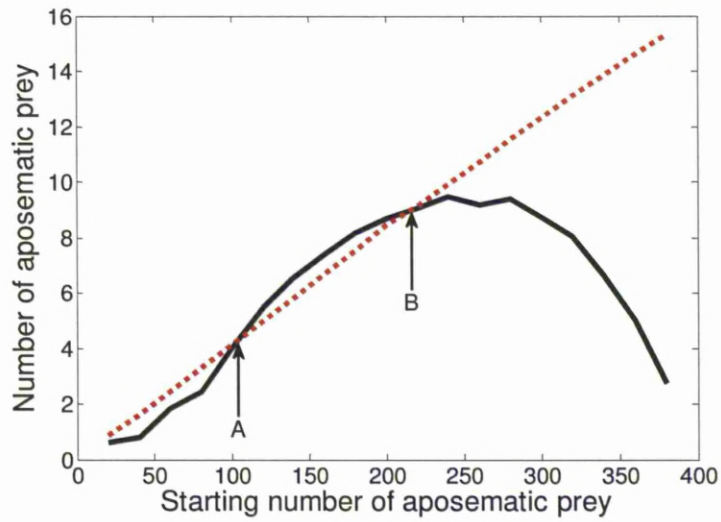
2.5 The dynamics of migration

2.5.1 Migration-selection balance in a habitat without backward migration

In order to better understand the dynamic effects of migration on the spread of the aposematic form, we modelled a series of simple scenarios, looking at the effects of selection and movement of prey on the net change in frequencies of prey forms. We start with a very simple scenario designed to examine the relationship between loss of aposematic forms through outward migration and gain from intergenerational selection. For simplicity we have not included backward migration (we deal with this later). We modelled the source habitat over 1 prey generation in order to determine whether the increase in abundance due to selection (i.e. the average increase in abundance of aposematic prey over 1 generation) was greater than the average removal of aposematic prey via out-migration for the same interval (and we subsequently extend the analysis to models that consider more habitats). We modelled a series of starting abundances for aposematic prey (20-380 aposematic prey in increments of 20) and ran the model for 10,000 repetitions to give a robust estimate of the average gain in abundance of aposematic forms due to selection and the average loss from migration. The models were tested with the following parameters (generations=*1*, $N_{c=}$400-N_a, $N_{a=}$*20-380, T=100,* c_a*=0.02,* c_c*=0.01,* α_c *=0.04,* α_a *=0.35,* $pred_{gen}$*=5,* DC_{num}*=random* [Table 2.1]). Each model was tested for three migration rates, N_{mig}=2, N_{mig}=5, N_{mig}=10.

When migration rates were set to N_{mig}=5, N_{mig}=10, the rate of change from selection was too weak to recoup the rapid loss in abundance due to outmigration. In contrast, where (N_{mig}=2, Figure 2.5) we observe that the number of aposematic prey gained by selection can be higher than that lost to out-migration, but only when they are at some intermediate frequency within the habitat (circa 110-210 individuals).

Figure 2.5 – Average net gain in abundance over 1 generation due to selection (solid line) vs. net loss to migration (dotted line) for varied starting abundances of aposematic prey (N_{mig}=2, generations=*1*, $N_{c=}$400-N_a, $N_{a=}$*20-380, T=100,* c_a*=0.02,* c_c*=0.01,* α_c *=0.04,* α_a *=0.38,* $pred_{gen}$*=5,* DC_{num}*=random [see Table 2.1]*).

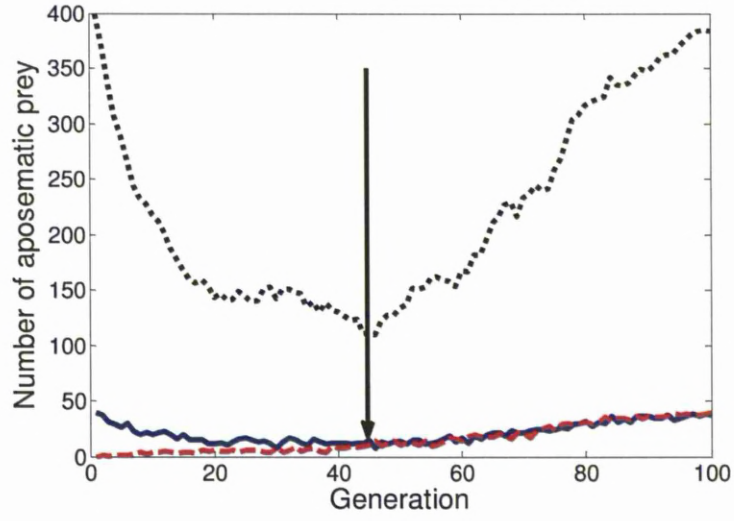


This result is explained by the fact that selection is weak toward the extremes of frequency distributions, so that at high or low frequencies of aposematic prey, the numbers replenished by natural selection are less than those removed by migration. The point on the X axis of 110 prey, (Label A in Figure 2.5) is a bifurcation point, below which prey move toward extinction and above which they increase in frequency to an equilibrium value at circa 210 aposematic forms (Label B in Figure 2.5). At this point outward migration and selection are in balance and the equilibrium is stable (values higher than this lead to reductions in aposematic numbers back to equilibrium, values lower than this lead to increases in their numbers up to equilibrium). In itself, however, this scenario is not sufficient to explain how the aposematic form can take over the entire range of the prey in a metapopulation, since it predicts a stable aposematic frequency somewhat less than fixation.

2.5.2 Migration-selection balance in a more realistic case

We next expanded our considerations to include both outmigration and inward migration from neighbouring habitats. For simplicity we consider a 6x6 square metapopulation grid with the centre sub-habitat starting at aposeme fixation (N_a=400), all individuals in the surrounding habitats were assigned to be cryptic. As with our larger metapopulation model, we again assume a torus effect at the edges of the lattice. The model was tested adopting the following parameters (generations=100, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.38, $pred_{gen}$=5, DC_{num}=random [Table 2.1], N_{mig}=5) and the abundance of aposematic prey in the source habitat was recorded for each generation as well as the number of aposematic prey lost to outmigration and the number of aposematic prey gained by in-migration (Figure 2.6).

Figure 2.6 - The effect of back-migration on a single source habitat at aposeme abundance (N_{mig}=5, generations=100, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.38, $pred_{gen}$=5, DC_{num}=random [see Table 2.1]). The dotted black line represents the overall abundance of aposematic prey within the source habitat, the red solid line represents the number of aposematic prey lost to out-migration and the dashed blue line represents the number of aposematic prey gained by back-migration. Note as aposematism becomes established in the sink habitats, we observe increasing in-migration until eventually in and out migration levels equalize and the overall abundance of aposematic prey in the source habitat approaches saturation.



The most important result is that changes in the frequency of the aposematic form in the source habitat are not monotonic. First, this morph declines in frequency due to outmigration. Selection favouring aposematism over crypsis is weak close to fixation, and so initially more prey leave the source via migration than return via selection (as in the simpler scenario above). Unlike the simpler scenario though, the aposematic form subsequently increases in frequency toward fixation. This is explained by the descendents of the original aposematic emigrants returning via inward migration from neighbouring habitats that increasingly convert to aposematism. Indeed, inward and outward migration of aposematic forms reach equilibrium, around generation 45, and subsequently natural selection favouring aposematism causes this prey form to increase to fixation (Figure 2.6). This analysis of optimal migration creates a specific simple rule which should promote outward migration from a source habitat:

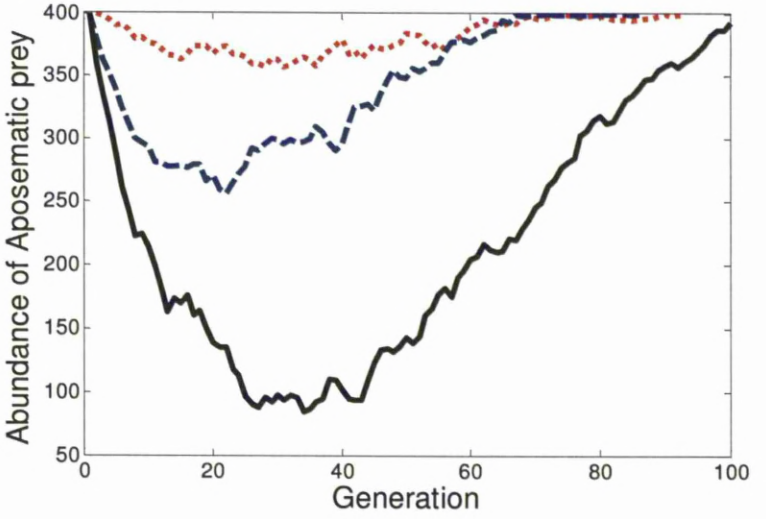
$$\Delta N_s + N_{in} >= N_{out} \qquad (2.2)$$

Where ΔN_s is the change in aposeme number due to selection in a given season, N_{in} represents the number of aposematic prey gained in the source habitat from back migration over 1 season and N_{out} represents the number of aposematic prey lost from the source habitat through out-migration over 1 season. Although our simplistic migration rule deals only with frequency dependent competition between competing prey morphs it draws similarity to habitat occupancy and colonization rules presented throughout the metapopulation literature (Hanski 1999).

These simulations help to explain why clustering of source habitats is so important for the evolution of aposematism across a metapopulation. Spatial proximity of "aposematic habitats" allows both inward and outward migration of aposematic forms. Clustering of source habitats should therefore reduce the net loss from source habitats due to in-migration from neighbouring or central source habitats, thus increasing N_{in}.

One prediction that follows is that habitats at the centre of a cluster should show less change than habitats at the fringes. To examine this prediction we next considered a clump of 9 source habitats arranged in a 3x3 grid, again placed at the centre of our 6x6 test metapopulation. Individuals in the surrounding sub-habitats were all cryptic. All other model parameters were as per the previous in/out-migration model (generations=100, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.38, $pred_{gen}$=5, DC_{num}=random [see Table 2.1], N_{mig}=5). We tracked frequency of aposematic forms in one of the border source habitats which fringes upon the cryptic populations, and similarly for the centre source habitat which is surrounded only by other source habitats (Figure 2.7). For comparison we also plot the tracked frequency of aposematic prey from the solitary source habitat model (corresponding to Figure 2.6).

Figure 2.7 – Change in abundance of aposematic prey in a source habitat over 100 generations. The solid line represents a single solitary source habitat, the dashed line represents a border habitat in a 3x3 source habitat clump and finally the dotted line represents the centre habitat in the 3x3 cluster with all source habitats lying at the centre of a 6x6 test metapopulation. (generations=100, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.38, $pred_{gen}$=5, DC_{num}=random [see Table 2.1], N_{mig}=5).



As predicted, the overall net loss of aposematic prey was much lower for both the border and central source habitats in the 3x3 cluster than in a single source habitat. It is evident therefore, that clustering of source habitats can provide extra protection from swamping of the source habitats, preventing the numbers of aposematic prey from dropping to levels where selection may become negative and extinction could occur (Puurtinen & Kaitala 2006). The results also show that the overall net loss of aposematic prey from the centre of the 3x3 cluster of source habitats is much less than in the border habitats (Figure 2.7).

In light of this finding we applied the 3x3 cluster of source habitats to our large 2500 habitat metapopulation framework to determine if larger cluster size could lower the critical avoidance learning rate for aposematic prey required for fixation throughout the entire metapopulation. We adopted the same parameters as our original models (generations=2000, T=100, c_a=0.02, c_c=0.01, α_c =0.04, $pred_{gen}$=5, mig_{gen}=1, $mutation_{rate}$=10^{-5}) with our optimal migration rate of N_{mig}=2. We found that where we assume 9 clumped source habitats begin at aposeme fixation (arranged in a 3x3 clump), the critical avoidance learning rate for aposematic prey required for total fixation was reduced to (α_a=*0.1*), considerably lower than the previous model in which we consider just 4 clustered source habitats and a migration rate of N_{mig}=2 where the critical level was found to be (α_a=*0.18*).

2.6 Predator Movement

In our next presentation of the model, we consider the effects of predator movement on the survival and spread of the aposematic morph. Our previous models consider a scenario in which a predator's foraging area exactly matches the area inhabited by a single sub-population. Here we relax this assumption and consider the effects of varying predator territory (Endler & Rojas 2009) . We consider two extensions: the first ("Increased Local Predator Foraging Area"), in which each sub-population suffers predation from several localized predators which are temporally spaced. In the second extension ("Random Predator Dispersal"), we consider a case in which predators are highly mobile and can move freely and randomly between sub-habitats, each prey season.

2.6.1 Increased Local Predator Foraging Area

In this model we allow for localized predator movement within the duration of a prey season. We again assume territoriality in that we only allow one predator to forage within a single sub-habitat at any given time. This is accomplished by dividing the time limit for a prey season (T=100 in all models) by the number of predators assumed to enter the habitat during the prey season (which in the case of all tested models was 4) hence each predator forages in the target habitat for 25 arbitrary time units. The predators selected to forage in the target habitat consist of the predator assigned to the habitat at the beginning of the prey season along with 3 others selected randomly from the neighbouring 8 habitats.

The model was tested initially in a 10x10 lattice model in which the centre 4 sub-habitats begin at aposeme fixation (although we showed previously that larger numbers of grouped source habitats make aposematism more likely to spread, we consider this to be a more realistic case). The parameters were as follows (generations=100, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.04-0.99, $pred_{gen}$=5, DC_{num}=random [Table 2.1], N_{mig}=5). The model was tested as per previous simulations with a range of avoidance learning rates for aposematic prey to determine the critical level required for aposeme fixation. We found that localized movement of predators and overlap between territories acted to prevent the spread of the aposematic morph, requiring an avoidance learning rate of value of α_a =0.8 (as opposed to α_a = 0.18 with fixed predators) .This result was subsequently verified in the 50x50 lattice model, requiring an avoidance learning rate of α_a =0.85 for aposeme fixation across the metapopulation.

2.6.2 Random Predator Dispersal

In this model we consider that each prey generation, predators are allowed to roam globally and settle in a new sub-habitat each prey generation. Computationally this is achieved by randomly shuffling the matrices containing the predator DC_{num} values as well as the corresponding memory counter data from previous experiences so that each predator carries it relevant counter data with it to its new destination. Again the model was tested with the following parameters (generations=100, T=100,

c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.04-0.99, $pred_{gen}$=5, DC_{num}=random [Table 2.1], N_{mig}=5), initially in a 10x10 lattice model. We found that when predators were allowed to globally disperse between prey generations, the critical avoidance learning rate required for the aposematic prey to take fixation was α_a = 0.35 (compared to 0.18 for the fixed predator model; this is valid for both the 10x10 and 50x50 lattice models). We then re-ran the 50x50 lattice model with a migration rate of N_{mig}=50, in which case we have total migration and dispersal of prey as well as predators, similar to the single habitat, changing predator model described by Lee et al, 2010. We found that the critical avoidance learning rate required for aposeme fixation across the metapopulation was α_a=0.8, similarly high compared with the required learning rate found for a single habitat of α_a=0.99 (Lee et al, 2010).

2.6.3 Predator Free Space

In the final presentation of our model we test the effect of reducing the overall number of predators present in the metapopulation. For brevity we consider only one treatment and reduce the number of predators across the metapopulation by half. This was achieved by adding a further 13 null predators (denoted by DC_{num}= 0) to the pool of predator values (see table 2.1), when a habitat receives a null predator, the predation process is skipped and stochastic re-population proceeds. In keeping with the chronology of the study, we applied this addition to the aforementioned random predator dispersal model to determine the effect on the critical learning rate required for aposeme fixation. We did however increase the duration of the model to consider 10,000 prey generations to determine whether drift might allow the aposematic morph to increase in abundance in the absence of predation (pilot simulations showed that drift alone, in the absence of predation can account for the fixation of the aposematic morph across the metapopulation and in all 100 repetitions, this occurred within 10,000 generations hence our decision to adopt this value). A series of avoidance learning rates for aposematic prey were tested (α_a=0.01-0.99). We found that when we reduce the number of predators by half, the critical avoidance learning rate required for fixation of the aposematic morph was raised to α_a=0.7 (in both the 10x10 lattice model) and α_a=0.75 (in the 50x50 lattice model).

2.7 Discussion

Previous theoretical approaches to modelling the evolution of aposematic warning displays neglect the effects that population structure may have on the first stages of aposematic evolution (Puurtinen and Kaitala 2006; Servedio 2000; Speed 2001; Thomas et al. 2004; Thomas et al. 2003; Yachi and Higashi 1998). In this paper we present the first theoretical metapopulation model of the evolution of aposematic warning displays from a receiver bias perspective, gaining insight into the process by which a novel conspicuous mutant may survive and spread throughout spatially distributed populations. Metapopulation theory encompasses a wide range of conceptual schemas (Hanski 1998). Our model adopts a spatially implicit discrete lattice approach which provides a robust framework on which to test the evolutionary effects of spatial structure while providing a reasonable approximation of real-life habitat patch structure. This approach is commonly used when modelling theoretical population dynamics (Hanski 1998; Hanski 1999; Sherratt 2006) and adheres closely to traditional metapopulation theory (Hanski 1999; Levins 1969). In the discussion we consider the main features of the model and how these parameters effect the survival of the novel aposematic prey.

Perhaps the most important and general finding of our models is that, other things being equal, the presence of migration between sub-habitats tends to decrease the likelihood that aposematism will persist and spread from rarity. The reason for this result lies in the fact that there is a critical abundance below which an aposematic prey will not be favoured by selection (Puurtinen & Kaitala 2006). When aposematic prey are too rare, predators are ignorant of the fact that they are unprofitable and they tend to be attacked at high rates because of their unfamiliarity and conspicuousness. It is easy to see why migration is problematic for the aposematic form. If we consider a novel aposematic mutant that arises within cryptic population in a single habitat, and that it subsequently increases in frequency because of for example random drift or predator wariness. In the absence of migration the aposematic prey may become sufficiently numerous so that it becomes favoured by selection. But with outward migration to neighbouring habitats its numbers will be diminished in the source habitat to the point that selection acts against it and hence it rapidly becomes extinct. Therefore, in our simple metapopulation model, aposematism is typically much less likely to evolve than it would in a single habitat

system. Since many prey populations exhibit nontrivial levels of migration, this appears to be an important conclusion. It has recently been argued that aposematism is rare relative to cryptic colouration across many animal taxa. We suggest that prey migration may be one of the most important ecological barriers to the establishment of this defensive trait. (Przeczek et al. 2008).

2.7.1 Importance of localization

With strong biases in predator behaviour favouring aposematic morphs over cryptic forms, fixation within one habitat could be sufficient to explain the successful export of aposematism across the whole population (see Table 2.1). We could, however, explain the spread of an aposematic trait most plausibly if we assumed that it initially rises to high frequencies within more than one habitat, especially if these habitats are clustered closely together prior to outmigration. This localised increase in abundance may be plausible in favourable conditions such as habitats which are temporarily predator-free combined with stochastic drift effects (Lee et al. 2010; Mallet & Singer 1987) or where resident predators show extreme wariness of novel coloured prey (Lee et al. 2010; Marples et al. 2005; Thomas et al. 2004; Thomas et al. 2003). The benefits from the clustering of source habitats for the spread of the aposematic form comes from reducing the overall net loss of aposematic prey from any given habitat within the source cluster, thus ensuring that numbers never drop below or near to the bifurcation point (point A, Figure 2.5) where there is a potential risk of extinction. Considering our 3x3 cluster of source habitats, a habitat in this cluster bordering the cryptic populations will suffer a lower net loss of aposematic prey in comparison to a single source habitat simply due to the immigration of aposematic prey from neighbouring "aposematic habitats". Similarly the habitat at the centre of the 3x3 source habitat clump will undergo an even lower net loss as it is surrounded only by "aposematic habitats" (Figure 2.7).

2.7.2 Predator Movement

Our models show that localized predator movement within a prey season, effectively modelling increased predator foraging area, acts to inhibit the survival and spread of the aposematic form. This result pertains because predators, although exhibiting dietary conservatism, do not consume enough of the aposematic prey to significantly learn to avoid them (see Lee et al. 2010) and as our initial models show, accelerated avoidance learning about aposematic prey is required for them to survive and spread in scenarios where predators significantly outlive their prey ($Pred_{gen}$=5). A similar result pertains, although to a much less detrimental level, when we allow random, global predator movement across the metapopulation, between prey generations. This difference in effect between local and globally mobile predators may arise due to local predators exhausting their wariness of the aposematic form quite quickly due to constant encounter (especially in the first instance whereby we assume four habitats begin the model at aposeme fixation). The effect of global predator movement ensures that, in most cases, a completely naive predator moves into the habitat and hence its DC wariness has not been eroded by experience.

We suggest that when aposematism arises in an isolated sub-population(s), it is most likely to spread to surrounding habitats when a single predator's patch approximately matches the area inhabited by a sub-population and when predators generally remain in that habitat for the duration of their lifetime. Although this presents a limiting scenario, we show that aposematism can indeed spread if predators are themselves geographically mobile although these conditions are found to be less favourable.

When we allow total prey migration along with globally moving predators then the model outcome is similar to the random predator, single habitat models described by Lee et al. (2010) in that the learning rate required for fixation of the aposematic morph is significantly raised, affirming the importance of habitat and population structure on the evolution of aposematic displays.

2.7.3 Predator Abundance

When the number of predators across the metapopulation was reduced, thus allowing some sub-habitats to remain predator free for a period of time, the probability of survival and spread of the aposematic morph was reduced. Although pilot studies showed that over long periods of evolutionary time, the unidirectional mutation towards the aposematic form and reproductive drift in the absence of predation could account for the fixation of the aposematic morph, our model shows that even with a significantly reduced number of predators in the modelled system, fixation is of the aposematic form was unlikely and required high levels of predator avoidance learning. This indicates that with free roaming predators, it is unlikely that a sub-population would escape predation long enough for drift to raise the abundance of the aposematic morph. Our model suggests that for drift to account for the fixation or at least increased abundance of aposematic prey, predation must be absent for sufficient periods of evolutionary time.

2.7.4 Mallet & Singer's Predictions

Our models show a shifting balance and moving clines as predicted by (Mallet & Singer 1987), in the sense that sufficient localisation in frequency of aposematic signals within a habitat could cause the destabilisation of crypsis in neighbouring habitats, and the outward movement of aposematism/crypsis clines. Because we have modelled asexual reproduction, we are of course not intending to describe the specific complications of sex or allelic dominance. It should also be pointed out that the random component in genetic drift comes in our models through stochastic repopulation of the prey between generations. None the less, even with these limitations, it can be seen that Mallet & Singer's general prediction, of localised evolution followed by shifting clines holds up well. The important insight from our models is, in our view, that although prey migration works to prevent the evolution of geographically widespread aposematism, localised clustering of aposematism across several habitats may effectively surmount the barrier posed by migration.

2.7.5 Stable Dimorphism and the persistence of aposematic prey

Our models show that where predator/prey lifespans are similar, and with moderate avoidance learning rates for aposematic prey (α_a=0.04-0.17), we observe stable co-existence of cryptic and aposematic morphs over the duration of the model, even when suffering attack from predators with large variation in their DC tendency. This result could not be demonstrated in single habitat models (Lee et al. 2010). Metapopulation structure and the inter-habitat movement of prey therefore appear to prevent extinction of the aposematic morph by outmigration and establishment in other sub-habitats where predators may be more wary of the novel immigrant. Examples of metapopulation dispersal acting to prevent complete extinction is well supported in the literature (Holyoak & Lawler 1996; Sabelis & Diekmann 1988) describing a 'hide and seek' phenomenon whereby prey migrate to a beneficial habitat with the effect that extinction is prevented. Although these studies describe asynchrony between predator and prey populations as the protecting mechanism preventing extinction, in our model 'havens' are created when a habitats contains a highly wary predator.

2.8 Conclusions

The models presented in this study provide insight into the effect that population structure may have had on the initial evolution and geographical spread of aposematism which previous studies have so far neglected. Aposematic warning displays clearly exist and must have spread throughout prey populations at some point in natural history. There are many examples of aposematic species which have been shown to conform to general metapopulation structure, from the red-spotted newt (*Notophthalmus viridescens*), (Shure et al. 1989) to the six-spot burnet moth (*Zygaena filipendulae*),(Menendez et al. 2002). Our model provides a missing insight into how such prey populations might have evolved conspicuous warning signals and how aposematism might have become established over time and geographic space. We re-iterate that initial localized increase in frequency may have been crucial to the evolution of warning colouration within such species followed by low levels of out-migration.

Chapter 3 - Honest signalling and the uses of prey colouration.

3.1 Overview

The first two chapters focus entirely on the initial evolution of warning signals and how the first aposematic prey may have survived and spread to fixation. This chapter begins the second major topic of the thesis looking more closely at optimal signalling, honesty and cheating in aposematism. Specifically, this chapter focuses on determining the conditions which might promote positive correlations to emerge between conspicuousness and defence as well as exploring the optimality of warning signals as an anti-predator adaptation.

A recent model by Blount et al. (2009) aimed to explain empirical data suggesting that there are positive correlations between toxicity and conspicuousness in certain aposematic species (Bezzerides et al. 2007; Cortesi & Cheney 2010; Summers & Clough 2001). In this model Blount et al. make a number of assumptions; first that warning display and toxicity compete for anti-oxidant molecules acquired from limited environmental resources, an assumption that is maintained in the models presented here. Second, that predators have pre-evolved biases towards warning colouration and hence that the probability of attack from predators declines multiplicatively with an individual's level of toxin and the strength of its warning display. Blount et al. also limited their simulations to only consider the part of the conspicuousness spectrum that acts as a warning display and did not consider that prey can evolve crypsis. In light of these limitations, this chapter extends the basic model to test the generality of the arguments put forward by Blount et al. (2009). In the first model extension, assumptions of predatory bias towards bright colouration are removed altogether and an alternative fecundity advantage to bright colouration is introduced. The results show that even in the absence of pre-existing predatory biases, positive brightness-toxicity correlations can still emerge from the system, as long as conspicuousness confers some other fecundity advantage. This finding has important implications for the initial evolution of warning signals in that it is plausible to imagine that prior to aposematism, if

conspicuousness evolved for some other adaptive reason and was a reliable indicator of prey toxicity, then predators would face selection to evolve to discriminate between prey based on conspicuousness (as outlined in the co-evolutionary model of Sherratt (2002). Aposematism may then be an example of an exaptation.

In the second extension of the Blount et al model, prey are allowed to evolve either warning display or crypsis, considering the full scale of prey appearances from very bright to very dull. The results show that where resources are scarce, it is optimal for prey to invest all available resources into crypsis with no defence, providing evidence that resource availability can influence the optimal defence strategies of prey. More importantly, this extension of the Blount et al. model did not affect the generality of the predictions for honest signalling for groups with higher resource availability, adopting aposematism as an optimal defence strategy.

In the previous two chapters, individual based stochastic models were used, allowing key ecological parameters to be easily and explicitly tested. When considering optimal traits and strategies, individual based stochastic simulations can become limited in that reaching a stable, defined optimal trait value can be time consuming given that reaching the optimum is reliant on stochastic mutations in trait values. Due to stochastic drift effects, the stability of such obtained "optimal" trait values would be difficult to identify, especially when considering multiple traits. The models presented in this and subsequent chapters adopt an existing, deterministic numerical optimization model framework which allows infinitely sized populations to evolve in frequency over a pre-mapped discretized trait landscape (Blount et al. 2009; Speed et al. 2010). This technique provides more stable optimal trait strategies, especially when considering co-evolution of several traits as is the case in the models presented here.

This work was devised from discussion between Thomas Lee, Mike Speed and Phil Stevens. Initial model design for development 1 was undertaken by Phil Stevens and developed by Thomas Lee and Mike Speed. Development 2 was designed by Thomas Lee with assistance from Mike Speed. Coding, implementation and testing were performed by Thomas Lee. Writing by Tom Lee (approx 2/3) with input from Mike Speed and Phil Stevens approx (1/3).

3.2 Abstract

Although signal reliability is of fundamental importance to the understanding of animal communication, the extent of signal honesty in relation to anti-predator warning signals has received relatively little attention. A recent hypothesis suggested that (aposematic) warning signals may often be honest, with brightness and toxicity positively correlated within prey populations. This hypothesis was represented in a theoretical model which assumed a physiological linkage between pigmentation and toxicity. Two critical shortcomings of that model were: (1) the requirement, among predators, for an innate aversion to brightly coloured prey and (2) the assumption that prey use pigments to generate warning colouration, rather than to develop crypsis. In this paper, we examine the importance of these assumptions for predictions regarding relationships between toxicity and warning colouration. Our models suggest that a positive brightness-toxin correlation can emerge if conspicuous prey colouration provides an additional fitness benefit unrelated to predation. Initially, this correlation could evolve for reasons unrelated to prey signalling; hence, aposematism might represent a striking example of exaptation. When investment in pigmentation could be diverted into crypsis or into warning colouration, and when warning colouration was associated with an additional benefit, prey generally invested in toxicity and aposematic display and honest signalling then emerged from the system. However, crypsis was favoured: (i) when available resources could confer a very low toxicity level and hence, aposematic advertisement of toxicity was ineffective; and (ii) when toxicity was extremely effective at protecting individuals compared to advertisement, in which case toxicity correlated positively with degree of cryptic colouration. Our results suggest that an underlying, physiological link between pigmentation and toxicity is robust in predicting the emergence of honest aposematic displays, but point to interesting conditions in which signal honesty is not predicted.

3.3 Introduction

Chemically defended prey often use distinctive, bright forms of (aposematic) colouration in order to advertise their unprofitability to predators (Ruxton et al. 2004). Aposematism is ecologically and taxonomically widespread, and has been intensively investigated as a case study in interspecies signalling and in predator-prey

coevolution (see reviews in Mappes et al. 2005; Marples et al. 2005). Though much is now understood about the evolution and function of aposematic signals, questions regarding the evolution of reliable aposematic signalling remain unresolved, both empirically and theoretically. One of the key ideas to explain the evolution of aposematic displays is that conspicuousness imposes costs of detection by predators, which cannot be borne by edible prey (Franks 2009; Grafen 1990; Sherratt 2002; Zahavi 1975). As a contrast to crypsis, therefore, conspicuousness could be seen as qualitatively honest, indicating unprofitability in prey (see especially Sherratt 2002). What is much less clear is whether variation in conspicuousness within or between aposematic species is itself a "quantitatively" honest cue, in which the more toxic individuals have brighter displays.

For some time it has been assumed that variation in aposematic displays is unlikely to act in a quantitatively honest manner. Several theoretical models, for example, predicted the converse: that it is optimal for more toxic prey to be less brightly coloured than those with lower levels of toxicity (Leimar et al. 1986; Ruxton et al. 2007, 2009; Speed & Ruxton 2005b, 2007). These models assume that, as prey become more toxic, individuals are better able to survive attacks and so gain less from costly aposematic signals. Indeed, there are some empirical data from Dendrobatid frog species to support the prediction that more toxic species may present less bright aposematic signals (Darst et al. 2006).

In contrast, other recent empirical work has reported apparent signal reliability in aposematic signalling within a population (of the Asian ladybeetle Harmonia axyridis, Bezzerides et al. 2007) and between species within a taxonomic group (e.g. dendrobatid frogs and marine opisthobranchs, Cortesi & Cheney 2010; Summers & Clough 2001). To explain these results, Blount et al. (2009) proposed a theoretical framework which would account for "quantitative" signalling honesty within a population of prey. Here, chemical defences and aposematic displays are assumed to deplete a common resource, so that resources allocated to toxicity reduce those available for signalling (and vice versa). Blount et al. considered that molecules needed for pigmentation (carotenoids, flavonoids, melanins, ommochromes, papiliochromes, pteridines and porphyrins) may have the capacity to function as antioxidants *in vivo*. If synthesis (or sequestration) and storage of toxins causes oxidative stress, then there may be competition between allocation of

molecules to pigmentation and to ameliorating the physiological costs of toxicity. This physiological linkage between display and the defence being advertised is key to the prediction of signal honesty.

Blount et al. further assumed that a prey's probability of surviving an attack is proportional to the product of the values of display (A) and toxicity (D). In this way a prey must have nonzero levels of toxicity and of aposematic signalling if it is to raise its probability of surviving an encounter above an assumed minimum. With this assumption, the probability of a prey surviving an attack is maximised when its available resource is split evenly between toxicity and display. For example, if the available resource value is 1, (and $A + D = 1$), then the product AD, and therefore the probability of surviving an attack, is highest when $A = D = 0.5$. If there are no other costs for either display or toxicity, then the optimal strategy is always to partition the resource equally into defence and display; the consequence is that, as the total available resource increases, so the total value of resource allocated to signalling and to toxicity is predicted to increase. In this way a positive correlation between the absolute values of toxicity and display emerges from the model.

It is often assumed that, by making prey more conspicuous to predators, signalling attracts an additional cost (Leimar et al. 1986; Ruxton et al. 2009). When this additional cost is added to the Blount et al. model, prey tend to invest less in signalling than in toxicity, and this is especially the case when resources are abundant, to the extent that signal honesty may break down altogether. Thus, at higher resource levels, Blount et al.'s model may predict a negative correlation between values of secondary defence and display, because individuals can become sufficiently toxic that they have less need for costly, bright signals. The result is that, depending on the availability of key resources, Blount et al.'s theoretical model can explain positive correlations between toxicity and conspicuousness (with resource limitation) and negative correlations (when resource limitations are relaxed see Darst et al. 2006).

Although the model of Blount et al. can predict signal honesty, it has a number of significant limitations. In particular, the model assumes that predators have some innate wariness of bright colouration, which interacts with the toxicity of an individual prey to determine the probability that the prey is killed during an

attack. This begs the question, why might predators be wary of brightly coloured prey in the first place? Some authors propose a process of coevolution leading to bright colouration in prey and wariness of bright colouration in predators (Lee et al. 2010; Sherratt 2002); this possibility could be profitably applied to the Blount et al. model. Another limitation of Blount et al.'s model is that it failed to consider the obvious possibility that prey might evolve cryptic colouration as an alternative to aposematic patterning. This is an important omission since it seems likely that pigments required for cryptic colouration could compete with toxicity for a shared resource.

In this paper, we build on the work of Blount et al. to examine the extent to which their findings were reliant on the key limitations of their model. We show that positive correlations between brightness and toxicity can be predicted even when predators have no initial bias towards the avoidance of brightly coloured prey, and that under many conditions in which cryptic or aposematic patterns are possible, reliable signalling (in the sense of a positive correlation between prey toxicity and brightness of colouration) is an emergent phenomenon. A key determinant of whether prey invest in crypsis or in aposematism is the efficacy of the toxins in protecting prey compared to that gained from aposematic advertising.

3.4 Methods

We first summarise the original model of Blount et al. (2009), before describing how we have adapted it to address more fundamental questions about the origins of warning colouration and the choice between warning and crypsis.

We set the initial prey population to consist of individuals allocated to one of n equally abundant resource groups. Individuals within group i ($i = 1 \ldots n$) have access to $R(i)$ resources, perhaps dictated by the characteristics of their local microhabitat. Prey evolve an optimal set of allocations between display (A) and secondary defence (D, assumed to be an internally stored toxin) for the available resource level. Individuals within the prey population allocate $R(i)$ resources available to them according to a trait, A ($0 \leq A \leq 1$), where A determines the proportion of available resources that are allocated to bright or conspicuous

colouration (meaning any colouration which enhances prey conspicuousness relative to its common background). The complement of A is D ($D = 1 - A$), the proportion of available resources that are allocated to secondary defences. Thus, for an individual with access to resources $R(i)$, trait A dictates investment in both aposematism and secondary defence. A is represented on a discrete grid to the nearest percentage point (A = 0.00, 0.01, 0.02 ...1.00) and the prey population is modelled deterministically by considering the proportion $f(i,A)$ of individuals in any resource group with any given trait value, where

$$\sum_{i=1}^{n} \sum_{A=0.00}^{1.00} f(i,A) = 1 \qquad (3.1)$$

The total population is N_0 and the total number of individuals in any resource group is $N_i = N_0/n$.

Predation imposes differential survival, $S(i,A)$ on individuals with different attributes (investment in aposematism and secondary defences) and this affects the relative proportions of different types of individual that are represented in the next generation. In the original model, survival is the only component of fitness that is affected by an individual's attributes (we modify this assumption later), such that the relative frequency of a given type of individuals after survival and breeding is given by

$$f'(i,A) = \sum_{A'=0.00}^{1.00} f(i,A').S(i,A').z \qquad (3.2)$$

where z is given by the indicator function

$$z = \begin{cases} 1-\varepsilon & A' = A \\ \varepsilon/100 & A' \neq A \end{cases}.$$

Equation (3.2) ensures that, at each generation, there is some low level of mutation, ε. Thus, every trait value loses ε of its potential representation in the next generation to mutation, and gains $\varepsilon/100$ of the potential representation of every other trait value within that resource group. This guarantees that solutions to the model are

evolutionarily stable by ensuring that every trait type always has the opportunity to invade from rare. As stated, equation (3.2) gives the relative representation of different traits in the next generation. This is rescaled to ensure that the total frequencies over all resource groups and traits sum to unity, equation (1), using

$$f''(i,A) = \frac{f'(i,A)}{n\sum_{A} f'(i,A)}$$

Survival of individuals in any generation is dependent on their resource group and A trait value. Specifically, survival depends on: the rate at which predators are encountered, $r(i,A)$; the probability of attack given an encounter, $p_1(i,A)$; and the probability of death given attack, $p_2(i,A)$. Survival is thus given by:

$$S(i,A) = e^{-r(i,A).p_1(i,A).p_2(i,A)} \tag{3.3}$$

The rate at which individual prey encounter predators is dependent on their relative conspicuousness. The absolute conspicuousness of any given individual is given by

$$c(i,A) = 1.5 - e^{-\alpha AR(i)} \tag{3.4}$$

where α is a constant that scales the rate at which conspicuousness increases with investment in aposematism. This gives a value between 0.5 (for zero investment in aposematism) and a maximum of 1.5 for higher investment in aposematism. The mean absolute conspicuousness across the whole prey population is

$$\bar{c} = \sum_{i=1}^{n} \sum_{A=0.00}^{1.00} c(i,A).f(i,A)$$

and the trait specific encounter rates are given by

$$r(i,A) = \frac{c(i,A)}{\bar{c}}. \tag{3.5}$$

The probability that a prey individual, once encountered is attacked, is assumed to depend on the mean level of secondary defences in the population as a whole, D^*. This is given by

$$D^* = \sum_{i=1}^{n} \sum_{A=0.00}^{1.00} (1-A)R(i).f(i,A).$$

The probability of attack is now

$$p_1(i,A) = 0.01 + 0.99\,e^{-0.1D^*} \tag{3.6}$$

where the 0.1 scales the exponent.

The probability that a prey individual dies as the result of an attack is assumed to decrease as a result of increased investment in both aposematism and secondary defences,

$$p_2(i,A) = 0.01 + 0.99\,e^{-0.1AR(i).(1-A)R(i)} \tag{3.7}$$

where the first term in the exponent is investment in aposematism and the second term is investment in secondary defence. This simulates a situation in which a predator is prepared by experience, evolution or sensory bias to "go slow" when handling brightly coloured prey (c.f. Endler 1993; Guilford 1994).

At the start of a simulation all possible allocation phenotypes are present in all resource groups and, when stability is reached, suboptimal allocation strategies have been removed from the population (to the limits imposed by the mutation-selection balance). Following Blount et al. we simulated evolution until stability was reached (defined where the summed absolute magnitudes of changes among frequencies of all trait types were less than 10^{-7} per generation); all results shown reflect this stability criterion. For the levels of mutation used, there was a single optimum value of A in each resource group and variance around that was negligible; consequently, only the mean value of A is shown. Unless otherwise stated, we use the values $n = 5$, $\alpha=0.01$, $\varepsilon=10^{-6}$ in our simulations.

Next, we describe our developments of the original model of Blount et al. (2009). Table 3.1 provides a summary of model details changed in each development.

Table 3.1 - Table comparing key features of the original model (Blount et al., 2009) and the two model developments presented in this paper.

Feature of the model	Original Model (Blount et al. 2009)	Development 1	Development 2
Pre-existing predatory aversion to warning colouration and defences	Depended on both level of colouration (A) multiplied by investment in defence [$R(i)$-A]:	No prior assumption of predatory aversion; see equations 3.9,3.12 & 3.13	As per original model
Fecundity advantage from bright colouration	No advantage assumed	Bright colouration assumed to improve fecundity (possibly due to sexual selection, thermoregulation, UV protection etc.); see equation 3.8	No advantage assumed
Evolution of cryptic colouration of prey	Not permitted	Not permitted	Considers cases where prey can invest in either cryptic (K) or aposematic (A) display with defence (D); see equation 3.14

3.5 Development 1: Origins of a signalling system

3.5.1 Origins of a signalling system: Model description

We begin by assuming that aposematic displays confer a small fecundity advantage unrelated to survival from predation. This advantage could emerge from one or more of thermoregulation, mate choice, or another mechanism (considered in more detail below). We use a logistic function to describe the relationship between reproductive success (v) and colouration (A):

$$v(i,A) = 1 + \frac{1}{1+e^{-\lambda[AR(i)-\mu]}}. \qquad (3.8)$$

This formula provides a convenient family of functional forms that cause v to vary from a minimum of 1 to a maximum of 2. The value of λ determines the steepness of the relationship between colouration and reproductive success; high values of λ create more of a step function, so that reproductive success increases relatively abruptly at some threshold of colouration. Lower values of λ generate smoother relationships between v and $AR(i)$. We investigated a full range of functional shapes, but for brevity report on one that provides typical results: gradually sigmoidal (λ = 0.1). The value of μ dictates the possible maximum reproductive success associated with different resource levels; values of μ are meaningful only to the extent that they are relative to the (arbitrary) scale of resource availabilities (and for the simulations that we report, we used $\mu = 1$). In order to avoid the predatory bias against brightly coloured prey, we revise equation (3.7) as follows,

$$p_2(i,A) = 0.01 + 0.99e^{-0.1(1-A)R(i)} \tag{3.9}$$

The probability that an individual survives an attack now depends only on its individual level of toxicity ($D = 1 - A$), and not on its colouration.

Frequencies of morphs after selection are now defined as the product of survival $S(i,A)$ and fecundity $v(i,A)$

$$f'(i,A) = \sum_{A'=0.00}^{1.00} f(i,A').S(i,A')v(i,A').z \tag{3.10}$$

where z is as defined in equation (3.2).

3.5.2 Origins of a signalling system: Results

The relationship between colouration, toxicity and resource availability, is affected by the overall quantity of resources (Fig. 1). When resources are relatively scarce, there is a positive correlation between appearance (A) and toxicity (D) (Fig.

1a). In contrast, when resource levels are relatively very high, there is no negative correlation between display and toxicity at the higher end of those resource values as found in the original Blount model (Fig. 1b).

This modified version of the model produces very similar results to those of the original Blount et al. model, in that it easily generates positive correlations between toxicity and brightness of appearance,but unlike the Blount model, it predicts no correlation at very high resource levels. At the heart of this version of the model is a direct trade-off between the benefits of fecundity which result from bright colouration (equation 3.8) and the benefits of survival from attacks (because of individual levels of toxicity, equation 3.9). In the range of the colour morphs in which the value of fecundity is increasing with the brightness of display, it is optimal to increase the absolute value of resource given to appearance as resources increase (Fig. 3.1a). In contrast, where the fecundity curve is essentially saturated (with high values of resources and high levels of brightness in Fig. 3.1b), then display becomes a poor investment relative to toxicity, providing very small marginal benefits, but more substantially increasing costs of detection by predators (Fig. 3.1b).

Figure 3.1a - Optimal toxicity (solid line) and displays (broken line) for prey with different levels of resources, generated using equations 3.8, 3.9, and 3.10. Here there are 5 resource states (n = 5) and μ = 30. Values of resource levels are: $R(i)$ = 6, 12, 18, 24, 30. (b) $R(i)$ = 75, 150, 225, 300, 375.

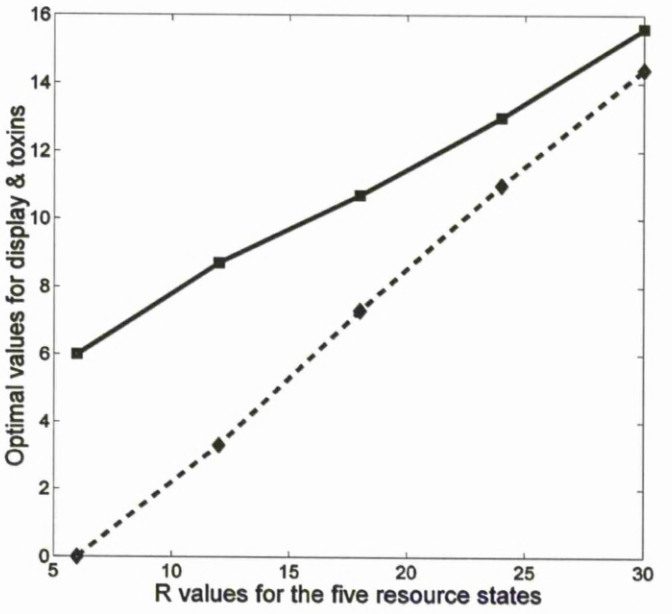
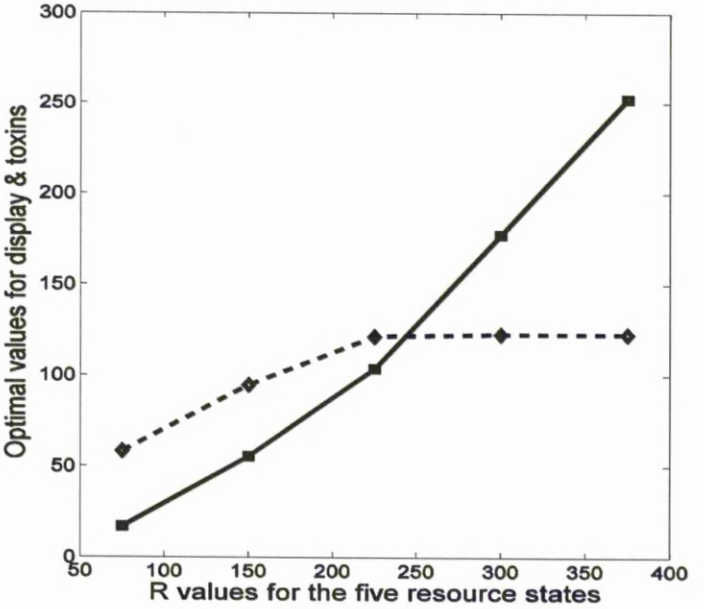


Figure 3.1b - Optimal toxicity (solid line) and displays (broken line) for prey with different levels of resources, generated using equations 3.8, 3.9, and 3.10. Here there are 5 resource states ($n = 5$) and $\mu = 30$. Values of resource levels are: $R(i) = 150$, 300, 450, 600, 750.



Although there is no component of this revised version of the Blount et al. model in which the bright colouration acts as a warning signal of chemical defence in the prey, some level of raised conspicuousness is associated with raised toxicity. Moreover, it is a relatively simple computational task to allow the evolved colouration to function as a warning signal to predators. For example, we could assume that predators make an estimate of the palatability of individual prey based on the average defence level associated with the value of its appearance (see Lee et al. 2010). The probability of attack, $p_1(i,A)$ (bounded between 0.01 and 0.99) would now decline with increases in the average defence level $\overline{D}_{AR(i)}$ associated with a particular value of appearance, $AR(i)$:

$$p(i,A) = 0.01 + 0.98exp(-0.1\overline{D}_{AR(i)}) \tag{3.11}$$

Owing to the correlation between bright colouration and toxicity that emerged from the system (e.g. Fig. 3.1a), the predators would tend to be wary of attacking brightly coloured prey and would, thus, treat brightness as a signal.

Alternatively, we could assume that the probability of death given attack is either the product or the sum of an individual's toxicity level $(1 - A)R(i)$ and the mean toxicity associated with a value of appearance $\overline{D}_{AR(i)}$. Here, the assumption is that the aggression with which a predator attacks a prey depends on its expectation of unpalatability, based on the value $\overline{D}_{AR(i)}$, and on precisely how nasty the prey turns out to be, $(1 - A)R(i)$. Formally, these alternatives can be written as either:

$$p_2(i,A) = 0.01 + 0.99e^{-0.1\overline{D}_{AR(i)}(1-A)R(i)} \tag{3.12}$$

or

$$p_2(i,A) = 0.01 + 0.99e^{-0.1\overline{D}_{AR(i)}+(1-A)R(i)} \tag{3.13}$$

With equations 3.11, 3.12 or 3.13 we get qualitatively the same set of results as in Fig. 3.1a, 3.1b. Hence we could explain the initial evolution of the signalling system as an exaptation (sensu Gould & Vrba 1982) by the prey of some other trait, which provides a fecundity benefit. Once bright colouration evolved, it could provoke coevolutionary responses in predators, resulting in the widely reported observation that they are wary of brightly coloured prey.

3.6 Development 2: crypsis or aposematic colouration?

3.6.1 Crypsis or aposematic colouration? : Model description

We now consider whether, when Blount et al.'s original model is extended to allow prey to generate cryptic or aposematic pigmentation, their original findings regarding signalling honesty are maintained. In this model we add a new parameter (K) representing the proportion of resources an individual allocates to cryptic display; K also competes with aposematic display (A) and defence (D) for available resources so that $A+D+K=1$. We limit the parameter space of the model to consider only values of A and K where $A \times K = 0$; hence cryptic and aposematic displays cannot be combined. The rate at which individual prey encounter predators is dependent on their relative conspicuousness. The absolute conspicuousness of an individual is given by

$$C(i,A,K)=1+0.5(e^{-\alpha_a.A.R(i)})-0.5(e^{-\alpha_k.K.R(i)}) \qquad (3.14)$$

This gives a default conspicuousness value of 1 for no investment in display. Investment in aposematism (A) increases conspicuousness to a maximum value of 1.5; alternatively, investment in crypsis (K) decreases the default conspicuousness to a minimum value of 0.5. α_a and α_k scale the relationship between the level of

investment in either display type (A or K) and encounter rate for each prey type respectively. Relative conspicuousness is calculated as in equation 3.5.

In the original model of Blount et al. individual prey could not gain raised survival from the benefits of their own toxicity unless they invested some resource into aposematic display. We relaxed this assumption by allowing nonzero levels of toxicity to increase prey survival compared to zero toxin values. To achieve this D_i is multiplied by a a coefficient, γ , which determines the minimum efficacy of toxicity in determining probability of survival. We amended equation 3.7 as follows:

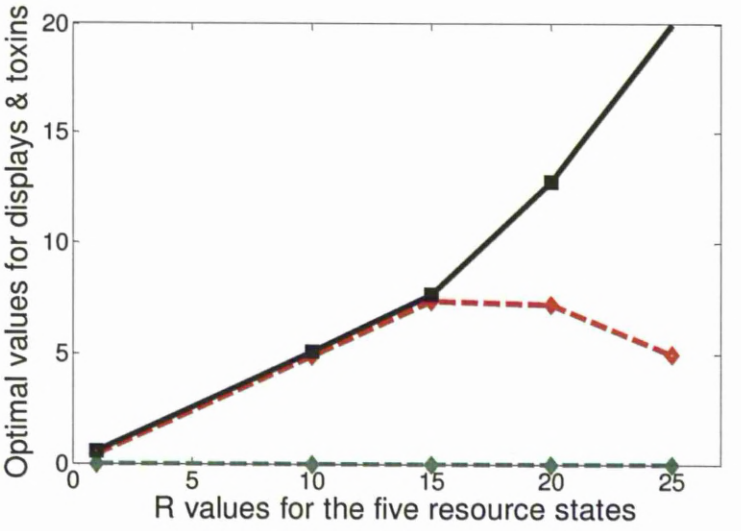
$$p_2(i,A) = 0.01 + 0.99e^{-0.1(AR(i)+\gamma).(1-A)R(i)} \tag{3.15}$$

3.6.2 Crypsis or aposematic colouration? : Results

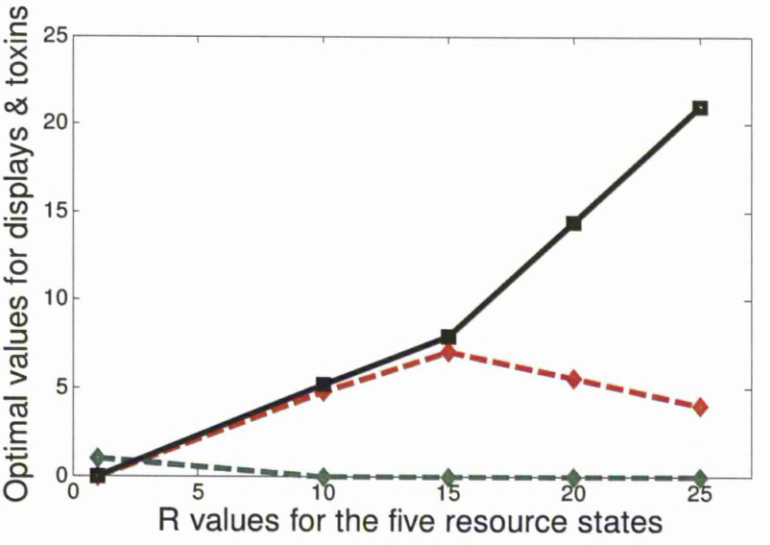
Where γ is set to a low value (0.1) and ($\alpha_a = \alpha_k = 0.01$), at no point was crypsis found to be the optimal strategy for any of the resource groups tested [$R(i)$ from 1 to 25] and our models yielded virtually identical results to those presented in Blount et al. (2009), (see Fig. 3.2a).

Figure 3.2 - Results of simulations in which prey evolve to allocate their resource between toxicity and cryptic or aposematic colouration Parameter values are: $R(i)$ = 1, 10, 15, 20, 25; (a) γ=0.1, α_a=0.01, α_k=0.01; (b) γ=0.1, α_a=0.1, α_k=0.1; (c) γ=10, α_a=0.1, α_k=0.1; Each scenario illustrates optimal values for toxicity (black line), aposematic display (red line) and crypsis (green line).

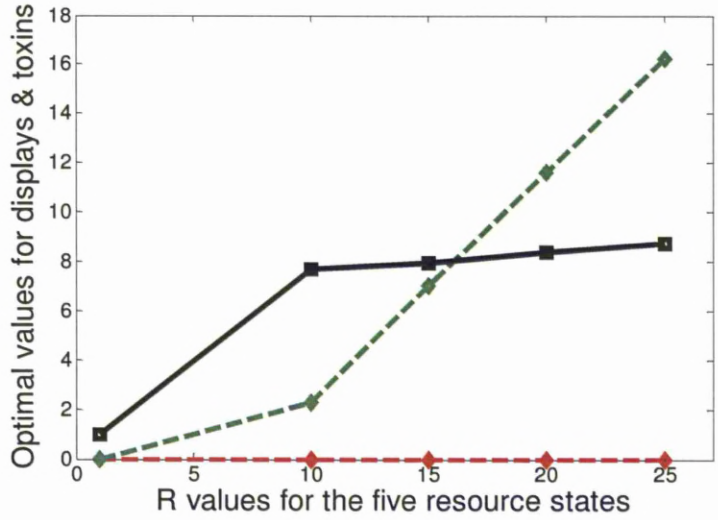
(a)



(b)



(c)



When we increased the weighting of the translation of display into prey conspicuousness (α_a,α_k = 0.1), we observed that for the group with the least available resource ($R(i)$ = 2), investment in crypsis is the optimal survival strategy of the prey (Fig. 3.2b). For all other resource groups (R_i = 10, 15, 20, 25) the results of Blount et al. pertained, in that a combination of warning signal and toxin investment was optimal (Fig. 3.2b). In this simulation then, crypsis was manifest only when prey lacked resources to generate an effective secondary defence.

We next increased the minimum level of effectiveness of toxicity substantially (γ=10), so that individual survival is greatly increased by a relatively small investment in toxicity. Now crypsis evolved as the optimal prey appearance for a wide range of values (Figure 3.2c). Here toxicity actually correlated positively with degree of cryptic pigmentation, and aposematism was not predicted. In this scenario, the toxin is sufficiently good at protecting the prey that it has no need of aposematic advertisement which, by contrast, provides relatively small survival benefits. As toxicity rises with resource levels, the curve defining survival from attack tends to saturate, at which point investment in crypsis becomes increasingly favoured.

We conclude that the general predictions of the Blount et al. model are supported, except in cases in which the intrinsic protection provided by the toxin greatly outweighs the survival benefits associated with bright colouration.

3.7 Discussion

Although signal honesty has been explored extensively with respect to sexual signals, there is a comparative lack of theoretical and empirical exploration of honesty within the study of anti-predator warning signals. Recently a number of theoretical models have begun to make predictions that prey may signal honestly to predators (Blount et al. 2009; Franks 2009; Lee et al. 2010) and there is some empirical support for these predictions (Bezzerides et al. 2007; Cortesi & Cheney 2010; Summers & Clough 2001). The model of Blount et al. (2009) is unique in assuming a physiological linkage between toxicity and display, in which a shared resource is depleted in the creation and maintenance of both traits. In this paper we sought to evaluate this theoretical framework to determine whether honest signalling can still be predicted when predators have no initial bias against brightly coloured prey, and when cryptic colouration can compete for the shared resource in the same way as aposematic colouration. In either case we confirm that a positive correlation between toxicity and display can be predicted in our extended version of the model. We discuss these results in light of the potential for conspicuousness to become correlated with toxicity in the absence of initial predator biases and with regard to the evolution of aposematic signalling as an alternative to cryptic colouration.

3.7.1 - Honest signalling and the origin of predator biases

In our first extension to the model of Blount et al. we assumed that predators had no initial biases to make them avoid brightly coloured prey. Instead, we assumed only that there was some alternative benefit to bright colouration and that individuals with higher toxicity levels were more likely to survive attacks than those with lower toxicity levels. Even though prey colour was not used as a signal by predators, bright colouration could become positively correlated with variation in toxicity levels in a

manner similar to that predicted by the model of Blount et al., if indeed bright colouration could incur some alternative fitness advantage. We found that, at high resource levels, the positive colour-toxicity correlation would reverse and become negative, as in the Blount et al. model.

The idea that bright colouration may be paired with toxicity in a prey animal for reasons other than aposematic signalling is not a new one. Notably, Guilford (1988) cautioned against the assumption that the pairing of bright colouration with toxicity necessarily indicates aposematism. Rather, Guilford argued that colouration which brings benefits other than predator deterrence, and which disrupts crypsis, may require effective secondary defences to compensate for the additional attention from predators caused by this colouration (Guilford 1988). The literature now contains numerous examples in which colouration that disrupts crypsis in defended species may have effects which are different to aposematism. Thermal melanism is one example (see discussions in Lindstedt et al. 2008; Williams 2007). Though it may not make animals very brightly coloured, such melanisation may prevent prey having a close colour match to their natural backgrounds, thereby raising conspicuousness (and see Friman et al. 2009).

Another benefit from bright colouration is from mate-choice. Some benefits may arise through sexual selection, in which females favour brightly coloured males. One reason may be sensory bias or, more generally, that brighter individuals capture the attention of mates more effectively and so have raised reproductive rates. In support of the general idea that mate choice provokes bright colouration that may acquire aposematic properties (or vice versa), a growing literature discusses the importance of sexual selection and aposematism for the evolution of conspicuous colour patterns in animals (Lewis & Cratsley 2008; Maan & Cummings 2008, 2009; Tazzyman & Iwasa 2010).

An intriguing example from intra-sexual selection has been recently reported(Crothers et al. 2011) in which variation in the bright dorsal colouration of the toxic poison arrow frog, *Dendrobates pumilio*, has been demonstrated to affect components of male-male competition.

These studies suggest that some of the recently reported positive correlations between toxicity and colouration (Bezzerides et al. 2007; Summers & Clough 2001) are not necessarily aposematic in origin, as Blount et al. assumed, but could reflect the capacity of well defended prey to make better use of other opportunities in their environments.

Even if the origin of bright colouration is not in predator defence, it remains possible that predators can use it as a signal of unprofitability. Hence, in our development of the model, we found the same qualitative results if we allowed predators to be more wary of prey as the average toxicity associated with their colouration increased. This leads to a system in which the primary cause of the correlation is not predation but, nonetheless, predators tend to treat the colouration as a signal and modify their wariness according to their expectations about the prey.

By modifying the framework of Blount et al., it is relatively easy both to envisage circumstances in which bright colouration could become associated with toxicity, and by which predators could then learn (or co-evolve) wariness of brightly coloured, toxic prey, that would stabilize honest aposematic signalling across many prey species. An important conclusion from our modelling, however, is that the primary reason for the observed correlation between colour and toxicity may be unrelated to aposematic signaling; rather, aposematism is an example of an exaptation. Of course, in some cases aposematism may be the only obvious explanation for a colour form because there is no clear sexual, thermal or other explanation (e.g. Cortesi & Cheney 2010), in which case the unmodified model of Blount et al. can explain signal-toxin correlations.

3.7.2 - Crypsis or Aposematism?

The original model of Blount et al. ignored the possibility that some prey may evolve to become cryptically coloured, rather than aposematic. It seems likely, however, that the assumption of resource competition between pigmentation and toxicity would apply equally to cryptic as well as to aposematic colouration. Our predictions were sensitive to assumptions about how effective a toxin could be in protecting an individual prey in the absence of an aposematic display. If this

protection was not very large then we found that (for the conditions simulated) crypsis was used only by prey with relatively low resource levels. In all other cases, prey used aposematic signalling combined with some nonzero level of toxicity, and signal honesty was predicted.

If the toxin could provide very substantial protection to prey in the absence of aposematic displays, then aposematism was not predicted; rather, prey invested in cryptic appearances such that toxicity correlated positively with the degree of crypsis of the prey. We can conclude that, for a large proportion of parameter space, the predictions of Blount et al. are robust, but that an interesting exception is seen when the minimum level of benefits from the toxin is very much greater than that from the display.

We have deliberately excluded the more complex examples in which prey may combine some elements of crypsis with elements of aposematic colouration. A number of authors have suggested that some examples of prey colouration may act to generate crypsis when viewed from a distance, yet have aposematic components that would provoke wariness from predators that are in close proximity (see data and discussions in Tullberg et al. 2005). This scenario is considerably more complex than that devised by Blount et al., because it requires explicit consideration of the distributions of relative positioning of predators and prey. We view this as an exciting possibility for future work.

3.8 - Conclusions

Consideration of the shared resources model of Blount et al. raises a number of important points. First, generalising the model to incorporate a more realistic evolutionary background, or a more realistic set of strategic options, did not limit the capacity of the model to make a general prediction of a positive correlation between conspicuousness of prey colouration and toxicity. Indeed, virtually all of the qualitative results in Blount et al. can be reproduced so that, for example, if we allow prey to become either increasingly cryptic or increasingly aposematic, it is optimal for prey with sufficient resources to become aposematic, and to show a positive colouration-toxicity correlation for a wide range of parameter space. The important

caveat here is that when toxins greatly outweigh advertisement in protecting individuals from attack, then toxicity can correlate positively with crypsis. Importantly, we have shown that a positive correlation between colouration and toxicity can be stable even if predators do not use prey colour as a signal. If prey colouration were to bring some additional and sufficiently large benefit, such as a mating or thermal benefit, then a positive correlation between toxicity and bright colouration can be predicted regardless of whether predators use colouration as a signal. There are likely to be more empirical tests of whether colour is related to toxicity in animals over the next few years. For signal honesty to be completely understood, other benefits of colouration must also be considered explicitly.

Chapter 4 - Modelling the ecological conditions that promote the combination of aposematic and cryptic displays in prey

4.1 - Overview

This chapter presents the first model of combined warning displays and crypsis in prey. There is growing empirical evidence to suggest that combining bright colouration with dull, disruptive markings can act to reduce prey detectability from a distance (Bohlin et al. 2008; Dimitrova et al. 2009; Stevens et al. 2006; Tullberg et al. 2005). Given the heterogeneity of pigment distributions adopted by many aposematic prey, it has been hypothesised that many aposematic species may combine their warning colouration with dull, disruptive patterns to conceal themselves from a distance by mean of disruptive camouflage and hence reduce mortality from naive predators while at the same time maintaining a warning signal for when encountered in close proximity by educated, wary predators (Bohlin et al. 2008; Dimitrova et al. 2009; Edmunds 1974a; Jarvi et al. 1981; Marshall 2000; Rothschild 1975; Stevens 2007; Tullberg et al. 2005). As discussed in chapter 1, the main disadvantage of aposematism as an anti-predator defence is that when predators are uneducated to the signal or vary in their willingness to eat aposematic prey, raised conspicuousness can be costly as it increases detection rates, hence mortality (Endler & Mappes 2004; Ruxton et al. 2004). It is reasonable to hypothesize that further adaptation which could assist in lowering the conspicuousness of an aposematic prey when viewed from a distance may be selected for, especially in light of evidence suggesting that detection distance makes little difference to the effectiveness of aposematic warning signals (Gamberale-Stille et al. 2009).

Despite empirical evidence suggesting that predator's visual systems can be susceptible to the effects of prey achieving disruptive concealment by combining seemingly conspicuous, non background matching colours with dull patterns (Dimitrova et al. 2009; Stevens 2007), and that prey exist which appear to adopt such displays, no theoretical analysis has previously been undertaken to attempt to understand the conditions which might promote combined use of crypsis and

aposematic colouration. This chapter presents a deterministic model in which prey can evolve a combination of cryptic display, warning display and chemical defence, with the relative effects of cryptic and warning display acting antagonistically to influence the overall perceived conspicuousness of the prey with varied weighting over a range of modelled distance intervals. The models build on the resource competition and allocation model of Blount et al. and those presented in chapter 3 in order to assess directly the implications of combined warning signals and crypsis on the predictions of signal honesty made in previous models.

The simulations presented explore a range of prey distributions modelled over two dimensional space to identify under what conditions a combination of crypsis an aposematism proves optimal. The results show that in habitats where prey adopt a random distribution, based on two dimensional area or where prey are often viewed from a significant distance (majority of prey reside at a greater than random distance from predators), that some combination of aposematic display, toxicity and crypsis proves to be optimal for the majority of resource states. Where the majority of prey are encountered in close proximity to predators, crypsis is never optimal and warning signal combined with defence is the optimal strategy for all resource values. Finally the results confirm that where aposematic prey can reduce detection by the possession of cryptic elements to their display, the positive correlation between warning signal strength and toxicity prevails over a greater range of resource values than presented in previous models (Blount et al. 2009). These results may have implications to how conspicuousness is quantified in empirical studies looking for correlations between conspicuousness and toxin levels in prey. It is suggested that in heterogeneously coloured aposematic prey, only the brightest elements of the display should be considered rather than the overall conspicuousness of the animal.

This work was devised and designed by Thomas Lee with advice from Mike Speed and Graeme Ruxton. Coding, implementation and testing were performed by Thomas Lee. Writing by Thomas Lee with minor editorial assistance from Mike Speed.

4.2 Abstract

Many prey species use bright warning displays to signal their toxicity to potential predators, an adaptation known as aposematism. This bright signalling can be costly as it can raise the overall conspicuousness of the prey, leading to increased detection and hence mortality from naive predators or from predators not-susceptible to the effects of the toxins used as a secondary defence. It has been suggested in the literature that some species may reduce this cost by combining the bright elements of their display with seemingly dull patterns in order to disguise themselves when viewed from a distance. Some recently published data supports this hypothesis. In addition, a recent model by Blount et al. (2009) suggested a mechanism by which aposematic displays may act as honest signals, yet considered only conspicuous displays, ignoring crypsis and the possibility of combined crypsis and aposematism. In this paper, we present an extension of the model of Blount et al., presenting the first theoretical model to test the effects of habitat structure and prey distribution on the optimal deployment of warning display, toxicity and cryptic display; assuming that the two display types act antagonistically with varied weighting over a range of modelled distances. We find that in habitats where prey adopt a random distribution, based on two dimensional area or where prey are often viewed from a significant distance, that a combination of aposematic display, toxicity and crypsis can be optimal over a range of resource levels. Further to this we show that as typical viewing distance increases, the level of crypsis adopted by individuals increases. Where the majority of prey are encountered in close proximity to predators, we report no cases of combined displays but pure aposematism. Finally in cases where resources are scarce and prey are more commonly observed from a significant distance, then pure crypsis without defence can be optimal. We suggest that the combination of aposematic and cryptic displays may be common and may have significantly influenced both the evolution and honesty of aposematic signals.

4.3 Introduction

Prey often adopt colouration as a means of defence against predators, whether in the form of crypsis, acting to reduce a prey's visibility against its background [which can include such techniques as background matching, masquerade and

disruptive colouration, see review in Ruxton et al. (2004)], or aposematism, acting as a conspicuous warning signal advertising the prey's unpalatability to predators (Poulton 1890; Wallace 1867). It is however, evident from observing the colouration patterns of animals that the two classifications may not always be mutually exclusive; prey may possess complex patterns which contain elements of both seemingly cryptic and conspicuous colouration, making simple classification of a prey's appearance inappropriate. Many documented and empirically confirmed aposematic species appear not to adopt uniform colouration, but instead combine bright elements with seemingly dull patterns, often in the form of stripes (such as the common wasp, *Vespula vulgaris* and many caterpillar species including the swallowtail butterfly larvae, *Papillio machaon*) and spots (examples including the seven spot ladybird, *Coccinella septempunctata* and the Monarch butterfly, *Danaus plexippus*). Indeed there are many aposematic species which adopt much more heterogeneous combinations of warning colouration and contrasting patterns, most notable in the genus *Dendrobatidae*, a prime example being the poison dart frog *Dendobates leucomelas* with its striking, irregular patches of contrasting yellow and black.

Researchers have speculated that the purpose of displays with strong contrast boundaries is to enhance the recognition and contrast of the aposematic signal preventing mistaken attacks, hence offering enhanced protection for the prey which adopt them; see summary in Aronsson and Gamerable-Stille (2009) and Stevens (2007). Theoretical models have shown that contrasting, repeating displays may be selected for because they are more easily identified by the visual systems of predators when in full or partial view (Kenward et al. 2004). Alternatively, some have speculated that these combined displays may provide an additional function and act to lower the overall conspicuousness of the animal when viewed from a distance, thus preventing detection by means of disruptive camouflage (Bohlin et al. 2008; Dimitrova et al. 2009; Edmunds 1974a; Jarvi et al. 1981; Marshall 2000; Rothschild 1975; Stevens 2007; Tullberg et al. 2005). Indeed there is supporting empirical evidence that conspicuous markings combined with contrasting element of display can aid in disguising prey when viewed from a sufficient distance in both humans (Bohlin et al. 2008; Tullberg et al. 2005) and avian predators (Dimitrova et al. 2009; Stevens et al. 2006).

The origin, and maintenance of aposematic signals has been the subject of active theoretical study over the last three decades (Ruxton et al. 2004). A striking omission of current models is that prey conspicuousness is assumed to hold a fixed value irrespective of viewing distance. Although models exist which consider that prey conspicuousness can vary over a range of values (Endler & Mappes 2004; Ruxton et al. 2009; Speed & Ruxton 2007), to our knowledge, there is no existing theoretical framework that considers the specific interaction between aposematic and cryptic markings and the effect this has on the detectability of a prey when viewed from various distances.

In light of empirical evidence exists suggesting that conspicuous colouration can aid to disguise prey when combined with contrasting patterns, and the lack of theoretical treatments of this phenomenon, we present the first theoretical model to explicitly consider the optimal combination of secondary defence (such as a toxin), warning display and cryptic colouration under a range of ecologically plausible conditions. We utilize a fully deterministic model in which prey can co-optimize their investment in these three traits, assuming that both prey defence and colouration draw from the same limited environmentally acquired resource (Blount et al. 2009), which could be considered to be energy or more specifically antioxidant molecules which can act either as pigments or in the prevention of oxidative stress caused by toxin storage.

Notably, the model of Blount et al. can be used to explain aposematic signal honesty (positive correlations between brightness and toxicity within or between species). Developing the model of Blount et al. (2009) allows us to explicitly test the implications of combined crypsis and aposematism on the predictions for signal honesty in aposematic prey. We explore the effects of prey distribution in two dimensional space and the antagonistic combined effects of warning displays and cryptic colouration over distance to determine the optimal combination of the three critical traits. We use this model to find plausible cases supporting the optimality of combined signals, and the conditions for which pure aposematism or pure crypsis is most likely. In our discussion, we address the implications of such displays to the predictions of signal honesty in aposematism and consider how combined displays could have facilitated the initial evolution of warning signals in prey.

4.4 Methods

Our model structure is based around an existing numerical optimization framework first presented by Blount et al., and we extend the framework to incorporate three anti-predator traits, cryptic display (C), warning display (W) and chemical defence (D). We further extend the model framework to incorporate distance dependent prey conspicuousness based on combined investment in cryptic and warning displays to determine the conditions under which combined displays prove to be optimal.

Within the prey population we assume there are five groups each containing an equal abundance of prey. Each group varies in the level of critical resource available to its constituents (e.g. antioxidant molecules present in food plant matter, see Blount et al, 2009). The strategy of an individual is to optimally allocate its available resource into its three critical traits, cryptic display (C), warning display (W) and chemical defence (D) in order to maximise its survival. For each resource group we therefore represent every possible prey strategy accounting for any investment combination of the three critical traits which does not exceed the resource quantity available to the group.

Investment in these critical traits (and the investment strategies of others it the prey population) affects prey survival. Investment in cryptic and/or warning display increases or decreases the perceived conspicuousness of a prey from an assumed default level based on the distance from which it is viewed by the predator. The rate at which an individual is encountered by predators increases with its level of perceived conspicuousness relative to the other members of the population, whereas the probability that such an encounter leads to an attack decreases with the mean level of investment in defences across the entire population. Finally, the probability that an attack on an individual causes its death declines multiplicatively with that individual's level of investment in defences and with the extent of their aposematic element of signalling. At the start of the model we assume that the frequency of all prey strategies in all resource groups sums to unity, with all prey strategies being equally abundant. The model proceeds in predation rounds with the relative frequencies of these strategies changing based on the their fitness level in each round

until a final stability criteria is met at which point the optimal strategy for each resource group is recorded.

4.4.1 Model Details

We assume that there are five equally abundant resource groups available (denoted R_i, where $i = 1, 2 \ldots 5$), and that prey are assigned randomly to one of these groups for the duration of their lifetime. The prey can 'choose' how to allocate its resource optimally between a combination of cryptic display (C), warning display (W) and secondary defence (D).

Individuals within the prey population allocate the R_i resources available to them according to two heritable traits represented as vertices on a square 2 dimensional matrix with N nodes per side (in all presented models N=101) with the horizontal vertex (j) representative of the proportion of resources allocated to warning display (W) and the vertex (k) representative of the proportion of resources allocated to cryptic display (C). These vertices are modelled on a discrete grid to the nearest percentage point ranging from 0 to 1 in N equal increments (0.00, 0.01, 0.02, ..., 1.00) f_{ijk} therefore represents the population frequency belonging to resource group R_i playing strategy (j,k) meaning that they possess W_{ij}, C_{ik} values are given by

$$W_{ij} = R_i\, j \qquad (4.1)$$

$$C_{ik} = R_i\, k \qquad (4.2)$$

However due to resource limitation we limit our consideration to values which adhere to the following rule

$$W_{ij} + C_{ik} \leq R_i \qquad (4.3)$$

Now that we know the investment in each visual trait for each individual, the level of investment in secondary defence (D) is given by

$$D_{ijk=} R_i - (W_{ij} + C_{ik}) \qquad (4.4)$$

At the start of the model, the frequency of prey across all resource groups and all strategies are set to sum to unity, hence the entire population is represented as

$$\sum_{i=1}^{5} \sum_{j=0.00}^{1.00} \sum_{k=0.00}^{1.00} f_{ijk} = 1 \qquad (4.5)$$

Hence the total population is N_0 and the total number of individuals in any resource group is $N_i = 0.2N_0$.

Simulations begin with a uniform distribution of individuals with all possible trait values [i.e. f_{ijk} is initially identical for all i,j and k]. Frequencies of individuals with different trait types are then assumed to evolve in response to selection imposed by predation. Predation imposes differential survival S_{ijk} on individuals with different attributes (investment in cryptic display, warning display and secondary defences) and this affects the relative proportions of individual strategies that are represented in the next generation. Strictly, we assume that survival is the only component of fitness that is affected by an individual's attributes, such that the relative frequency of a given type of individuals after survival and breeding is given by

$$f'_{ijk} = \frac{f_{ijk}\, S_{ijk}}{\sum_{i=1}^{5} \sum_{j=0.00}^{1.00} \sum_{k=0.00}^{1.00} f_{ijk}\, S_{ijk}} \qquad (4.6)$$

Note that we divide by the sum of the updated frequencies so that the sum of all frequencies over all resource groups and strategies again sums to unity. To ensure that evolved optimal strategies were stable against invasion from all other strategies

we introduce a small level of mutation between generations within each resource group.

$$f''_{ijk} = \frac{\sum_{j=1}^{N}\sum_{k=1}^{N} f'_{ijk}\,\varepsilon}{N^2} + (1-\varepsilon)f'_{ijk} \qquad (4.7)$$

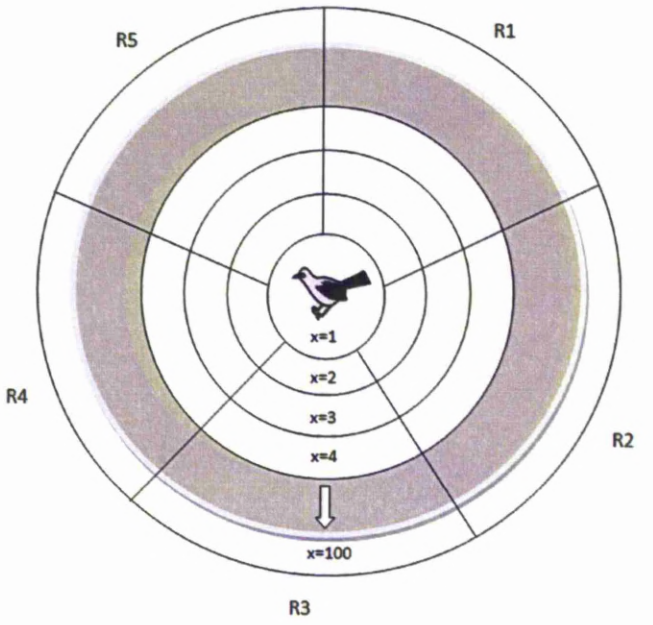
Equation (4.7) ensures that, at each generation, there is some low level of mutation ε. Mutation from any trait value to any other trait value is equally likely. Thus, every trait value loses ε of its potential representation in the next generation to mutation, and gains ε/N^2 of the potential representation of every other trait value within that resource group. This guarantees that our optimally derived strategies are stable by ensuring that every trait type always has the opportunity to invade from rare.

Survival of individuals in any generation is dependent on the resource group they belong to and the strategy they play in term of the proportion of the resource invested in any combination of cryptic display, warning display and chemical defence. Specifically, survival depends on: the rate at which predators are encountered r_{ijk}, the probability of attack given an encounter $P1_{ijk}$ and the probability of death given attack $P2_{ijk}$. Survival is thus given by

$$S_{ijk=}\, e^{-r_{ijk}\cdot P1_{ijk}\cdot P2_{ijk}} \qquad (4.8)$$

The rate at which individual prey encounter predators (r_{ijk}) is dependent on their relative conspicuousness. Because we are interested in understanding the conditions for which combined displays might be optimal, at this stage we consider our prey population to be distributed over two dimensional space (Figure 4.1).

Figure 4.1 – Modelled habitat structure showing the concentric zones of the predator's assumed field of vision (x=1:100). Note, for the purpose of our model, we assume from a game theoretic sense that all resource availabilities are available in all distance zones.



We divide the predator's range of vision into 100 distance zones Z_x where (x=1,2,3....100) extending radially outwards from the predator. These visual zones can be thought to be concentric rings enclosing the predator and hence as distance from the predator increases, the area of habitat contained in the visual zone increases with radial distance. Due to the fact that prey are now distributed over a number of distance intervals we create a frequency distribution representing the proportion of prey frequency contained in each distance zone based on the two dimensional area of the zone.

$$f_x = \frac{\pi(x^2 - (x-1)^2)x^\lambda}{\sum_{x=1}^{100} \pi(x^2 - (x-1)^2)x^\lambda} \qquad (4.9)$$

note, we divide by the sum of all prey frequencies over all distance zones to ensure that the prey frequencies in the system sum to unity. We also provide a facility for biasing this random distribution so that the majority of prey can be shifted to reside in closer, or further proximity to the predator. To achieve this, we introduce a power

term where λ controls the frequency shift towards or away from the predator. When λ <0, the prey are shifted to be in closer than random proximity to the predator, when λ >0 the frequency distribution is shifted away from the predator. When λ =0, the default area based random distribution prevails. This shifting of prey frequency could represent a number of behavioural characteristics of the prey or predators in the modelled habitat as well as ecological constraints, for instance where we shift prey to be in closer proximity to the predator λ <0, this could be caused by space constraints in the habitat or equally be caused by visually heterogeneous habitats in which predators only encounter prey in close proximity as they forage around obstacles. Similarly where we shift frequency away from the predator this could represent scenarios where prey can better distance themselves from predators by early detection of impending threat.

Next, we distribute prey over the distance zones by multiplying the frequency of each strategy in each resource group (which sum to 1) by the frequency distribution for each distance zone (which over all distance zones Z_x sum to 1)

$$f_{xijk} = f_{ijk} f_x \qquad (4.10)$$

We then calculate the perceived conspicuousness of each prey phenotype for each distance zone from which it could be viewed by the predator. We assume that the influence of investment in warning display on the overall conspicuousness of a prey holds the strongest weighting at the closest distance interval i.e. holds a value of 1 and declines by a negative exponential relationship towards 0 as distance increases. We assume that crypsis is opposite, and has a weighting of 0 at the closest distance which increases with distance by a positive exponential to a maximum of 1.

$$v_{xijk} = 1 + \left[(0.5e^{-\alpha_w W_{ij}})e^{-\beta\frac{100-x}{100}}\right] - \left[(0.5e^{-\alpha_c C_{ik}})e^{-\beta\frac{x}{100}}\right] \qquad (4.11)$$

Where β represents a scaling constant for the exponential weighting of distance set to β=0.05 in all presented models.

Next we calculate the average conspicuousness over all strategies in all resource groups over all distance zones given by

$$\bar{v} = \sum_{x=1}^{100} \sum_{i=1}^{5} \sum_{j=1}^{N} \sum_{k=1}^{N} v_{xijk} f_{xijk} \qquad (4.12)$$

Finally we calculate the encounter rate (r) for each strategy and for each resource group over all distance zones. We first determine the encounter rate for each strategy at each distance interval

$$r_{xijk} = \frac{v_{xijk}}{\bar{v}} \qquad (4.13)$$

Then finally we sum over all distance intervals to give a mean encounter probability for each strategy over each resource group weighted by the abundance of prey at each distance interval

$$r_{ijk} = \sum_{x=1}^{100} r_{xijk} f_x f_{ijk} \qquad (4.14)$$

Finally the mean relative encounter rate (r) for any individual is given by

$$r_{ijk} = \frac{v_{ijk}}{\bar{v}} \qquad (4.15)$$

Pilot runs showed that calculating encounter rate based on relative encounter rate (as shown in equaton 4.15) or absolute values v_{ijk} made no difference to the outcome of the model.

The probability that a prey individual, once encountered, is attacked, is assumed to depend on the mean level of secondary defences in the population as a whole given by

$$\overline{D} = \sum_{i=1}^{5} \sum_{j=0.00}^{1.00} \sum_{k=0.00}^{1.00} D_{ijk} f_{ijk} \qquad (4.16)$$

Consequently, the overall probability of attack is given by

$$P1_{ijk} = 0.01 + 0.99e^{-0.1\overline{D}} \qquad (4.17)$$

Where 0.1 scales the exponent. Here we assume that the probability of attack once detected is the same for all prey individuals and is bounded between 0.01 and 1.00 (to ensure that no type of individual is completely invulnerable to attack), the probability of attack increases as the mean toxicity in the population decreases.

We follow the assumptions of Blount et al. and consider that the predator is prepared by evolution to handle brightly coloured prey with care. This assumption forms the primary reason that toxic prey use aposematic displays and it is well supported in the empirical literature (Gamberale-Stille & Tullberg 1999). We also assume that secondary defences can increase the probability of survival at this stage (Skelhorn & Rowe 2006; Wiklund & Jarvi 1982). Thus, the probability that a prey individual dies as the result of an attack is assumed to decrease multiplicatively as a result of increased investment in both the warning element to display W and secondary defences D as given by

$$P2_{ijk} = 0.01 + 0.99e^{-(\gamma + W_{ijk})D_{ijk}} \qquad (4.18)$$

Where γ represents a minimum level of warning signal possessed by an individual.

At this stage detection has already occurred and the decision to attack the prey has been made, hence our assumption here is that the probability of survival is based on the individual prey's level of toxicity and level of investment in the warning signal element of display, which upon capture, is fully evident to the predator. This assumption in our view seems well founded in light of evidence that aposematic prey do not appear to gain in terms of survival from being detected from a greater distance (Gamberale-Stille et al. 2009).

We tested the model with a range of resource levels within a single population (R_1=5, R_2=20, R_3=50, R_4=100, R_5=200) with a mutation rate of ($\varepsilon=10^{-6}$) until a stability criteria was reached (so that the summed absolute magnitudes of changes among frequencies of all trait types were less than 10^{-8} per generation. In each case we modify the distribution of prey across the modelled habitat varying the parameter λ. We tested a range of distributions from λ=-5 to λ=20 in increment of 1 where $\lambda<0$ shifts prey to reside in a closer than random distribution to predatory threat and $\lambda>0$ shifting prey to reside at a greater than random distance to the predators. In all models a stable optimal phenotype was reached for each resource group which was recorded and plotted.

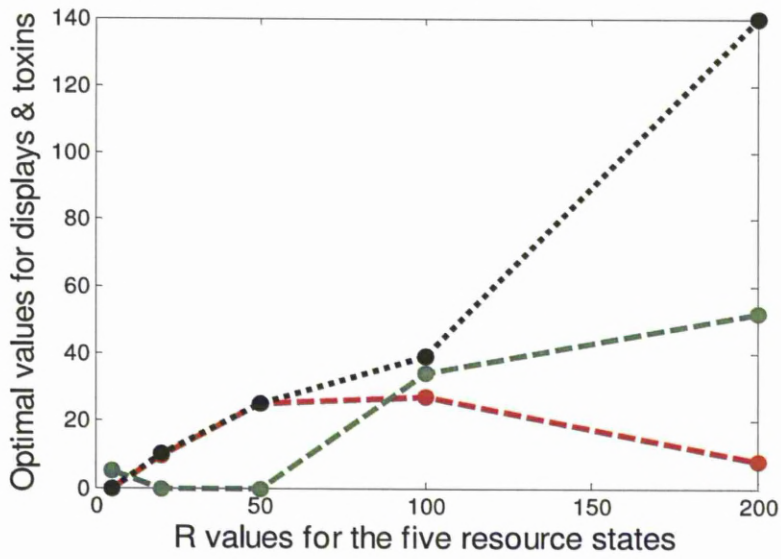
4.5 Results

4.5.1 Random prey distribution

In our first presentation of the model, we assume that prey are randomly distributed across the zones of the modelled habitat based on increasing two dimensional area (λ=0, Fig.1). This distribution could be analogous to a homogenous habitat in which prey are free living and randomly dispersed in open sight. In this example, the majority of prey individuals reside towards the maximum visual range of the predator due to the fact that this zone covers the largest physical area. Our results show that where resources are scarce (R=5, Figure 4.2), the optimal strategy of the prey is to invest solely in cryptic display to prevent detection. Where resources

are more abundant (R=20-50), pure crypsis is no longer an optimal strategy and instead, investment in warning signal combined with toxicity becomes optimal. Where resources are very abundant (R=50-200), we observe that investment in a combination of warning signal, cryptic display and toxicity becomes the optimal strategy. For our highest resource group, we note that the warning element of the display is reduced and the optimal strategy is to invest heavily in crypsis and toxicity. In this case, the prey holds some non-zero value of warning signal so that if detected, even though its appearance is overwhelmingly cryptic, its probability of surviving an attack is increased (see equation 4.14).

Figure 4.2 – Optimal level of investment in warning signal, cryptic display and toxicity for each of the modelled resource groups. Here we assume random prey dispersal across the distance zones based on increasing 2 dimensional area (λ=0). The red line represents investment in warning display, the green line investment in cryptic display and the black line investment in toxicity.



4.5.2 Increased distance between predator and prey

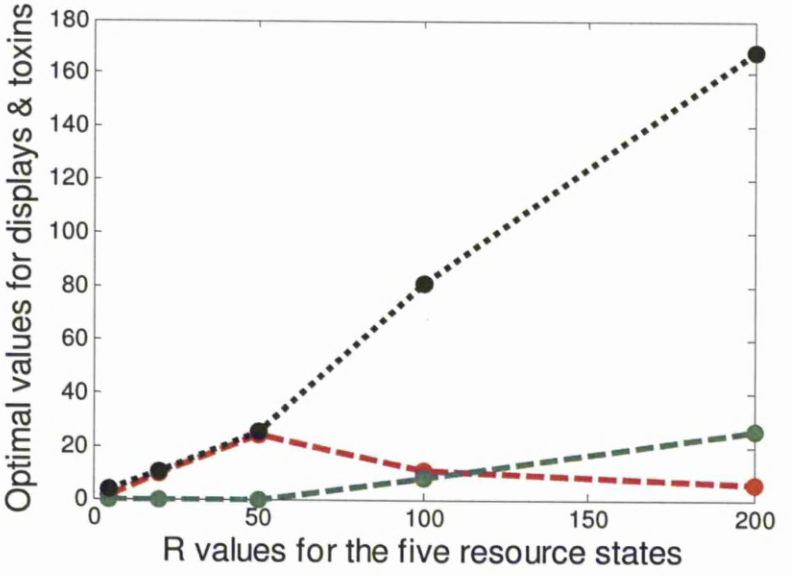
We extended the random prey distribution model to shift the prey distribution so that a greater than random number of individuals reside at the maximum distance interval from the predator (λ=1 to λ=20 in single increments). This scenario could represent cases where the prey can actively distance themselves from predatory

threat. Other reasons why prey might reside in distant proximity to predators might be habitat constraints whereby the predator may be airborne and prey land-bound, ensuring prey detection distance is significantly large. In all tested cases (λ=1 to λ=20 in single increments), our results were qualitatively identical to that of the random predator distribution model (λ=0, see Figure 4.2). We did though observe that for prey with access to high levels of resource (R=200) that as the population was shifted to reside further from the predator, investment in crypsis marginally increased.

4.5.3 Decreased distance between predator and prey

Next, we shifted the prey distribution so that more prey are viewed from a shorter distance than if they were randomly distributed (λ=-2). Here, one could imagine a case in which habitat constraints force predators and prey to reside in closer proximity, simply due to habitat size or geographical constraints. In this model, we observe that for the lowest resource group (R=5, Figure 4.3) crypsis is no longer the optimal strategy. Instead, for resource groups (R=5 to 50) we observe that a combination of warning display and toxicity is optimal. As resources become more abundant we again observe that a combination of all three traits becomes optimal (R=50-200) with the optimal strategy for the highest resource group (R=200) to be mostly cryptic while maintaining some investment in aposematic display and investing heavily in toxicity to ensure that if detection and attack does occur, that the prey's chances of survival is high (see equation 4.14).

Figure 4.3 – Optimal level of investment in warning signal, cryptic display and toxicity for each of the modelled resource groups. Here we assume prey reside or are often viewed in closer proximity to the predator than if they were randomly distributed and in open sight (λ =-2). The red line represents investment in warning display, the green line investment in cryptic display and the black line investment in toxicity.

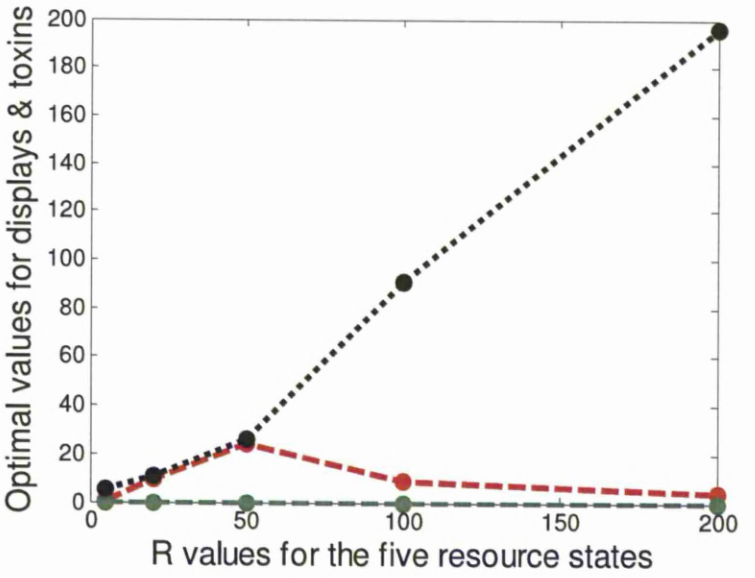


4.5.4 Minimal distance between predator and prey

In our final application of the model, we shift the distribution of prey to model a situation where prey and predators typically encounter each other in very close proximity (λ=-5). This result could apply to situations where predators and prey share space-limited habitats or indeed in highly heterogeneous environments where prey are often hidden and only encountered upon active foraging (one could imagine that this could also be attributed to prey behaviour i.e. behavioural crypsis and hiding). In this model we observe that crypsis is never an optimal strategy (see Figure 4.4). This is due to the fact that cryptic colouration is unable to reduce detection from such a short distance and hence presents a poor economic strategy. For all resource groups tested, some combination of toxicity and warning signal is optimal. This result is identical to that presented by Blount *et al.* in which for lower

to intermediate resource groups, signal honesty pertains and breaks down as resources become more abundant due to the costs of raised encounter rate.

Figure 4.4 – Optimal level of investment in warning signal, cryptic display and toxicity for each of the modelled resource groups. Here we assume prey reside or are often viewed in very close proximity to the predator (λ=-5). The red line represents investment in warning display, the green line investment in cryptic display and the black line investment in toxicity.



4.6 Discussion

All previous theoretical models consider prey conspicuousness to hold a fixed value dependant on investment in a single display trait. However, the results of our model and indeed general observation of many aposematic species indicate that this is an oversimplification of a complex phenomenon; the conspicuousness of a prey's appearance may be highly dependent on the overall heterogeneity of the display and the distance from which it is viewed (Bohlin et al. 2008; Cott 1940; Marshall 2000; Schaefer & Stobbe 2006; Stevens 2007; Stevens et al. 2006). Such combinations of bright displays and seemingly disruptive, dull markings are widespread in aposematic species and across taxa. Our models are the first to consider the combination of aposematic and cryptic elements of prey colouration and make

important predictions as to the ecological conditions which may give rise to such adaptations.

Our models predict that the use of combined displays is highly dependent on the distance from which prey are typically viewed by predators (and of course that the perceptive systems of the predators themselves are subject to such combined blending effects).We further show that when prey are distributed randomly in a habitat based on increasing two dimensional area with radial distance from the predator, that the optimal strategy for prey with low resource availability is to invest solely in crypsis (R=2). For low to intermediate resource availabilities (R=20 to 50), we predict that aposematism without combined cryptic display is optimal. Finally for prey with higher levels of resource availability, the optimal survival strategy is to invest a modest amount in warning signalling, enough to raise the probability of surviving an attack without incurring too much cost to overall conspicuousness, then to invest more heavily in toxins and cryptic colouration. This pattern of majority crypsis with less prevalent aposematic marking seems common in the species thought to adopt such combined displays such as the Swallowtail butterfly larvae *Papillio machaon.* We show that the optimality of combined displays was robust over a range of prey distributions (λ=-2 to λ=20 in single increments).

4.6.1 Predictions for pure aposematism or pure crypsis

Pure crypsis was rarely observed in the simulations. Only when prey have limited access to resources and adopt a random distribution in the modelled habitat (R=2 in Figure 4.2), do we observe pure crypsis as an optimal strategy. Here, prey maximise survival by investing all of their relatively meagre resources into cryptic colouration to prevent detection. Pure aposematism, on the other hand was predicted for all resource levels when prey resided in very close proximity to their predators (Figure 4.4). In this condition, prey detection is likely and from such short distances, investment in crypsis would do little to prevent detection, hence the optimal strategy for a prey is to invest all of its available resources in a combination of warning signal and toxicity to deter attacks after detection. In other conditions (λ=-2 to λ=20 in single increments) it pays for prey to combine some level of crypsis with some level of aposematism (Figures 4.2, 4.3), but notably prey tend to invest more resource in

crypsis as the overall level of resources increases, and often (absolutely) less in aposematic display. Hence a general prediction is that crypsis becomes an increasingly important component of colouration of defended prey as their access to resources increases.

4.6.2 Implications for conspicuousness-toxin correlations

Our model considers that the conspicuousness of a prey can vary depending on the distance from which it is viewed. This in our view may have implications to how positive correlations between prey conspicuousness and toxicity should be assessed. Our model suggests that the addition of cryptic colouration increases the resource range over which there is a positive correlation between toxicity and warning signal strength. In Figure 4.2 (random dispersal of prey, combined crypsis-aposematism observed) we note a positive correlation between toxicity and warning signal strength between R=5 and R=100; however, in Figure 4.4 (very close proximity, no crypsis observed) the positive correlation is now less extensive, ranging from R=5 to R=50. The results suggest that if crypsis is combined with aposematism, the overall conspicuousness of the prey declines as crypsis is included, but the brightness of the signalling component of the prey's appearance continues to increase with toxicity over a greater range. This finding has potentially important implications for empirical studies which increasingly look for associations between toxicity and conspicuousness (Bezzerides et al. 2007; Cortesi & Cheney 2010; Summers & Clough 2001; Wang 2011), suggesting that in addition to measures of overall conspicuousness, the correlation between brightness of signalling components and toxicity needs to be examined separately.

4.6.3 Implications for the evolution of aposematism

The evolution of warning signals still remains a topic of active theoretical debate within the literature (Franks & Noble 2004; Lee et al. 2010; Puurtinen & Kaitala 2006; Ruxton et al. 2004), the main evolutionary paradox being that any individual deviating away from crypsis would suffer raised conspicuousness thus

causing detection and likely death from naive predators. Perhaps in an already defended, cryptic population, the addition of a small conspicuous body part or pattern would not necessarily incur the enhanced detection previously thought (Lindstrom et al. 1999) thus allowing number of these mutants to accrue beyond a critical abundance after which they will be selected for (Puurtinen & Kaitala 2006). Combined displays may therefore present as an intermediate step in warning signal evolution for some species.

4.6.4 Future developments

The models presented in this study do not explicitly consider the detailed perceptual systems of predators which may be exploited by the combination of bright warning signals and disruptive markings. Models in which predators are represented in simulations in terms of specific retinal and cognitive properties would provide a fascinating development of this work to determine the type of pattern which might evolve to exploit such modelled perceptual systems. These patterns could then be compared to those of real prey to determine if similarities exist. The use of artificial neural networks as a modelling tool could provide such a framework, for example, recent models by Kenward et al. (2004), assess how predator visual systems may select for certain pattern combinations in prey against a range of backgrounds or when view is limited. It is conceivable that such modelling techniques could be adapted to explicitly consider distance and the interplay between cryptic and bright displays.

4.7 Conclusions

Our aim was to extend the theoretical framework of Blount et al, (2009) to consider the full scale of prey conspicuousness, more specifically the combination of cryptic and warning displays. We find theoretical support for the benefits of such displays, affirming the supporting empirical evidence (Bohlin et al. 2008; Dimitrova et al. 2009; Marshall 2000; Stevens et al. 2006; Tullberg et al. 2005). Our model further predicts the conditions under which such displays might be selected for rather

than pure crypsis or aposematism and provides insight into how the existence of combined crypsis and warning signals may have influenced the evolution and honesty of aposematic signals. We suggest that the combination of aposematic displays with disruptive, cryptic patterns may be a widespread and common adaptation which invites further empirical and theoretical study.

Chapter 5 - Co-evolutionary change in secondary defences and mimetic resemblance.

5.1 Overview

The final chapter of this thesis presents the results of a co-evolutionary model of mimicry between prey species in which prey can vary and evolve in appearance as well as toxicity level. The previous four chapters have explored the evolution and optimality of warning signals in prey species. This chapter examines how the optimal defence levels of mimetic species can drive further post-mimetic selection on toxin levels and how, in turn, this affects the dynamics of mimicry.

Mimicry of warning signals between prey species presents some of the most compelling cases of co-evolution in the natural world. As such the phenomenon of mimicry has received considerable theoretical study over recent decades (Ruxton et al. 2004). A major omission of current models is that they consider only evolution of the visual signal and assume that toxicity is fixed for the duration of the simulations (Sherratt 2008). Though the importance of signal and toxin evolution was suggested in the early literature (Brower & Brower 1964; Huheey 1976), no model framework exists which allows for the evolution of both signal and defence levels in mimetic prey. Current models also consider pre- and post-mimetic fitness change as a measure of the mutualistic or parasitic nature of the evolved relationship (Kokko et al. 2003; Turner et al. 1984). Though this measure of post mimetic parasitism or mutualism is methodologically justified, as the simulations in this chapter show, this post-mimetic state may be altered by one or more species evolving in toxicity levels driven by the newly afforded protection or indeed parasitism of mimicry, thus further changing the nature of the initial mimetic relationship from mutualism to parasitism or *vice versa*.

The simulations presented in this chapter make a series of novel predictions about the nature and dynamics of mimetic relationships and present a range of outcomes simply by varying the respective abundances and marginal costs of toxicity for each prey species. From these results, three crucial predictions are made. Firstly, it is predicted that Müllerian co-mimics may gain an additional advantage from

mimicry in that they can reduce their toxin investment in their post-mimetic state, an advantage so far unreported in the literature. Second, that increased toxicity of the model, rather than shifting patterns may be a common outcome of Batesian mimicry which again has so far been unreported. Finally, as mentioned above, the post-mimetic evolution and optimization of toxin levels in both species can change the dynamics of the relationship from mutualistic to parasitic even to the extent of an initially mutualistic (traditionally Müllerian) co-mimic evolving to become a fully parasitic Batesian mimic. Further to these main predictions and in the context of the second major heading of the thesis, the results of the simulations presented in this chapter highlight the presence of within-species defection and cheating. The results show that often, when both species evolve to a stable post-mimetic state, that this state is not one which maximises fitness for all members of the population. Hence individual fitness could be improved further by all members of the population cooperating and adopting an alternative toxicity level. It is this within species cheating that facilitates the post mimetic adjustment of toxin levels and hence changes in the nature of the final mimetic state.

Overall, the results presented in this chapter could promote a re-appraisal of the causes and consequences of mimetic evolution and shape future empirical and theoretical treatments of mimicry.

This work was devised from discussion between Thomas Lee, Hannah Rowland, Graeme Ruxton, Johanna Mappes and Mike Speed. Model design was undertaken by Thomas Lee with advice from Mike Speed & Graeme Ruxton. Coding, implementation and testing were performed by Thomas Lee. Initial drafts were written by Thomas Lee (presented here) with the final version contributed to by all authors.

5.2 Abstract

Defensive mimicry, in which different species use similar warning signals, serves as a paradigm case study in co-evolution, including both antagonistic arms races (in parasitic Batesian mimicry) and evolved mutualisms (in Müllerian mimicry). Existing literature focuses only on adaptive change in mimetic signals, and does not consider that the edibility and toxicity of the prey may similarly evolve. Here we describe a theoretical framework within which both appearance and toxicity can co-evolve within mimetic species, and in which prey species may co-evolve in relation to each other. When prey can evolve in toxicity and appearance rather than just appearance, then the course of their evolution may be profoundly altered: for example being mutualistic for one phase of evolution, but parasitic for another phase. Individuals derived from an ancestral defended species can reduce toxin investment as mimicry evolves, switching from a state of initial mutualism to pure parasitism in the process. Traditionally, Müllerian mimicry is seen as a means for defended prey forms to reduce mortality costs of predator learning. We suggest an additional advantage: that members of Müllerian species can reduce their investment in secondary defences. Finally, it is commonly appreciated that the evolutionary response of models to their parasitic Batesian mimics is to attempt to shift their aposematic pattern to escape the burden of mimicry. Our model can predict an alternative response, in which the model species increases its investment in toxins when it is parasitized by an edible species.

5.3 Introduction

Mimetic resemblance between prey species has, for over a century, been an actively studied phenomenon, not least because it represents a powerful case study for the mechanisms of natural selection and provides key examples of mutualism and parasitism between species (Forbes 2009). Mimetic resemblance between two or more aposematic prey species is traditionally divided into two distinct categories. In Batesian mimicry a palatable mimic resembles an unpalatable model, in this case the advantage is to the mimic and the detriment to the model in terms of fitness induced by the education of and avoidance by predators (Bates 1862). In Müllerian mimicry, two or more unpalatable species share the same visual appearance and, according to

Muller's original hypothesis, individuals of both species benefit by sharing the mortality cost of educating predators (Müller 1879). Where Batesian mimicry appears to provide a clean-cut case of parasitism, the umbrella term Müllerian mimicry now encompasses a wide range of alternative hypotheses which have distanced themselves from and call into question aspects of Muller's original hypothesis; that the visual resemblance of defended prey should always be mutualistic (Sherratt 2008). A number of empirical studies have shown that predator avoidance learning is not based solely around a fixed number of encounters with prey, hence questioning the numerical basis for Muller's assumptions (Rowland et al. 2010a; Sherratt 2008). Other theories question in more detail the specific learning mechanisms and receiver biases of predators, and how these may be affected by inequality in the defence levels of the mimetic species in question (Speed 1999; Turner 1987). It seems therefore, that less well defended prey may in some circumstances act in a parasitic manner, diluting the protection of their better defended co-mimics (so called quasi-Batesian mimicry, see Kokko et al. 2003; Marshall 1908; Rowland et al. 2010b; Sherratt et al. 2004; Speed 1993; Speed et al. 2000; Turner & Speed 1999).

Aside from mimicry, there has been a recent focus in the theoretical literature on understanding the optimal toxicity level that defended prey species should adopt in order to maximise fitness (Broom et al. 2005, 2006). These studies acknowledge the evidence that toxins can be costly to prey which adopt them (Longson & Joss 2006); hence there is a trade-off between the advantages of toxicity, in terms of predator avoidance and rejection, and the marginal fitness costs incurred by the possession of the toxin itself. This optimal level of toxin investment has been found to vary significantly depending on other factors affecting overall prey fitness, including levels of predation (Longson & Joss 2006), life history stage, and the presence of other anti-predator defences such as aposematic colouration (Blount et al. 2009; Lee et al. 2011). Although there are a host of theoretical studies aiming to understand the evolution and maintenance of mimetic relationships, focusing on the evolution of signals (reviews in Mallet & Joron 1999; Ruxton et al. 2004; Sherratt 2008; Turner 1977), I feel that a major omission of these models is that they do not consider the evolution of toxicity levels in the prey populations that they model.

Such a constraint seems difficult to justify given that there seems to be no valid reason to expect that prey cannot evolve to alter their defences (Rausher 2001).

Though the importance of signal and toxin evolution was suggested in the early literature (Brower & Brower 1964; Huheey 1976), there have been no attempts to produce a coherent theoretical framework within which toxicity can evolve with mimicry. In this paper we present a novel deterministic framework in which two (or more) prey species are free to co-evolve visual resemblance and levels of defensive toxins and in which predators generalise around prey forms and follow rules of density dependent learning. Our results are the first to show that the evolution of prey defences in mimetic systems may be crucial to fully understanding the dynamics of mimicry in terms of how stable evolved mimetic relationships are and whether these relationships prove to be ultimately mutualistic or parasitic in nature. We argue that the ecology and evolution of mimicry can only be fully understood when prey toxicity levels can evolve with mimetic signals.

5.4 Methods

Table 5.1 - Key model variable and parameter values

Parameter	Description	Value
$\mathbf{V}$	Visual trait	n/a
$\mathbf{D}$	Defence trait	n/a
$\mathbf{V_{min}}$	Minimum value for visual trait (V)	0
$\mathbf{V_{max}}$	Maximum value for visual trait (V)	100
$\mathbf{D_{min}}$	Minimum value for defence trait (D)	0
$\mathbf{D_{max}}$	Maximum value for defence trait (D)	1
f_{ijk}	Prey frequency for any trait combination in a species	Variable, sums to unity for each species (i)
$\mathbf{B}$	Number of discrete intervals into which each trait is divided	101
$\mathbf{N}_i$	Absolute number of prey present in each species	Variable – see individual model description
$\mathbf{A}_{ijk}$	Absolute number of prey for any given trait combination in a species	This is given by $N_i f_{ijk}$
α_L	Inflection point of the density dependent learning function	1000
β_L	Gradient of the density dependent learning function	200
α_{PD}	Inflection point of the probability of death from attack function	0.5
β_{PD}	Gradient of the probability of death from attack function	0.2
min_{PD}	Minimum value for the probability of death form attack function	0.5
α_D	Inflection point of the cost to defence function	0.5
β_D	Gradient of the cost to defence function	0.1
α_E	Inflection point of the encounter probability function	10000
β_E	Gradient of the encounter probability function	2000

α_{S1}	Inflection point of the probability of attack function	0.04
β_{S1}	Gradient of the of the probability of attack function	0.01
γ_1	Scaling factor for the cost of defence for species 1	variable
γ_2	Scaling factor for the cost of defence for species 2	variable
σ_g	Standard deviation for the normal generalization function	4
σ_m	Standard deviation for the normal mutation functions	1
0	Mutation rate	10^{-12}
Generations	Number of seasons simulated	50,000

We evaluated scenarios for the co-evolution of prey appearance and toxicity in a fully deterministic system (see Blount et al. 2009) where predators can learn to avoid prey based on the mean toxicity levels associated with a particular visual trait and the absolute number of prey with that particular appearance, while also allowing predators to generalize across alternative prey appearances.

We consider populations of two prey species; species 1 is represented as i=1 and species two as i=2. Members of both species are assumed to be able to exploit the same trait space, which consists of a visual trait (V_j) and a chemical defence trait (D_k) represented as vertices on a two dimensional matrix. An individual of species (i) with trait values j and k has defence level and visual trait given by

$$V_{ij} = j - 1 \qquad (5.1)$$

and

$$D_{ik} = \frac{(k-1)}{B-1} \qquad (5.2)$$

Without limiting generality, we assume that both appearance and toxin levels are limited to a discrete number of equally spaced levels (within $V_{min} = 0$, V_{max}=100 and D_{min}=0, D_{max}=1), with j and k taking integers between 0 and B (where B=101, the total number of discretized trait nodes). Each individual must exist at one of the nodes in a two dimensional trait space, that position defining both its appearance and its toxicity.

Species 1 begins all simulations with monomorphic visual trait (V=40) and species 2 with monomorphic visual trait (V=60). The two populations are therefore initially distinguishable from each other. We assume for simplicity of presentation that variation in the visual trait does not affect detection rates, so that all starting positions in this trait are initially equivalent with respect to fitness, in the absence of any effects of mimicry (but see Speed & Ruxton 2010). At the start of a generation we ensure that all prey frequencies within each species sum to unity.

$$\sum_{j=0}^{B}\sum_{k=0}^{B} f_{ijk} = 1 \qquad (5.3)$$

Since survival terms are dependent on absolute and not simply relative abundances of prey each species is assigned an absolute number of prey individuals N_i. The absolute abundance of prey belonging to a species i possessing a combination of traits j and k is given by

$$A_{ijk} = N_i f_{ijk} \qquad (5.4)$$

Next we consider how fitness is calculated using a number of component functions.

5.4.1 Encounter

The probability than any one prey individual is encountered by the predator during a season is assumed to decline as the total absolute number of prey increases according to a sigmoid function

$$E_{ij} = 1 - 0.99\left(\frac{1}{1 + \frac{\sum_{i=1}^{2} e^{-N_i - \alpha_E}}{\beta_E}}\right) \qquad (5.5)$$

Where α_E represents the inflection point of the curve (α_E = 10000) and β_E the gradient (β_E = 2000). We set the inflection point of this curve (described by equation 5.5) relatively high, since pilot runs showed that selection for mimicry was strongest when encounter rates were high.

5.4.2 Probability that an encounter is converted into an attack

Next, we consider the probability that once encountered, a predator decides to attack the prey in question. In calculating this probability we assume that the predator(s) can gain knowledge of the mean toxicity level associated with each visual trait value, *j*, from past encounters with the prey. Predator knowledge is thus weighted by a logistic density dependent function so that knowledge about the mean toxicity level associated with a particular visual trait is weighted by the absolute density of prey with that visual phenotype and their respective toxicity levels and is also amended to include generalisation across prey appearances.

5.4.2.1 Mean defence level for each visual trait

There is growing empirical evidence to support the suggestion that predators base per encounter attack probabilities on the mean toxicity levels of prey presented to them (Lindström et al. 2006; Rowland et al. 2010b; Speed et al. 2000), hence we calculate the mean defence level (*i*) associated with each visual phenotype (*j*). Note that mean toxicity is weighted by the absolute abundance of prey at each of the defence levels (*i*).

$$\overline{D}_j = \frac{\sum_{i=1}^{2}\sum_{k=0}^{B} A_{ijk}D_{ijk}}{\sum_{i=1}^{2}\sum_{k=0}^{B} A_{ijk}} \qquad (5.6)$$

5.4.2.2 Density Dependent Learning

In our model, naive predators assume that novel prey are edible and hence always attack them. If prey are defended, predators can learn to avoid them and tend to do so more quickly as the defence level of the prey increases. Positive correlations between learning rates and intensity of reinforcing stimuli are well established within the animal learning literature (Rescorla 1971; Speed 1993). Predator knowledge about the defence levels of a prey of any given appearance increases as a product of prey number and the defence level of each individual prey with that appearance. This ensures that prey with high toxin levels and/or high abundance support higher levels of predator knowledge than those with low toxicity levels or low abundance. Fully Batesian mimics with a toxicity level of zero cause no change from the naive attack probability.

$$L_j = \frac{1}{1 + \frac{e^{-(\sum_{i=1}^{2}\sum_{k=0}^{B} f_{ijk}N_iD_{ijk})-\alpha_L}}{\beta_L}} \qquad (5.7)$$

Where α_L represents the inflection point of the logistic function and β_L the gradient. With this equation it is possible that if the model numbers are insufficient to complete avoidance learning, an influx of abundant, less defended mimics can increase the predator's knowledge state and hence act in a mutualistic manner, despite inequality in defence levels. Hence, we can predict both mutualism and parasitism when there are inequalities in defence levels between defended mimetic species (Sherratt et al. 2004).

5.4.2.3 Predator Generalization

Predators generalize between prey such that the attack probability on prey of a given appearance is most strongly influenced by the attack rates on prey of similar appearance (Balogh et al. 2010; Balogh & Leimar 2005; Ruxton et al. 2008; Speed & Ruxton 2010). The total protection received by an individual with a particular visual appearance (V) is therefore based on the mean toxicities and learning states associated with that specific visual appearance and on the mean toxicity and learning states of prey of other visual appearances, weighted by visual similarity by the predators' (Gaussian) generalization function. The mean defence level for each visual trait, adjusted for learning effects and predator generalization (Z) can now be given as

$$Z_{ijk} = \sum L_{ij}\bar{D}_{ij} \frac{\frac{1}{\sigma_g\sqrt{2\pi}} e^{-\frac{((1,B)-j)^2}{2{\sigma_g}^2}}}{\sum_0^B \frac{1}{\sigma_g\sqrt{2\pi}} e^{-\frac{((1,B+1)-j)^2}{2{\sigma_g}^2}}} \qquad (5.8)$$

We ensure that all weights in our generalization distributions sum to unity. If a species lies sufficiently close to the maximum or minimum values of the visual trait we truncate their Gaussian distributions, representing generalisation at a stimulus boundary (see method in Speed & Ruxton 2010). To minimise this complication, we positioned the prey some distance from this boundary such that truncation effectively had no influence on our results. Finally, the overall probability of attack can be given by

$$S1_{ijk} = 1 - \left(0.01 + 0.99\left(\frac{1}{1 + \frac{e^{-z_{ijk}-\alpha_{S1}}}{\beta_{S1}}}\right)\right) \qquad (5.9)$$

Where the latter term describes a series of j normal curves with a standard deviation σ_g centred around each visual trait V which are summed to give the overall survival after generalization effects have been considered.

5.4.3 Probability that an attack leads to the death of the prey

This is assumed to decline with increasing investment in chemical defence (D) as described in equation 5.5, since chemical defences can aid in preventing the death of a prey individual. Many species combine aposematic displays and toxic secretions/tissues on peripheral, non life critical body areas (Wiklund & Jarvi 1982) and peck marks have for example commonly been observed on the wing tips of aposematic butterflies (Edmunds 1974b) suggesting taste-rejection in the wild. Hence, the probability that an attack leads to death is given by,

$$P\_D_{ijk} = 1 - min_{PD}\left(0.01 + 0.99\left(\frac{1}{1 + \frac{e^{-D_{ijk} - \alpha_{PD}}}{\beta_{PD}}}\right)\right) \qquad (5.10)$$

where α_{PD} is the inflection point of the function (set to 0.5) and β_{PD} the gradient set to 0.2 and min_{PD} is the minimum value that the function can take (so that even for a prey with a high toxicity level, an attack from a predator can still cause some injury and a nontrivial chance of death). Without imposing this limit selection for mimicry and indeed any form of a defence would be much weaker with prey surviving the majority of attacks.

5.4.4 Marginal costs of chemical defence

Toxin storage and synthesis can be costly to prey individuals in a number of ways (for a review see Ruxton et al. 2004). Hence in our model we include a logistic function to describe the fitness costs associated with possession of chemical defence (D) see equation 5.11.

$$C\_D_{ijk} = 1 - \gamma_i\left(\frac{1}{1 + \frac{e^{-D_{ijk} - \alpha_D}}{\beta_D}}\right) \qquad (5.11)$$

where α_D is the inflection point of the function and β_D represents the slope of the function. We introduce a scaling factor γ_i which scales the severity of the fitness loss as toxicity increases. We vary the costs of toxicity for both species in our tests (γ_1, γ_2).

5.4.5 Reproduction

The overall survival for any trait combination in each species is given by

$$S_{ijk} = (e^{-E_{ijk}P\_A_{ijk}P\_D_{ijk}})C\_D_{ijk} \qquad (5.12)$$

Hence, at the end of each season we update the frequencies of prey as follows

$$f_{ijk}{}' = f_{ijk}S_{ijk} \qquad (5.13)$$

5.4.6 Mutation

We allow mutation in both the visual and defence trait space. We do this by taking a small proportion of prey frequency from each of our trait nodes and distributing them to neighbouring nodes (Blount et al. 2009) but weighting the distribution of mutations by two Gaussian distributions centred around the trait value being mutated, one for visual mutations and one for defence mutations as described below (see Balogh & Leimar 2005).

Mutation in the visual axis (V):

$$f_{ijk}'' = f_{ijk}'(1-\theta) + \sum f_{ijk}'\theta \frac{\frac{1}{\sigma_m\sqrt{2\pi}} e^{-\frac{((1,B)-j)^2}{2\sigma_m{}^2}}}{\sum \frac{1}{\sigma_m\sqrt{2\pi}} e^{-\frac{((1,B)-j)^2}{2\sigma_m{}^2}}} \qquad (5.14)$$

Mutation in the defence axis (D):

$$f_{ijk}''' = f_{ijk}''(1-\theta) + \sum f_{ijk}'\theta \frac{\frac{1}{\sigma_m\sqrt{2\pi}} e^{-\frac{((B,1)-k)^2}{2\sigma_m{}^2}}}{\sum \frac{1}{\sigma_m\sqrt{2\pi}} e^{-\frac{((B,1)-k)^2}{2\sigma_m{}^2}}} \qquad (5.15)$$

After mutation, all frequencies within a species (i) sum to unity, we hence apply the following equation to morph frequencies

$$f_{ijk}'''' = \frac{f_{ijk}'''}{\sum_{j=1}^{B}\sum_{k=1}^{B} f_{ijk}'''} \qquad (5.16)$$

Initial parameter testing and visual inspection of the key functions of the model were performed prior to the presented results set. For brevity we present results for the model as using a set of plausible example parameter values (Table 5.1). Our model explorations, however, make us confident that the qualitative phenomena described in the results section are robust to plausible perturbations both to computational implementation (especially of the initial conditions and process of mutation) and the values given to parameters.

Initially, the frequency of prey within each species is equally distributed across all possible defence levels. From this starting point we first allow toxicity to evolve toward its optimal level in the absence of mimicry (constraining the visual trait to a constant value without mutation for 1000 generations). We subsequently allow mimicry to evolve (by allowing mutation in V after generation 1000) and we trace the trajectory of the population over time from non-mimetic to mimetic states. This is analogous to starting the simulation with both species being visually distinct with no overlap in the predator's generalization around each signal. Rather than explicitly modelling a larger visual trait range and setting the initial prey species to reside in greater distance within the trait space V, to save on computational time we opted to simply prevent evolution in the visual trait for the first 1000 generations. Pilot studies in which mutation in visual appearance was permitted from generation 1 and where species 1 were initially set to monomorphic V=100 and species 2 were set to monomorphic V=900 (over a total range of 1000) showed that the model predictions remained unchanged.

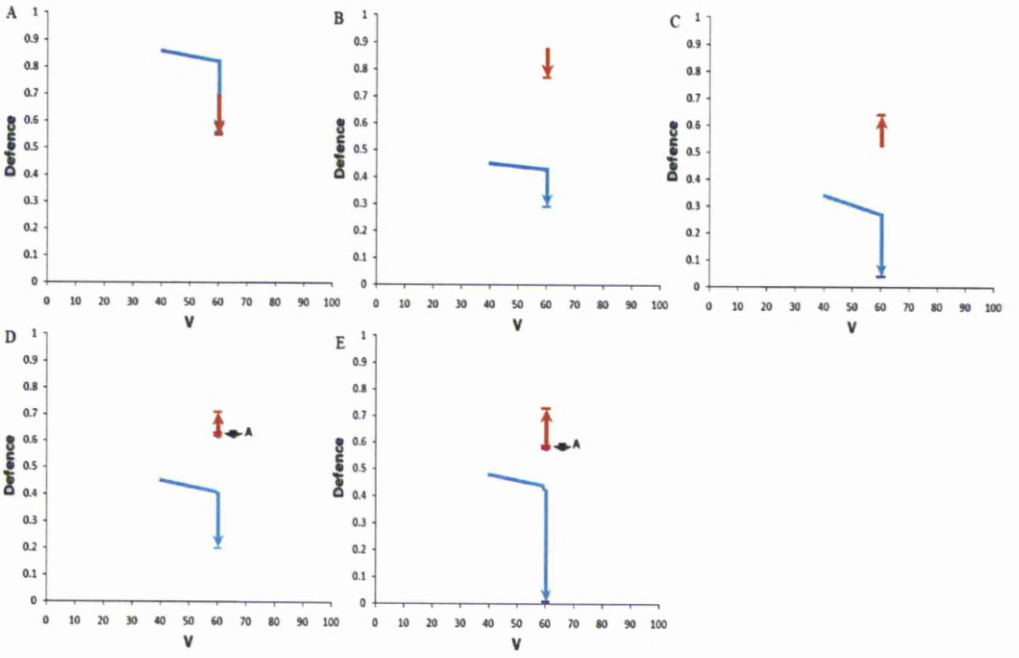
For clarity in our figures, we do not show the first 1000 generations where each species is allowed to evolve toxicity prior to mutation being allowed in the visual trait. There is rapid convergence to a very narrow distribution around a single modal value for investment in defence and for visual appearance. In the figures presented in the main text, we therefore consider the modal trait combination for each species. In line with contemporary theory (Mallet 1999) our simulations predicted advergence of one species appearance toward another, rather than convergence by both prey species to an intermediate mimetic phenotype (see appendix for further details)

5.5 Results

We evaluated scenarios for the co-evolution of prey appearance and toxicity in a fully deterministic system where predators can learn to avoid prey based on the mean toxicity levels associated with a particular visual trait (V) and the absolute number of prey with that particular appearance, while also allowing predators to generalize across alternative prey appearances. We present five scenarios in which we vary the marginal costs of toxicity for each species (γ_1 and γ_1) and the starting

abundance of each species (N_1 and N_2), these being the key parameters affecting the model outcome. For further details, please refer to our methods section.

Figure 5.1 - Arrow plot showing the modal phenotype for each species over evolutionary time. The blue arrow represents species 1 and the red arrow represents species 2. **A.** (N_1=1000, N_2=3000, γ_1=0.3, γ_2=0.3). The appearance of the more toxic "model" does not change over time, but the appearance of the less toxic "mimic" does change to resemble the model. Further, once perfect mimicry is achieved, both species are selected to reduce their investment in defences to an equal level. **B.** (N_1=2000, N_2=1000, γ_1=0.6, γ_2=0.3). The less toxic species 1 evolves to visually resemble the more toxic species 2 and once this resemblance has occurred, both species decrease their level of toxin investment, indicating a mutualistic relationship. **C.** (N_1=3000, N_2=6000, γ_1=0.6, γ_2=0.3). Members of the less toxic species 1 evolve to visually resemble those of the more toxic species 2. However, once visual resemblance is established, individuals of species 1 reduce their investment in defensive toxins while those of species 2 increase investment in toxins to compensate for the parasitism of the quasi-Batesian mimic (species 1). **D.** (N_1=2000, N_2=2000, γ_1=0.6, γ_2=0.3). Individuals of the less toxic species 1 evolve to visually resemble the more toxic individuals of species 2 and in doing so, individuals of species 1 reduce investment in defensive toxins. The modal defence level for species 2 initially reduces as the mimic engages (labelled A in the figure) but then increases as members of the mimetic species 1 further reduces their defensive toxin investment indicating a switch from mutualism to parasitism. **E.** (N_1=2000, N_2=3000, γ_1=0.6 γ_2=0.3). Individuals of the less toxic species 1 evolve to visually resemble the more toxic individuals of species 2 and in doing so, individuals of species 1 reduce investment in defensive toxins to approximately zero. We observe that the modal defence level for species 2 initially reduces as the mimic engages but then increases as members of the mimetic species 1 further reduces their investment in toxins indicating a switch from mutualism to fully parasitic Batesian mimicry. See appendix figures A5.1-A5.5 for corresponding mean fitness and toxicity plots for each presented scenario.



5.5.1 Scenario 1 – Mimetic mutualism with equal toxicity

We first set the absolute abundances of the species such that species 2 is three times as common as species 1 (N_1 = 1000, N_2 = 3000) and assume that both species pay the same marginal costs of toxicity (γ_1=0.3, γ_2=0.3). When only the defence trait can initially evolve (and therefore in the absence of mimicry), the less numerous species 1 evolves a higher level of toxicity than species 2. This difference reflects the fact that predators know less about species 1 with which they have fewer encounters, than they do about the more numerous species 2 (equation 5.7). The rarer species 1 needs therefore to invest more in toxicity to compensate for the fact that predators know less about its defences.

When the visual trait is also allowed to evolve (after generation 1000) there is advergence of the more toxic, but less abundant mimic to the less toxic but more abundant model (Figure 5.1 panel A). In this mutualistic scenario, an individual's risk of attack is reduced when it takes on perfect mimicry; hence its need to invest in defence is similarly reduced. We also observed a net increase in population level fitness for both species once mimicry engages, confirming the mutualistic nature of the relationship (see **Appendix** Figure A5.1).

5.5.2 Scenario 2 - Mimetic mutualism with unequal toxicity

Next we set the abundances of the species to be N_1=2000 and N_2=1000. We assume that species 1 pays a higher marginal cost to toxicity, twice that of species 2 (γ_1=0.6, γ_2=0.3). Prior to mimetic evolution, species 1 is both more abundant and less toxic than species 2. When mimicry is allowed to evolve, species 1 adverges to resemble the less abundant but more toxic species 2 (Figure 5.1 panel B). Again as in scenario 1, both species experience a net reduction in toxin investment indicating that the resulting mimetic relationship yields pure mutualism for both species (and see confirmation in Appendix Figure A5.2). Mutualism, even with differences in toxicity levels occurs because the nastier species is relatively rare, and hence predators have partial knowledge that it is defended. Addition of a mimic increases the knowledge state substantially (see equation 5.7) more than offsetting the fitness cost caused from a reduction in mean toxicity for the model-mimic group (equation 5.6).

5.5.3 Scenario 3 - An example of pure parasitism (quasi-Batesian mimicry)

We next increased the abundance of both species to N_1=3000 and N_2=6000, and now predators have relatively accurate knowledge about the toxicity of both species before mimicry evolves. The cost coefficient for species 1 is again double that of species 2 (γ_1=0.6, γ_2=0.3) so that prior to mimetic evolution, species 1 is both less abundant and less toxic that species 2. Once engaged as a mimic, individuals of species 1 reduced investment in toxicity due to the new mimetic protection (Figure 5.1 panel C). Species 2 however now has a parasitic burden from a quasi-Batesian mimic (species 1), but rather than attempt to shift its warning signal away from that of the mimetic parasite, individuals of species 2 increase their levels of toxicity to compensate for the costs of parasitism. Notably this co-evolutionary response by the parasitized species benefits the parasitic mimetic form by increasing its protection further. This scenario therefore shows a case of purely parasitic quasi-Batesian mimicry, (see Appendix Figure A5.3). In extensive pilot work we did not find that the parasitized model species evolved its appearance away from its original trait

value unless mutation rates and breadth of predator generalization were set exceptionally high (see appendix for further details); hence co-evolutionary chase in mimetic signals was not observed under reasonable conditions. The primary force preventing a model moving away from its parasitic mimic is our density dependent learning function (equation 5.7), which punishes rare forms with high attack rates.

5.5.4 Scenario 4 - A switch from Müllerian mimicry to quasi-Batesian mimicry

Not all mimetic relationships remain simply mutualistic or parasitic as can be seen when we give the species equal abundance (N_1=2000 and N_2=2000) and assume that species 1 pays double the marginal cost of toxicity as species 2 (γ_1=0.6, γ_2=0.3). For a period of time from when the mimic (species 1) engages the model (species 2), we observe a net reduction in toxicity for both species indicating a period of mutualism (see Figure 5.1 panel D, black arrow labelled A represents the initial reduction in toxicity of species 2). Next, as species 1 continues to reduce investment in toxins, species 2 switches to begin increasing investment in defensive toxins, indicating that the relationship has switched from mutualistic to parasitic (Appendix Figure A5.4). When species 1 evolves to resemble species 2 there is initially a mutual reinforcement of protection as both species have invested relatively heavily in toxicity in their pre-mimetic state. Such mutualism is destabilised however when individuals of species 1 continue to reduce their toxicity level (due to paying a higher cost). Such defection changes the mimetic relationship from mutualism to parasitism and forces individuals of species 2 to begin to invest more heavily in toxins.

5.5.5 Scenario 5 - A switch from Müllerian to parasitic Batesian mimicry

A similar, but more extreme result pertains if we set the absolute abundances of the species to be N_1=2000 and N_2=3000 (again assuming that the cost coefficient of species 1 is twice the marginal cost of toxicity of species 2, γ_1=0.6, γ_2=0.3) so that species 1 benefits by advergence to species 2 (Figure 5.1 panel E). In order to demonstrate removal of all toxicity from the initially mutualistic, defended mimic,

we assumed that the marginal survival benefit from the possession of toxins is lower than in other simulations (equation 5.10, pleases see Appendix Figure A5.8). The resulting outcome of this scenario is similar to the previous one, with species 1 adverging to resemble species 2 followed by a period of declining toxicity for both species (mutualistic phase). As in Fig.1d, there is a continued reduction in toxin investment for species 1 and a switch to increasing toxin levels for species 2 indicating a parasitic phase (see Figure 5.1 panel E). The mimic (species 1) reduces investment in toxicity to a mean value of 0.01 over a period of 50,000 generations (see appendix figure A5.5). This likely represents an optimal value of zero, but kept very slightly higher by mutation-selection balance. To check this we began a simulation with all members of species 1 as visual mimics of species 2 (V=60) and all with a toxicity of zero, after 10^5 generations the modal toxicity was negligibly different to zero (9×10^{-3}), again explained by mutation-selection balance.

5.6 Discussion

Our simulations represent the first model to attempt to integrate the co-evolution of signal mimicry and investment in secondary defences. We argue that the evolution of signal mimicry between the prey species can act as a driver for adjustment of the optimal levels of defence, with mean toxicity of a population increasing when it is under parasitic load and decreasing when gaining from mimetic mutualisms. Three predictions arise from our model. 1. Müllerian co-mimics may gain an additional advantage from mimicry in that they can reduce their toxin investment in their post-mimetic state. 2. That increased toxicity of the model, rather than shifting warning patterns may be a common outcome of Batesian mimicry. 3. That the post-mimetic evolution and optimization of toxin levels in both species can change the dynamics of the relationship from mutualistic to parasitic. These results give reason to re-appraise the causes for the evolution and maintenance of Müllerian mimicry, which could be seen as a means of reducing the costs of investment in chemical defences. Next, we consider the key findings of the model in more detail.

5.6.1 Mutualism or Parasitism?

Investigations of previous models of mimicry have often focussed on the fitness change between pre- and post-mimetic engagement (convergence or advergance of the signal) as a measure of the mutualistic or parasitic nature of the relationship (Kokko et al. 2003; Turner et al. 1984). However, our results indicate that this initial post-mimetic state often only represents a transitory state subject to further modification in the toxicity levels of the respective prey species. In some cases we show that the fundamental nature of mimetic relationships can remain qualitatively unchanged. For example, in scenario 1 we show that an initially mutualistic relationship is maintained, even after both species reduce their investment in toxicity due to improved survival from the mimetic resemblance. In alternative cases, however, we show that a state of mimetic mutualism can shift to parasitism as members of one prey species are able to reduce investments in toxicity so much that it damages its co-mimic partner (scenarios 4 & 5). Whether or not we observe mutualism, parasitism or a combination of both is dependent on the abundance of prey in the populations of each species and the marginal cost of toxicity that those individuals pay. Mutualism is predicted when both of the species are at sufficiently low abundance that predators have relatively incomplete knowledge of their toxicity in the absence of mimicry (see equation 5.7 above). In this case predators are prone to attack the species as if there is a high chance that individuals are not toxic. When the appearance of species 1 adverges to resemble species 2, however, there is now a much larger abundance of individuals with the same appearance. Predators now have a more complete knowledge that the prey are defended and so reduce attacks, to the benefit of both species. In the case of equal toxicity costs (scenario 1, Figure 5.1a), both species converge on the same toxicity value. However we can explain different optimal toxicities within a pair of mutualistic co-mimics by assuming that the marginal costs of toxins are different between the species. For example if species 1 pays higher marginal costs to toxicity, this species tends to a lower level of toxicity than species 2 after mimicry evolves (Figure 5.1b).

In contrast, parasitism is often the outcome of the system when the abundance of one or both species is very high. When mimicry evolves there is now little to gain

from density dependent learning, since predator knowledge about defence is already very good (equation 5.10). Now fitness is much more strongly affected by the inequality in defence, in which less well defended individuals decrease mean toxicity and hence increase per encounter attack rates (Figure 5.1c,d). Hence a major determinant of whether mimicry is predicted to be mutualistic or parasitic is the relative influence of predator learning state (which favours mutualism) vs. discrepancies between defence levels (which favours parasitism). We further show that under alternative conditions where toxic prey are less likely to survive attacks from predators and the mimic pays much higher marginal costs for their toxins, that an initially mutualistic model/mimic relationship can lead to parasitic quasi-Batesian mimicry (a parasitic relationship between two defended types) and then full Batesian mimicry as the mimic reduces its investment in toxicity to zero (scenario 5).

As we noted earlier, the literature tends to focus very specifically on the evolution of appearances in mimetic prey, but there are examples in which enough is known about colour and chemical defence to provide evidence for this switch in mimicry status. A good example is found in the Tiger beetles (genus *Cidinela*) (Vogler & Kelley 1998). One species (*C. rufiventris*) is cited as a candidate that transformed its status from Müllerian to Batesian mimicry through loss of toxicity. Similarly Rutland's studies of the Viceroy (*Limenitis archippus*) and Florida queen (*Danaus gilippus*) show considerable variation in the relative toxicity of the species perhaps providing candidate populations in which parasitism is the outcome whereas elsewhere it is mutualism (Ritland 1991, 1994). Although we do not consider such extensions explicitly in our present model, one might expect the opposite case to also be possible, that a Batesian species that acquires toxins, either because they are cost free (e.g. digestive presence of toxins from a new host plant) or indeed by adaptive change that makes toxin possession less costly, might switch to from being a Batesian to fully mutualistic Müllerian mimic (see Huheey 1976).

5.6.2 Absence of coevolutionary chase

We found little evidence for co-evolutionary chase, in which models and mimics engage in cycles of phenotypic change (Gavrilets & Hastings 1998). Only when mutation rates were set extremely high ($\theta < 0.01$) and the standard deviation of

the predator's generalization was sufficiently large (σ_g>8) could modal shift in the model's visual trait away from that of the mimic be observed (see appendix for further details). In all other tests of our model we found no evidence of co-evolutionary chase in visual appearance, even where the evolved mimetic relationship was traditionally Batesian and fully parasitic to the model species. One reason for this is, as hypothesised in the literature, that new mutant forms of the model that are sufficiently distinct from the parasitic mimic will initially be very rare in an environment and likely to be attacked by ignorant predators. Hence very strong density dependence makes the model resistant to evolutionary change (Nur 1970). Rather, a novel prediction of our simulations is that the model species is expected here to compensate for the costs of parasitic mimicry by increasing investment in toxicity. In this case parasitism pays off doubly, since the parasitic mimic wins even greater protection because of the harm that it initially does to the model species. If there are strong density dependent constraints on change in mimetic patterns, then we suggest that a frequent response to mimetic parasitism may well be an increase in individual levels of toxicity within a prey population.

5.7 Conclusions

The evolution of mimetic signals is important because it provides clear, visible evidence of adaptive evolutionary change. It is therefore understandable that the vast majority of mimicry studies explore change in appearances of prey species, and neglect the possibility of change in levels of secondary defence. One major conclusion that can be drawn from this work is that comprehension of mimicry as an adaptation also requires the consideration of changes in the levels of secondary defences of mimetic species. This view of mimicry may be rather different to that generally considered: mimetic mutualisms may arise because they allow levels and therefore costs of toxicity to reduce; such mutualisms may often be conditional, with some likelihood of a switch to parasitism by one member of a mimetic pair. Finally, the common response to Batesian parasitism may not be change in appearance of the model species in an attempt to disengage the mimic, rather compensation by increasing toxin levels may be a common response of Batesian models.

OVERALL CONCLUSIONS

This thesis presents a series of models aimed at providing a fresh approach to answering some of the remaining pertinent questions associated with warning signal evolution and optimality. In order to achieve this, these models re-appraise more closely existing theory, taking into account new ideas, modelling techniques and empirical data to present a series of novel and updated findings and hypotheses. Often, in pursuit of the answers to a given problem, theoretical models create further questions. Indeed the theoretical models and results presented in this thesis provide a list of unresolved questions and directions for future work. It is this cycle of empirical observations and theoretical testing which advances our understanding of these complex processes. If this work only acts only to influence and provide further direction of study within the field then it has served its purpose well.

FUTURE WORK

As outlined in the introduction, the pursuit of using theoretical models to understand how evolution has acted to promote certain traits and outcomes often leads to further questions being raised. Below is a summary of the key questions and directions for future work that this study has raised and partial suggestions on how these questions might be answered.

Chapters 1 and 2

- More empirical study is needed on the nature of dietary wariness in predators. Current data suggests that there is large variation in dietary conservatism tendency between individual predators. What is not known is why such large amounts of variation exists within populations of predators at any given time. Perhaps previous experiences with novel, aversive food items acts to increase future wariness of novel foods. It is reasonable to expect that hunger or ingested toxin levels of individuals might influence willingness to sample new prey items. There may be some hereditary

link to behavioural boldness. Further study is also needed to determine whether dietarily conservative predators learn to avoid distasteful prey at a faster rate than less conservative predators. When more is understood about the function of dietary wariness in predators, more realistic state-dependent models applied to evolving prey populations such as those presented in chapters 1 & 2 will make more realistic predictions as to the extent dietary wariness might select for novel conspicuous prey.

Chapter 4

- The model presented in chapter 4 which examines combined displays in prey could be extended to remove the limitation of resource competition between defence types as this is unlikely to apply to all aposematic species (indeed this is only a theoretical hypothesis and remains to be empirically tested). The predictions for the optimality of combining aposematism with elements of crypsis could then be tested in a more general context.
- As described in the discussion, an explicit model accounting for the specific retinal and cognitive abilities of known predators would allow us to test the mechanisms by which "concealment by conspicuousness" can operate. This could be extended to allow the evolution of patterns which exploit these shortcomings of visual perception in predators. The resulting patterns could then be compared to the patterns of real life prey thought to adopt some level of combined crypsis and warning signal to determine if any similarities exist

Chapter 5

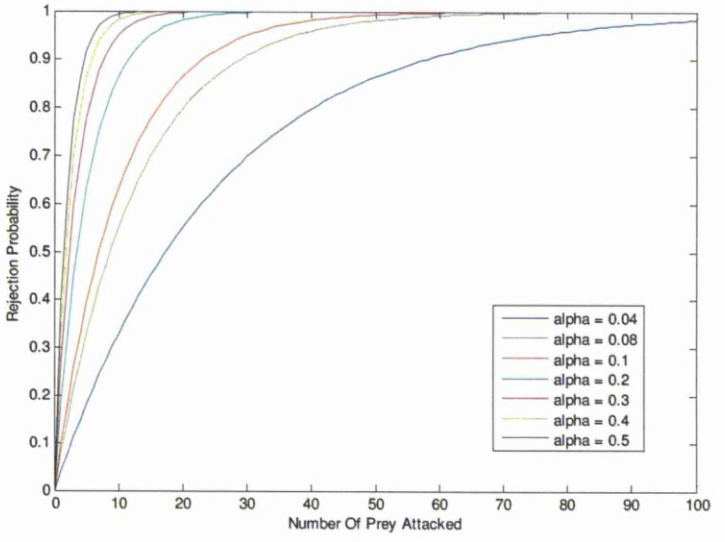
- In chapter 5, it is assumed that all values of visual trait (V) hold the same conspicuousness value. A valid extension of this work would be to scale prey conspicuousness with this visual trait. Such a model could be used to further explore the possibility of co-evolutionary chasing and shifting of aposematic patterns.
- The mimicry/toxin co-evolution model presented in chapter 5 considers no interspecific competition between the mimetic species. Recent empirical studies have shown that the trade off between competition for niche space and mimetic mutualism

can promote the coexistence of ecologically undifferentiated species (Alexandrou et al. 2011) and hence the implementation of competition the model would be a worthy addition in order to determine how the dynamics of mimicry might be influenced by ranging degrees of niche overlap between species and how mimetic resemblance might affect the outcome of competition, perhaps preventing competitive exclusion in cases where in the absence of mimetic mutualism, it may be expected.

APPENDIX

A1 - Appendix for chapter 1

Figure A1.1 - How rejection probability varies with number of encounters for various values of avoidance learning rate (α)



A1.1 Exponentially weighted moving average method for modelling DC

It is possible that decisions about when to begin sampling are sensitive to the rate at which animals encounter novel food items. To simulate this a variable S is established which varies between 0 and 1 based upon a weighted moving average equation taking into account the order, type and time of prey encounters. Initially the predator is assumed to exhibit full dietary conservatism and hence is assigned an S value of 0, and we take a threshold, DC_{rate}, to determine when dietary conservatism is applied. For example, if DC_{rate} =0.5, then if S <= 0.5 then D.C. is "on" and the probability of the predator rejecting the aposematic prey is 1 ($R=1$), if S > 0.5 then

the rate of encounter of the novel prey is high and dietary conservatism is turned off (i.e. $R=0$). S is calculated as an exponentially weighted moving average:

$$S = z.Y + (1-z).S_{t-1} \quad \text{(A1.1)}$$

Here (S) represents the exponentially weighted moving average, (Y) represents the observation (1 when an aposematic prey has been encountered, 0 when no prey or a cryptic prey has been encountered) and (z) represents a memory factor for weighting the current observation at time (t). The higher the value of z, the greater the influence of current events on decision making.

A1.2 Dietary conservatism via the weighted moving average method

We repeated the simulations described in the first section of the main paper, but changed the method of dietary conservatism to the weighted average method, with DC_{rate} set to 0.5, and z (equation A1.1) initially set to z=0.2. With other conditions the same as previous simulations and with no difference in learning rate ($N_{c=399}$, $N_{a=1}$, $T=100$, $generations=4000$, $c_a=0.02$, $c_c=0.01$, $\alpha_a=0.04$, $\alpha_c=0.04$) the outcome was similar, in that in 182 of the 1000 runs, dynamic equilibrium was reached and in no cases did the aposematic mutant reach fixation. With the weighted average method, this dynamic equilibrium could not be achieved in the presence of biased avoidance learning for aposematic prey in any trials (α_a=0.08-0.99); instead any short period of coexistence observed was eventually forced to fixation within the 4000 generation time scale.

Next we consider $z = 0.01$ whereby recent events have a smaller impact on the weighted average (see equation A1.1), and in the conditions used, the predator is more resistant to losing its wariness of the aposematic form. Now the aposematic form reaches fixation in 190 of 1000 runs (see e.g. Figure A1.2), and it never stays in the kind of dynamic equilibrium seen in Figure 1.1a in the main text. Individual

parameter tests were also conducted using the weighted average DC method and are presented in Figures A1.3a,b,c,d,e.

Fig A1.2 – Example of Fixation of the aposematic morph in the single habitat, single predator model with weighted average dietary conservatism z=0.01 (DC_{rate}=0.5, N_c=399, N_a=1, T=100, generations=4000, c_a=0.02, c_c=0.01, α_a =0.04, α_c =0.04)

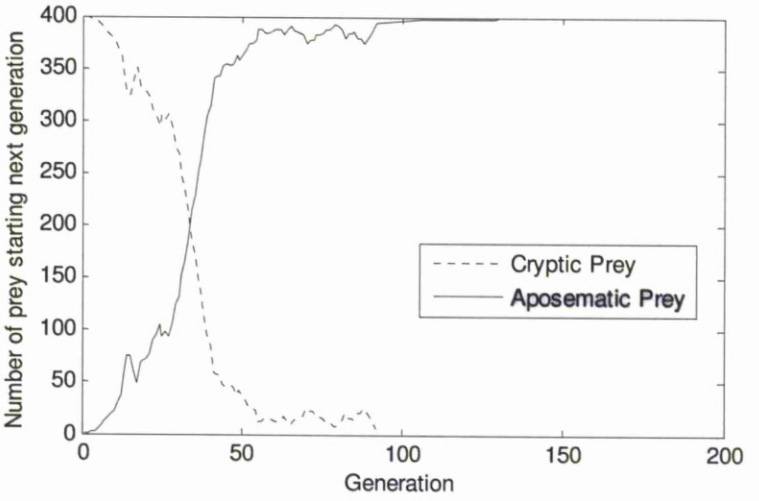


Figure A1.3a – Number of trials (out of 100) in which the novel aposematic morph reached fixation or equilibrium for varied levels of (Z) in the Moving Average DC model (DC_{rate}=0.5, $N_{c=399}$, $N_{a=1}$, T=100, generations=4000, c_a=0.02, c_c=0.01, α_a =0.04, α_c =0.04).

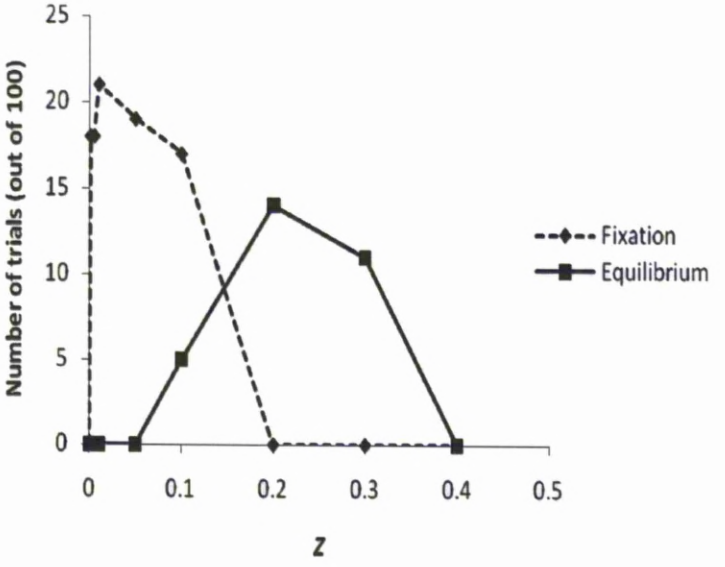


Figure A1.3b – Number of trials (out of 100) in which the novel aposematic morph reached fixation or equilibrium for varied levels of (*N*) in the Moving Average DC model (DC_{rate}=0.5, ***Z=0.2***, $N_{a=1}$, *T=100, generations=4000,* c_a***=0.02,*** c_c*=0.01,* α_a *=0.04,* α_c *=0.04*).

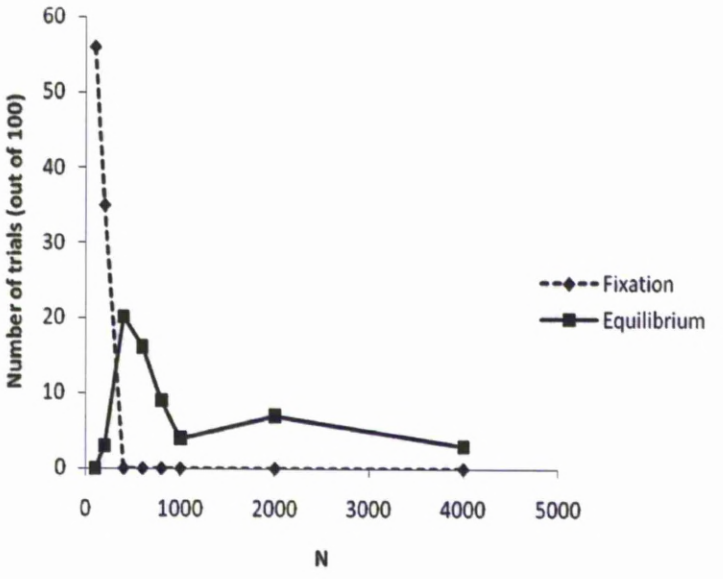


Figure A1.3c – Number of trials (out of 100) in which the novel aposematic morph reached fixation or equilibrium for varied levels of (C_a) in the Moving Average DC model (DC_{rate}=0.5, ***Z=0.2,***N_c*=399,* $N_{a=1}$, ***T=100, generations=4000,*** c_c*=0.01,* α_a *=0.04,* α_c *=0.04*).

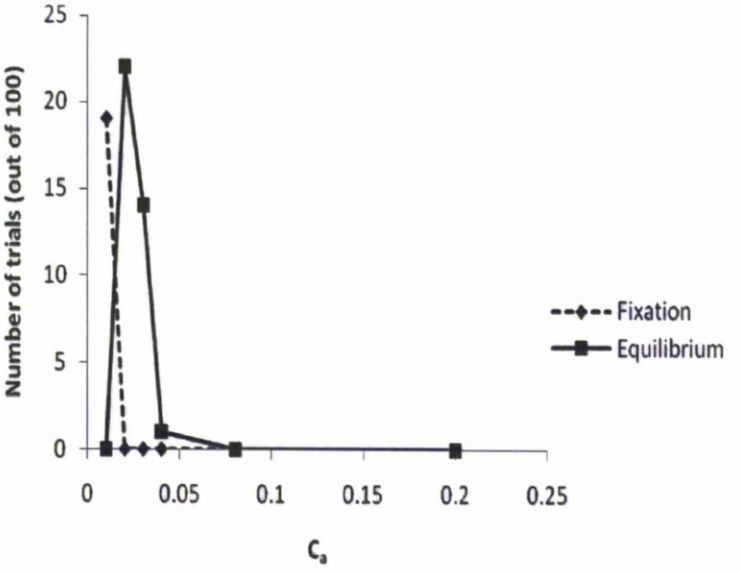


Figure A1.3d – Number of trials (out of 100) in which the novel aposematic morph reached fixation or equilibrium for varied levels of (α_a) in the Moving Average DC model (DC_{rate}=0.5, ***Z=0.2***,N_c=*399*, $N_{a=1}$, ***T=100, generations=4000***,c_c=*0.01*,c_a=*0.02*, α_a =*0.04*, α_c =*0.04*).

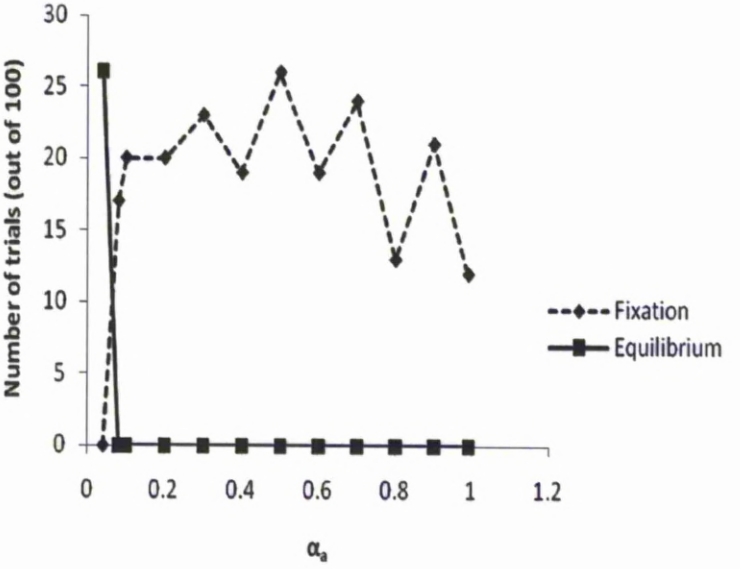


Figure A1.3e – Number of trials (out of 100) in which the novel aposematic morph reached fixation or equilibrium for varied levels of (*T*) in the Moving Average DC model (DC_{rate}=0.5, ***Z=0.2***,N_c=*399*, $N_{a=1}$, *generations=4000*, c_c=***0.01***, c_a=*0.02*, α_a =*0.04*, α_c =*0.04*).

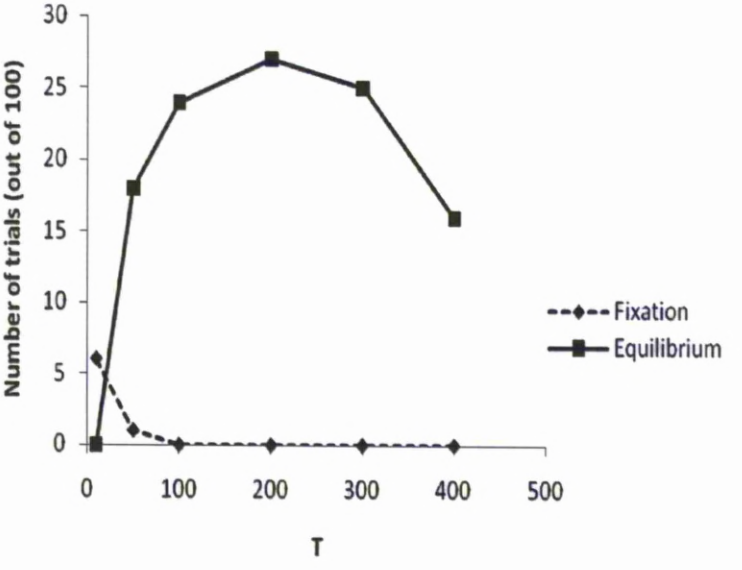
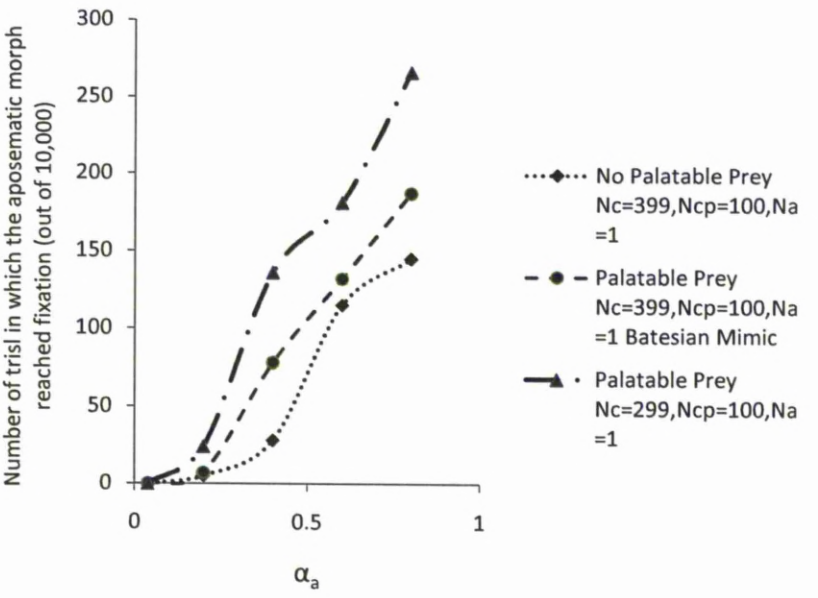
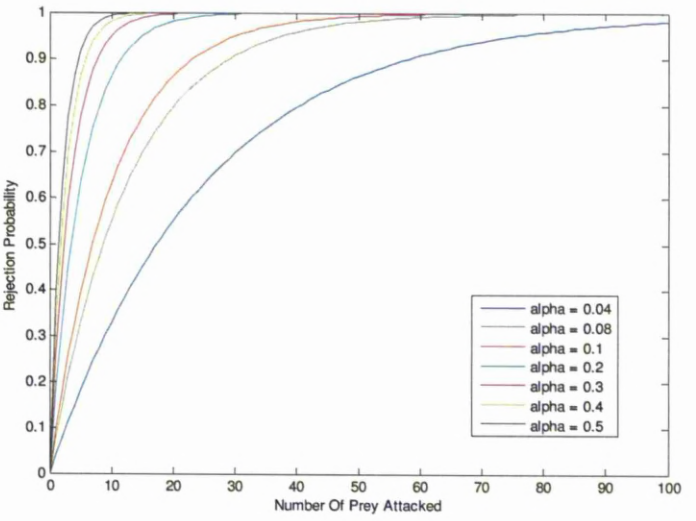


Figure A1.4 – Addition of palatable prey in the predator replacement model showing the number of trials (out of 10,000) in which the novel aposematic morph reached fixation where (α_a *=0.04,* α_a *=0.2,* α_a *=0.4,* α_a *=0.6,* α_a *=0.8)* in the fixed DC predator replacement model (DC_{num}=random (table 1.2 in the main text), N_a*=1,* *T=100, generations=4000,* $pred_{gen}$*=5,* c_a*=0.02,* c_c*=0.01,* α_c *=0.04).*



A2 - Appendix for chapter 2

Figure A2.1 - How rejection probability varies with number of encounters for various values of avoidance learning rate (α)



A2 Additional simulations

A2.1.1 Null model 1 - no D.C. with equal avoidance learning rates

In the first presentation of the model we ran a simple null test to determine if the aposematic prey morph could invade from rare where avoidance learning rates were equal for both prey types and where the predators show no dietary conservatism. The model adopts the standard parameters (see table 2.1 in the main text) other than where explicitly stated. Here, the predators showed no D.C tendency (DC_{num}=0 for all predators). Predator avoidance learning rate for both prey types was set to be equal (α_a=0.04 and α_a=0.04). We allowed 10 prey to migrate to each of the surrounding habitats each prey generation (N_{mig}=10) giving 80 total migratory prey. Migration began in the second generation of the model (mig_{gen}=2). In this model we assumed that the predators and prey live for equal lengths of time ($pred_{gen}$=1).

A2.1.2 Results

At no point in the simulation did the aposematic morph increase in abundance showing that without predators showing dietary conservatism and the benefits of accelerated avoidance learning, a more conspicuous mutant in a cryptic prey population rapidly and repeatedly becomes extinct.

A2.2.1 Null model 2 - no D.C. with bias avoidance learning rates

The model was re-tested with the same parameters as in null model 1, however we now increased the avoidance learning rate for aposematic prey to (α_a=0.99) to determine whether fixation could occur if the predator learned to avoid the aposematic morph more quickly than its cryptic conspecific due to the combined effect of the warning signal and its toxicity.

A2.2.2 Results

As predicted by the previous single habitat models of Lee et al. (2010), at no point in the simulation did the aposematic morph increase in abundance. Increased avoidance learning alone provides insufficient protection for the novel aposematic prey to allow any increase in abundance.

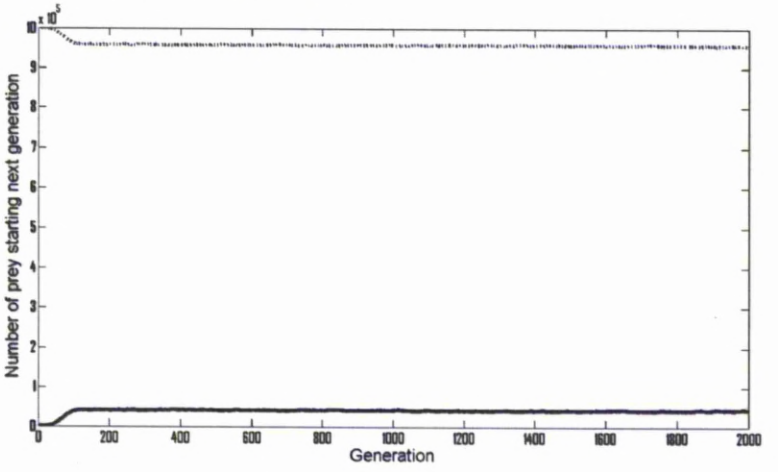
A2.3.1 D.C. model 1 – dietary conservatism with equal avoidance learning

Next, we introduce predator dietary conservatism to determine whether initial avoidance of the aposematic morph can cause any increase in abundance within the cryptic prey population. Each predator now assumes a DC level drawn randomly

from the pool of DC values (Table 2.2 in the main text) for the duration of its lifetime. We use the same parameter set as null model 1 whereby we assume equal learning rates (α_a *=0.04* and α_c *=0.04)* and equal predator-prey life spans ($pred_{gen}$*=1*).

A2.3.2 Results

Figure A2.2 –Dynamic equilibrium between the aposematic and cryptic morphs (generations=2000,, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.04, predgen=1, mig_{gen}=2, $mutation_{rate}$=10-5, DC_{num}=random (Table 2.2 in the main text), N_{mig}=10). The solid line represents the number of aposematic prey and the dotted line represents the number of cryptic prey.



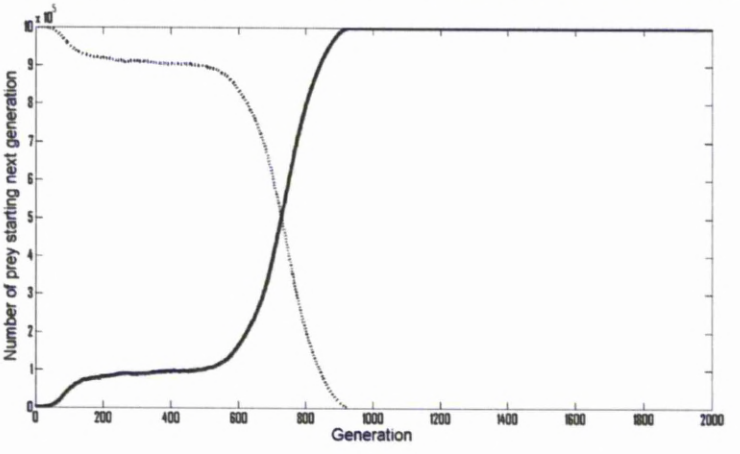
Where predators show dietary conservatism and with equal avoidance learning rates for both cryptic and aposematic prey we now yield an increase in abundance of the aposematic morph (Figure A2.2). The aposematic prey reach an observed fixed abundance of approximately 50,000 individuals and sustained that abundance over the 2000 generation duration of the model in a state of dynamic equilibrium (correlation coefficient r= 0.164).

A2.4.1 D.C. model 2 - dietary conservatism with bias avoidance learning

Adopting the same parameters as D.C. model 1, we then tested increasing levels of avoidance learning rates for aposematic prey (α_a) to determine whether the state of dynamic equilibrium between competing morphs could be replicated with biased avoidance learning and to determine if a critical avoidance learning rate for aposematic prey existed which would allow fixation to occur.

A2.4.2 Results

Figure A2.3–Fixation of the aposematic morph (generations=2000, T=100, c_a=0.02, c_c=0.01, α_c =0.04, α_a =0.18, predgen=1, mig_{gen}=2, $mutation_{rate}$=10-5, DCnum=random (Table 2.2 in the main text), N_{mig}=10). The solid line represents the number of aposematic prey and the dotted line represents the number of cryptic prey.



For values of avoidance learning rate for aposematic prey (α_a =*0.04-0.17*) we did not yield fixation of the aposematic morph but instead we again observe dynamic equilibrium between the competing prey morphs. Increasing α_a between these limits had the effect of increasing the abundance of aposematic prey at which dynamic

equilibrium occurred (see Figure 2.1 in main text). We observe that the aposematic morph reaches dynamic equilibrium at an increased abundance of approximately 80,000 individuals compared to 50,000 for equal avoidance learning rates (Figure A2.1).Increasing the avoidance learning rate for aposematic prey (α_a>=0.18) yielded fixation of the aposematic morph (Figure A2.3).

A2.5.1 DC Model 3 – increased predator lifespan

In previous simulations we have shown that in the metapopulation habitat as described above with the predator life span set to be equal to that of the prey we observe dynamic equilibrium ($\alpha_a < 0.18$) and fixation of the aposematic morph ($\alpha_a >= 0.18$). Next we consider a model whereby predators now live for 5 prey generations ($pred_{gen}$=5) in order to determine whether dynamic equilibrium or fixation can occur under such conditions. We assume the same parameters as D.C. Model 1 and tested a range of values of avoidance learning rate for aposematic prey (α_a =0.04-0.99).

A2.5.2 Results

In all trials, the aposematic morph did not increase in abundance at any point during the simulation indicating that when aposematic individuals arose by mutation, they rapidly became extinct. Lee et al. (2010) showed that under the same conditions in a single habitat model, fixation of the aposematic morph could be demonstrated where the avoidance learning rate for aposematic prey was just ($\alpha_a = 0.35$). Our models show therefore, that the inter-habitat movement of prey at the levels tested ($N_{mig}=10$) had a detrimental effect on the viability of the aposematic morph.

A5 - Appendix for chapter 5

Systematic examination of the key model functions and parameter testing of a range of prey abundances (N_i) and marginal toxicity costs for each species (γ_1, γ_2) were performed prior to the final test cases presented in the main text which we believe represent the 5 most pertinent outcomes of the model. The results presented represent robust cases for which the nature of the results are not sensitive to minor differences in the key parameters N_i and γ_1, γ_2. We provide a series of supplementary figures (see appendix Figures A5.1-A5.9) displaying plots of the key modal functions and additional results from the simulations presented in the main text.

A5.1 Convergence Vs Advergence

Our models predict that in all but cases where marginal costs of toxicity and abundances are equal in both species, advergence of one species to resemble the other (as opposed to convergence of both species to a novel appearance) is predominant in all simulations, even when both species benefit from mimetic resemblance. In pilot runs of our model, even when the marginal costs of toxicity and abundance of both species are equal we did not observe convergence at all. Indeed even when the two species begin the simulations with a greater degree of overlap in predator generalization (V=45, V=55), convergence could not be demonstrated. This dominance of advergence over convergence occurs because even the degree of protection from being mistaken for both established species cannot outweigh the fitness of the established visual signals given that prey abundance and hence learning state is so low at the point at which the two generalization curves overlap. There should exist a critical abundance of prey at the point at which the generalization curves overlap (V=50 in this case), which if met, will favour convergent evolution.

A5.2 Individual vs. group optimal strategies

In our model, the final, stable outcome is not that which maximises fitness for individuals of either species since selfish individuals defect from the group optimum, reducing their investment in toxicity and hence the stable level of toxicity within a group. Our final steady state solution is much closer to the concept of an evolutionary stable state, and shows the set of two strategies , one for each species where no individual would improve its fitness by unilaterally adopting another strategy. In fact, if all individuals switched to other strategies it would be possible for them all to improve their fitness (as our simulation shows straight after mimetic resemblance evolves, see figures A5.1-A5.5) but such a cooperative solution would be unstable against invasion and (if allowed to) the system would evolve back to the equilibrium that we show as the long-term solution presented in the main results.

A5.3 Co-evolutionary chase

With the standard model parameters (see Table 5.1 in the main text) we observed no cases of co-evolutionary chase in prey appearance, even in traditionally Batesian, fully parasitic scenarios e.g. (N_1=2000, N_2=2000, γ_1=0.99 γ_2=0.1). As discussed in the main text, this is a result of strict density dependence (see equation 5.7 in the main text). In this case, the model is unable to escape the parasitism of the mimic due to the fact that any individuals of the model population deviating from the ancestral appearance will be rare and hence selected against due to predators having no knowledge of the toxicity level of the new appearance. To determine if evolutionary chase was attainable within our model framework we re-tested the same fully Batesian model as described above (N_1=2000,N_2=2000, γ_1=0.99 γ_2=0.1) with a range of increased mutation rates and predator generalization standard deviations.. Only when mutation rates were set extremely high (θ >0.01) and the standard deviation of the predator's generalization was sufficiently large (σ_g>8) could modal shift in the model's visual trait, away from that of the mimic be observed. This chase however was short-lived and the mimic soon engaged in the same appearance, after which appearances of both population did not change over 100,000 generations.

A5.4 Appendix Figures for Chapter 5

Figure A5.1 – Scenario 1 - Mean defence level and mean fitness (S_i) of species 1 and 2 for each generation. N_1=1000, N_2=3000, γ_1=0.3, γ_2=0.3. The dotted line represents species 1 with the solid line representing species 2. The x axis begins from the 1100th generation before any mimetic relationship has evolved and after a stable pre-mimicry fitness has been established. As species 1 adverges to resemble species 2 (approx. 1300th generation), we observe a net increase in fitness and net loss of toxicity for both species indicating a purely mutualistic relationship.

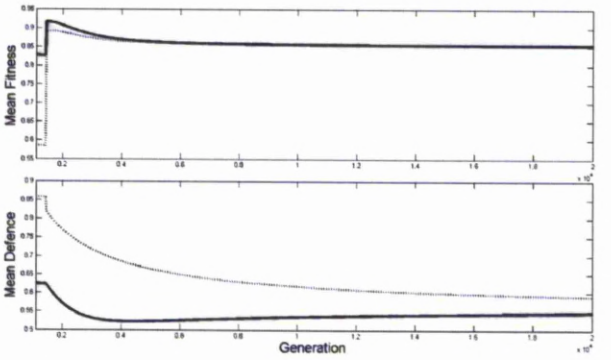


Figure A5.2 -Scenario 2 - Mean defence level and mean fitness (S_i) of species 1 and 2 for each generation. N_1=2000, N_2=2000, γ_1=0.6, γ_2=0.3. The dotted line represents species 1 with the solid line representing species 2. The x axis begins from the 1100th generation before any mimetic relationship has evolved and after a stable pre-mimicry fitness has been established. As species 1 adverges to resemble species 2 (approx. 2000th generation), we again observe a net increase in mean fitness and net loss of mean toxicity for both species indicating a purely mutualistic relationship. Here we observe that both species final mean toxicity levels differ due to differences in the marginal costs both species pay.

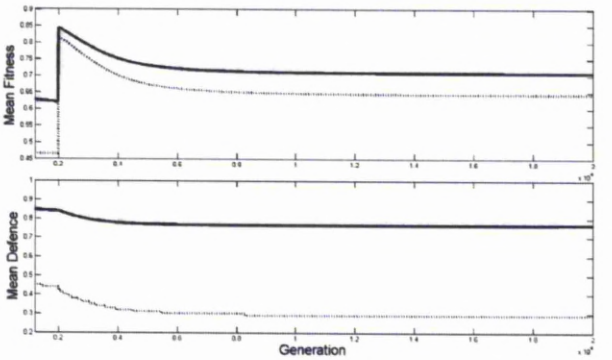


Figure A5.3 - Scenario 3 - Mean defence level and mean fitness (S_i) of species 1 and 2 for each generation. N_1=3000, N_2=6000, γ_1=0.3, γ_2=0.3. The dotted line represents species 1 with the solid line representing species 2. The x axis begins from the 1100th generation before any mimetic relationship has evolved and after a stable pre-mimicry fitness has been established. As species 1 adverges to resemble species 2 (approx. 1500th generation), we observe that species 1 benefits from a net increase in mean fitness and net loss of mean toxicity while species 2 suffers a net loss in mean fitness and net gain in mean toxicity indicating a purely parasitic relationship has formed.

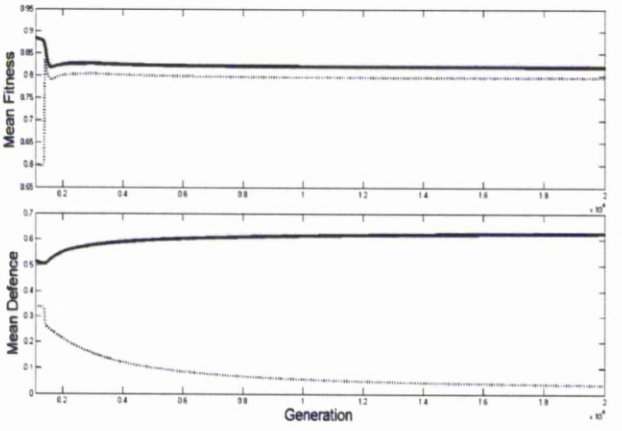


Figure A5.4 - Scenario 4 - Mean defence level and mean fitness (S_i) of species 1 and 2 for each generation. N_1=2000, N_2=2000, γ_1=0.6, γ_2=0.3. The dotted line represents species 1 with the solid line representing species 2. The x axis begins from the 1100th generation before any mimetic relationship has evolved and after a stable pre-mimicry fitness has been established. As species 1 adverges to resemble species 2 (approx. 1200th generation), we observe that initially, both species experience an increase in mean fitness and a decrease in mean toxicity. At around the 1500th generation we observe that as species 1 continues to reduce investment in toxicity, species 2 begins to reduce in mean fitness and increase investment in toxicity to a point where fitness is lower than its pre-mimetic state indicating a switch from a mutualistic to parasitic phase.

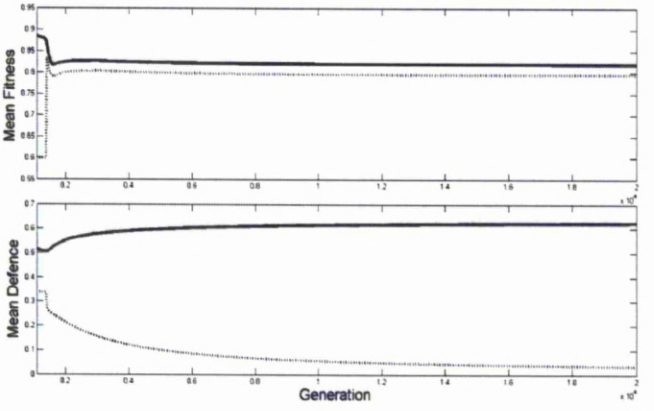


Figure A5.5 - Scenario 5 - Mean defence level and mean fitness (S_i) of species 1 and 2 for each generation. N_1=2000, N_2=3000, γ_1=0.6, γ_2=0.3. The dotted line represents species 1 with the solid line representing species 2. The x axis begins from the 1100th generation before any mimetic relationship has evolved and after a stable pre-mimicry fitness has been established. Here, we observe the same mutualism to parasitism switch as in scenario 4 (Figure 5.1 in the main text and A5.4 in the appendix), but now we observe that the mimetic species (species 1) reduces toxin investment to 0, becoming a fully edible Batesian mimic.

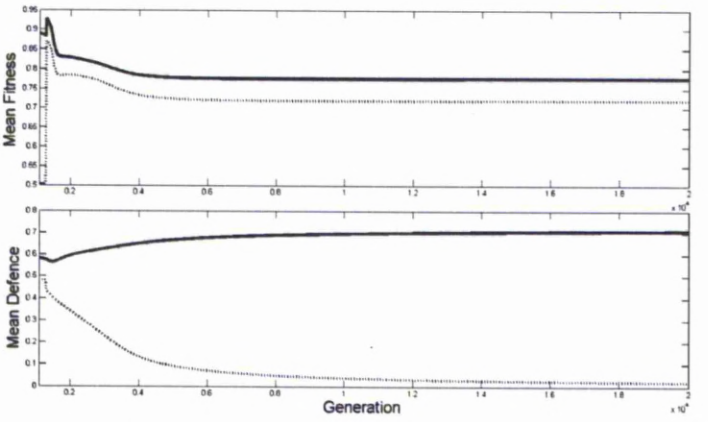


Figure A5.6 - Plot showing the density dependent learning coefficient function for $0 \leq N_j \leq 2000$, see equation 5.7 in the main text.

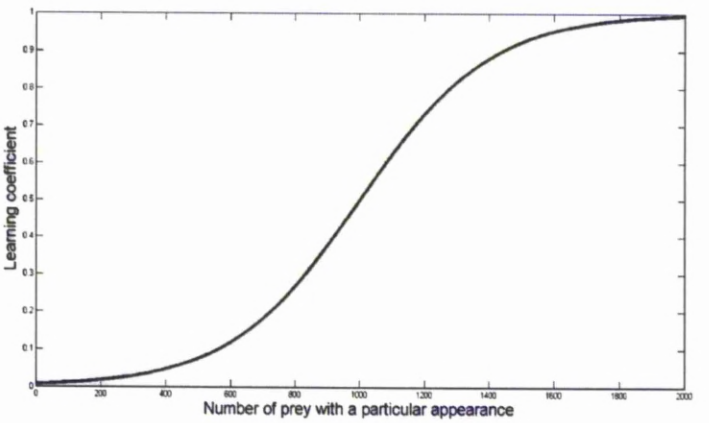


Figure A3.7- Standard predator generalization curves around our starting populations. The dotted line represents generalization around species 1, solid line species 2.

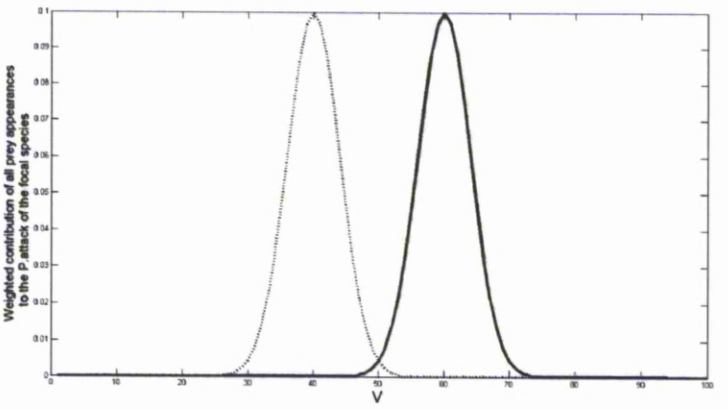


Figure A5.8- Plot of the probability of death from attack function for $0 \leq D \leq 1$, see equation 5.10 in the main text. The solid line represents the standard function (β_{PD} =0.2), the dotted line represents the modified function where Müllerian to Batesian mimicry can be demonstrated ($\beta_{PD} = 0.15$), see scenario 5 in the main text.

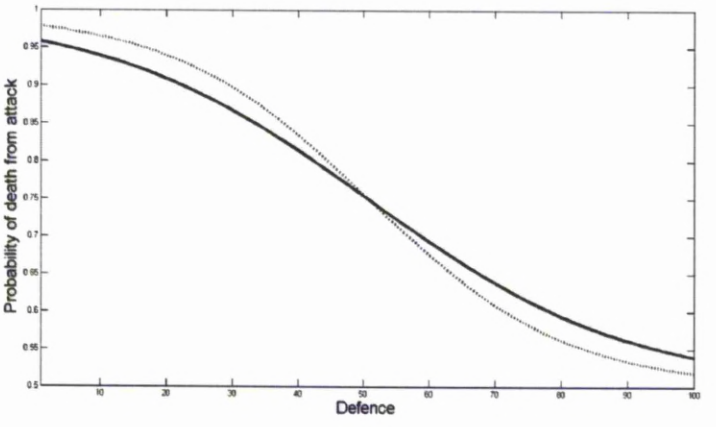
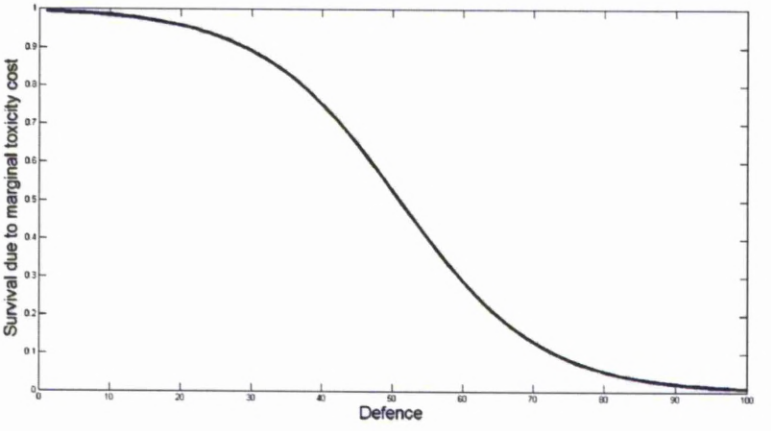


Figure A5.9- Plot of the marginal cost of toxicity function and the effect on prey survival for $0 \leq D \leq 1$, see equation 5.11 in the main text.



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