Antipredator Defence and the Evolution of Senescence in Lepidoptera

by

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A thesis submitted to the Faculty of Graduate and Postdoctoral Affairs in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Biology

Carleton University
Ottawa, Ontario

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Abstract

Senescence, defined as a reduction in physiological function, fecundity or survivorship with age, is a nearly ubiquitous phenomenon which nonetheless presents an apparent evolutionary paradox, since individuals that did not senesce would seemingly have a selective advantage over those that do. This paradox is resolved by evolutionary theory in terms of life history trade-offs, since selection is expected to favour benefits early in life even if they are associated with costs late in life. In this research project, I explored one of the fundamental predictions of the evolutionary theory of senescence: that increased predation should select for increased rates of senescence and therefore reduced longevity. In particular, I tested the prediction that aposematism (conspicuous coloration paired with chemical defence) should be associated with lower predation rates than other strategies such as crypsis (or camouflage), and this should lead to lower senescence rates and higher longevity in aposematic species.

My research program addressed two broad questions: first, are there differences in predation-related mortality between cryptic and aposematic prey, and second, what are the implications of these differences (or lack thereof) for the evolution of senescence? Predation rates were compared using field experiments with artificial prey and wild predators, and included a test of a specific theory of post-reproductive senescence based on kin selection. My results indicated that there was little support for the kin selection mechanism of post-reproductive senescence, however there was some support for the more general mechanism of differential predation rates between aposematic and cryptic prey.

The relationship between defence and senescence was explored in two ways: by fitting senescence models to demographic data from Lepidoptera, and by building a simulated population which evolved longevity in the presence of predation. Senescence was present in all 22 species of Lepidoptera for which demographic data was available, and aposematic species had significantly higher longevities than non-aposematic species, although not significantly lower senescence rates. Results from the evolutionary simulations indicated that aposematic prey do not necessarily evolve higher longevity than cryptic prey, unless certain behaviours (namely cautious sampling and neophobia) are present in the predator population.

Acknowledgements

I would like to thank several people without whose help and support this thesis would not have been possible. Firstly, I need to thank my supervisor, Dr. Tom Sherratt, not only for the opportunity to work in his lab, but also for encouraging me to take a chance and transfer into a Ph.D. Tom is a tireless researcher with a keen eye for interesting questions, and his passion and personality make him an engaging supervisor. I am tremendously grateful for his insights, as well as his guidance and support. Much of my current interest in statistics and computer models is also due to his excellent courses in those subjects.

Thanks are also due to the members of my committee: Dr. Jayne Yack and Dr. Howard Rundle. Their comments and guidance were instrumental in shaping my research, particularly in the early days when I was tentatively exploring the topics which would eventually become my research project. I am also grateful to them for their support during the transition into my Ph.D., and for believing that my research questions were interesting enough to support four more years of research.

I need to thank several members of my lab, without whom I would have been hard-pressed to complete this thesis. Richard Webster and Ian McLean helped me to design and carry out my field experiments, and their discussions and brainstorming sessions throughout this process have been invaluable. Dr. Chris Hassall and Dr. Kevin Abbott were both enormously knowledgeable resources, and were always helpful and easy to talk to. Tom Hossie provided help with a particularly difficult analysis, and valuable advice during the writing of one of my chapters. Jordan Pleet and Jennifer Kong

deserve special thanks for the hard work they contributed to my field research, often in unpleasant conditions.

I also need to thank my family and friends for their support throughout this process. Special thanks go to my parents, who have supported me in many ways throughout my education, and always encouraged me to challenge myself. Thanks also to my Uncle Kieran, who helped spark my interest in science at an early age, and whose conversations over the years have encouraged me to pursue a career in research.

Finally, I would like to thank my wife Elena for so many things. For encouraging me to take the leap and move to Ottawa for grad school in the first place, and then encouraging me again to take the equally big leap into a Ph.D. For supporting me throughout my research, not just emotionally, but physically as well, working two full field seasons and spending many sunny Sunday afternoons in the lab. For patiently listening to all of my rambling conversations about my thesis, and for being so supportive during the writing process. I don't think I could have done this without her.

Preface – Co-authorship statement for Chapters 2 and 3

Of the four data chapters included in this thesis, Chapter 2 has been published in the *Biological Journal of the Linnean Society*, and Chapter 3 has been accepted for publication in the *Journal of Zoology*. Dr. Tom Sherratt and I collaboratively developed the research questions and experimental design used in Chapter 2, and Dr. Sherratt programmed the human predation model. I performed data collection for the human predation experiment, and data collection in the field was carried out with the help of Elena Korshikov, Ian McLean and Jordan Pleet. I analyzed the data from both the human and field predation experiments, and drafted the manuscript with help from Ms. Korshikov. Dr. Sherratt and Ms. Korshikov both provided editorial suggestions to improve the manuscript.

I developed the research questions and experimental design for Chapter 3 in collaboration with Dr. Sherratt, and performed data collection with the help of Elena Korshikov and Jennifer Kong. I analyzed the data, with help from Tom Hossie designing the stratified Cox proportional hazards model. I drafted the manuscript, and Dr. Sherratt and Mr. Hossie both provided editorial suggestions to improve the manuscript

For the purpose of this thesis, I often make use of the pronouns “I” and “my”; I completely acknowledge the contributions of all co-authors, and hope that they will allow me this discretion.

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Chapter 1 – General Introduction

One of the fundamental questions in biology is: why do we age? Ageing, or senescence, is characterized by a reduction in physiological function, fecundity or survivorship over time, and this process of age-related deterioration presents an evolutionary paradox, since individuals that did not senesce would appear to have a selective advantage over those that do. There are several evolutionary theories that seek to resolve this apparent paradox, and many of them propose that there are trade-offs between function early and late in life, for instance between fecundity and longevity. It has been suggested that the nature and intensity of these trade-offs may also be responsible for the high amount of variability in longevity among species.

This thesis will investigate one of the fundamental (yet controversial) predictions of evolutionary theory: that increased predation (as well as other extrinsic sources of mortality) should select for increased rates of senescence and reduced longevity. In particular, I will expand on previous work with Lepidoptera, which suggests that species employing aposematism (conspicuous coloration paired with distasteful chemicals) as a defensive strategy live longer than those employing crypsis (or camouflage). Two inter-related questions will be addressed: first, are there differences in predation-related mortality between cryptic and aposematic prey, and second, what are the implications of these differences (or lack thereof) for the evolution of senescence?

To draw together the seemingly distantly-related topics of senescence and antipredator defence, I will begin by reviewing previous studies of senescence and longevity in Lepidoptera and other species, and discussing the importance of quantifying

senescence. I will then summarize the physiological mechanisms and evolutionary theories of senescence, and the reasoning behind the prediction that predation should select for higher senescence rates. Finally, I will introduce aposematism and crypsis as defensive strategies, and summarize the current evidence supporting the link between defence and longevity, including an alternative evolutionary theory based on senescence in the post-reproductive phase.

1.1 Senescence

Senescence is an intrinsic process of age-related deterioration that is widespread in nature (for reviews, see: Hughes & Reynolds, 2005; Bonsall, 2006; Williams *et al.*, 2006; Sherratt & Wilkinson, 2009), and can be defined either at the individual level (as a loss of physiological function or fecundity over time) or at the population level (as a decline in survivorship with chronological age). An early and often repeated prediction regarding senescence is that animals do not live long enough to experience senescence in the wild (Medawar, 1952; Hayflick, 2000; Kirkwood & Austad, 2000). There have been many studies that have since refuted this assertion, demonstrating reduced survivorship with age in a large number of species (for a review, see Nussey *et al.*, 2013). However, relatively few studies have measured this age-related decline in survivorship in insects (but see Bonduriansky & Brassil, 2002; Dukas, 2008; Zajitschek *et al.*, 2009; Sherratt *et al.*, 2010, 2011). Previous to the work presented here, only one study had quantified rates of senescence in Lepidoptera (and only for a single species; Gotthard *et al.*, 2000).

Delayed senescence is usually indicative of long life. However, senescence is defined as a *change* in survivorship with chronological age, while longevity (whether

expressed as the mean of a sample or its maximum) is simply a consequence of mortality, and will arise even in the absence of senescence. Despite the lack of studies directly measuring senescence, several studies have demonstrated a high level of variability in adult longevity among Lepidoptera, and found correlations with several factors, including defensive strategy, geographic distribution and adult diet (Dunlap-Pianka *et al.*, 1977; Karlsson & Wiklund, 2005; Molleman *et al.*, 2007; Beck, 2008; Beck & Fiedler, 2009). It seems natural to assume that this difference in longevity represents variation in rates of senescence, and that the relationships between longevity and various ecological factors could be used to test hypotheses about the evolution of senescence. However, senescence has not been broadly quantified in Lepidoptera, and it is important to note that longevity could conceivably vary without a corresponding variation in senescence rates if species possessed different underlying fixed rates of mortality. Likewise, two populations could senesce at different rates and yet have the same mean longevity. For these reasons, it is important to directly measure senescence in Lepidoptera, and this can only be done by fitting demographic models of senescence to survivorship data.

One of the most common demographic models of senescence is the Gompertz model (Gompertz, 1825), which assumes that the rate of mortality changes exponentially with age. The Gompertz model can be fitted to demographic data using maximum likelihood estimation (MLE), as can other models such as the logistic, which incorporates a deceleration term to account for late-life mortality plateaus in some species (Pletcher, 1999). Since these models are partially nested (the Gompertz model, for instance, corresponds to a special case of the logistic where the deceleration term is zero), their fitted likelihoods can be compared using likelihood ratio tests (Hilborn & Mangel, 1997)

to determine which best fits the demographic data. Likewise, since the Gompertz can be simplified to produce a non-senescent model with constant mortality, likelihood model fitting can be used to determine whether senescence is present in a population and quantify the rate of increase in mortality; indeed, this has been done previously to test whether senescence arises in several species of damselfly (Sherratt *et al.*, 2010; 2011). Overall, this approach can be used to elucidate the relationship between senescence rate and longevity in insect populations, and to compare senescence rates between species.

Two important questions remain, however: how (if at all) should predation and senescence be inter-related, and when should aposematic prey experience reduced senescence compared to cryptic prey? To answer these questions, it is necessary to explore both the physiological mechanisms that produce senescence, and the evolutionary theories that seek to explain its origin.

1.1.1 Physiological Mechanisms of Senescence

Senescence is characterized by a process of age-related physiological deterioration, and there are several theories which seek to explain the mechanisms behind this process. Some theories, such as the free radical hypothesis (Harman, 1956) and the metabolic signalling hypothesis (Kenyon, 2001), propose that senescence is a consequence of metabolic activity. Indeed, there is evidence that free radical molecules produced as a byproduct of oxygen metabolism are associated with age-related cellular damage (Balaban *et al.*, 2005), and insulin-related signalling pathways have been shown to regulate both longevity and reproduction (Kenyon, 2001; Longo & Finch, 2003). Genetic mechanisms may also impose a limit on the number of replications a cell can

undergo (the Hayflick limit; Hayflick & Moorehead, 1961). According to the telomere theory, this limit is determined by repeating elements at the ends of chromosomes (telomeres), which shorten during each bout of replication, eventually becoming exhausted and leaving the cell vulnerable to genetic damage which causes cell death (for a review, see Monaghan, 2010). These mechanisms may also be inter-related; many longevity-enhancing mutations in the insulin signalling pathway also upregulate antioxidant genes (Guarente & Kenyon, 2000), and there is evidence that rates of deterioration in telomeres vary with the amount of oxidative stress (Passos & Ziglinicki, 2005).

Although there has been extensive research into the mechanisms of physiological deterioration associated with senescence, a fundamental question remains: why has evolution not led to better protections against these sources of deterioration, for instance by lengthening telomeres or increasing the production of antioxidants? In other words, since individuals that did not experience this age-related deterioration would appear to have a selective benefit over those that do, why is senescence such a widespread phenomenon? There are several (inter-related) theories of senescence that attempt to answer this question.

1.1.2 Evolutionary Theories of Senescence

Senescence poses an apparent evolutionary paradox, since individuals that did not senesce would appear to have a selective advantage over those that do. Several evolutionary theories have been proposed which attempt to address this apparent problem, and to understand how selection could favour a trait which seems to have such a

negative effect on fitness. August Weismann (1889) provided one of the earliest evolutionary theories of senescence, suggesting that species evolved finite life spans so that older individuals could leave room for younger ones, benefitting the population as a whole. This theory, based on what we would now consider group selection principles, has the weakness that selection would favour individuals that "cheated" by living longer, as well as being unable to account for high levels of variation in longevity both within and among species.

The modern, individual-based evolutionary theory of senescence originated with Medawar (1952), who suggested that senescence was caused by a sort of "benign neglect". He recognized that selection would act strongly to remove deleterious mutations that had an effect early in life, but that there would be far less selection to purge deleterious mutations that acted late in life because so few individuals would be around to experience their harmful effects. A similar theory based on genetic trade-offs was proposed by Williams (1957), who suggested that deleterious traits could be actively selected for if their cost occurred late in life, as long as they had an associated benefit early in life. This was later termed the antagonistic pleiotropy theory (Rose, 1991). Several other theories have also explored potential age-related evolutionary trade-offs: the disposable soma theory (Kirkwood 1977;1996), for instance, characterizes senescence as a resource-based trade-off between reproduction early in life and maintenance later in life, while the reliability theory of ageing (Gavrilov & Gavrilova, 2001; Laird & Sherratt, 2009) suggests that variability in rates of senescence is due to differential investment in redundant physiological protection or repair mechanisms. These theories all follow the basic assumptions first outlined by Medawar (1952), namely that selection favours

beneficial traits more strongly when they occur early in life, and disfavours deleterious traits more weakly when they occur late in life. They also predict that if extrinsic factors cause high rates of mortality, then trade-offs involving reduced longevity, for instance in exchange for higher fecundity, will be favoured by selection.

1.1.3 Extrinsic Mortality

One of the fundamental predictions of evolutionary theories of senescence is that high rates of extrinsic mortality (i.e. from external sources such as accidents, disease and predation) should result in increased rates of senescence, expressed through higher rates of intrinsic mortality (from innate sources such as organ failure or cancer). Whenever extrinsic mortality is high, selection will favour trade-offs that result in a "live fast and die young" strategy, since few individuals will avoid mortality long enough to pay the cost associated with the early benefit (Williams, 1957; Hamilton, 1966). Conversely, when extrinsic mortality is low, selection should remove late-acting deleterious traits, resulting in organisms that are "built to last". Despite general support (see Sherratt & Wilkinson, 2009), at least one recent study has provided evidence that contradicts this prediction (Reznick *et al.*, 2004), and others have raised important theoretical caveats. For example, density-dependent population growth can lead to conditions where extrinsic mortality selects for decreased senescence, for instance if increased density reduces survival of older individuals (Abrams, 1993). The same result is predicted if individuals experience condition-dependent susceptibility to external sources of mortality early in life (Williams & Day, 2003). The effect of extrinsic mortality on senescence is therefore complex, since both density dependence and condition dependence can lead to a

relationship between extrinsic mortality and senescence that is positive, negative or even neutral (Abrams, 1993; Williams & Day, 2003). Caution should also be exercised when distinguishing between intrinsic and extrinsic sources of mortality, since they may often not be independent. For instance, intrinsic age-related deterioration could cause increased vulnerability to predation and disease, and predation or disease-related damage could in turn increase an organism's vulnerability to starvation or accident. Indeed, it may not be possible to fully disentangle extrinsic and intrinsic sources of mortality (Abrams, 1993).

Despite these caveats, there is considerable evidence that high rates of extrinsic mortality are correlated with high rates of senescence (for a review, see Hughes and Reynolds, 2005). One of the most common sources of mortality is predation (Ruxton *et al.*, 2004), and species that are best able to avoid or deter predators would therefore be expected to evolve lower rates of senescence than other species (Williams, 1957). Indeed, there is evidence that birds (Holmes and Austad, 1995; Holmes *et al.*, 2001) and flying or gliding mammals (Austad and Fischer, 1991; Holmes and Austad, 1994) live longer than terrestrial species. Similarly exceptional longevities have been also been documented in subterranean species (Buffenstein, 2008) and populations which have become geographically isolated from their predators (Austad, 1993). Since the presence (or absence) of predators seems to have a strong influence on the evolved longevities of a range of species, it stands to reason that defensive strategies employed in the presence of predators might have a similar effect.

1.2 Aposematism and Crypsis

Because of the high mortality costs of predation, many species have evolved defensive strategies to avoid or deter predators, and two of the most common strategies are aposematism and crypsis. Aposematism is a common strategy among insects, and is widespread and well-studied in Lepidoptera (Nishida, 2002; Mappes *et al.*, 2005). Aposematic species employ conspicuous coloration paired with unpleasant or painful secondary defences such as toxins or distasteful chemicals (Poulton, 1890). Predators that attack aposematic prey soon learn to avoid similar looking prey because of this association between conspicuous colour patterns and secondary defences (Mappes *et al.*, 2005). In contrast, cryptic prey avoid predation by reducing predators' ability to detect them. This is accomplished by adopting colour patterns which match their background ("background matching"; Endler, 1984) or using contrasting markings to break up their outlines ("disruptive coloration"; Cott 1940; Merilaita & Lind, 2005). It is important to note that these two types of crypsis (background matching and disruptive coloration) are not exclusive, and may be present in a single individual (Endler, 1984; Merilaita & Lind, 2005).

It has been suggested that aposematic species should live longer than non-aposematic species, since predators often learn to avoid aposematic prey, and this may cause aposematic species to experience lower predation rates than other species (Blanco & Sherman, 2005; Molleman *et al.*, 2007; Beck & Fiedler, 2009). There is some evidence from fish, snakes and amphibians that chemically defended species live longer than those without defensive chemicals (Blanco & Sherman, 2005), and from Lepidoptera that aposematic species live longer than non-aposematic species (Blest,

1963; Beck & Fiedler, 2009). However, it should be noted that species with chemical defences do not always live longer than related species that lack defensive chemicals (Hossie *et al.*, 2013), and other factors such as geographical distribution and adult diet may also play a role in shaping longevity (Dunlap-Pianka *et al.*, 1977; Molleman *et al.*, 2007; Beck & Fiedler, 2009).

There is some evidence from studies using captive predators that aposematic prey experience lower predation rates than cryptic prey (Sillen-Tullberg, 1985; Alatalo & Mappes, 1996; Halpin *et al.*, 2008), however relative predation rates on aposematic and cryptic prey have not yet been compared using wild predators. It should also be noted that aposematic prey are not always expected to experience less predation than cryptic prey. Conspicuous coloration can lead to high rates of predation if prey are rare or if predators are naive, since such prey are easily found, and in both cases predators would not have learned to avoid similar looking prey (Lindstrom *et al.,* 2001; Ruxton *et al.,* 2009; Marples & Mappes, 2011). For these reasons, it is important to directly measure the relative effectiveness of aposematism and crypsis in a field setting using wild predators, in order to effectively evaluate the key assumption behind the theoretical prediction that aposematic prey should evolve lower senescence rates than cryptic prey. Furthermore, the same method can be used to test an alternative theory that also predicts the evolution of different longevities between aposematic and cryptic prey, based on post-reproductive senescence.

1.2.1 Post-Reproductive Senescence

An interesting alternative to the individual selection-based theory of Medawar and Williams was proposed by Blest (1963). He suggested that the observed differences in longevity between aposematic and cryptic Lepidoptera could be caused by variation in the rate of senescence after the end of reproduction. He argued that aposematic species should evolve long post-reproductive life spans, since their presence would teach predators to avoid similar looking prey. Conversely, cryptic species should have short post-reproductive life spans, to keep predators from forming a search image for similar looking prey. Because selection at the individual level necessarily ceases after reproduction, this post-reproductive senescence theory was originally proposed based on the principles of group selection, and had the same weakness as other group selection-based theories of senescence, namely that it would be vulnerable to invasion by "cheaters". However, in one of his classic papers, Hamilton (1964) restated the theory in terms of kin selection, recognizing that individual selection could favour the evolution of a post-reproductive senescence trait if it provided a benefit to relatives carrying the same trait.

Although selection for post-reproductive senescence may seem less likely than selection for senescence during the reproductive phase, there is indeed evidence that some animals experience accelerated senescence following reproduction (Robertson & Wexler, 1962; Wodinsky, 1977). Conversely, post-reproductive longevity is high in humans, and a kin selection mechanism has been proposed to account for this whereby humans that have finished reproduction benefit relatives by caring for grandchildren (Lahdenpera *et al.*, 2004). It is also important to note that the post-reproductive theory of

senescence in Lepidoptera is not incompatible with the individual selection-based evolutionary theory of senescence, and in fact both may be acting within a given species to shape the rate of senescence. In order to distinguish between them, it is necessary to test Blest's original assumption regarding predation, namely that the presence of conspecifics reduces predation on aposematic prey, but increases predation on cryptic prey.

1.3 Chapter Outline

In this thesis, two inter-related questions were addressed: first, are there differences in predation-related mortality between cryptic and aposematic prey, and second, what are the implications of these differences (or lack thereof) for the evolution of senescence? Chapters 2 and 3 focus on the first question, while chapters 4 and 5 focus on the second.

Chapter 2 investigates the effect of similar-looking conspecifics on predation rates in cryptic and aposematic prey, using both human subjects and wild birds as predators. Results from these experiments were used to test an interesting question posed by Blest (1963) and later re-formulated by Hamilton (1964): do aposematic prey experience a benefit from the presence of conspecifics by teaching predators to avoid similar looking prey? And conversely, do cryptic prey experience a cost by allowing predators to form a search image? If so, predation could produce differences in post-reproductive longevity through the mechanism of kin selection.

In chapter 3, similar methods were used to more generally measure predation by wild predators on cryptic and aposematic prey in the field. Both background-matching

and disruptive prey were used, as well as aposematic prey with two levels of chemical defence. These results were used to test the more general evolutionary hypothesis that aposematic and cryptic prey experience different levels of predation throughout their adult lives, and that this difference in mortality could select for different rates of senescence in species with each defensive trait.

Chapter 4 sought to determine whether senescence is present in Lepidoptera, and to characterize and quantify the variation in senesce rates among species. The relationship between senescence rate and longevity was also explored to determine whether the widespread use of longevity as a proxy for senescence is justified. Finally, both longevity and senescence rate were compared between aposematic and non-aposematic species to test the prediction that aposematic species experience reduced senescence compared to other species.

In chapter 5, a simulated population based on a genetic algorithm was designed in which a longevity trait evolved under different mortality regimes, including simulated predation. Different defensive strategies and assumptions regarding predator behaviour were tested, and competition between different prey morphs was also simulated. The results were used to investigate the conditions under which aposematism would be expected to outcompete crypsis (and *vice versa*), and how longevity would evolve in the resulting populations.

Senescence is a nearly ubiquitous feature of biological systems; however, rates of senescence are highly variable, and shaped by many complex and inter-related genetic and environmental factors. A deeper understanding of the nature of the selective trade-offs that shape senescence provides both direction for future research, and a context in

which to interpret new findings about the mechanisms that drive senescence. The modern individual selection-based theory of senescence provides a framework which predicts much of the observed variation in senescence both among and within species, and predation has long been cited as an important ecological factor in the evolution of senescence. However, recent theoretical and empirical studies have raised questions about this prediction. By exploring the link between predation and senescence, and particularly the role of aposematism and crypsis, the work presented in this thesis attempts to better explain the ecological context in which rates of senescence evolve, and highlights ways in which the relationship between predation and senescence is more complicated than was previously thought.

Chapter 2 – Post-reproductive senescence in moths as a consequence of kin selection: Blest's theory revisited

2.1 Abstract

In 1963 Blest reported that cryptic, palatable moth species had faster rates of post-reproductive senescence than conspicuous, unpalatable (aposematic) moth species. He argued that these defence-dependent differences could be explained as a consequence of selection to reduce predation on conspecifics in both cases; this hypothesis was later reformulated by Hamilton in terms of kin selection. Here we re-analyze Blest's original data, and test his underlying assumption that the presence of conspecifics affects predation rates on similar-looking prey using a combination of laboratory work on humans and field work with wild birds. The collective evidence for Blest's theory is weak at best, and we propose a more general hypothesis that post-reproductive senescence rates in cryptic and aposematic prey are a by-product of extrinsic mortality imposed by predation. This work has been published in the *Biological Journal of the Linnean Society*.

Carroll, J., Korshikov, E., Sherratt, T.N. 2011. Post-reproductive senescence in moths as a consequence of kin selection: Blest's theory revisited. *Biol. J. Linn. Soc.* **104,** 633-641.

2.2 Introduction

The struggle was over. The insignificant little creature now knew death. As I looked at the dead moth, this minute wayside triumph of so great a force over so mean an antagonist filled me with wonder. Just as life had been strange a few minutes before, so death was now as strange.

Virginia Woolf (1942), The Death of the Moth.

Traditionally, evolutionary theory has characterized senescence, or ageing, as a by-product of reduced selection at advanced age classes (Medawar, 1952; Williams, 1957). Thus, late acting deleterious mutations are traditionally thought to accumulate in genomes, either as a consequence of mutation-selection balance (Medawar, 1952), or as a consequence of trade-offs between early and late life (Williams, 1957) such as reproduction or repair (Kirkwood, 1977). While this explains many characteristics of senescence, including its widespread occurrence in nature, comparative studies have revealed a range of phenomena that require specific explanation. Many migratory species for example, including birds and some insects, are unusually long lived compared to non-migratory species (Møller, 2007; Møller, 2008; Herman & Tatar, 2001). There is also evidence that some species may have evolved accelerated rates of senescence as a response to environmental pressures such as disease and resource shortage (Longo, Mittledorf & Skulachev, 2005; Mitteldorf & Pepper, 2009). Further, a number of species have unusual patterns of senescence in the post-reproductive phase, either having remarkably short lives after the end of reproduction (Wodinsky, 1977; Uematsu *et al.*,

2010), or conversely, as in humans, having extended post-reproductive life stages (Bourke, 2007; Lahdenpera *et al.*, 2004; Lee, 2003).

Variation in the rate of post-reproductive senescence is particularly intriguing, since individual-based selection should cease to act once reproduction is over. One way in which a post-reproductive senescence trait could evolve is if there was some benefit to other individuals carrying the same trait. In an early and influential study, Blest (1963) demonstrated differences in the post-reproductive longevity of male moths, based on whether the species were cryptic (palatable and camouflaged) or aposematic (chemically defended and brightly coloured). Blest theorized that cryptic individuals should evolve high rates of post-reproductive senescence, since their presence after the end of reproduction would allow predators to form a search image for that type of prey, and increase the chance of finding similar looking conspecifics. Aposematic individuals, on the other hand, might be expected to evolve low rates of post-reproductive senescence, since by their presence they could teach predators to avoid similar looking conspecifics.

Although Blest's original theory for post-reproductive senescence in moths was formulated using a general mechanism of group selection, it was soon presented by Hamilton from a gene-level perspective in terms of kin selection (Hamilton, 1964). Indeed, examples such as Blest's provided considerable encouragement to Hamilton when formulating his theory of inclusive fitness (Harman, 2010). If individuals in the post-reproductive phase could benefit relatives carrying similar post-reproductive traits, then selection on post-reproductive senescence could take place, despite the trait being expressed after the end of reproduction. As Hamilton recognized, a major condition of this theory is that the population concerned would need to be highly structured, such that

there is a higher than average chance of nearby individuals sharing the same post-reproductive senescence traits.

All else being equal, species that experience higher overall rates of extrinsic mortality should evolve higher rates of senescence through increased selection for early fecundity at the cost of longevity, while those that experience lower rates of mortality should evolve lower rates of senescence (Williams, 1957; but see Abrams, 1993; Williams & Day, 2003; Williams *et al.*, 2006 for important caveats). If members of cryptic species are attacked more frequently than members of aposematic species, then the differences in post-reproductive longevity that Blest observed could also be explained as an indirect consequence of a general difference in rates of senescence mediated by extrinsic mortality. Indeed, there is evidence of differences in longevity between many species of fishes, reptiles and amphibians based on the presence or absence of chemical defence (Blanco & Sherman, 2005), with chemically defended species tending to live longer. Similarly, there is some evidence that the presence of chemical defence may account for some of the observed variation in the longevity of adult Lepidoptera (Beck & Fiedler, 2009; Dunlap-Pianka, Boggs & Gilbert, 1977). More generally, recent studies have since demonstrated a link between extrinsic mortality and rates of senescence in fish (Buston & García, 2007) and insects (Stearns *et al.*, 2000), although in size-structured populations, older individuals may have higher survival rates than younger individuals, and under such conditions, extrinsic mortality and senescence will not always be linked in simple ways (Reznick *et al.*, 2004).

Blest's original analysis involved a qualitative comparison of the life-table data for males (and subsequently females) of different species of moth, and comparing

between them using rank correlation methods. In this paper, we begin by re-analyzing Blest's data to formally assess the strength of evidence for senescence and to obtain maximum likelihood estimates of relevant demographic parameters associated with post-reproductive senescence (see Appendix A). We then present two experiments which test the effect of conspecifics on predation in cryptic and aposematic prey. The first experiment used human predators to determine whether the presence of conspecifics in a computer simulation affects predation rates on cryptic prey. Humans have been used to successfully evaluate a range of theories relating to warning signals and crypsis. For example, Webster *et al.* (2009) conducted a computer experiment in which they varied the orientation of moth images on tree trunks and found that the optimal orientation matched the orientation of real moths in the field. Likewise, a study by Beatty *et al.* (2005) used humans to repeat a great tit (Parus major) study by Alatalo & Mappes (1996), and found comparable results. Nevertheless, to test Blest's theory in a more natural setting we also performed a second experiment to measure predation rates on individuals (and groups) of cryptic and aposematic artificial moths in the field. This general method has been used to measure predation rates by wild avian predators on both individual cryptic (Cuthill *et al.*, 2005; Stevens *et al.*, 2006) and aposematic prey (Speed *et al.*, 2000), but not both at once, and not in groups. Clearly, if the presence of one cryptic prey item enhances the likelihood of attack on other cryptic prey items (and the presence of one conspicuous defended prey type reduces the per capita attack rate on others) then one would expect to see a positive relationship between group size and predation rate. Conversely, if there is "safety in numbers" and attacking one aposematic

prey item reduces the attack rate on other aposematic prey, then one would expect a negative relationship between group size and per capita predation rate.

2.3 Methods

2.3.1 Human Predation

We developed a Visual Basic 2008 program that generated virtual cryptic prey and presented them to human subjects as groups with a range of sizes. Our program was based on an earlier program used by Jackson *et al.* (2005) to quantify overall detection rates of groups of different sizes (the survivorship of individual prey items was not investigated in this earlier study). In the current study, the arena over which prey were distributed was a square with a side length of 400 pixels, which was filled with a proportion of white and green pixels in a random pattern (90% white and 10% green). Prey items were squares with a side length of 10 pixels, and contained a slightly higher proportion of green pixels (80% white and 20% green; see Appendix B-1). A total of 112 different human subjects were presented with a series of prey arenas, which had an 80% chance of containing a prey group of 1-6 individuals (chosen at random from a uniform distribution to avoid assumptions regarding grouping behaviour), and a 20% chance of containing no prey. This within-subject design was used rather than a between-subject design (in which each human subject would encounter prey groups of a single size) to reduce the predictability of the foraging environment, and better measure the relative cost of group size on individual prey survival. Whenever prey were displayed in the arena, they were randomly distributed within that arena, subject to the condition that prey not overlap each other or the arena edge. Finding and clicking on prey items was rewarded

with a sound and the addition of a point to the subject's score. The subjects could continue searching until they found every prey item in a group, but they could also move to a new prey area at any time. The subjects were able to move to a new prey area by pressing a button, and there was no maximum or minimum time limit for a single prey area. Trials ran for five minutes.

2.3.2 Field Predation

Field work was conducted in two areas near Ottawa, Ontario, Canada, from June to August 2009: Stony Swamp (latitude 45.3 degrees, longitude -75.82 degrees) and Gatineau Park (latitude 45.47 degrees, longitude -75.8 degrees). There were two experimental sites in each area (separated by 4 km in Stony Swamp and 6 km in Gatineau), and in each of the two sites, two types of artificial prey were presented. In one of the sites, artificial prey were cryptic (palatable and camouflaged) and aposematic (chemically defended and brightly coloured). In the other, the chemical defence treatment was switched, so that cryptic prey were chemically defended, and conspicuous prey were palatable. While of considerable interest in evaluating the combined effects of signal and defence, the results for these two complementary treatments (cryptic defended and conspicuous palatable prey) are not of direct relevance to evaluating Blest's theory, so for the sake of brevity they are presented in Appendix C.

Artificial prey targets made from pastry dough were pinned to trees with paper "wings" to simulate moths (see Appendix B-2). Chemically defended prey were rendered distasteful by the addition of quinine hydrochloride and ground mustard seed to the pastry bait (1.25g and 2.5g per 500g of pastry, respectively; see Speed *et al.*, 2000),

while palatable prey used pastry baits containing only butter and flour. Targets were pinned to tree trunks at a height of 2m in groups of 1, 2 or 5.

Each week, two transects were laid in each of the two sites in either Stony Swamp or Gatineau Park (alternating each week). Within each transect, prey groups were pinned to trees with a minimum distance of 3m between each tree. Each transect included 10 replicates of each prey treatment and group size, for a total of 60 prey groups per transect (120 prey groups per site). Only deciduous trees with a diameter greater than 10 cm were used.

Prey targets were surveyed at 2, 4, 24 and 48 hours for signs of predation by avian predators, following the methods of Cuthill *et al.* (2005) and Stevens *et al.* (2006). Predation was inferred if the pastry bait was partly or completely missing, or from the presence of beak marks in the paper target. Trials were repeated a total of nine times over the summer (five trials in Stony Swamp and four in Gatineau Park), and transects laid in the same sites always had the same prey treatments to promote learning in predators, although an effort was made to not reuse the same trees more than once.

2.4 Results

2.4.1 Human Predation

The proportion of prey groups attacked by each subject was analyzed by fitting a general linear model (response: arcsin transformed proportion of prey groups attacked, fixed factor: group size, random factor: human subject). Both group size and human subject had a significant effect on the number of prey groups attacked ($F_{5, 521}=49.191$, $P<0.0001$ for groups, $F_{110, 521}=1.966$, $P<0.0001$ for human subjects). A Tukey's post-

hoc test was performed to determine which groups contributed to the overall significance. Single prey were attacked less often than larger groups, and groups of two were attacked less often than groups of four and five, but not groups of three or six (Figure 2-1).

The proportion of prey attacked within each group was also analyzed by fitting a general linear model (response: arcsin transformed proportion of prey attacked, fixed factor: group size, random factor: human subject). Both group size and human subject had a significant effect on the overall arcsin proportion of prey items attacked per screen ($F_{5, 3213}=6.916$, $P<0.0001$ for groups, $F_{110, 3213}=4.155$, $P<0.0001$ for human subjects). A Tukey's post-hoc test was performed to determine which groups contributed to the overall significance. Including prey groups that were not attacked, single individuals were attacked less than individuals in groups of two or three, and there was no significant difference in the proportion of individuals attacked in groups of two, three, four and five (Figure 2-2). A significantly lower proportion of individuals in groups of six were attacked compared to groups of two, three and four, but not compared to groups of five or one.

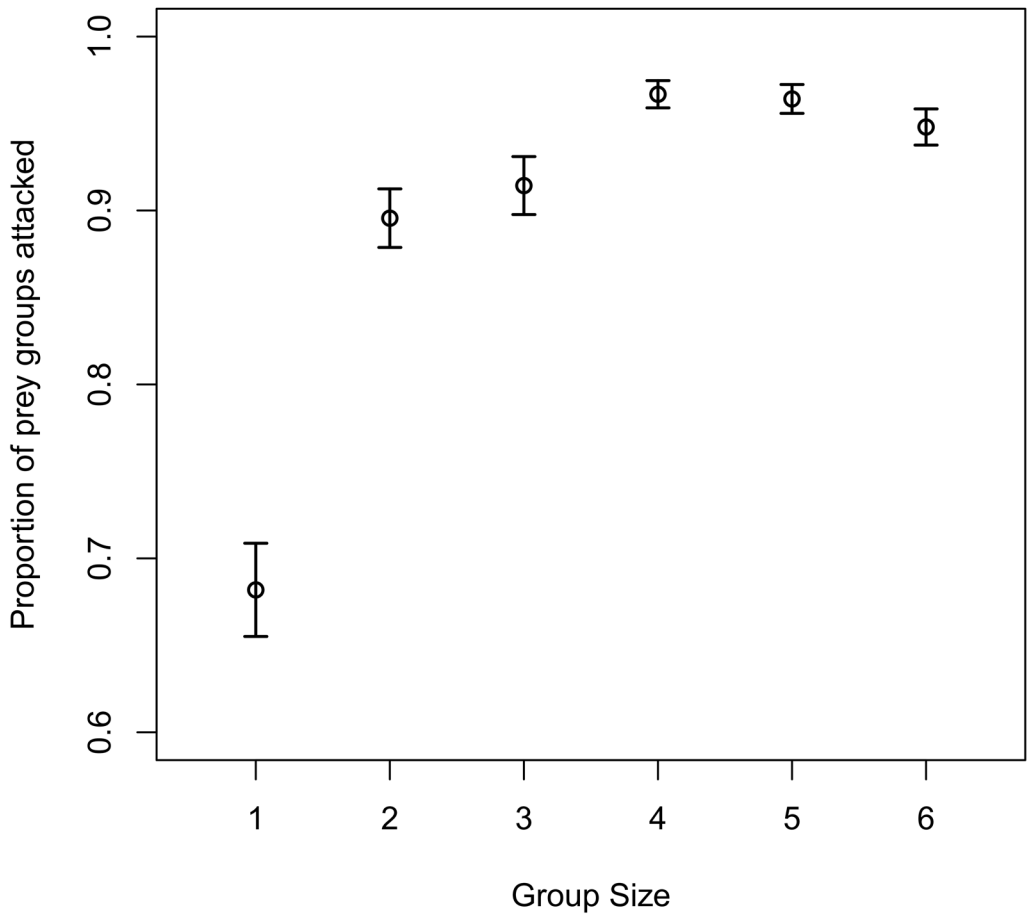


Figure 2-1 Proportion of prey groups attacked by human subjects vs. group size ± standard error. Single prey were attacked significantly less often than larger groups.

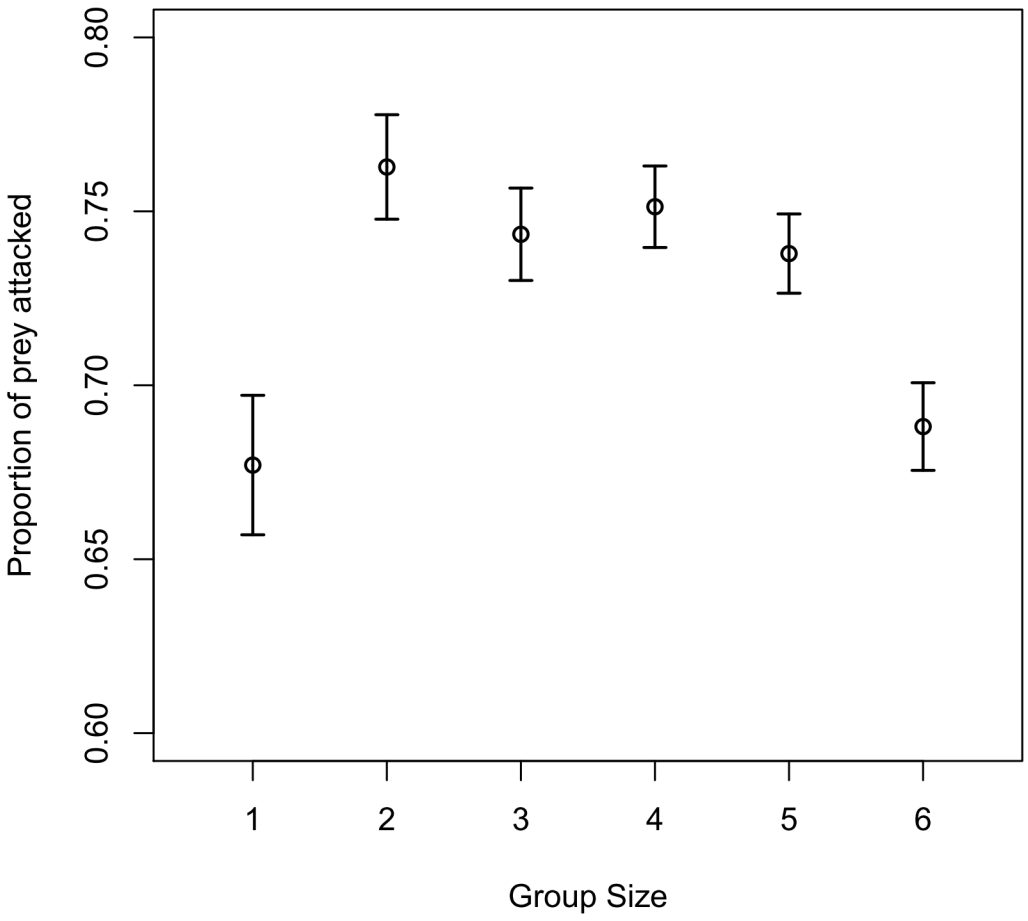


Figure 2-2 Proportion of prey attacked in each group vs. group size ± standard error. A significantly lower proportion of single prey were attacked compared to groups of two and three. A significantly lower proportion of prey in groups of six were attacked compared to groups of two, three and four.

2.4.2 Field Predation

Results from the two areas (Stony Swamp and Gatineau) were analyzed separately. Attack rates on groups as a whole were analyzed using a Cox proportional hazard model (Cuthill *et al.*, 2005; Stevens *et al.*, 2006), with three predictor variables (defence, group size and trial number; see Table 2-1). Predation was deemed to have occurred when at least one individual in a group was attacked. Results from Gatineau were non-significant for defence ($P=0.137$), group size ($P=0.513$) and trial number ($P=0.712$), as well as interactions between predictors. In Stony Swamp, there was a significant effect of both defence ($P=0.033$) and trial number ($P<0.0001$) on group attack rates, but there was no significant effect of group size ($P=0.208$) (Figure 2-3). Here, aposematic prey groups were attacked less than cryptic ones, and prey groups were attacked more in later trials than earlier ones.

Table 2-1 Cox proportional hazard models of attacked prey groups, with three main effects (defensive strategy, group size and trial number) and all interactions. Test statistics reported for the models: W (d.f.), significance (***p<0.001, **p<0.01, *p<0.05 n.s. p>0.05).

Model Factors	Stony Swamp	Gatineau
Defence	4.528(1)*	2.211(1)n.s.
Group Size	3.145(2)n.s.	1.366(2)n.s.
Trial Number	49.099(4)***	1.372(3)n.s.
Defence*Group Size	2.560(2)n.s.	0.297(2)n.s.
Defence*Trial	9.371(4)n.s.	1.059(3)n.s.
Group Size*Trial	3.512(8)n.s.	6.323(6)n.s.
Defence*Group Size*Trial	4.521(8)n.s.	3.227(6)n.s.

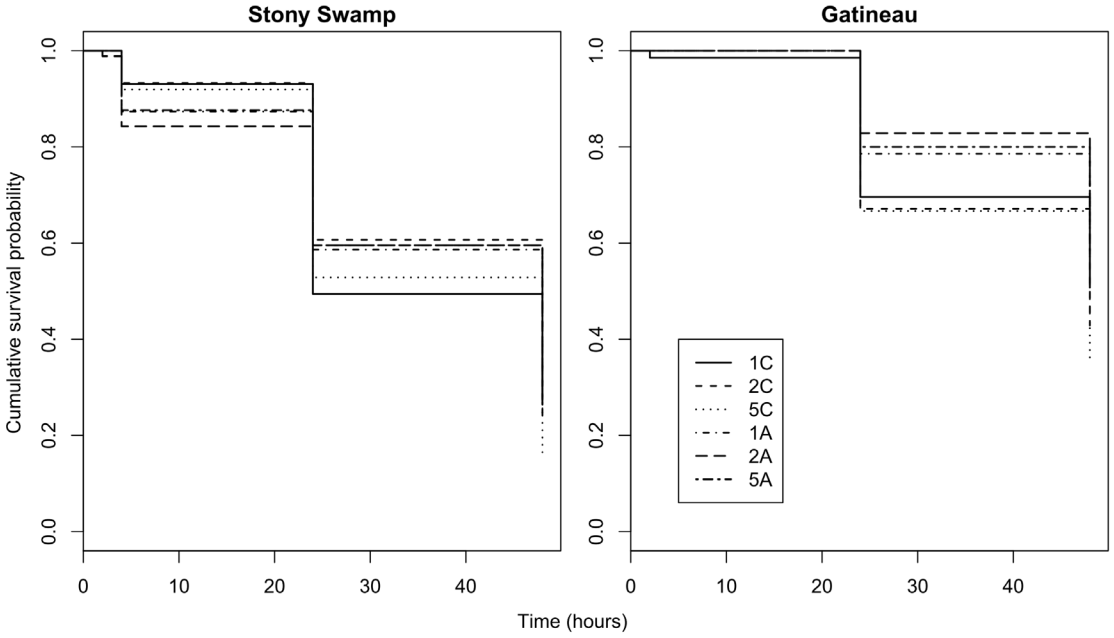


Figure 2-3 Cumulative survival probability for groups of artificial prey. Prey were in groups of 1, 2 or 5, and either cryptic (C) or aposematic (A). There are no significant differences in predation between different group sizes, however aposematic prey in Stony Swamp were attacked significantly less than cryptic prey.

The proportion of prey attacked in each group was also analyzed by fitting a general linear model (response: arcsin proportion of prey attacked, fixed factors: defence, group size and trial number; see Table 2-2). In Gatineau, there was a significant effect of both defence ($P<0.0001$) and trial number ($P<0.0001$) on the proportion of prey attacked within a group, with a lower proportion of aposematic prey attacked compared to cryptic prey (Figure 2-4), and a higher proportion of prey attacked in later trials compared to earlier trials (Figure 2-5). In Stony Swamp, there was a significant effect of trial number ($P<0.0001$) but not defence ($P=0.332$), with a higher proportion of prey attacked in later trials (Figure 2-5). There was also a significant interaction between defence and trial number ($P=0.001$; Table 2-2). There was no significant effect of group size in either Gatineau ($P=0.857$) or Stony Swamp ($P=0.881$), and no significant interactions with group size.

Table 2-2 General linear models of arcsin transformed proportion of prey attacked, with three main effects (defensive strategy, group size and trial number) and all interactions. Test statistics reported for the models: Fs(d.f.), significance (***p<0.001, **p<0.01, *p<0.05, n.s. p>0.05).

Model Factors	Stony Swamp	Gatineau
Defence	0.942(1, 498)n.s.	26.559(1, 394)***
Group Size	0.126(2, 498)n.s.	0.155(2, 394)n.s.
Trial Number	78.409(4, 498)***	21.244(3, 394)***
Defence*Group Size	0.185(2, 498)n.s.	0.735(2, 394)n.s.
Defence*Trial	4.958(4, 498)**	0.839(3, 394)n.s.
Group Size*Trial	0.226(8, 498)n.s.	1.778(6, 394)n.s.
Defence*Group Size*Trial	0.845(8, 498)n.s.	0.676(6, 394)n.s.

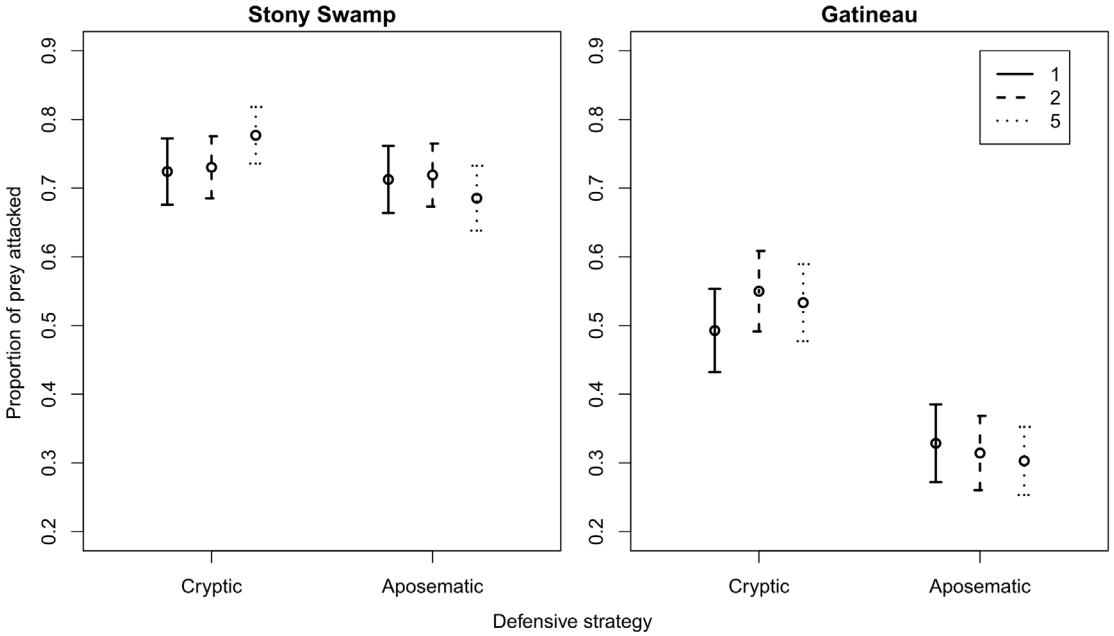


Figure 2-4 Proportion of prey attacked vs. defensive strategy and group size, ± standard error. There are no significant differences in the proportion of prey attacked between group sizes, however a significantly lower proportion of aposematic prey were attacked compared to cryptic prey in Gatineau.

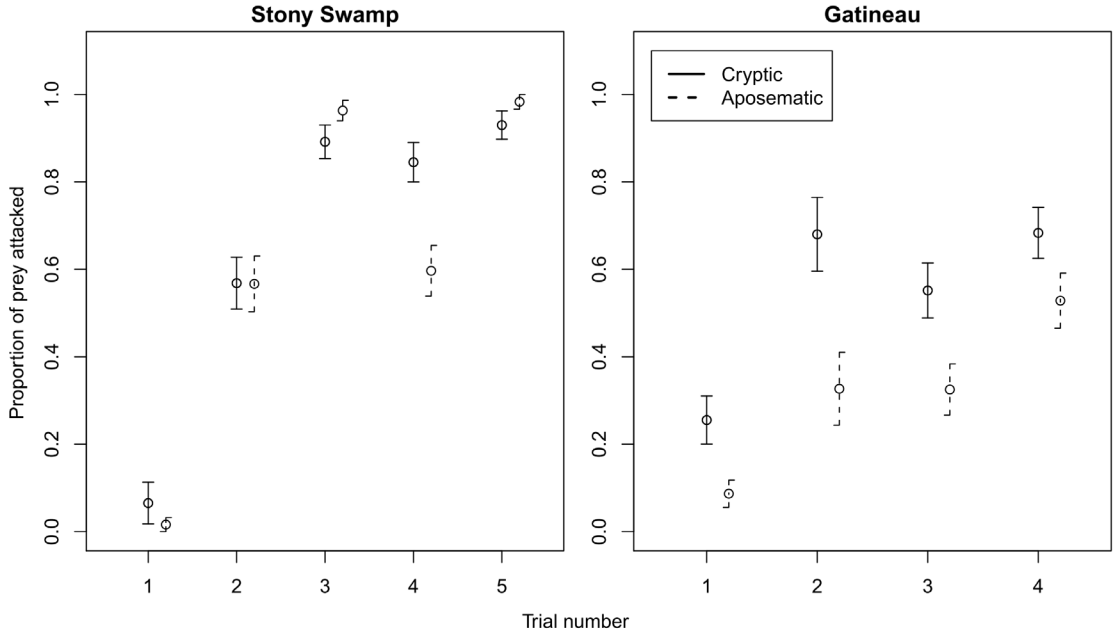


Figure 2-5 Proportion of prey attacked vs. trial number and defensive strategy, ± standard error. In both sites and for both prey types, a significantly higher proportion of prey were attacked in later trials compared to earlier ones.

2.5 Discussion

We conclude that there is little support for Blest's theory of evolution of post-reproductive senescence. The human predation experiment indicates that Blest's proposed mechanism is at least plausible however, since individual cryptic prey were attacked less often when alone than when in groups of two. This is what one would expect if the presence of conspecifics following reproduction increased other individuals' chances of being detected by a predator. Nevertheless, individual prey did not experience significantly lower per capita predation than groups of four, five or six individuals, and groups of six individuals experienced significantly lower per capita predation than groups of two, three and four. This dilution effect may well have arisen as part of a foraging strategy by human subjects, where because of the low cost of moving between groups, it became profitable for them to move on after attacking only the most conspicuous individuals in a group of prey (Charnov, 1976). Overall, the results of the human predation experiment seem to indicate that the predation cost due to the presence of conspecifics is highest when numbers of nearby conspecifics are small. For example, the difference in overall predation rates was higher between groups of one and two than groups of five and six.

In the field predation experiment, the presence of conspecifics did not significantly affect group attack rates or per capita predation of either cryptic or aposematic prey. Even if there was no effect of group size on predation of cryptic prey, it is surprising that there was no effect of group size on predation of aposematic prey, since previous studies using captive avian predators in "novel world" experiments have demonstrated that defended prey benefit from the presence of conspecifics, reducing the

overall per capita predation rate (Gamberale & Tullberg, 1998; Lindström *et al.*, 2001). Nevertheless, a number of studies have shown that predators frequently sample more unpalatable prey when there are more presented (Greenwood, Wood & Batchelor, 1981; Lindström, Alatalo & Mappes, 1999; Beatty, Beirinckx & Sherratt, 2004; Sherratt in press), which may act to reduce the benefit of co-occuring with unpalatable conspecifics. Avian predators have also been shown to attack aggregated aposematic prey more forcefully than individual aposematic prey (Skelhorn & Ruxton, 2006). It is therefore possible that the effect of group size is very small for aposematic prey, or that the number of individuals in a group needs to be significantly larger for a strong biological effect to be observed.

Issues of spatial scale are especially important to bear in mind when considering the learning of avian predators, which are highly mobile. Throughout the experiments, groups were defined at a very small scale (a single tree trunk), with groups of different sizes occurring on different trees within each site. This was done intentionally to maximize the difference in visual signals between groups, but groups could have been defined in other ways, for instance as the population density within a site, or at even larger scales (Fisher, 1930; Ruxton & Sherratt, 2006). It is clearly possible that avian predators develop and retain search images over much wider scales than we were able to evaluate in these experiments.

Previous studies using similar predator trials have used a randomized block design, with different experimental locations for each block, to explicitly minimize learning (Cuthill *et al.*, 2005; Stevens *et al.*, 2006). Some wild avian studies have been performed with longer predator trials (Cuthill, Hiby & Lloyd, 2006; Allen, Raison &

Weale, 1998; five and six days, respectively), but they still lacked the long-term replication over several weeks that was present in this experiment. The temporal changes in predation rates observed over the entire course of the experiment were somewhat unexpected. Although the observed increase in predation rates on cryptic prey was not surprising, it was anticipated that predation rates on aposematic prey would decline over successive weeks of exposure to predators. On the contrary, the predation rates on aposematic prey increased at a similar rate to cryptic prey. This might indicate that aposematic prey were insufficiently distasteful, however the distastefulness recipe used was previously shown to be effective (Speed *et al.*, 2000), and our results indicate that aposematic prey were preyed on significantly less than cryptic prey in at least one site in each analysis. Of course, the lower predation rates on aposematic prey could also have been influenced by neophobia due to their conspicuous coloration. However, the results from appendix C indicate that when chemically defended prey were cryptic, they experienced lower predation rates than palatable, conspicuous prey (see Appendix C-3 and C-4), so clearly, any neophobia towards conspicuous prey was overcome. Although we do not know why the attack rates on aposematic prey continued to increase over time, other factors such as seasonal changes in predator abundance could have contributed to the long-term temporal changes.

The results from the human and avian predators provide little evidence for Blest's theory of group predation and post-reproductive senescence. Other factors also make Blest's argument unlikely, at least as it applies to Lepidoptera. In particular, in loosely structured populations (e.g., highly mobile moths), it is unlikely that traits beneficial to conspecifics will spread exclusively by kin selection, because there is a good chance that

such traits will have an equal chance of benefitting an individual that doesn't carry them. As Hamilton (1964) noted: "the selective forces . . . will be strongest when the average relationships of neighbours is highest, which will be in the most viscous populations". Many moth species are mobile and have high rates of gene flow (Peterson & Denno, 1998), which typically removes population genetic structure. For example, studies of highly dispersive corn borer populations (Lepidoptera: Crambidae) have shown no spatial genetic structure across a 720 km range (Kim *et al.*, 2009). We therefore feel that it is unlikely that species of Lepidoptera occur in sufficiently structured populations to facilitate kin selection (Stevens, Turlure & Baguette, 2010), although we note that the Saturniids studied by Blest may disperse less than other Lepidoptera, due to their lack of adult feeding and subsequently short adult lifespan (Beck & Kitching, 2007). Collectively, our results suggest that the beneficial effect of post-reproductive senescence patterns is too small, and the mobility of moth populations too high, for the observed patterns of senescence to be explained by kin selection.

Our observations that aposematic prey groups were attacked less than cryptic prey groups (Stony Swamp), and a lower proportion of aposematic prey were attacked compared to cryptic prey (Gatineau), are both consistent with the proposal that cryptic prey experience higher mortality than aposematic prey (Blanco & Sherman, 2005). This suggests that traditional evolutionary theories of ageing based on mutation accumulation (Medawar, 1952) and antagonistic pleiotropy (Williams, 1957) could explain the observed differences in longevity between chemically defended and undefended species. It could also account for the differences that Blest observed, since any selection for a senescence trait is likely to affect overall rates of senescence, including both the pre and

post-reproductive phases of an individual's life cycle. More studies characterizing the effectiveness of aposematism and crypsis are clearly needed. Life history traits such as larval host specificity and adult feeding strategies have also been shown to affect adult moth longevity (Dunlap-Pianka *et al.*, 1977; Beck & Kitching, 2007; Beck, 2007). Studies comparing the longevity of cryptic and aposematic Lepidoptera will need to account for these types of life history traits to characterize the relative importance of predation strategies in the evolution of senescence. Future studies should also measure rates of senescence rather than longevity, both to confirm the presence of senescence in populations of lepidoptera, and to provide quantitative estimates of senescence which can be directly compared between species.

Chapter 3 – A direct comparison of the effectiveness of two anti-predator strategies under field conditions

3.1 Abstract

Aposematism and crypsis are two widespread defensive strategies that have evolved in organisms to reduce attacks by predators. However, although both have been studied extensively, predation rates on unpalatable conspicuous prey have seldom been directly compared to those on palatable cryptic prey, and never in the field. In this study we use established methods to compare the effectiveness of both defensive traits, by presenting artificial prey targets on trees where they were subject to attack by wild avian predators in a natural field setting. When partially consumed prey and those that had been completely removed were both treated as attacked by predators, there were no differences in attack rates between targets with the two defensive strategies. However, aposematic prey were completely consumed less often than cryptic prey, and partially consumed more often. This suggests that predators engage in taste rejection of unpalatable prey and/or feed on conspicuous prey more cautiously ("go-slow" predation). We also observed significant differences in predation among experimental sites, in spite of their similarity and relatively close proximity, and among trials, which suggests that prey may experience highly variable predation in the wild. If aposematic prey are capable of surviving attacks by predators, then this represents a potential defensive benefit of aposematism over crypsis. This work has been accepted for publication in the *Journal of Zoology*.

Carroll, J., Sherratt, T.N. *In Press.* A direct comparison of the effectiveness of two anti-predator strategies under field conditions. *J. Zool.*

3.2 Introduction

Many insects experience high rates of predation in the wild, and because of this, species have evolved a range of defensive strategies to avoid detection and/or deter predators when encountered (Poulton, 1890; Cott, 1940). One way that insects avoid detection is by adopting colour patterns that resemble their backgrounds (Endler, 1984). Another (potentially complementary, see Fraser *et al.,* 2007) strategy is disruptive coloration (Cott, 1940; Cuthill *et al.,* 2005). Disruptively-patterned individuals employ contrasting markings to break up their outlines, for instance by bisecting their bodies with dark lines or breaking up their edges with irregular blotches, thereby hindering recognition (Merilaita & Lind, 2005; Stevens & Merilaita, 2009). Note that the two above camouflage mechanisms are not mutually exclusive, and both may be present in a single prey individual (Endler, 1984; Merilaita & Lind, 2005).

Nevertheless, not all insects have evolved camouflage as a response to predation. Many insects, including many species of Lepidoptera (Nishida, 2002; Mappes *et al.,* 2005), are aposematic. Aposematism is a defensive strategy in which characteristics that render prey unprofitable to attack (for instance stings or toxins) are coupled with conspicuous signals such as bright colour patterns (Poulton, 1890). Predators that attack aposematic individuals soon learn to avoid similar looking prey due to unpleasant or painful secondary defenses such as defensive chemicals (Mappes *et al.,* 2005). However, developing chemical defences can be costly (Nishida, 2002; Mappes *et al.,* 2005), and high levels of conspicuousness can potentially lead to aposematic prey experiencing higher attack rates than cryptic prey, especially at low population densities and in the

presence of naïve predators (Lindstrom *et al.,* 2001; Ruxton *et al.,* 2009; Marples & Mappes, 2011).

Avian predators are often considered the model receivers when quantifying predation on cryptic and aposematic prey because they are common predators of insects and because they are primarily visual predators which respond to the colour based cues involved in both defensive strategies (Endler, 1978, 1981; Cuthill *et al.,* 2005). Many studies have separately quantified the effectiveness of either crypsis or aposematism in reducing predation by wild avian predators (Cuthill *et al.,* 2005; Stevens *et al.,* 2006; Skelhorn & Rowe, 2009, 2010; Speed *et al.,* 2000), and there is some evidence from captive predation studies that aposematic prey experience lower predation rates than cryptic prey (Alatalo & Mappes, 1996), even when both prey types are chemically defended (Sillen-Tullberg, 1985; Halpin *et al.,* 2008). However, although some studies have directly compared the fates of cryptic and conspicuous prey (Thomas *et al.*, 2004; Saporito *et al.*, 2007; Stevens *et al.*, 2008), to our knowledge the survival rates of palatable cryptic and unpalatable conspicuous (aposematic) prey have never been directly compared using wild predators under field conditions.

In this study we modified methods from Cuthill *et al.* (2005) and Stevens *et al.* (2006), which used artificial cryptic prey placed on tree trunks to measure predation by wild avian predators, to include aposematic prey (see, for example, Speed *et al.*, 2000 and Skelhorn & Rowe, 2010). While crypsis and aposematism both vary continuously in terms of their effectiveness in deterring predation (Turner *et al.,* 1984), the question of relative effectiveness (albeit at arbitrarily low and high values of defence) has important implications for the life histories of organisms that co-evolve with these defences. For

example, Blanco & Sherman (2005) found that chemically protected species from a range of taxa had overall higher longevities than unprotected species, and proposed that these observations could be explained by chemically protected species evolving under lower overall extrinsic mortality than unprotected species (see also Hossie *et al.*, 2013). We were interested in testing this assumption, and our expectation was that aposematic prey would experience reduced predation compared to cryptic prey, particularly at high levels of chemical defence.

3.3 Methods

Field work was conducted during July and August 2010 in four sites in Gatineau Park, near Gatineau, Quebec, Canada, which were separated by at least 1.7 km (see Appendix D-1). Prey were made from pastry dough (360g flour, 210g lard, 30g water) which was stapled to tree trunks underneath a triangle of "Rite in the Rain®" waterproof paper (http://www.riteintherain.com/) to simulate wings. In each site, five types of artificial prey were presented. There were two palatable cryptic prey types (with either uniform grey wings or wings with a cryptic colour pattern), two aposematic prey types (with conspicuous wings and different levels of unpalatability), and a white palatable control (Appendix D-2). The white palatable control was included to provide a prey target that did not benefit from either crypsis or aposematism, since it was both palatable and conspicuous, but lacking typical warning coloration.

To create the high and low crypsis targets, reflectance measurements were taken from samples of sugar maple bark (*Acer saccharum*) using an Ocean Optics S2000 spectrometer. Two colours were chosen which approximated relatively low and high

reflectance values within the sample measurements (see Appendix D-3 for a comparison of reflectance values between the two colours and sugar maple bark). The wings of the "high crypsis" targets were produced by printing triangles of "Rite in the Rain®" paper with the two colours in a pattern based on sugar maple bark, which was created by manipulating photographs of sugar maple bark to produce monochrome images, and substituting the two cryptic prey colours. The wings of the "low crypsis" targets were uniformly printed with the lighter colour. The high crypsis targets were expected to be more cryptic than the low crypsis targets since they better matched the background, and were also potentially disruptive due to the presence of edge-intersecting patches (Stevens & Cuthill, 2006). The pastry in both the high and low crypsis targets was dyed with 1ml of black Wilton® gel icing colour (http://www.wilton.com/) per 500g pastry. The wings of the white palatable controls had no colour pattern printed on them, and the pastry (white in colour) was not dyed.

The remaining two prey types were modified to have either a low (0.6g quinine hydrochloride, 1.2g ground mustardseed, 0.012g Bitrex per 500g pastry) or high (1.5g quinine hydrochloride, 3g ground mustardseed, 0.3g Bitrex per 500g pastry) level of unpalatability. Quinine hydrochloride has been shown to be aversive to wild avian predators when combined with pastry (Speed *et al.,* 2000), and is chemically similar to quinine compounds found in species of aposematic insects, arachnids and other arthropods (Eisner *et al.,* 2005). Quinine compounds are not toxic to birds, but are bitter tasting, and elicit an emetic response at high doses (Alcock, 1970). Bitrex is a bitter tasting chemical that has been shown to elicit an aversive response in birds (Skelhorn & Rowe, 2009, 2010), but is not toxic or emetic even at very large doses (Schafer *et al.,*

1983), so its only role was to provide an unpleasant or aversive taste to predators. The low and high unpalatability treatments were given conspicuous wings coloured either red or yellow depending on the site, to control for possible pre-existing predator colour preferences. In sites 1 and 2, the prey with a low level of unpalatability were given yellow wings while highly unpalatable prey were given red wings; these colours were reversed in sites 3 and 4. Both types of unpalatable pastry were dyed with 1ml of orange Wilton® gel icing colour per 500g pastry.

Trials were conducted for five weeks. Each week, one transect was laid in each of the four sites. Each transect contained 12 replicates of the five prey types, for a total of 60 prey items per transect, or 240 per week over all four sites. Individual prey targets were stapled to tree trunks at a height of 2m, with the paper wings covering the pastry bodies. Only deciduous trees with a diameter greater than 10 cm were used, and trees with prey targets were a minimum of 3m apart. Transects were left out for four days, and prey targets were surveyed at 24, 48, 72 and 96 hours for signs of predation by avian predators. Two types of predation events were recorded: the time until the pastry bodies were damaged or partly removed from the prey targets, and the time until complete removal of the pastry bodies. Targets with damaged or partly removed pastry were left on the tree, and subsequent complete removal of the pastry bodies was also recorded. Data was censored if there was evidence of attacks by invertebrates such as ants or slugs, which were detectable through the presence of numerous small bite marks and slime trails, respectively. Targets censored in this way were considered to have survived only until they were damaged by invertebrates, but were not counted as having been attacked by predators. Non-avian predators (chipmunks and squirrels) were also present in the

study sites, but we observed beak marks in the pastry bodies of the targets, and small holes and tears in the paper wings, which suggest that avian predators were responsible for much of the observed predation.

Predation was analyzed using a Cox proportional hazards regression (Cox, 1972), which has been used in similar predation studies with censored data and non-uniform predation risk (Cuthill *et al.,* 2005, 2006; Stevens *et al.,* 2006). Analyses were conducted using the survival library (Therneau, 2013) in R (R Development Core Team, 2008). Preliminary analyses indicated that hazard rates differed significantly between the four sites used in the study, as well as between each trial (see Figure 3-1 and Appendix D-4). Given this variability, and because we had no a priori hypotheses regarding the effect of trial or site on predation, the analyses were stratified, which allowed hazard rates to be fitted separately for each trial and site. Defensive strategy was included as a factor in the fitted model, but tree type was not, since the majority of trees used (1053 out of 1200) were sugar maple, and preliminary analyses showed that there was no significant effect of tree type on hazard rates. Overall significance was measured using the Wald test, and pairwise contrasts were used to compare specific treatments.

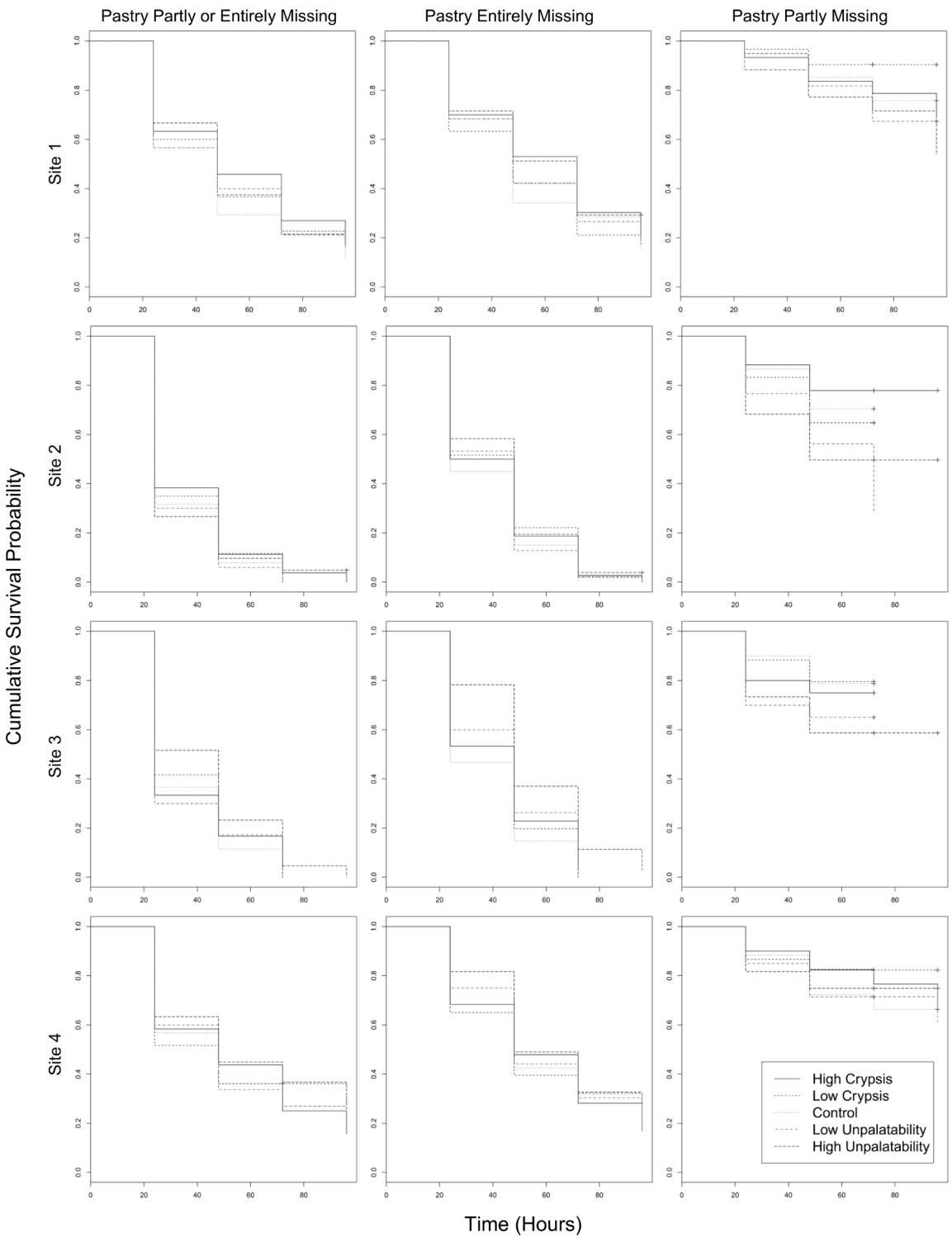


Figure 3-1 Cumulative survival probability for each prey type, separated by experimental site (rows) and predation measure (columns). There were significant differences between sites for all three predation measures.

3.4 Results

Predation was assumed to have occurred if either part or all of the pastry was removed from the target. Predation rates over the total 96 hour collection period ranged from 33% to 92% (mean ± SE: 77 ± 3.7%), and there was no significant effect of defensive treatment on overall predation rates (fit of stratified Cox model: Wald = 6.01, df = 4, P = 0.1985). To differentiate between exploratory attacks and complete consumption by predators, the above two measures were also analyzed separately.

When predation was assumed to have occurred only if the pastry bodies were entirely removed from the targets, there were significant differences in mortality between defensive treatments (Wald = 17.08, df = 4, P = 0.0019). The pastry bodies were entirely removed from highly unpalatable targets at a significantly lower rate than high crypsis (Wald = 7.99, df = 1, P = 0.005), low crypsis (Wald = 10.55, df = 1, P = 0.001) and white (Wald = 12.44, df = 1, P < 0.001) targets, but not significantly less than targets with low unpalatability (Wald = 1.97, df = 1, P = 0.161). There were no other significant differences between defensive treatments (all P > 0.7043).

When predation was assumed to have occurred only in the unusual instance of the pastry bodies being partly removed from the targets (and targets with pastry entirely missing were censored by considering them as surviving up until that point, but not attacked by predators), there were again significant differences in mortality between defensive treatments (Wald = 21.38, df = 4, P < 0.001). The pastry bodies were partly removed from highly unpalatable targets significantly more than low crypsis (Wald = 14.3, df = 1, P < 0.001) and white (Wald = 8.84, df = 1, P = 0.0029) targets, but not significantly more than high crypsis targets (Wald = 2.18, df = 1, P = 0.1403) or targets

with low unpalatability (Wald = 0.01, df = 1, P = 0.95). There were no other significant differences between defensive treatments (all P > 0.1679).

3.5 Discussion

To our knowledge, this is the first time that predation rates on cryptic and aposematic prey have been directly compared in a field setting using wild predators. When prey were considered killed if either part or all of the pastry body was removed, the total mortality rate at the end of the four day survey period was high (77%), however we found no significant difference in survivorship between defensive treatments over the course of our experiment. This was an unexpected result, especially considering that we also found no significant difference in overall survivorship between the different defensive treatments (cryptic and aposematic) and the white palatable control, or between the different cryptic treatments. The white palatable control was assumed to be conspicuous but not warning coloured; however it is possible that the white coloration was aversive to predators (see Lyytinen *et al.,* 1999). Likewise, we did not observe a significant effect of high crypsis targets on predation by wild birds compared to low crypsis targets, even though the presence of edge-intersecting patches made them putatively disruptive (Stevens & Cuthill, 2006), and differences in predation between similar disruptive and monochrome prey have been demonstrated in previous studies (Cuthill *et al.,* 2005; Stevens *et al.,* 2006). One possible reason for this is that the colours on our high crypsis targets may have had insufficient contrast (Schaefer & Stobbe, 2006), and therefore failed to achieve a disruptive effect.

It is important to note that we observed significant variation in hazard rates between our experimental sites and between trials (Figure 3-1 and Appendix D-4, respectively), which could have masked differences in the effectiveness of our defensive treatments. Even though the experimental sites contained similar types of trees, and were separated by a maximum of 5km, there were clear differences in the overall amount of predation and the types of prey which were attacked most often (although many of the differences within each site were non-significant). There could be several reasons for these differences, including predator experience (Skelhorn & Rowe, 2007), availability of alternative prey, or even light quality, which can affect target conspicuousness (Endler, 1993). We did not collect quantitative data on potential predator populations in our study sites, but based on personal observations, the most common species included black-capped chickadees (*Poecile atricapillus*), white-breasted nuthatches (*Sitta carolinensis*), northern cardinals (*Cardinalis cardinalis*), american crows (*Corvus brachyrhynchos*), american robins (*Turdus migratorius*) and yellow-bellied sapsuckers (*Sphyrapicus varius*). Whatever the cause, the observed variation between sites and trials clearly reflects the diverse selection pressures that are likely to be experienced by prey in the wild, at least in the short term.

In contrast to the combined analysis, we found a significant effect of defensive treatment on predation rates when prey targets with pastry completely removed and partly removed were considered separately. Previous experiments with wild avian predators have generally considered predation to have occurred if the edible portion of the prey target was either partly or entirely missing (Cuthill *et al.,* 2005, 2006; Schaefer & Stobbe, 2006; Stevens *et al.,* 2006; Rowland *et al.,* 2008). In doing so, no distinction is made

between exploratory attacks and complete consumption by predators (but see Hossie & Sherratt, 2012), which is of considerable interest when comparing defensive strategies such as crypsis and aposematism. Indeed, when we analyzed our predation measures separately (i.e. predation was defined as the entire pastry being removed vs. part of the pastry being removed), they produced very different results. Specifically, when attacks were considered only if they resulted in the complete removal of the pastry, highly unpalatable prey experienced significantly less predation than high crypsis, low crypsis and white prey, but not significantly less than prey with low unpalatability. Conversely, when only partial removal of the pastry was considered, highly unpalatable prey experienced significantly more predation than low crypsis and white prey, but not significantly more than high crypsis prey or prey with low unpalatability. These results suggest that predators may have been sampling highly unpalatable prey at higher rates than cryptic prey and controls, but consuming them at a lower rate. It should be noted that we were not able to distinguish between single and multiple attacks by predators, and indeed it is possible that completely "consumed" prey targets were simply attacked multiple times by different predators. However, the fact that the observed differences were in the anticipated direction (unpalatable targets consumed less often than palatable ones) suggests that the distinction between partially and completely eaten prey is a valid one.

The lack of significance between prey with high and low levels of unpalatability may indicate that low levels of unpalatability have an intermediate effect on predation. Indeed, it may be profitable for aposematic prey to invest in lower levels of unpalatability in light of the metabolic costs of chemical defenses (Nishida, 2002; Mappes *et al.,* 2005).

However, the lack of significance in predation rates between low unpalatability prey and cryptic prey suggests that there is a benefit to being more unpalatable, particularly since predators may strategically consume aposematic prey based on factors such as hunger and toxin load (Sherratt *et al.*, 2004; Skelhorn & Rowe, 2007). The lack of significance between the two types of unpalatable prey could also have been caused by predators moving between sites, since in two of the sites the colour treatments were reversed and this may have confused predator learning. However, we consider this unlikely, since both conspicuous prey types possessed some level of chemical defence, and the colour treatments were never reversed within a single site.

The differences between complete and partial consumption of cryptic and aposematic baits are readily explained by "go-slow" predation (Guilford, 1994), a strategy in which predators cautiously sample aposematic prey and reject those that are unpalatable without necessarily killing them. This allows predators to avoid the cost of consuming chemically defended prey, while still being able to sample novel or rare conspicuous species; at the same time, aposematic individuals may avoid the disproportionately high mortality rates that are often a consequence of conspicuousness. Go-slow predation may therefore represent a potential defensive advantage of aposematism over crypsis, especially since aposematic insects can survive sampling and rejection by both captive and wild avian predators (Wiklund & Jarvi, 1982; Sillen-Tullberg, 1985; Pinheiro, 1996). Go-slow predation can also help to explain the evolution and spread of novel aposematic species, which has been traditionally considered problematic due to the presence of anti-apostatic (positive frequency-dependent) selection (Endler, 1988; Skelhorn & Ruxton, 2007), by providing a benefit

for honest signalling (Holen & Svennugsen, 2012). To date, several experiments with captive avian predators have demonstrated the presence of go-slow predation in response to novel aposematic prey (Sillen-Tullberg, 1985; Gamberale-Stille & Guilford, 2004; Skelhorn & Rowe, 2006a, 2006b; Halpin *et al.,* 2008), but as far as we are aware it has not yet been documented in wild predators.

It is important to note that our results could also have been caused by simple taste-rejection behaviour. Taste-rejection differs from go-slow predation in that predators reject prey based solely on palatability, and do not exhibit cautious attack behaviour when chemically defended prey are conspicuous. This means that taste-rejection behaviour could be exploited by both cryptic unpalatable and aposematic prey. In retrospect, it would have been interesting to include a cryptic unpalatable control in our defensive treatments, to better distinguish between go-slow predation and taste-rejection by predators. However, while experiments with captive avian predators have shown that taste-rejection occurs with both cryptic and conspicuous chemically defended prey, predators were more likely to learn cautious sampling or outright avoidance when chemical defence was paired with conspicuous coloration (Sillen-Tullberg, 1985; Halpin *et al.,* 2008).

Our results have demonstrated a potential defensive advantage for aposematic prey that is consistent with go-slow predation. Although aposematic and cryptic prey are attacked at similar rates, aposematic prey are consumed less often, indicating that they may be more often rejected by predators after sampling. This could represent an important benefit of aposematism as a defensive strategy, and may have played a role in the evolution of aposematism in the face of significant metabolic and signalling costs.

Chapter 4 – A comparative analysis of ageing in Lepidoptera

4.1 Abstract

One of the fundamental predictions of the evolutionary theory of senescence (albeit with caveats) is that high extrinsic mortality should select for high rates of senescence. Since predation is a common source of mortality, it has been suggested that species which employ aposematism (conspicuousness paired with chemical defence) should evolve reduced rates of senescence. Indeed, previous studies with Lepidoptera using longevity as a proxy for senescence have found that aposematic species live significantly longer than other species. However, very few studies have quantified rates of senescence in Lepidoptera, and none have compared rates of senescence between aposematic and non-aposematic species. In this study, we quantified senescence rates in 22 species of Lepidoptera by fitting demographic senescence models to adult survivorship data drawn from the literature. Senescence was present in all 22 species, and rates of senescence were negatively correlated with mean longevity, although the relationship was non-linear. Aposematic species had lower estimated rates of senescence and higher mean longevities than non-aposematic species, although the relationship with senescence rates was non-significant, potentially due to the relatively small number of species compared. Our results clearly indicate that Lepidoptera show increases in mortality with chronological age, and provide further tentative support for the hypothesis that aposematic species of Lepidoptera experience reduced senescence compared to non-aposematic species.

4.2 Introduction

Senescence (or ageing) is defined in demographic terms as a change in the probability of death and/or fecundity with chronological age. Despite its ubiquity, senescence poses one of the fundamental questions of biology, since individuals that do not senesce would seem to have an evolutionary advantage over those that do (Sherratt & Wilkinson, 2009). The modern (individual-based) evolutionary theory of senescence is rooted in the mutation accumulation hypothesis proposed by Medawar (1952), who recognized that selection would act least strongly to remove deleterious mutations that had an effect late in an individual's life. Several elaborations of this theory have since been proposed which highlight a variety of trade-offs in the shaping of longevity, such as the antagonistic pleiotropy (Williams, 1957), disposable soma (Kirkwood, 1997) and reliability (Gavrilov & Gavrilova, 2001; Laird & Sherratt, 2009) theories of senescence.

One of the fundamental predictions of the evolutionary theory of senescence is that high levels of mortality from external sources should select for high rates of senescence (Williams, 1957; Hamilton, 1966). However, there are important caveats to this prediction. For example, when population growth is density-dependent and increased density reduces the survival of older individuals, increased extrinsic mortality should select for a reduction in the rate of senescence rather than an increase, and if increased density affects survival uniformly across age classes, extrinsic mortality should be selectively neutral (Abrams, 1993). High extrinsic mortality could also select for a reduced rate of senescence if individuals experience condition-dependent susceptibility to external sources of mortality early in life (Williams & Day, 2003). The relationship between extrinsic mortality and senescence is complex, since both density dependence

and condition dependence can, under certain conditions, lead to results in which the correlation between extrinsic mortality and senescence is positive, as well as neutral or negative (Abrams, 1993; Williams & Day, 2003).

Despite these caveats, organisms that experience low levels of extrinsic mortality over evolutionary time are generally expected to evolve low rates of senescence. Since predation is a common source of mortality in wild populations, species that are able to avoid predation would be expected to evolve lower rates of senescence. Two common defensive strategies are crypsis, in which prey avoid detection by adopting colour patterns that match their backgrounds (Endler, 1984), and aposematism, in which prey advertise an unprofitable trait such as stings or toxins through conspicuous coloration (Poulton, 1890). It has been suggested that aposematic prey may experience reduced predation compared to cryptic prey, and that this could lead to the evolution of different longevities and rates of senescence between aposematic and cryptic species (Blanco & Sherman, 2005; Molleman *et al.*, 2007; Beck & Fiedler, 2009). Indeed, there is some evidence from butterflies and moths that aposematic species live longer than other species (Blest, 1963; Beck & Fiedler, 2009), although other factors such as geographical distribution and adult diet also play a role (Dunlap-Pianka *et al.*, 1977; Karlsson & Wiklund, 2005; Molleman *et al.*, 2007; Beck & Fiedler, 2009).

Although previous analyses have examined butterfly longevity, no study has directly compared rates of senescence in different species of Lepidoptera. Since senescence reflects a change in mortality with chronological age, and longevity is more a reflection of the underlying rate of mortality, longevity might vary among species without variation in senescence and *vice versa*. In order for senescence to be present,

rates of mortality need to be shown to change with age. To describe this change, the shape of the mortality curve can be characterized by fitting certain functions. In this study, we fit one of the most well-known, simplest and commonly used senescence functions, the Gompertz model (Gompertz, 1825), which assumes an exponentially increasing or decreasing rate of mortality with age, and compare it to a non-senescent model with constant mortality.

Senescence has been detected and characterized in a range of vertebrates, including mammals (Promislow, 1991; Gaillard *et al.*, 2003) and birds (Ricklefs, 1998; Reed *et al.*, 2008). While it has been argued that wild animals do not live long enough to grow old (Kirkwood & Austad, 2000), some studies have found senescence in short-lived adult insects (Bonduriansky & Brassil, 2002; Dukas, 2008; Zajitschek *et al.*, 2009; Sherratt *et al.*, 2010, 2011). So far, it appears that very few studies have fitted senescence models to demographic data from Lepidoptera (Gotthard *et al.*, 2000; Carroll *et al.*, 2011), and none have compared rates of senescence between species. In this paper we use maximum likelihood methods (Pletcher, 1999; Sherratt *et al.*, 2011) to fit senescence models to laboratory and mark-recapture survivorship data from 22 species of Lepidoptera. We use these results to answer three complementary questions: first, how widespread is senescence in Lepidoptera? Second, what is the relationship between senescence and mean longevity? And third, is there evidence for different rates of senescence between aposematic and non-aposematic species?

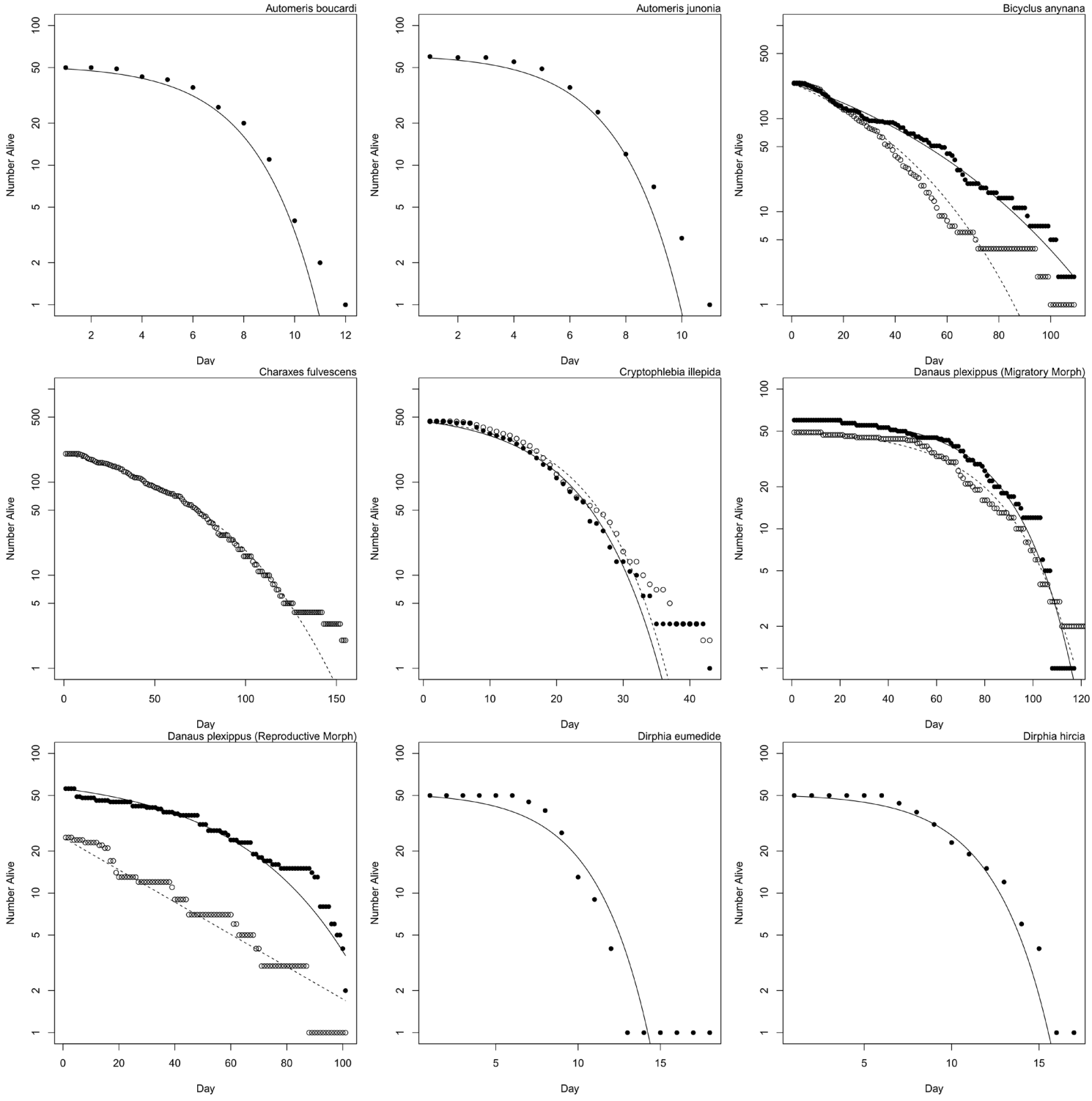
4.3 Methods

Daily survivorship data were collected both from laboratory survivorship curves and from field based mark-recapture studies of Lepidoptera. Data were extracted using the GetData graphical data digitization software package (http://getdata-graph-digitizer.com/) on high-resolution images, a technique that has been previously shown to have high repeatability (Sherratt *et al.*, 2011). Figures 4-1 and 4-2 show semi-log plots of cumulative survivorship per day for each species, with data from both sexes plotted separately when available. In the case of monarch butterflies (*Danaus plexippus*), data were available for both migratory and reproductive morphs, and the two morphs were therefore analyzed separately.

Senescence models were fitted using maximum likelihood (Pletcher, 1999; Sherratt *et al.*, 2011). To determine whether senescence was occurring in each species, two models (the Gompertz model and a non-senescent model) were fitted using the bbmle package (Bolker, 2012) in R (R Development Core Team, 2008). The non-senescent model (with the hazard function $\mu = a$) assumes a constant mortality rate a throughout an individual's life, while the Gompertz model (with the hazard function $\mu = ae^{bx}$) assumes that the mortality rate a increases at an exponential rate b as an individual ages (time x). Parameter estimates for both models were derived by maximum likelihood estimation (MLE). Given that the models are nested (the non-senescent model is effectively a Gompertz model with $b = 0$), the best-fitting model for each species was identified using likelihood ratio tests (Hilborn & Mangel, 1997). Cumulative survivorship curves were calculated using the cumulative probability density of the best fitting senescence model, and the curves were plotted against cumulative survivorship

data (Figures 4-1 and 4-2). Instantaneous hazard functions were also plotted against the estimated hazard rate for each species (Figures 4-3 and 4-4).

Neither parameter in the Gompertz model fully captures the shape of the senescence curve, since *a* represents initial mortality, while *b* represents the rate of exponential increase in mortality rate (Ricklefs & Scheuerlein, 2002). Overall senescence rates were therefore compared by combining the parameter estimates (*a* and *b*) from the fitted Gompertz models to create a single index of the rate of ageing ($\omega = (ab)^{1/2}$). This index has units of time^{-1}, and is equal to the square root of the slope of the relationship between mortality rate and age at age 0 (Ricklefs & Scheuerlein, 2002). We used general linear models to examine patterns between the rate of ageing and mean longevity, and to test the assumption that senescence rates vary with defensive strategy. Our data set did not include enough species to test for the effect of defence on senescence using a phylogenetically controlled comparative analysis. This is unfortunate, since defensive traits such as aposematism are often highly phylogenetically conserved, and this can affect the outcome of comparative studies (Hossie *et al.*, 2013); our results should therefore only be considered preliminary.


Automeris boucardi
Automeris junonia
Bicyclus anynana
Charaxes fulvescens
Cryptophlebia illepida
Danaus plexippus (Migratory Morph)
Danaus plexippus (Reproductive Morph)
Dirphia eumedide
Dirphia hircia
Number Alive
Day

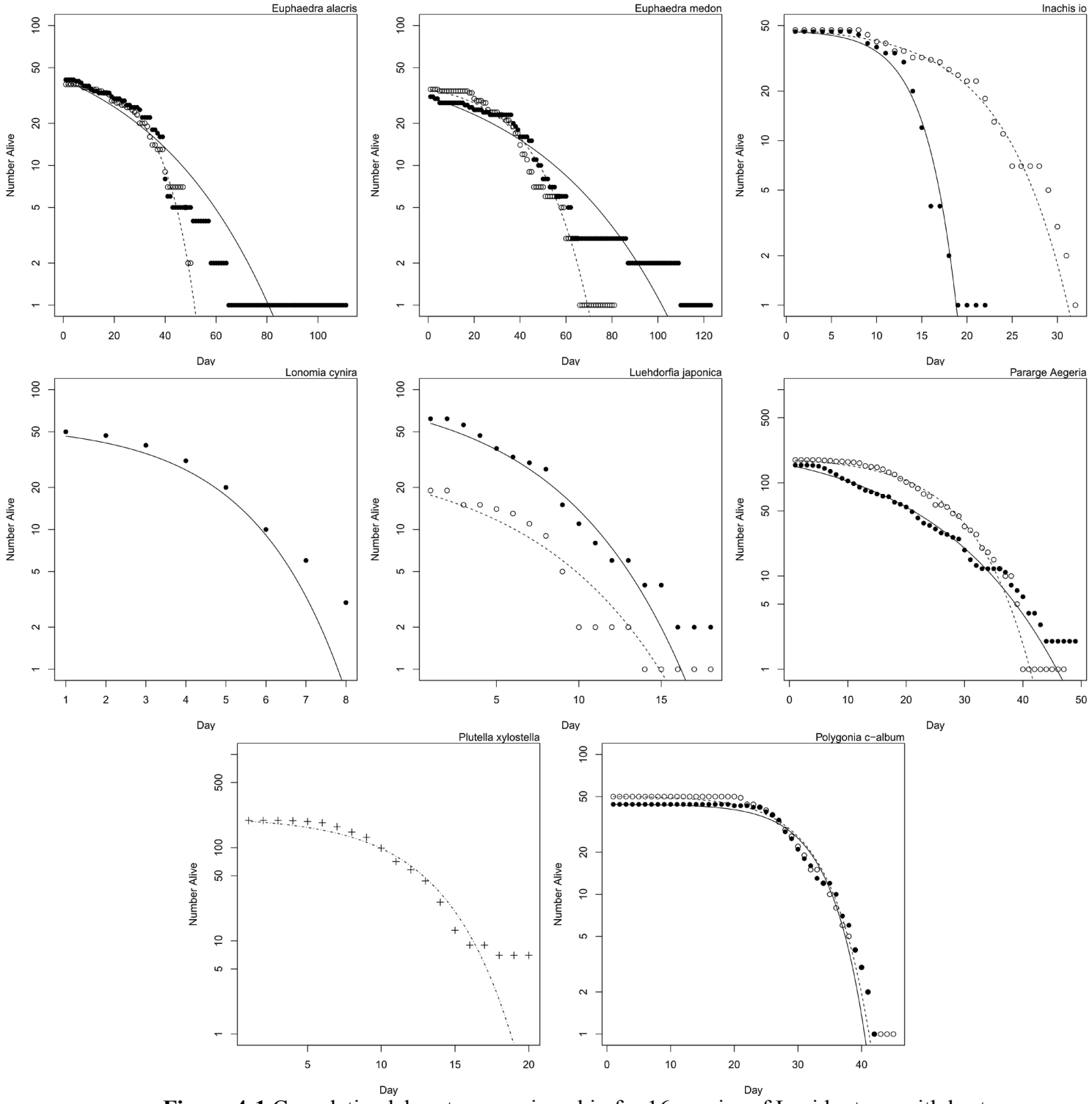


Figure 4-1 Cumulative laboratory survivorship for 16 species of Lepidoptera, with best fit models (Gompertz or no senescence; see Table 4-1 for a list of models and model parameters). Open circles indicate females, closed circles indicate males, and crosses indicate samples where sex data was not provided. For demographic models, dashed lines indicate females, solid lines indicate males, and dash-dotted lines indicate samples where sex data was not provided.

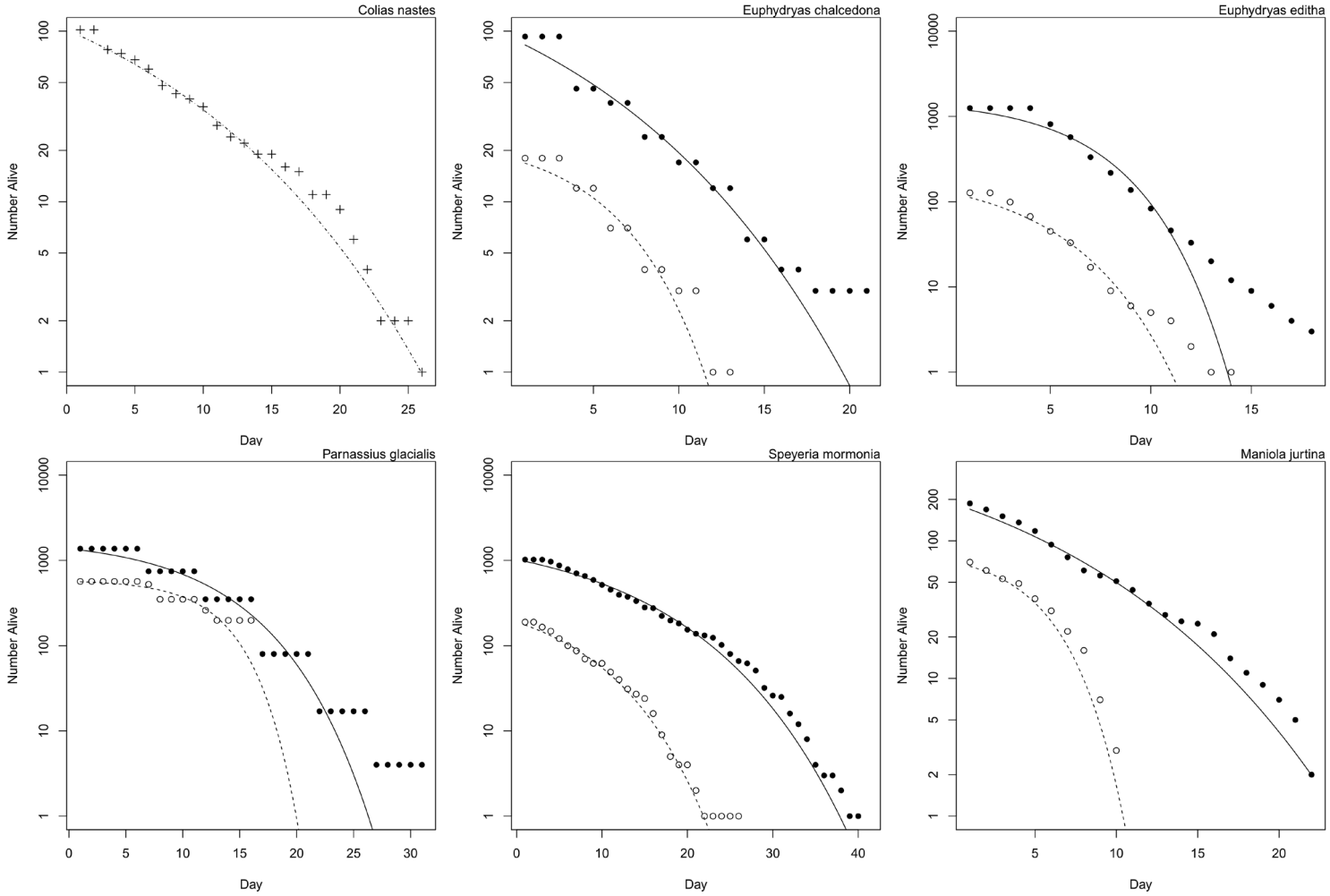
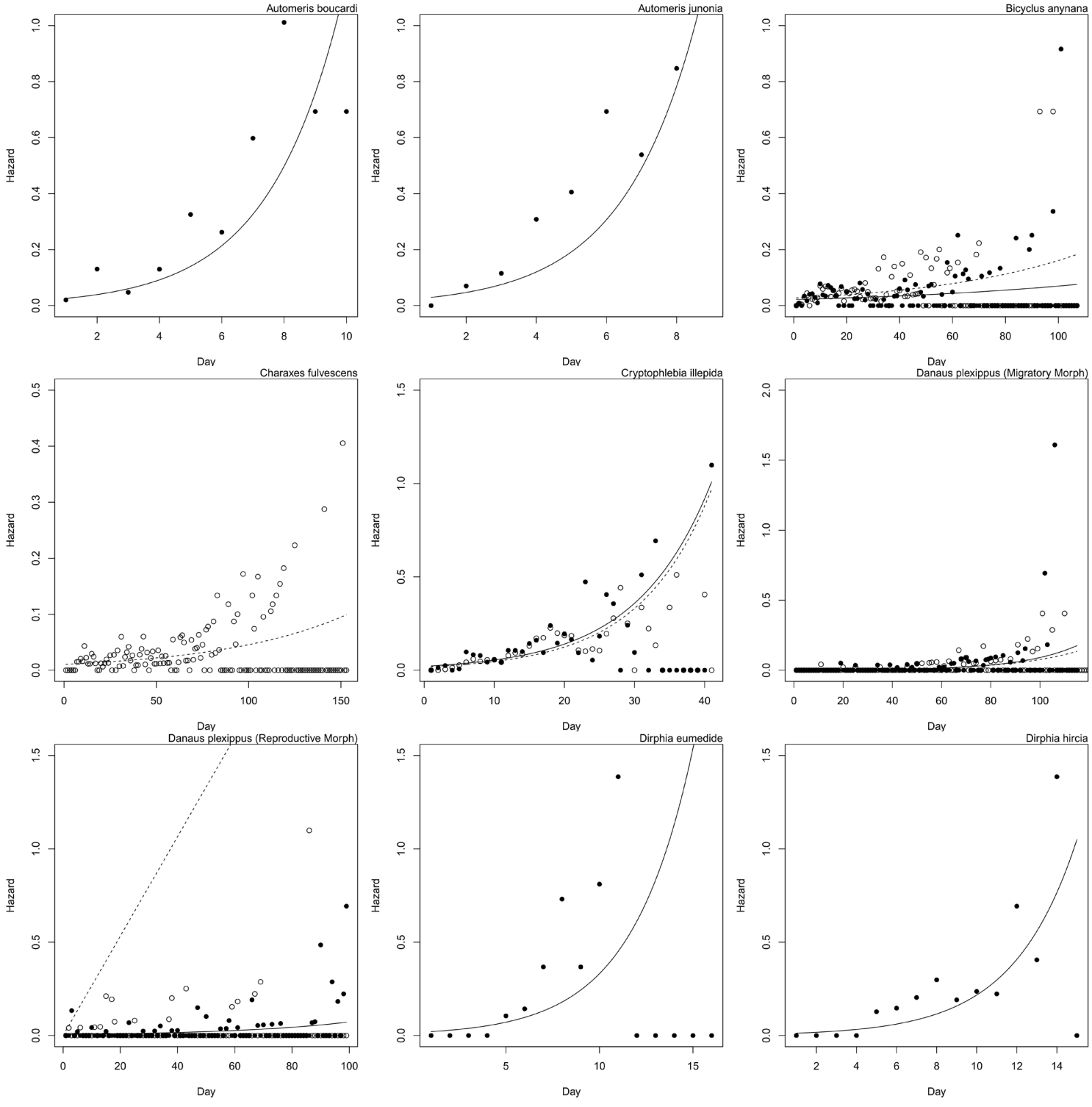


Figure 4-2 Cumulative field survivorship for 6 species of Lepidoptera, with best fit models (Gompertz or no senescence; see Table 4-2 for a list of models and model parameters). Open circles indicate females, closed circles indicate males, and crosses indicate samples where sex data was not provided. For demographic models, dashed lines indicate females, solid lines indicate males, and dash-dotted lines indicate samples where sex data was not provided.


Automeris boucardi
Automeris junonia
Bicyclus anynana
Charaxes fulvescens
Cryptophlebia illepida
Danaus plexippus (Migratory Morph)
Danaus plexippus (Reproductive Morph)
Dirphia eumedide
Dirphia hircia
Hazard
Day

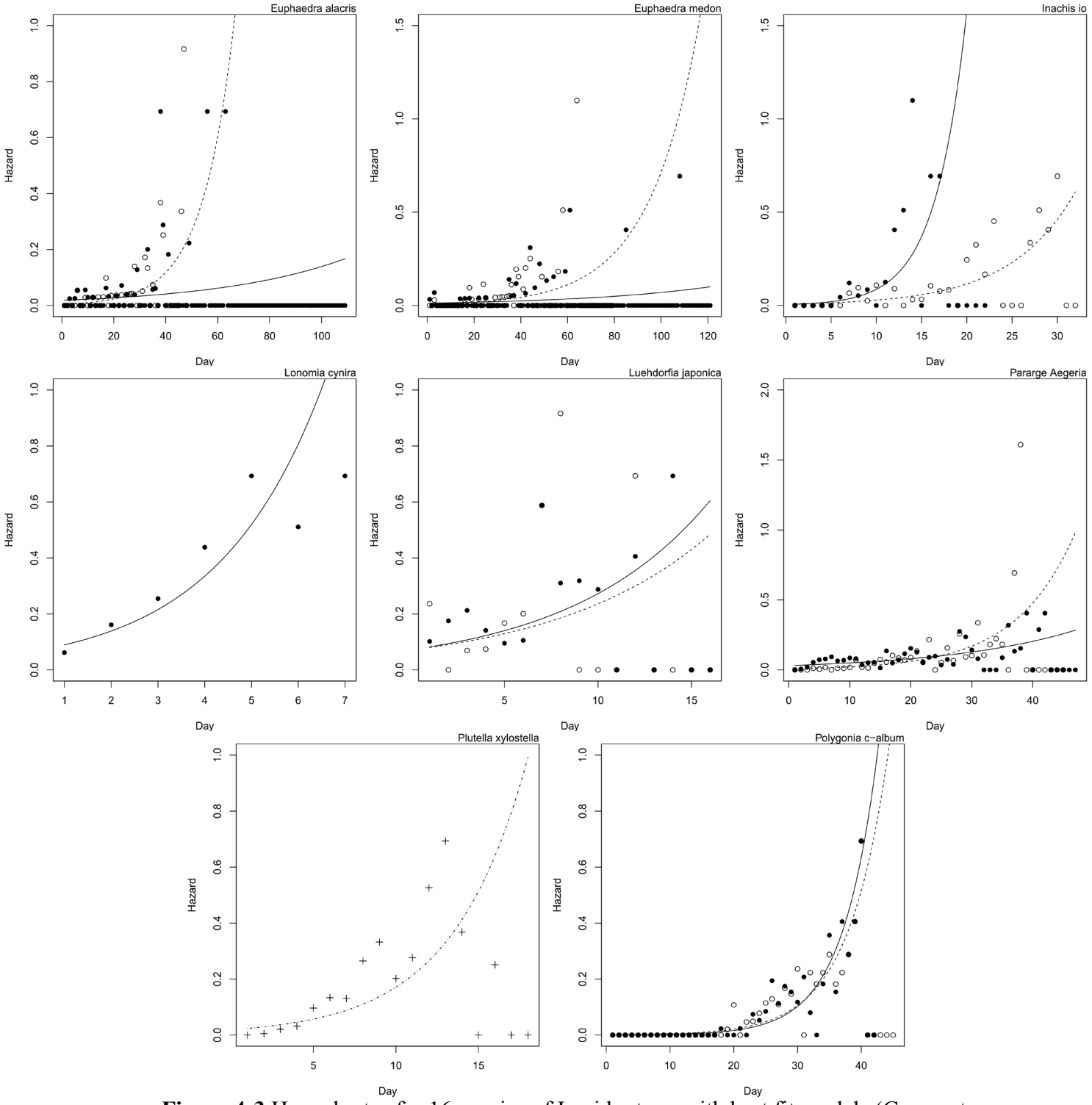


Figure 4-3 Hazard rates for 16 species of Lepidoptera, with best fit models (Gompertz or no senescence; see Table 4-1 for a list of models and model parameters). Open circles indicate females, closed circles indicate males, and crosses indicate samples where sex data was not provided. For demographic models, dashed lines indicate females, solid lines indicate males, and dash-dotted lines indicate samples where sex data was not provided.

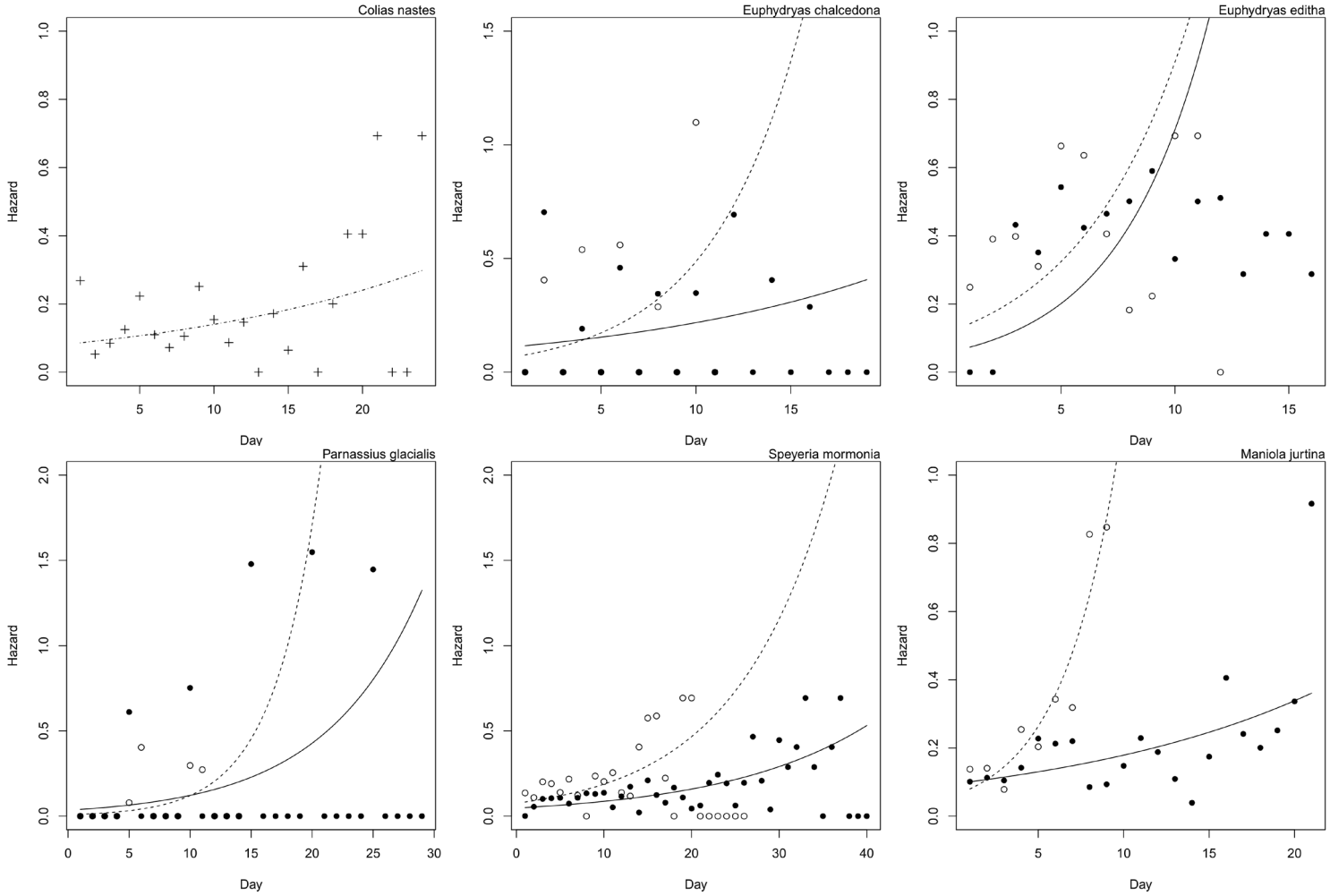


Figure 4-4 Hazard rates for 6 species of Lepidoptera, with best fit models (Gompertz or no senescence; see Table 4-2 for a list of models and model parameters). Open circles indicate females, closed circles indicate males, and crosses indicate samples where sex data was not provided. For demographic models, dashed lines indicate females, solid lines indicate males, and dash-dotted lines indicate samples where sex data was not provided.

Although mean adult longevity has been shown to be resistant to biases caused by small sample size (Moorad *et al.*, 2013), previous studies with Lepidoptera have instead used median and maximum longevity as proxies for senescence (Molleman *et al.*, 2007; Beck & Fiedler, 2009). It has also been suggested that ω should not be used as a proxy for senescence rates, since the parameters a and b in the Gompertz model can co-vary without changing the value of ω, which could potentially lead to populations with different initial mortalities and rates of increase having similar ω values (Giaimo, 2013). To explore these potentially confounding factors, preliminary analyses were carried out which examined patterns between senescence parameters (ω, a and b) and measures of longevity derived from the survival data (mean, median and maximum; see Appendix E-1 and E-2). Analyses were also conducted to test whether the effects of defensive strategy and sex were consistent across different senescence parameters and measures of longevity (Appendix E-3).

4.4 Results

In almost every case, the Gompertz model provided a significantly better fit than the non-senescent model to the available daily survivorship data (see Tables 4-1 and 4-2 for parameter estimates and likelihood ratio significance values of laboratory and mark-recapture data, respectively), indicating the presence of senescence in all 22 species of Lepidoptera. The Gompertz model did not provide a significantly better fit than the non-senescent model for the female reproductive morph of the monarch butterfly (*Danaus plexippus*). Rates of senescence were highly variable among species, and between males and females of the same species in several cases.

Table 4-1 Parameter estimates of best fit models (Gompertz or constant mortality) for 16 species of Lepidoptera by sex (where available) based on laboratory survivorship data, obtained using R (R Development Core Team 2008). Likelihood ratio tests were performed to determine which model provided the best fit to the data.

Species	Sex	Sample Size	a (95% LCI, UCI)	b (95% LCI, UCI)	Log-Likelihood, Best Model and LR Significance	References
Automeris boucardi	Male	50	0.0170 (0.0076, 0.0347)	0.4224 (0.3177, 0.5291)	-113.16, Gompertz, P<0.0001	Blest (1963) Fig. 1
Automeris junonia	Male	60	0.0184 (0.0090, 0.0351)	0.4688 (0.3661, 0.5728)	-128.56, Gompertz, P<0.0001	Blest (1963) Fig. 1
Bicyclus anynana	Female	240	0.0269 (0.0221, 0.0325)	0.0179 (0.0120, 0.0235)	-999.24, Gompertz, P<0.0001	Molleman *et al.* (2008b) Fig. 1
Bicyclus anynana	Male	240	0.0218 (0.0176, 0.0268)	0.0116 (0.0064, 0.0166)	-1066.97, Gompertz, P<0.0001	Molleman *et al.* (2008b) Fig. 1
Charaxes fulvescens	Female	202	0.0105 (0.0082, 0.0133)	0.0147 (0.0107, 0.0185)	-971.52, Gompertz, P<0.0001	Molleman *et al.* (2008a) Fig. 1
Cryptophlebia illepida	Female	451	0.0175 (0.0145, 0.0210)	0.0979 (0.0882, 0.1075)	-1549.05, Gompertz, P<0.0001	Jones & Aihara-Sasaki (2011) Fig. 1
Cryptophlebia illepida	Male	450	0.0213 (0.0178, 0.0254)	0.0941 (0.0840, 0.1039)	-1535.31, Gompertz, P<0.0001	Jones & Aihara-Sasaki (2011) Fig. 1
Danaus plexippus (migratory morph)	Female	49	0.0018 (0.00078, 0.0036)	0.0377 (0.0281, 0.0475)	-228.46, Gompertz, P<0.0001	Herman & Tatar (2008) Fig. 2
Danaus plexippus (migratory morph)	Male	60	0.0011 (0.00047, 0.0023)	0.0441 (0.0340, 0.0548)	-276.17, Gompertz, P<0.0001	Herman & Tatar (2008) Fig. 2
Danaus plexippus (reproductive morph)	Female	25	0.0267 (0.0175, 0.0385)		-115.62, Constant, P=0.075	Herman & Tatar (2008) Fig. 1
Danaus plexippus (reproductive morph)	Male	56	0.0060 (0.0032, 0.0105)	0.0249 (0.0152, 0.0349)	-266.92, Gompertz, P<0.0001	Herman & Tatar (2008) Fig. 1
Dirphia eumedide	Male	50	0.0154 (0.0081, 0.0279)	0.3070 (0.2430, 0.3685)	-122.73, Gompertz, P<0.0001	Blest (1963) Fig. 1
Dirphia hircia	Male	50	0.0093 (0.0040, 0.0195)	0.3153 (0.2416, 0.3915)	-128.31, Gompertz, P<0.0001	Blest (1963) Fig. 1
Euphaedra alacris	Female	38	0.0045 (0.0018, 0.0101)	0.0816 (0.0558, 0.1088)	-148.12, Gompertz, P<0.0001	Molleman *et al.* (2009) Fig. 1

Euphaedra alacris	Male	41	0.0182 (0.0111, 0.0287)	0.0204 (0.0082, 0.0310)	-178.43, Gompertz, P=0.0017	Molleman *et al.* (2009) Fig. 1
Euphaedra medon	Female	35	0.0069 (0.0032, 0.0133)	0.0465 (0.0296, 0.0629)	-148.40, Gompertz, P<0.0001	Molleman *et al.* (2009) Fig. 1
Euphaedra medon	Male	31	0.0123 (0.0065, 0.0217)	0.0173 (0.0056, 0.0280)	-143.94, Gompertz, P=0.0046	Molleman *et al.* (2009) Fig. 1
Inachis io	Female	47	0.00718 (0.0032, 0.0148)	0.1388 (0.1009, 0.1781)	-157.88, Gompertz, P<0.0001	Wiklund *et al*. (2003) Fig. 2
Inachis io	Male	46	0.0047 (0.0020, 0.0106)	0.2906 (0.2281, 0.3532)	-122.02, Gompertz, P<0.0001	Wiklund *et al.* (2003) Fig. 2
Lonomia cynira	Male	50	0.0576 (0.0298, 0.1041)	0.4397 (0.3047, 0.5752)	-101.65, Gompertz, P<0.0001	Blest (1963) Fig. 1
Luehdorfia japonica	Female	19	0.0709 (0.0309, 0.1462)	0.1202 (0.0250, 0.2049)	-53.16, Gompertz, P=0.015	Matsumoto (1984) Fig. 7
Luehdorfia japonica	Male	62	0.0720 (0.0455, 0.1100)	0.1331 (0.0771, 0.1861)	-169.89, Gompertz, P<0.0001	Matsumoto (1984) Fig. 7
Pararge aegeria	Female	176	0.0077 (0.0053, 0.0109)	0.1033 (0.0886, 0.1180)	-630.23, Gompertz, P<0.0001	Gotthard *et al.* (2000) Fig. 1
Pararge aegeria	Male	155	0.0312 (0.0235, 0.0408)	0.0470 (0.0331, 0.0604)	-571.10, Gompertz, P<0.0001	Gotthard *et al.* (2000) Fig. 1
Plutella xylostella	-	196	0.0190 (0.0138, 0.0259)	0.2196 (0.1914, 0.2476)	-543.38, Gompertz, P<0.0001	Soufbaf *et al.* (2010) Fig. 2
Polygonia c-album	Female	50	0.00094 (0.00033, 0.0024)	0.1588 (0.1267, 0.1923)	-165.23, Gompertz, P<0.0001	Wiklund *et al.* (2003) Fig. 2
Polygonia c-album	male	44	0.00043 (0.00010, 0.0014)	0.1824 (0.1435, 0.2251)	-140.75, Gompertz, P<0.0001	Wiklund *et al.* (2003) Fig. 2

Table 4-2 Parameter estimates of best fit models (Gompertz or constant mortality) for 6 species of Lepidoptera by sex (where available) based on field survivorship data, obtained using R (R Development Core Team 2008). Likelihood ratio tests were performed to determine which model provided the best fit to the data.

Species	Sex	Sample Size	a (95% LCI, UCI)	b (95% LCI, UCI)	Log-Likelihood, Best Model and LR significance	References
Colias nastes	-	102	0.0816 (0.0583, 0.1118)	0.0539 (0.0217, 0.0850)	-312.11, Gompertz, P=0.0013	Roland (1982) Fig. 7
Euphydryas chalcedona	Female	18	0.0614 (0.0225, 0.1423)	0.2069 (0.0724, 0.3384)	-45.88, Gompertz, P=0.0031	Murphy *et al.* (1986) Fig. 8
Euphydryas chalcedona	Male	93	0.1087 (0.0780, 0.1483)	0.0694 (0.0286, 0.1078)	-258.74, Gompertz, P=0.0011	Murphy *et al.* (1986) Fig. 8
Euphydryas editha	Female	127	0.1155 (0.0858, 0.1533)	0.2064 (0.1505, 0.2591)	-289.06, Gompertz, P<0.0001	Murphy *et al.* (1986) Fig. 8
Euphydryas editha	Male	1254	0.0569 (0.0515, 0.0628)	0.2526 (0.2384, 0.2664)	-3003.29, Gompertz, P<0.0001	Murphy *et al.* (1986) Fig. 8
Maniola jurtina	Female	70	0.0590 (0.0341, 0.0971)	0.2994 (0.0341, 0.0971)	-163.3, Gompertz, P<0.0001	Brakefield (1982) Fig. 1
Maniola jurtina	Male	187	0.0946 (0.074, 0.1193)	0.0637 (0.0365, 0.0902)	-543.47, Gompertz, P<0.0001	Brakefield (1982) Fig. 1
Parnassius glacialis	Female	567	0.0085 (0.0066, 0.0109)	0.2652 (0.2442, 0.2867)	-1579.24, Gompertz, P<0.0001	Matsumoto (1985) Fig. 4
Parnassius glacialis	Male	1372	0.0347 (0.0314, 0.0383)	0.1257 (0.1174, 0.1337)	-4198.22, Gompertz, P<0.0001	Matsumoto (1985) Fig. 4
Speyeria mormonia	Female	189	0.0475 (0.0427, 0.0527)	0.0604 (0.0530, 0.0677)	-3420.93, Gompertz, P<0.0001	Boggs *et al.* (1987) Fig. 3
Speyeria mormonia	Male	1023	0.0756 (0.0589, 0.0960)	0.0909 (0.0637, 0.1170)	-548.97, Gompertz, P<0.0001	Boggs *et al.* (1987) Fig. 3

There was a significant negative correlation between ω and mean longevity (r = 0.554, $F_{1, 36}$ = 46.963, P < 0.0001). Since the relationship was non-linear (Figure 4-5), a polynomial regression was fitted which included a linear, quadratic and cubic term using Type I sums of squares to test for the significance of each additional term (r = 0.978, $F_{1, 34}$ = 20.412, P < 0.0001 for the cubic term). The relationships between senescence parameters (ω, a and b) and different measures of longevity (mean, median and maximum) were qualitatively similar, and significantly negatively correlated in all cases (see Appendix E-1 and E-2).

Although ω was lower in aposematic species than non-aposematic species, the difference was borderline non-significant (P = 0.079). There was also no significant difference between males and females (P = 0.472), and no significant interaction (P = 0.664; see Table 4-3). Aposematic species had significantly higher mean longevity than non-aposematic species (P < 0.001), but there were no significant differences in longevity between males and females (P = 0.163) and no significant interaction (P = 0.366; see Table 4-3). When similar analyses were performed using other senescence parameters (a and b) and measures of longevity (median and maximum), the results were qualitatively similar (see Appendix E-3).

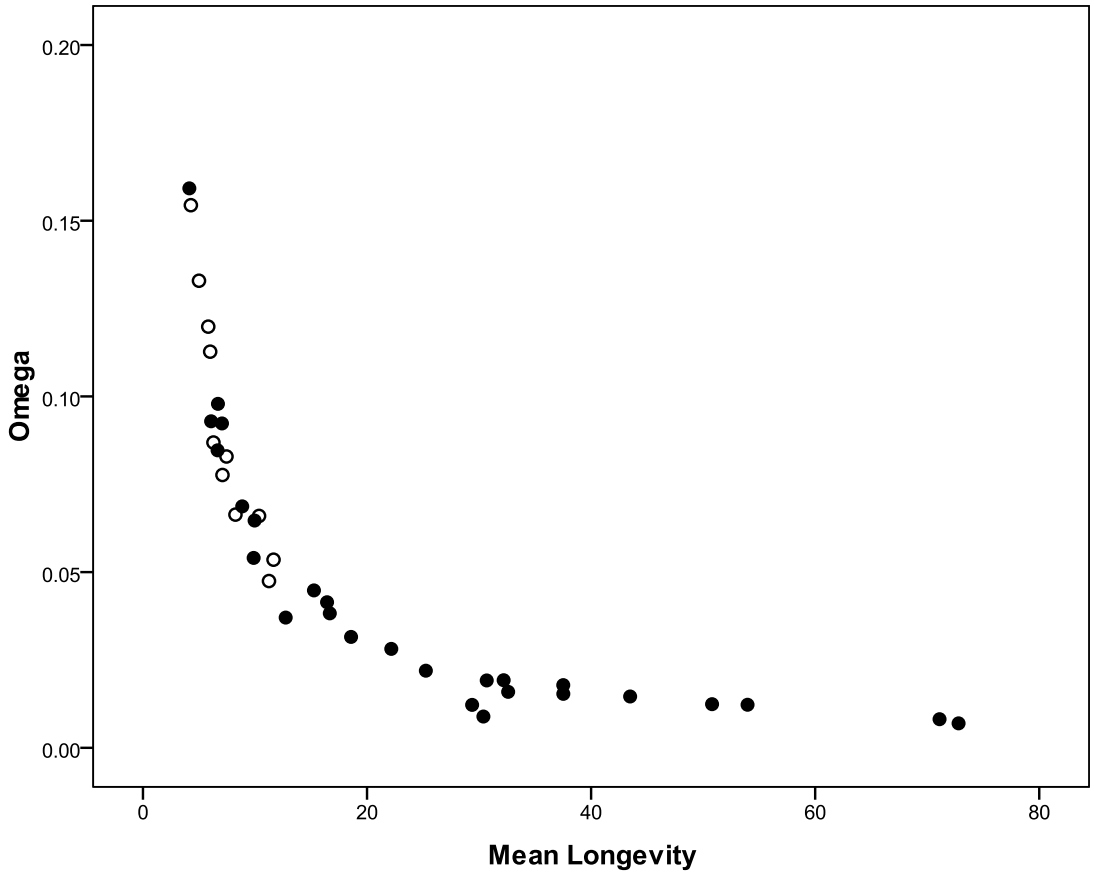


Figure 4-5 Omega (ω) vs. mean longevity for 22 species of Lepidoptera. Open circles indicate field survivorship, while closed circles indicate laboratory survivorship.

Table 4-3 Results from two general linear models with rate of senescence (ω) and mean longevity as dependent variables. In both models, there were two factors (defence and sex) as well as an interaction term. Aposematic species had significantly higher mean longevity than non-aposematic species, but there was no significant difference in the rate of senescence (ω). There were no significant differences between males and females in either model, and no interactions between aposematism and sex.

Dependent Variable	Model Factors	*F*	d.f.	Sig.
Rate of Senescence (ω)	**Aposematism**	3.302	1, 32	P = 0.079
	Sex	0.530	1, 32	P = 0.472
	Aposematism * Sex	0.192	1, 32	P = 0.664
Mean Longevity	**Aposematism**	13.279	1, 32	P < 0.001
	Sex	2.037	1, 32	P = 0.163
	Aposematism*Sex	0.840	1, 32	P = 0.366

4.5 Discussion

Senescence was present in all of the species surveyed, except for females of the reproductive morph of the monarch butterfly (*Danaus plexippus*). This is an important finding, since only two studies have previously fitted senescence models to demographic data from Lepidoptera, and both used a small number of species: Gotthard *et al.* (2000) fitted logistic models to a single species, the speckled wood (*Pararge aegeria*), while Carroll *et al.* (2011) fitted Gompertz models to five species of Saturniid moth (both data sets were also included in this analysis). In contrast, we have demonstrated the presence of senescence across a range of Lepidoptera with diverse habits and life histories.

Our results also indicate that rates of senescence are highly variable both within and among species. Senescence rates were higher (and mean longevities were lower) in mark-recapture studies compared to laboratory studies, which is not surprising since wild species were presumably exposed to additional sources of mortality such as predation. Previous studies of Lepidoptera have also demonstrated that factors such as geographical distribution and adult diet are correlated with observed variations in longevity (Dunlap-Pianka *et al.*, 1977; Karlsson & Wiklund, 2005; Molleman *et al.*, 2007; Beck & Fiedler, 2009), but none have directly compared senescence rates. Longevity is often used as a proxy for senescence, but it is unclear to what extent this assumption is justified. Our results indicate that there is a significant correlation between senescence and longevity across a range of Lepidoptera, although the relationship is non-linear (Figure 4-5). The negative correlation between senescence and longevity is significant across a range of senescence parameters and measures of longevity, which supports the assumption that longevity is an appropriate proxy for senescence rate (Appendix E-1 and E-2).

Previous studies have also demonstrated significant differences in longevity between chemically defended and non-defended species in a range of taxa (Blanco & Sherman, 2005; Hossie *et al.*, 2013), and between aposematic and non-aposematic species of Lepidoptera (Blest, 1963; Dunlap-Pianka, 1977; Beck & Fiedler, 2009). We did not find a significant difference in senescence rates between aposematic and non-aposematic prey, even when analyzing the senescence parameters *a* and *b* separately, although values of all three senescence parameters were lower for aposematic prey. This may be at least partially because our data set included only three aposematic species: monarch butterflies (*Danaus plexippus*; Beck & Fiedler, 2009) and two species of Saturniid moth (*Dirphia eumedide* and *Dirphia hircia*; Blest, 1963). However, longevity values taken from the survivorship data (mean, median and maximum) were significantly higher in aposematic than non-aposematic species, which lends support to the theory that aposematic species evolve lower rates of senescence, particularly in light of the consistently negative relationship that we observed between senescence parameters and all three measures of longevity.

Because of the small number of species for which data were available, we did not include defensive strategies such as mimicry or eye-spots in our analysis. It would be interesting to include these defensive strategies in future analyses, since at least one study has detected a significant positive relationship between longevity and the presence of eye-spots in Lepidoptera, while failing to find an effect of Batesian mimicry (Beck & Fiedler, 2009). Our data set was also not large enough to test for the effect of aposematism using a phylogenetically controlled comparative analysis, and we did not control for potentially confounding factors such as body size, which makes our

conclusions regarding aposematism tentative. However, they agree with results from two large phylogenetically controlled studies, which have found that the presence of chemical defence and aposematism are significantly correlated with increased longevity (Hossie *et al.*, 2013; Beck & Fiedler, 2009).

Overall, we have demonstrated the presence of senescence in several species of Lepidoptera, as well as quantifying the high level of among-species variability in senescence rates. Our results have provided tentative support for the hypothesis that aposematic species live longer than non-aposematic species, as well as exploring the relationship between senescence and longevity.

Chapter 5 – The coevolution of senescence and antipredator defence under predation

5.1 Abstract

An early prediction of the evolutionary theory of aging, albeit with caveats, was that high levels of extrinsic mortality should select for high rates of senescence. Since predation is an important source of mortality for many species, it has been suggested that different antipredation strategies could result in different rates of senescence. In particular, it has been proposed that aposematism (chemical defence paired with a conspicuous signal) may be more effective at reducing predation than crypsis (or camouflage), and that this could explain some of the observed differences in longevities among species of fish, reptiles and Lepidoptera. Although many studies have attempted to model senescence, and others have explored the evolution of antipredator defences, here we present a model of the coevolution of senescence with a defensive trait. Using a simple genetic algorithm, we explore conditions under which senescence evolves due to predation, and how different defensive strategies and assumptions about predator behaviour affect this relationship. Our results confirm the prediction that high levels of predation select for reduced longevity, and suggest that unpalatable prey evolve higher longevity than palatable prey when both are relatively conspicuous. However, aposematic prey do not evolve higher longevity than highly cryptic prey, unless behaviours such as cautious sampling or neophobia are present in the predator population.

5.2 Introduction

Senescence, or ageing, is a near-universal phenomenon which poses an apparent evolutionary paradox, since it appears that individuals that do not senesce would gain a reproductive advantage over those that do. Several theories have been advanced which seek to explain why senescence arises in the face of natural selection. One of the earliest individual-based evolutionary theories of senescence, the "mutation accumulation" theory, was proposed by Medawar (1952), who recognized that selection would act most strongly to remove deleterious mutations that had an effect early in an individual's life. In contrast, there would be far less selection to purge deleterious mutations that acted late in life because so few individuals would survive long enough to experience their harmful effects. By the same token, a heritable trait that is deleterious late in life could be actively selected for if it was beneficial early in life, a theory now called "antagonistic pleiotropy" (Williams, 1957; Rose, 1991). Although several refinements and elaborations have been proposed, such as the "disposable soma" theory (Kirkwood, 1977; 1996) and the "reliability" theory of ageing (Gavrilov & Gavrilova, 2001; Laird & Sherratt, 2009), the above arguments form the basis for the majority of evolutionary theories of senescence.

The theories of mutation accumulation and antagonistic pleiotropy both predict that mutations which provide a fitness benefit later in life will be only weakly selected for, since external sources of mortality make it likely that most individuals will not survive to reap the benefits of such late-acting beneficial mutations. It follows from this prediction that the overall chance of dying due to "extrinsic" factors (starvation, drought, predation, accident, etc.) could have a strong influence on the evolved intrinsic longevity

of a species (Williams, 1957). In particular, species which experience high levels of extrinsic mortality would be expected to evolve shorter lives (and earlier reproduction), while species which experience low levels of extrinsic mortality would be expected to evolve longer lives. There are some important caveats to this prediction, however. In particular, both density-dependent population growth and condition-dependent mortality can lead to conditions under which extrinsic mortality is selectively neutral, or even selects for reduced senescence (Abrams, 1993; Williams & Day, 2003).

One of the most widely investigated sources of extrinsic mortality is predation, and lower predation rates have been cited as a possible cause of increased longevity both in birds (Holmes & Austad, 1995; Holmes *et al.*, 2001) and flying or gliding mammals (Austad & Fischer, 1991; Holmes & Austad, 1994), as well as subterranean species (Buffenstein, 2008) and geographically isolated populations (Austad, 1993). Many species have evolved traits to mitigate predation-related mortality, and two of the best known defensive strategies are crypsis and aposematism. Cryptic species avoid detection by adopting colour patterns that resemble their backgrounds (Endler, 1984), while aposematic prey possess toxins (or some sort of active defence), which are advertised with conspicuous colour patterns (Poulton, 1890). It has been suggested that aposematic prey may experience reduced predation compared to cryptic prey, and that this could lead to the evolution of different longevities between aposematic and cryptic species (Blanco & Sherman, 2005; Molleman *et al.*, 2007; Beck & Fiedler, 2009). Indeed, there is some evidence from butterflies and moths that aposematic species live longer than other species (Blest, 1963; Beck & Fiedler, 2009), and from fish, snakes and frogs that chemically defended species live longer than those without chemical defenses (Blanco &

Sherman, 2005), even when accounting for phylogeny (Hossie *et al.*, 2013). However, other factors including geographical distribution and adult diet also play an important role in shaping longevity (Dunlap-Pianka *et al.*, 1977; Karlsson & Wiklund, 2005; Molleman *et al.*, 2007; Beck & Fiedler, 2009).

While numerous models have been presented which explore the evolution of senescence (Hamilton, 1966; Abrams, 1993; Penna, 1995; Travis, 2004; Mittledorf, 2006; Laird & Sherratt 2009), and many others have attempted to better understand the evolution of crypsis and aposematism (Turner, 1984; Speed, 1993, 2001; Sherratt, 2002, 2011; Franks & Noble, 2004; Sherratt & Franks, 2005; Lee & Speed, 2010; Lee *et al.*, 2010; Holen & Svennugsen, 2012), here we combine both approaches by asking if and how longevity can co-evolve with anti-predator defence. This approach is important, not just to understand why chemically protected species tend to live longer than cryptic species (Blest, 1963; Blanco & Sherman, 2005; Beck & Fiedler, 2009), but also to help characterize the inter-relatedness between these superficially disparate phenomena.

We began by creating a simple model based on a genetic algorithm (Holland, 1975) to capture the decline in the intensity of selection with chronological age. This model is similar to the Penna model of ageing (Penna, 1995), although for the sake of simplicity we assume discrete generations and a fixed population size. In its simplest form, the model simulates the evolution of a longevity trait within a prey population at a fixed level of extrinsic mortality. Once characterized, we develop this model further by introducing a more explicit representation of extrinsic mortality in the form of predation, with a population of predators feeding on prey with fixed defensive attributes. For comparative purposes, the population of predators we have invoked were assumed to

forage in a manner represented by a "Pavlovian predator" that has been thoroughly explored by Speed (1993; 2001). Our final model explores the coevolution of longevity with a defensive trait comprised of two distinct evolving colour morphs (cryptic and conspicuous). Collectively, these approaches help us understand how the nature of defence is likely to shape both longevity and the strength of selection for one morph type over another.

5.3 Methods

Our simulation models were developed and executed using R (R Development Core Team, 2008).

5.3.1 Fixed Extrinsic Mortality

We consider a population of individuals with a fixed number of genes, all with two alleles (0 and 1). These genes code for the continued survival (1) or death (0) of an individual at each time step. Individuals experience intrinsic mortality at the earliest time step in which they have a mortality gene, so that in the absence of extrinsic mortality an individual with the genome (1111100101 . . . 00110), for example, would live for 5 time units, while an individual with the genome (1111111010 . . . 10001) would live for 7 time units (any ones after the first zero have no effect on the phenotype, and are therefore latent). Individuals in the initial population are randomly assigned survival or mortality genes for each potential time step (see Appendix F-1A for a representation of a starting population). The population size is fixed, as is the maximum possible lifespan (defined as the length of the genome). There is a fixed mutation rate that can cause a survival

gene to become a mortality gene, and *vice versa* (forward and backward mutation rates are equal in all versions of the model).

Each generation, intrinsic mortality times are calculated for each individual, although a proportion of the population die due to extrinsic mortality before intrinsic mortality occurs. An extrinsic mortality time is also calculated for each individual based on a fixed chance of experiencing extrinsic mortality at each time step. Each individual's actual survival time is defined as the smaller of their extrinsic and intrinsic mortality times. To allow for selection, each individual is given a "lottery ticket" for every time step that they were alive, and the population for the next generation is drawn from this pool of lottery tickets, so that individuals undergo reproduction proportional to their survival. For simplicity, pre- and post-reproductive life stages are not modeled. Mutation is probabilistically imposed at the genome level during reproduction, and the simulation runs for a fixed number of generations.

5.3.2 Pavlovian Predator: fixed prey palatability and single colour pattern

Fixed rates of extrinsic mortality only crudely characterize mortality caused by predators, since predators may change their attack rates based on factors such as prey palatability (for example, learning to avoid unpalatable prey or to attack palatable prey more often). To simulate extrinsic mortality from predation more directly, we adapted the "Pavlovian predator" model from Speed (1993), with predators feeding on prey with fixed defensive attributes. Speed's approach was in turn based on the Monte Carlo predator described by Turner *et al.* (1984). In this model, there are a fixed number of predators that can potentially attack prey every time step, and which learn to increase or

decrease their attack rates based on the palatability of prey they have previously attacked. Predators begin each generation with a fixed probability of attacking prey on encounter, which in most simulations is set at 0.5 as in Speed (1993). Prey have a fixed palatability value that can vary from 0 to 1, with 0 indicating a highly unpalatable individual, and 1 indicating a highly palatable individual. When a predator encounters a prey individual, it determines whether to attack based on its current attack probability. If the prey is attacked, the prey dies and the predator's attack probability (P) is modified based on the prey's palatability, according to the difference equation:

$$\Delta P = \alpha_l(\lambda_l - P)$$

where α_l indicates the rate of learning, and λ_l indicates the prey individual's palatability. The rate of learning (α_l) is set by the equation:

$$\alpha_l = 0.5 + |\lambda_l - 0.5|$$

This learning algorithm assumes that the change in attack probability is proportional to the difference in palatability from 0.5 and is complete after a single attack when prey are either extremely palatable ($\lambda_l = 1$) or unpalatable ($\lambda_l = 0$).

Predators also gradually "forget" their learned attack probability over any time steps in which they do not attack a prey individual, according to the equation:

$$\Delta P = \alpha_f(\lambda_f - P)$$

The value of λ_f is equal to the initial attack probability (usually 0.5), and the value of α_f is fixed at 0.2, as in Speed (1993).

As we are interested in the evolution of senescence in both palatable cryptic prey and aposematic prey, we consider prey with fixed palatability and conspicuousness. Prey are therefore given a visibility value which, as a probability of detection on "encounter",

can vary between completely invisible ($\gamma = 0$) and entirely visible ($\gamma = 1$). The overall probability of attacking a given prey type on encounter is therefore the probability of detection multiplied by the probability of attack on detection.

To simulate cautious sampling by predators, we also explored cases in which predators' initial attack probability is not set at 0.5, but is instead inversely (and linearly) related to prey visibility (when prey have a visibility of 0.1, predators' initial attack probability is 0.9; conversely, when prey have a visibility of 0.9, predators' initial attack probability is 0.1). This assumption is based on evidence from wild predators that predators have an innate aversion to traditionally aposematic colours such as orange and yellow, as well as a propensity to attack prey with traditionally cryptic colours such as green and brown (Smith, 1977; Schuler & Hesse, 1985; Ruxton *et al.*, 2004). Predators increase or decrease their attack rates based on prey palatability after encountering (and choosing to attack) prey, as above.

5.3.3 Pavlovian Predator: fixed prey palatability and two colour patterns

To investigate how longevity coevolves with signalling under predation, we again use Pavlovian predators, but we include two distinct prey morphs. This model is based on Speed (2001), which expanded the "Pavlovian predator" model from Speed (1993) to include two types of unpalatable prey (cryptic and conspicuous) as well as concepts from receiver psychology such as increased memorability and discriminability of conspicuous prey (Guilford, 1990; Guilford & Dawkins, 1991). The model includes two prey morphs (cryptic and conspicuous) that share a fixed level of palatability ($\lambda_l = 1$ for palatable prey and $\lambda_l = 0$ for unpalatable prey). Predators are aggressive (initial attack probability = 1),

and are able to perfectly distinguish between prey morphs, meaning that they have a separate attack probability for each morph. Experiments with captive avian predators have shown that avoidance learning rates are significantly higher with aposematic prey compared to unpalatable cryptic prey (Gittleman & Harvey, 1980; Sillen-Tullberg, 1985, Roper & Wistow, 1986). Because of this, conspicuous prey morphs are always highly visible ($\gamma = 1$) and predators have higher associated learning rates ($\alpha_l = 0.7$), while cryptic prey morphs can have a range of visibilities ($0 < \gamma < 1$) and predators have lower associated learning rates ($\alpha_l = 0.1$). There is a uniform forgetting rate associated with both prey morphs ($\alpha_f = 0.005$). These learning values ($\alpha_l = 0.7$ for conspicuous prey and $\alpha_l = 0.1$ for cryptic prey), which were taken from Speed (2001), are in turn based on measurements from captive trials with avian predators (Gittleman & Harvey, 1980).

The two prey morphs (cryptic and conspicuous) have a simple binary colour gene which can mutate between the two forms (forward and backward mutation rates are equal in all simulations), and prey individuals in the initial population are assigned a colour morph randomly, although initial populations that were entirely made up of either cryptic or conspicuous prey were also explored. In addition to a higher rate of learning, conspicuous prey also provoke a neophobic response in predators. Neophobia causes each predator to reject a fixed number of conspicuous prey encountered (ε), after which predation and learning occur normally. Neophobia has been recorded in a range of taxa (Mappes *et al.*, 2005). Experiments with avian predators have shown that novel prey items are often rejected in favour of familiar prey (Marples, *et al.*, 1998; Thomas *et al.*, 2004), possibly as a consequence of an optimal risk-taking strategy (Sherratt, 2011), and

that conspicuously coloured prey are rejected more often than cryptic prey (Smith, 1977; Sillen-Tullberg, 1985).

5.4 Results

5.4.1 Fixed Extrinsic Mortality

In the absence of extrinsic sources of mortality, simulated populations evolve the maximum possible longevity, bounded by the length of the genome. Imposing fixed rates of extrinsic mortality causes populations to evolve a lower mean and maximum longevity compared to no extrinsic mortality, while mean longevity in the simulated population is (as expected) significantly lower than maximum longevity (Appendix F-1). Although several values were explored for the population parameters, we report results from a standard simulation with a population size of 1000 individuals, a genome of length 100, and forward and backward mutation rates of 0.005. In most simulations, populations evolved a stable mean longevity after 200 generations, but all simulations were continued for 1000 generations to ensure stability (Appendix F-2). As expected, mean evolved longevity decreases dramatically with increasing levels of extrinsic mortality (Figure 5-1).

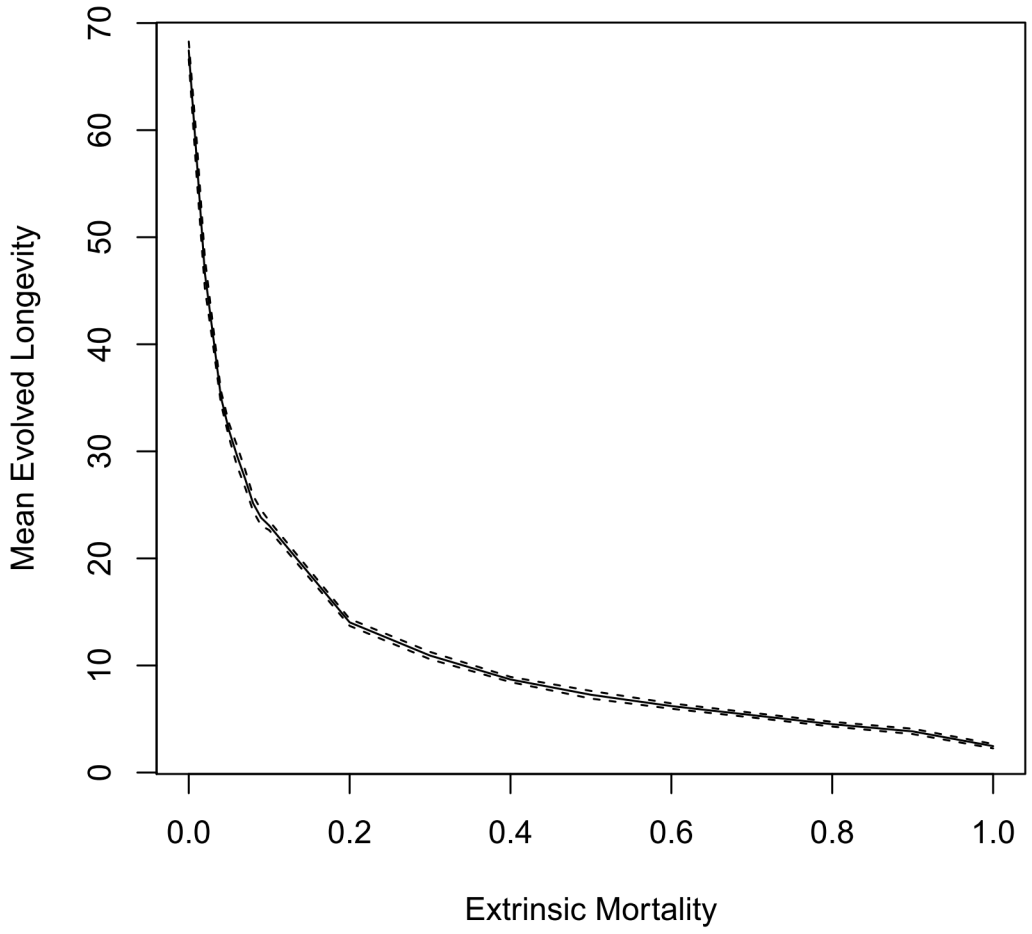


Figure 5-1 Mean evolved longevity vs. extrinsic mortality. Dotted lines represent 95% confidence values. As predicted by Medawar (1952), higher extrinsic mortality results in lower evolved longevity. When extrinsic mortality is absent, evolved longevity approaches the maximum value allowed by the length of the genome, because long lived individuals can receive more "lottery tickets" for the next generation. Number of individuals = 1000, maximum genome length = 100, forward and backward mutation rates = 0.005, number of generations = 1000.

5.4.2 Pavlovian Predator: fixed prey palatability and single colour pattern

When individuals are subject to extrinsic mortality due to predation, several trends become apparent. Lower visibility and lower palatability are both correlated with higher mean evolved longevity (Figure 5-2A), but the effect of prey palatability on longevity is only present at higher visibilities. When prey are very cryptic (visibility values below 0.2), the effect of palatability on evolved longevity is negligible. When predator aggressiveness (initial attack probability) is inversely related to prey visibility, palatability is still negatively correlated with evolved longevity (Figure 5-2B). Prey visibility also has an effect on evolved longevity, but the relationship is more complex. Highly cryptic prey (low visibility) have high longevity regardless of palatability, while highly conspicuous prey (high visibility) also have high longevity, but only when unpalatable. In both models, predator attack rates are inversely related to evolved longevity (Figure 5-3). Contrary to expectations, aposematic prey experience increased predation and reduced longevity compared to highly cryptic prey, at least when conspicuousness is not free to evolve. Even when cautious sampling by predators is included in the model, predation rates and evolved longevities of aposematic prey are comparable to highly cryptic prey.

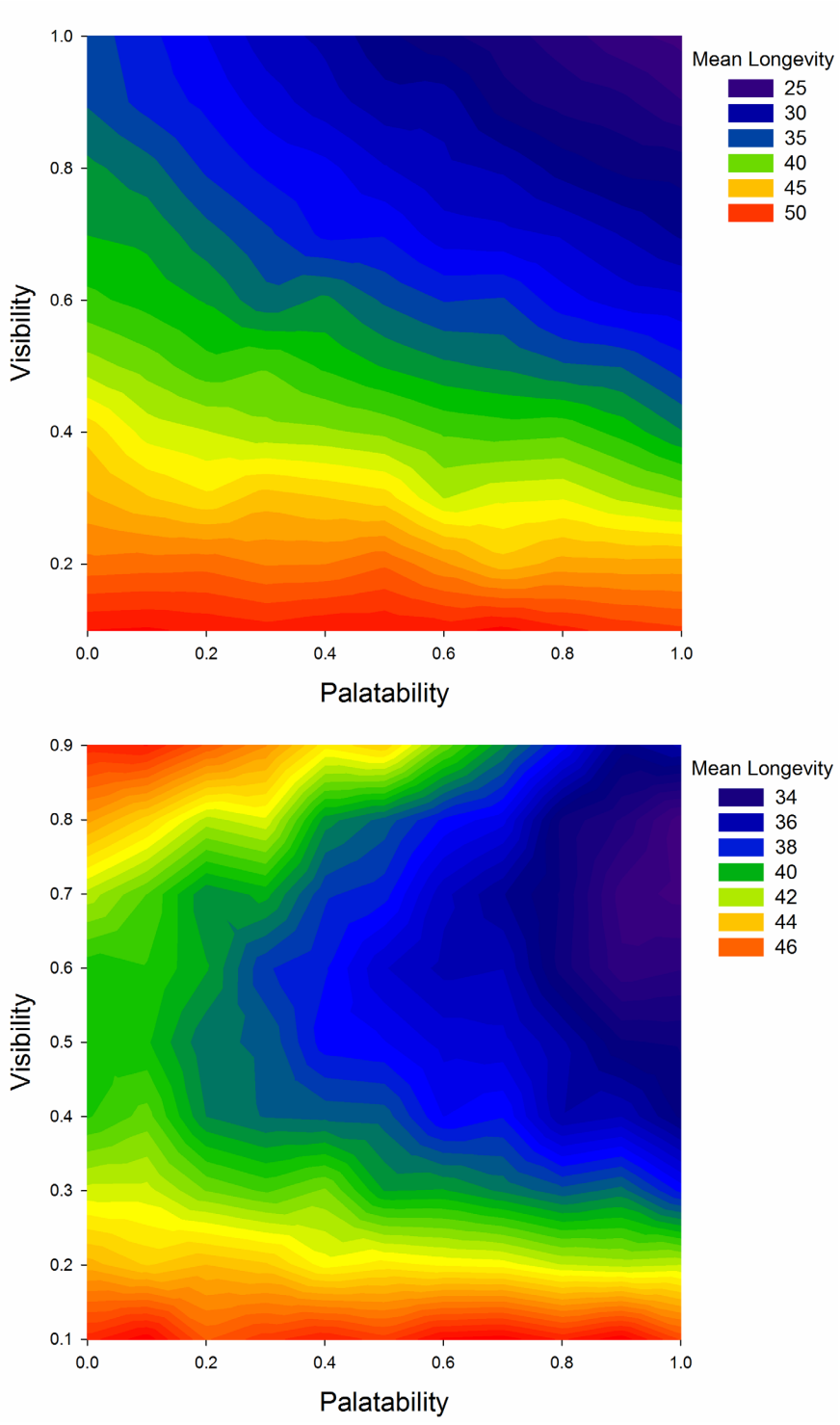


Figure 5-2 Mean evolved longevity vs. prey palatability and visibility. In (A), predators have an initial attack probability of 0.5, while in (B) initial attack probabilities are equal to 1-visibility, so that naive predators are more likely to attack cryptic prey than conspicuous prey. In both cases, predators learn to increase or decrease their attack rates based on prey palatability after encounters with prey. Parameter values as in Figure 5-1.

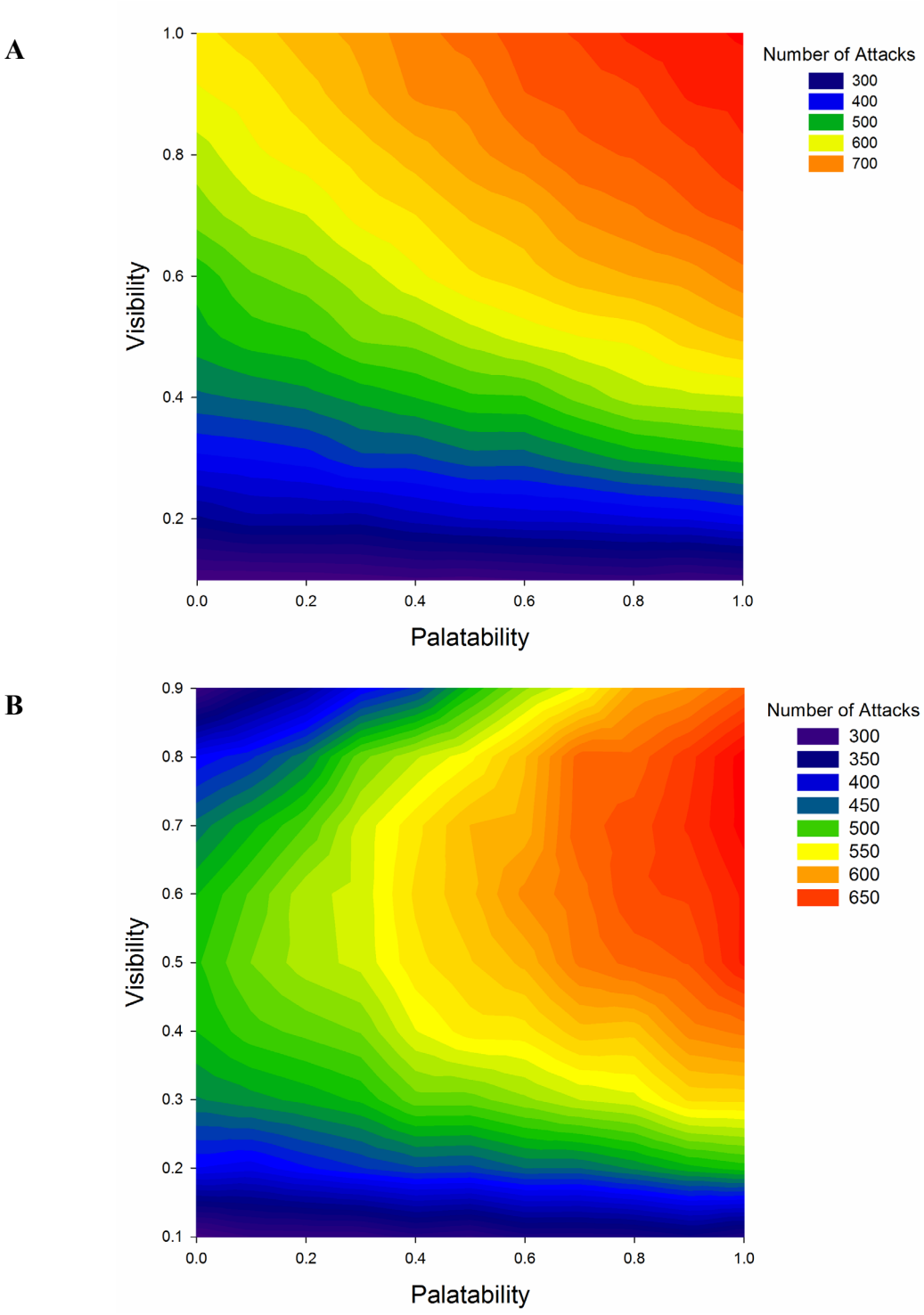


Figure 5-3 Number of attacks by predators vs. prey palatability and visibility. In (A), predators have an initial attack probability of 0.5, while in (B) initial attack probabilities are equal to 1-visibility, so that naive predators are more likely to attack cryptic prey than conspicuous prey. In both cases, predators learn to increase or decrease their attack rates based on prey palatability after encounters with prey. Parameter values as in Figure 5-1.

5.4.3 Pavlovian Predator: fixed prey palatability and two colour patterns

In simulations with two prey morphs, both cryptic prey palatability and predator neophobia have an effect on evolved longevity. There is also frequency-dependent selection, as evidenced by outcomes being dependent on which morph is most common in the population. When prey are palatable ($\lambda_l = 1$) and neophobia is absent ($\varepsilon = 0$), cryptic morphs evolve to dominate the population at all levels of cryptic prey visibility (γ); conspicuous morphs are dominant only at higher levels of neophobia (Figure 5-4). When prey are unpalatable, populations dominated by conspicuous prey are more common, even without neophobia, although in the absence of neophobia, conspicuous morphs are only dominant when crypsis is a poor strategy ($\gamma > 0.5$). In all populations, unpalatable prey evolve higher longevity than palatable prey, although this effect is largest when conspicuous morphs are common and neophobia is low (Figure 5-5). Unpalatable prey also experience fewer attacks by predators than palatable prey, and the difference is largest when conspicuous morphs are common and neophobia is low (Figure 5-6). Although unpalatable prey evolve higher longevity than palatable prey and experience lower predation rates, conspicuousness does not evolve in unpalatable prey unless crypsis is a poor strategy or predators possess some level of neophobia. Higher levels of neophobia lead to the evolution of conspicuousness even in palatable prey, and in unpalatable prey even when crypsis is highly effective.

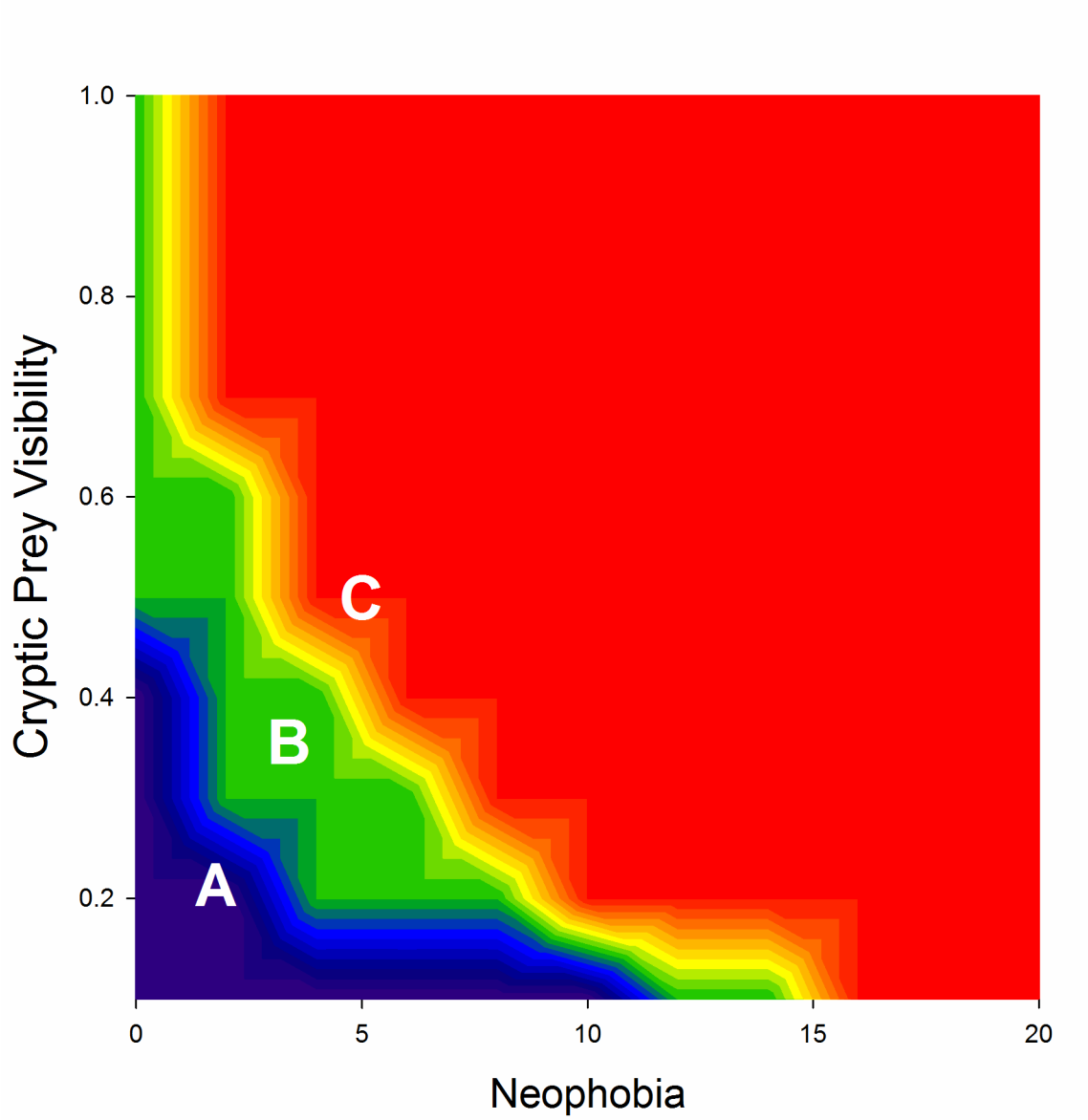


Figure 5-4 Most common prey morphs in unpalatable and palatable populations vs. neophobia and cryptic prey visibility. (A) At low levels of cryptic prey visibility and neophobia, prey evolve crypsis in both palatable and unpalatable populations. (B) At intermediate levels of cryptic prey visibility and neophobia, palatable prey evolve crypsis, while unpalatable prey evolve aposematism. (C) At high levels of cryptic prey visibility and neophobia, both palatable and unpalatable prey evolve aposematism. Parameters as in Figure 5-1; $\lambda_I = 1$ for unpalatable populations, $\lambda_I = 0$ for palatable populations, conspicuous prey visibility = 1.

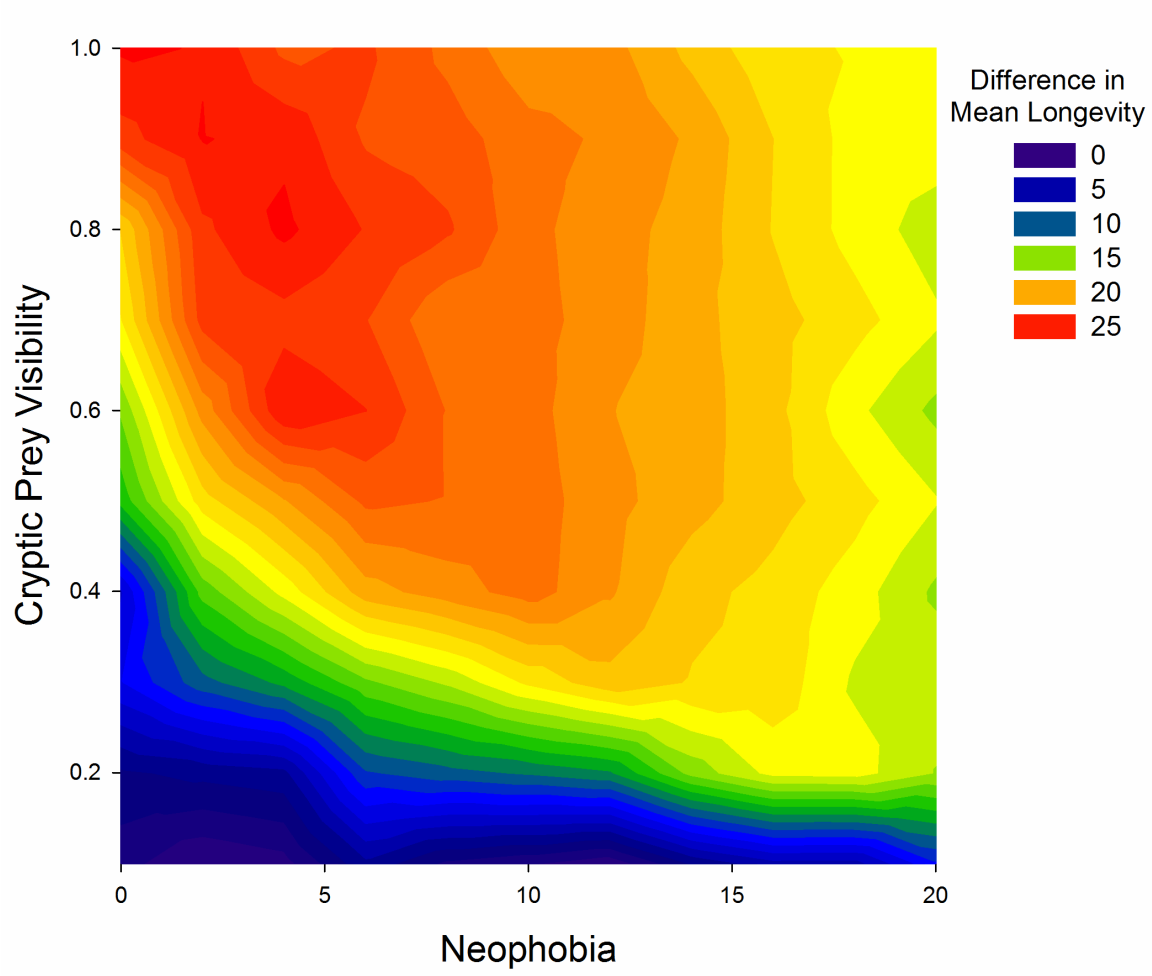


Figure 5-5 Differences in mean evolved longevity of unpalatable and palatable populations vs. neophobia and cryptic prey visibility. Unpalatable prey evolve higher longevity than palatable prey in all populations, but the difference is greatest when cryptic prey visibility is high and neophobia is low. Parameters as in Figure 5-4.

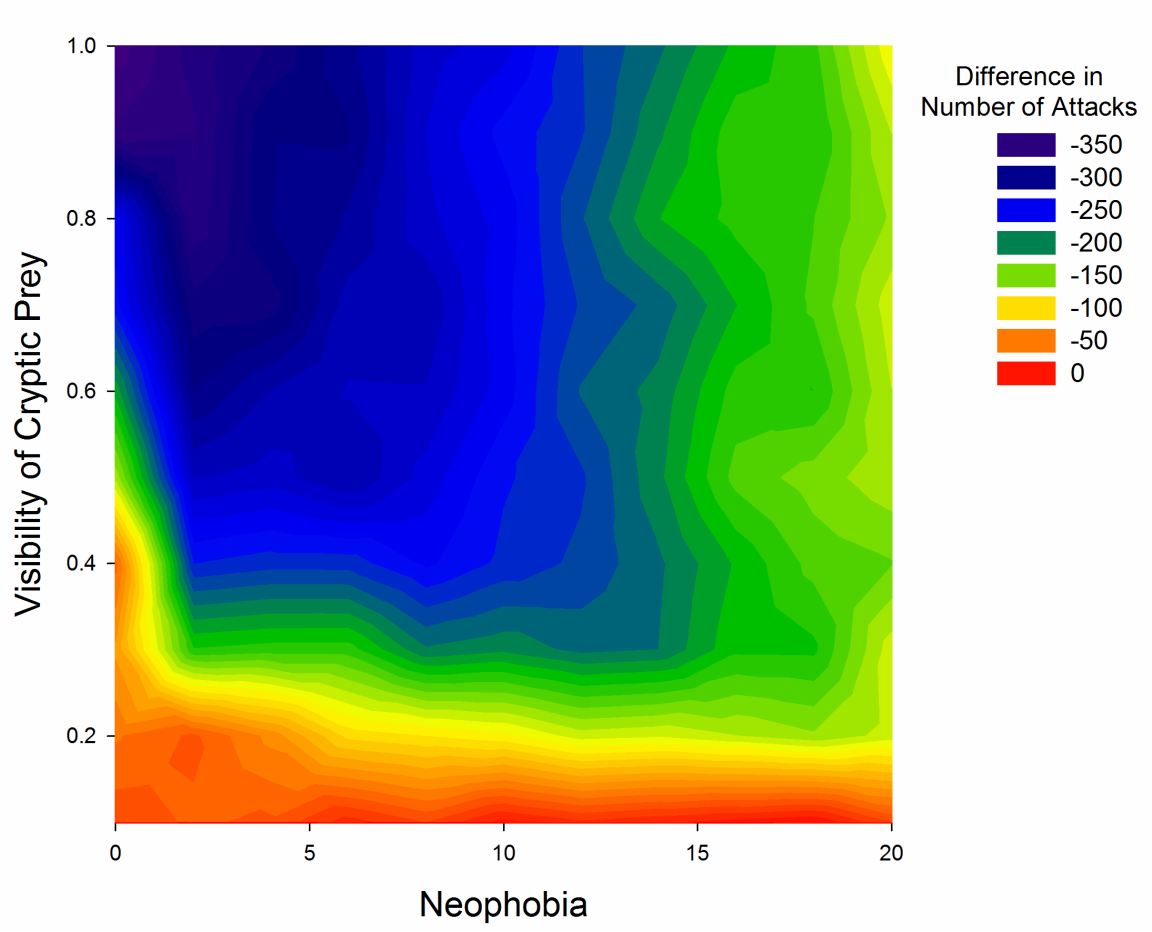


Figure 5-6 Differences in number of attacks by predators between unpalatable and palatable populations vs. neophobia and cryptic prey visibility. Unpalatable prey experienced fewer attacks than palatable prey in all populations, but the difference is greatest when cryptic prey visibility is high and neophobia is low. Parameters as in Figure 5-4.

In both palatable and unpalatable populations, morph frequencies generally evolve to a stable level quickly (ie, under 200 generations), although simulations were continued for 1000 generations as above to ensure stability (Appendix F-3). Differences in evolved longevity between palatable and unpalatable prey are also consistent over time, although they evolve more gradually.

5.5 Discussion

One of the fundamental predictions of the evolutionary theory of ageing is that high levels of extrinsic mortality should select for high rates of senescence and decreased longevity (Williams, 1957; but for important caveats, see Abrams, 1993; Williams & Day, 2003). Our first goal when building this model was to confirm that our simple genetic algorithm produces the pattern of longevity that is predicted by the evolutionary theory of ageing, and indeed, there is a clear relationship between extrinsic mortality and evolved longevity in the model (Figure 5-1).

By adapting the "Pavlovian predator" from Speed (1993; 2001), we explored the association between defensive strategies and the evolution of longevity under predation. Previous studies comparing longevity in a range of taxa have assumed that aposematism represents a defensive advantage over crypsis, and suggested that reduced mortality of aposematic prey relative to cryptic prey is (at least in part) responsible for observed differences in longevity (Blanco & Sherman, 2005; Beck & Fiedler, 2009). However, other factors such as geographical distribution and adult diet also play an important role in shaping longevity (Dunlap-Pianka *et al.*, 1977; Karlsson & Wiklund, 2005; Molleman *et al.*, 2007; Beck & Fiedler, 2009), and the association between defence and longevity

may be complex. For example, the tenebrionid beetle *Stenomorpha marginata* is a harmless (Batesian) mimic of the chemically defended tenebrionid model *Eleodes obscura* and they therefore share similar phenotypes. However individuals of the mimic species tend to live for approximately three months as adults, whereas the models live up to 4 years (Hetz & Slobodchikoff, 1990).

Our results suggest that aposematism is not necessarily more effective than crypsis in reducing predation, unless predators possess behaviours such as cautious sampling or neophobia. When our predation model included a single prey morph (see section 3.2, Pavlovian predator), conspicuous prey evolved lower longevity and experienced higher predation than cryptic prey, even when highly unpalatable (Figure 5-2A and 5-3A, respectively). Even when cautious sampling by predators was included in the model, aposematic (highly visible and unpalatable) prey evolved longevities and had predation rates that were comparable to highly cryptic prey (Figure 5-2B and 5-3B).

A co-evolutionary model was necessary to ensure a degree of self consistency, so that (for example) when considering the longevity of an aposematic species we can be confident that an aposematic strategy would have evolved under the conditions specified. In the coevolutionary model (see section 5.4.3), conspicuous colour morphs outcompete cryptic morphs when neophobia and cryptic prey visibility are high. Unpalatable populations evolve conspicuousness more often than palatable populations, but at low levels of visibility (cryptic prey were very cryptic) and neophobia (naive predators did not avoid conspicuous prey), crypsis evolves even in unpalatable populations (Figure 5-4). This is somewhat surprising, however there are some examples of crypsis and chemical defence occurring together in the wild. The butterfly *Dryas julia*, for example,

has relatively cryptic larvae, possibly because predators vary in their learning rates or their resistance to defensive chemicals (Endler & Mappes, 2004).

When prey are palatable, neophobia is necessary for the evolution of conspicuous colouration, even when cryptic prey are highly visible. As expected, conspicuous morphs are more competitive with cryptic morphs in unpalatable populations than palatable ones. However, even when prey are unpalatable, a relatively high level of neophobia is required for conspicuous morphs to outcompete very cryptic morphs (although the values of neophobia used in our model are still lower than those in Speed, 2001, where neophobia was set at 24 individuals). These results largely agree with the predictions made by Speed (2001), namely that in spite of higher associated learning rates, the ability of conspicuous unpalatable prey to outcompete cryptic unpalatable prey is dependent on both cryptic prey visibility and the presence of neophobia. It is noteworthy, however, that even in cases where Speed (2001) predicted that the relative survival benefit of conspicuousness over crypsis in unpalatable prey should be small (i.e., no neophobia and high or intermediate cryptic prey visibility), conspicuous prey morphs consistently outcompete cryptic morphs. Also of note, although conspicuous prey were less competitive in palatable populations, the presence of sufficiently high levels of neophobia still conferred a significant advantage over cryptic prey, leading conspicuous morphs to dominate the population.

Even though conspicuous morphs do not always outcompete cryptic morphs in unpalatable populations, unpalatable prey always evolve higher mean longevity than palatable prey (Figure 5-5). The difference is largest when neophobia is small, which is not surprising, since the benefit of unpalatability should be largest when other protections

such as neophobia are weak. As with single prey morph simulations, predator attack rates in both palatable and unpalatable populations are inversely related to evolved longevity (Figure 5-6). Unpalatable prey always experience fewer attacks than palatable prey, and the difference is largest at low levels of neophobia and intermediate levels of crypsis.

Our results demonstrate that the prediction made by Williams (1957), namely that extrinsic mortality should select for increased senescence and reduced longevity, holds true for a simple evolving population built using a genetic algorithm. When predators were introduced to the model, we found a strong negative correlation between predation rate and mean evolved longevity, however aposematism does not always result in reduced predation or senescence compared to crypsis, unless some other mechanism such as cautious sampling or neophobia is present. In simulations with coevolving senescence and colour traits, the evolution of conspicuousness was highly dependent on the presence of neophobia in the predator population, especially at low levels of cryptic prey visibility. However, unpalatable prey evolved higher mean longevity than palatable prey in both conspicuous and cryptic populations. Our results therefore provide conditions under which aposematic morphs would be expected to outcompete cryptic morphs (and vice versa), as well as highlighting the importance of predator behaviour for the evolution of senescence in aposematic and cryptic prey.

Chapter 6 – General Discussion

Although senescence and anti-predator defence may at first seem like distantly related topics, hypotheses regarding their relationship are almost as old as the modern evolutionary theory of senescence. Williams (1957) and Hamilton (1966) both recognized the important role that extrinsic sources of mortality such as predation would play in the evolution of senescence, and that defensive traits that evolved to mediate predation would play an important role in shaping selection for longevity. Lepidoptera provide an ideal system for the study of predation and senescence, since they exhibit a variety of defensive strategies, including crypsis and aposematism, and there is well documented variability in their longevity. For these reasons, this thesis focused on predation, longevity and senescence in Lepidoptera; however, the results may be more broadly applicable, since predation is a common source of mortality, and both aposematism and crypsis are widely employed as defensive strategies.

The questions addressed in this thesis fall into two broad categories: first, are there differences in patterns of predation experienced by aposematic and cryptic prey? Second, what are the implications of these differences (if any) for the evolution of senescence in aposematic and cryptic species? Related sub-questions include: do predation patterns in groups of cryptic and aposematic prey support the evolution of post-reproductive senescence? Do wild avian predators display the same aversion to aposematic prey that has been demonstrated using captive predators? Is senescence widespread in adult Lepidoptera? What is the relationship between senescence rate and longevity? Do aposematic species experience lower rates of senescence than non-

aposematic species as well as higher longevities? And finally, what role does predator behaviour such as cautious sampling and neophobia play in the evolution of senescence in aposematic and non-aposematic species? A variety of methods were used to study these questions, including field predation experiments, senescence model fitting and computer simulations.

In chapter 2, I investigated an interesting post-reproductive senescence theory that was first proposed by Blest (1963) and later formalized by Hamilton (1964) in terms of kin selection: that aposematic prey might benefit relatives by living longer after reproduction and teaching predators to avoid similar-looking prey, while cryptic prey should die quickly after reproduction, to keep predators from forming a search image for similar-looking prey. Using wild avian predators, I tested the basic predictions behind the theory, namely that aposematic prey should experience reduced predation in the presence of conspecifics, while cryptic prey should experience increased predation. The prediction regarding higher mortality in cryptic prey was also tested using human subjects hunting for virtual prey on a computer. Although kin selection can operate at a variety of spatial levels, I chose to consider a conservative case where groups were defined as a single cluster of prey, either on a given tree or in a given computer image. In human trials, there was a significant effect of group size on *per capita* predation in cryptic prey, with groups of prey being attacked significantly more often than individuals, and experiencing lower *per capita* mortality. However, the effect was strongest at small group sizes, and groups larger than two individuals experienced no additional cost in attack rate or mortality. Surprisingly, there was no effect of group size on predation in either aposematic or cryptic prey in the field experiments, even in the control site, where

palatabilities were reversed (conspicuous prey were palatable, and cryptic prey were unpalatable). This raises the possibility that the levels of unapaltability and crypsis used in the artificial prey were not sufficiently high to avoid or deter predation. However in one of the two experimental sites, aposematic prey experienced significantly lower predation than cryptic prey, and in both of the control sites, (cryptic) unpalatable prey experienced significantly lower predation than (conspicuous) palatable prey. These results imply that the defensive treatments were at least partially effective, and that aposematic prey may be more effective at avoiding predation than cryptic prey. While there is little evidence for the kin selection-based mechanism proposed by Blest and Hamilton, there is some evidence for the more general prediction that aposematic prey experience lower overall predation rates relative to cryptic prey, albeit in only one experimental site.

In chapter 3, I again used wild avian predators, this time to test the prediction that aposematic prey should experience lower predation rates than cryptic prey. This experiment was designed to measure differences in the types of attacks experienced by aposematic and cryptic prey in addition to overall predation rate. Exploratory attacks (in which the edible portion of the prey target was damaged or partially removed) and complete consumption (in which the edible portion of the prey target was completely removed) were recorded and analyzed separately. The experiment was also designed to verify the results from chapter 2 using an improved type of cryptic prey that was spectrally matched to the background, and to quantify the effect of different levels of defence. In this experiment, there were five defensive treatments: a low and high crypsis treatment, a low and high aposematism treatment, and a white palatable control that was

neither cryptic nor unpalatable. Surprisingly, there was no significant difference in overall predation rates between aposematic and cryptic prey, and no significant difference between any of the other defensive treatments and the control. However, predation rates were highly variable among both experimental sites and trial weeks, and this high level of variability could have obscured some significant differences in the overall predation results. When different types of attacks were analyzed separately, the results were quite different. Highly aposematic prey were partially consumed more often other types of prey, except for prey with low unpalatability, which experienced an intermediate level of attack. Conversely, highly aposematic prey were completely consumed less often than other types of prey, again with low unpalatability prey being consumed at an intermediate rate. These results are consistent with "go-slow" predation (Guilford, 1994), a strategy whereby predators cautiously sample prey that are conspicuous, while more readily consuming cryptic prey. It is important to note that they are also consistent with simple taste-rejection, in which predators reject distasteful prey after attack independently of their visual signalling. If the results were caused by go-slow predation rather than taste-rejection, they represent a potential benefit of aposematism over crypsis (particularly if aposematic prey are able to survive the initial attack by predators and subsequently escape), and may support the prediction that aposematic species should experience lower predation-related mortality than cryptic species.

In chapter 4, adult survivorship data were collected from the literature, and models of senescence were fitted both to determine whether senescence is present in Lepidoptera, and to quantify variation in senescence rates between species. The relationship between senescence and longevity was also explored using the results from

the fitted models, and the longevities and senescence rates of aposematic and non-aposematic species were compared. Senescence was present in all 22 species of Lepidoptera surveyed, except in one case (females of the reproductive morph of the monarch butterfly *Danaus plexippus*), indicating that it is a widespread phenomenon in this group. As expected, there was a strong negative (although non-linear) relationship between senescence rate and mean longevity, providing support for the use of longevity as a proxy for senescence in comparative studies. Mean longevity was significantly higher in aposematic species than non-aposematic species, which was consistent with previous comparative studies of Lepidoptera. However, although senescence rates were lower in aposematic species than non-aposematic species, the difference was non-significant, potentially due to the small number of aposematic species in the data set. Overall, these results demonstrated the widespread occurrence of senescence in adult Lepidoptera and explored the relationship between senescence and longevity. They also provided support for the theory that aposematic species evolve higher longevity than non-aposematic species.

In chapter 5, a simulated population based on a genetic algorithm was programmed that allowed a longevity trait to evolve under different extrinsic mortality regimes, including both the simple case of a fixed mortality rate and the more complex case of a "Pavlovian predator" (Speed 1993; 2001), which modified its attack rate based on prey palatability. Two types of simulations were performed with the Pavlovian predator. In one, longevity was allowed to evolve in populations with fixed palatability and conspicuousness traits. In the other, the longevity trait was allowed to coevolve with a prey coloration trait. As predicted by evolutionary theory, the results demonstrated a

clear negative relationship between extrinsic mortality and mean evolved longevity. However, both Pavlovian predator models (single prey morph and two competing prey morphs) demonstrated that aposematic prey did not evolve higher longevities than cryptic prey unless certain conditions regarding predator behaviour were met. In particular, aposematic prey evolved lower mean longevity compared to cryptic prey unless cautious sampling was included in the model (predators were initially more hesitant to attack conspicuous prey than cryptic prey). When prey morphs were allowed to coevolve, unpalatable prey evolved higher mean longevity than palatable prey, but conspicuous morphs did not outcompete cryptic morphs unless neophobia (initial rejection of a fixed number of conspicuous prey by naive predators) was included in the model. When cryptic morphs were highly cryptic (their visibility to predators was low), high levels of neophobia were necessary for conspicuous morphs to outcompete cryptic morphs. These results highlighted several important factors that may affect the evolution of longevity in aposematic and cryptic prey.

Although many studies have studied senescence in wild populations, senescence in insects has been much less thoroughly explored (Nussey *et al.*, 2013). Established methods such as mark-recapture have been used to quantify senescence in the wild (see Sherratt *et al.*, 2010; 2011), but the potentially complex relationships between senescence and ecological factors such as predation and geographical distribution have not yet been explicitly tested, despite several interesting comparative studies of longevity (Dunlap-Pianka *et al.*, 1977; Karlsson & Wiklund, 2005; Molleman *et al.*, 2007; Beck & Fiedler, 2009). Many groups of insects are also highly diverse, and this presents opportunities for phylogenetically controlled comparisons of senescence rates. Controlling for shared

ancestry can be important when comparing senescence or longevity between species, since species which share traits such as chemical defence are often closely related (Hossie *et al.*, 2013). By combining existing research on insect ecology with measurements of senescence, further insights can be gained into the forces which shape evolutionary trade-offs, and how they vary in natural populations.

In addition to the general research directions that have been highlighted, a number of specific questions emerge from the work presented in this thesis. Chapter 3 presented some evidence for the presence of go-slow predation, however further experiments that include an unpalatable cryptic control would be required to disentangle the effect of palatability from that of conspicuousness. This would allow go-slow predation by predators to be distinguished from simple taste-rejection. Chapter 4 demonstrated the presence of senescence in Lepidoptera, but in order to detect the relationship between senescence and aposematism, a much larger analysis is required to allow for a phylogenetically-controlled comparison. Another potentially profitable avenue of research would be to compare longevities or senescence rates in Lepidoptera to concentrations of defensive chemicals, since species with higher concentrations of defensive chemicals might be expected to experience reduced predation, and therefore reduced senescence. Chapter 5 raised several interesting questions about the role of predator behaviour and learning, and further models could explore systems with coevolving palatability, as well as modeling the physiological costs of chemical defence. Another potentially interesting addition to the model would be the inclusion of pre- and post-reproductive life stages in the prey population, which could be accomplished by simply giving reproductive "lottery tickets" only to individuals between certain ages in

the longevity genome. This would allow the model to test the post-reproductive senescence predictions made by Blest (1963) and Hamilton (1964), as well as exploring how selection acts on senescence during different life stages. These results could then be incorporated into models which test both life-history and predator behaviour hypotheses. If there is a single message that emerges from this research, it is that the link between senescence and predation is complex. Future work will need to draw together insights from a number of areas, including predator and prey behaviour, chemical entomology, population biology and demography to untangle this relationship.

Appendices

Appendix A – A re-analysis of Blest's data using maximum likelihood methods of model fitting

When Blest (1963) originally analyzed the longevity of captive moths, he compared average longevities, not rates of senescence. Indeed, he did not demonstrate that senescence was occurring in his sample organisms. By re-analyzing his data using maximum likelihood methods of model fitting, and a commonly accepted model of senescence based on an exponentially increasing hazard function (the Gompertz model), we can answer two questions: first, can the presence of senescence be detected in Blest's demographic data? Second, what are the coefficients of the senescence models for each species? Do they conform to the hypothesis laid out by Blest (chemically defended species live longer/senesce more slowly)?

Methods

Using VistaMetrix (www.skillcrest.com), data were collected from the moth survivorship plot in Blest (1963). These data were transformed from percent survivorship into mortality times (in days) for each individual.

There were five species of moth in the data*: Lonomia cynira, Automeris junonia, Automeris boucardi, Dirphia eumedide* and *Dirphia hircia*. Two models of senescence, defined using the probability densities of hazard functions, were fitted to the data for each species using maximum likelihood estimation (**A-1**). The first model, the null or no senescence model, assumes a constant hazard function over an individual's lifetime. The second model, the Gompertz or senescence model, assumes that the hazard function increases exponentially.

Likelihood model fitting was carried out using the bbmle package in R, and the fitted null and Gompertz models were compared using both likelihood ratio testing (since the Gompertz model reduces to the null model as a special case) and Akaike Information Criteria (AIC).

Results and Discussion

All five moth species fit the Gompertz model significantly better than the null model, indicating that senescence was present in the captive populations of each species (**A-2**). The parameters in the Gompertz model were combined using $\omega = (a*b)^{1/2}$ to provide a single measure of the rate of senescence (Ricklefs & Scheuerlein, 2002).

Lonomia cynira is a cryptic leaf mimic, while both *Automeris* species possess eyespots, and both *Dirphia* species are chemically defended (Blest, 1963). Based on the presence of different modes of defense in each species, Blest predicted that the order of post-reproductive senescence rates should be *Lonomia*<*Automeris*<*Dirphia*, and this is precisely what we see.

It is impossible with such a small sample size to address the issue of statistical significance, and we need to be wary of phylogenetic factors influencing the results, but these results seem to indicate that a larger analysis across species of Lepidoptera may be of value.

A-1 Hazard functions and probability density functions for two models of senescence (Null and Gompertz).

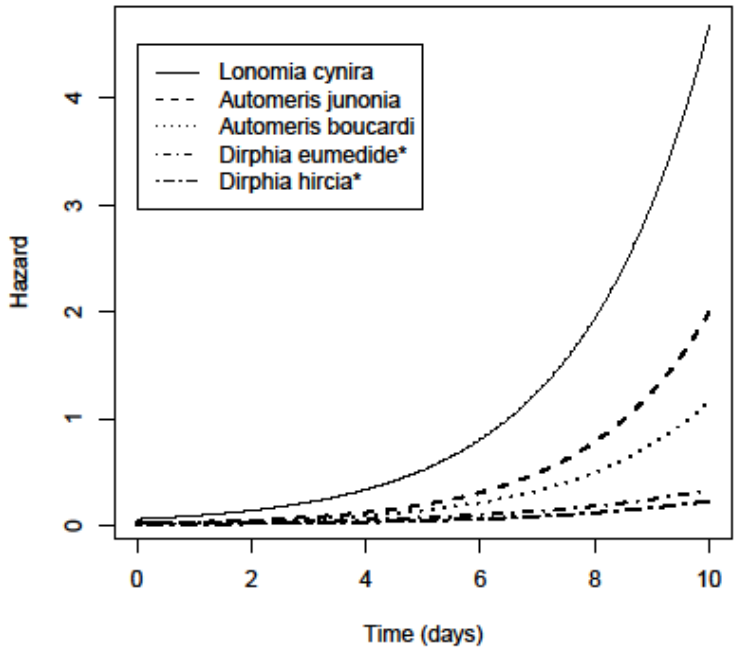
Model	Hazard Function	Probability density of time to death ***f(x)***
No Senescence	$\mu_x = a$	ae^{-ax}
Gompertz	$\mu_x = ae^{bx}$	$a\ exp[bx - a/b(e^{bx} - 1)]$

A-2 Results of likelihood ratio and AIC tests between null and Gompertz models for five species of Saturniid moth (* indicates chemically defended species).

Species	Likelihood Ratio Test		AIC		
	P-value	Recommended	Null	Gompertz	Recommended
Lonomia cynira	$4.78*10^{-10}$	Gompertz	244.07	207.30	Gompertz
Automeris junonia	$2.2*10^{-16}$	Gompertz	338.67	261.12	Gompertz
Automeris boucardi	$1.78*10^{-15}$	Gompertz	291.61	230.32	Gompertz
Dirphia eumedide*	$2.2*10^{-16}$	Gompertz	320.15	249.45	Gompertz
Dirphia hircia*	$2.2*10^{-16}$	Gompertz	330.65	257.25	Gompertz

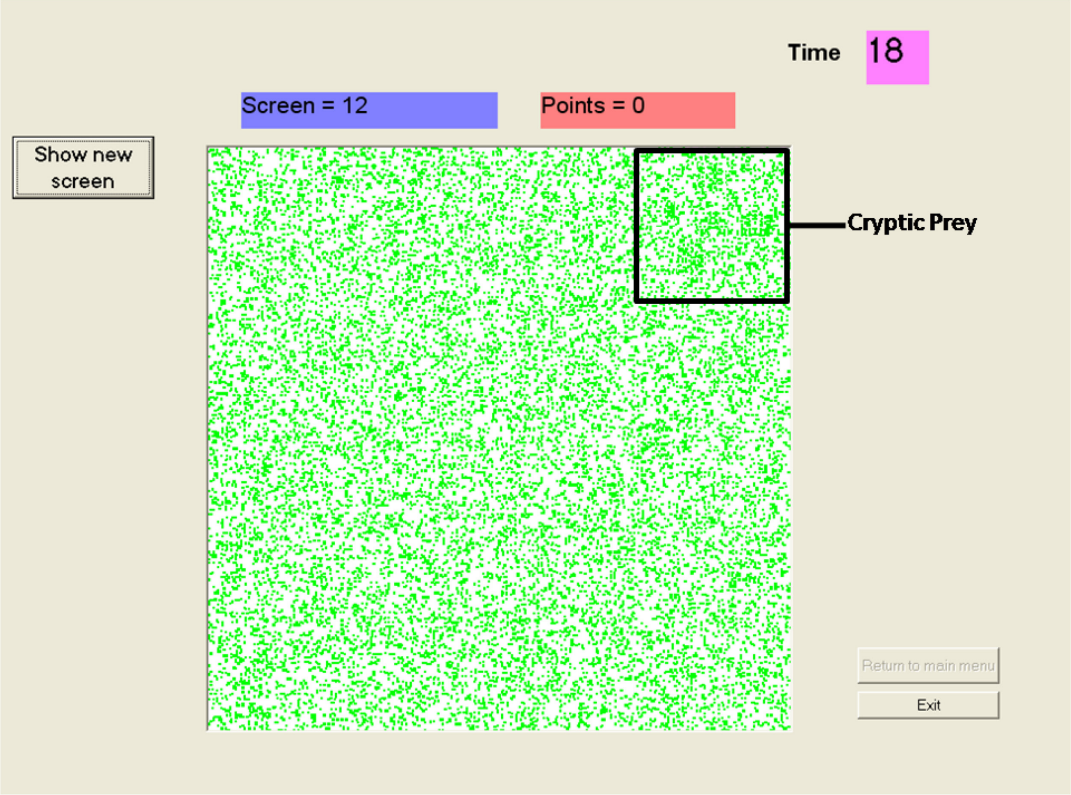
A-3 Parameter estimates of Gompertz senescence models for five species of Saturniid moth, and the combined senescence rate estimate (* indicates chemically defended species).

Species	Parameter Estimates		
	a	b	ω
Lonomia cynira	0.05764	0.4397	0.159199
Automeris junonia	0.01856	0.4676	0.093159
Automeris boucardi	0.01707	0.4214	0.084813
Dirphia eumedide*	0.01548	0.3064	0.06887
Dirphia hircia*	0.008204	0.3324	0.052221



A-4 Gompertz hazard functions for 5 species of Saturniid moth (* indicates chemically defended species).

Appendix B – Supplementary figures for chapter 2



B-1 User interface of human predation program with cryptic prey shown.

B-2 Cryptic and conspicuous artificial prey targets

Appendix C – Results from field trials on cryptic defended and conspicuous palatable prey

Results from the two sites (Stony Swamp and Gatineau) were analyzed separately. Attack rates were analyzed using a Cox proportional hazard model (Cuthill *et al.*, 2005; Stevens *et al.*, 2006), with three predictor variables (defense, group size and trial number; **C-1**). However, since individual prey targets within groups were not independent, analysis was performed on group predation, which was defined as occurring when at least one individual in a group was attacked. In both sites, there was a significant effect of trial number on group attack rates, with more prey groups attacked in later trials compared to earlier ones (**C-3**). There was no significant effect of defense or group size.

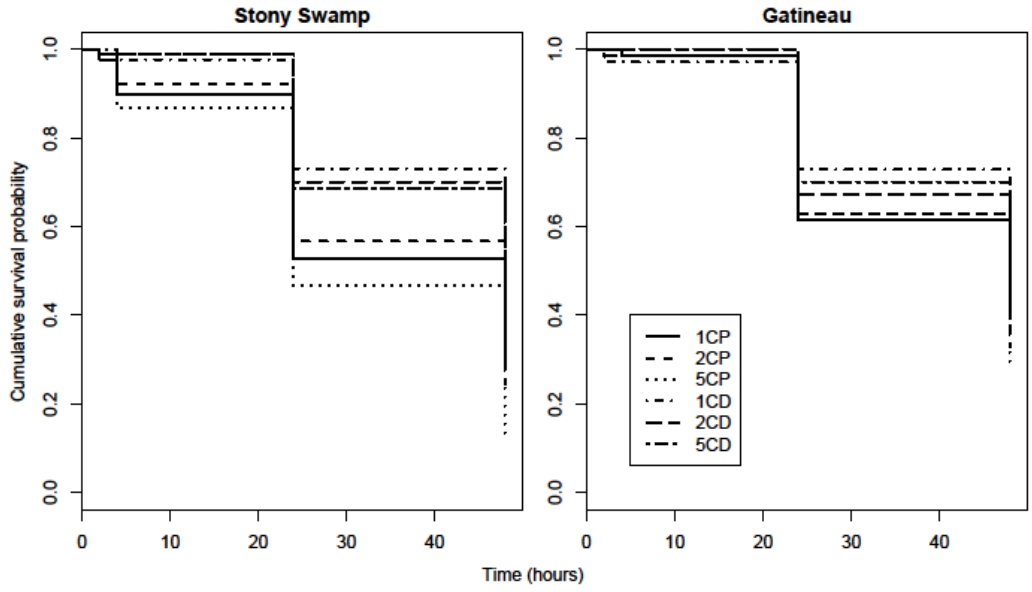
The proportion of prey attacked in each group was also analyzed by fitting a general linear model (response: arcsin proportion of prey attacked, fixed factors: defense, group size and trial number; **C-2**). In both sites, there was a significant effect of both defense and trial number on the proportion of prey attacked within a group, with a lower proportion of cryptic defended prey attacked compared to conspicuous palatable prey (**C-4**), and a higher proportion of prey attacked in later trials compared to earlier trials (**C-5**). In Stony Swamp, there was also a significant interaction between defense and trial number (**C-2**). There was no significant effect of group size in either site.

C-1 Cox proportional hazard models of attacked prey groups, with three main effects (defensive strategy, group size and trial number) and all interactions. Test statistics reported for the models: W (d.f.), significance (***p<0.001, **p<0.01, *p<0.05 n.s. p>0.05).

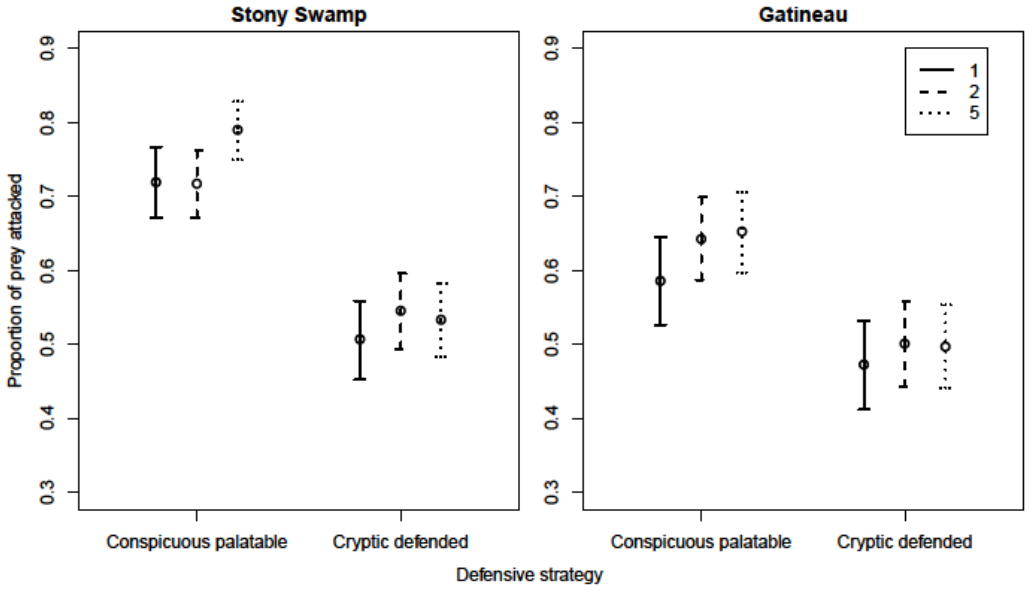
Model Factors	Stony Swamp	Gatineau
Defense	2.461(1)n.s.	1.002(1)n.s.
Group Size	0.179(2)n.s.	1.384(2)n.s.
Trial Number	9.688(4)*	11.928(3)**
Defense*Group Size	0.454(2)n.s.	1.022(2)n.s.
Defense*Trial	0.670(4)n.s.	1.347(3)n.s.
Group Size*Trial	2.239(8)n.s.	4.045(6)n.s.
Defense*Group Size*Trial	2.949(8)n.s.	5.235(6)n.s.

C-2 General linear models of arcsin transformed proportion of prey attacked, with three main effects (defensive strategy, group size and trial number) and all interactions. Test statistics reported for the models: Fs (d.f.), significance (***p<0.001, **p<0.01, *p<0.05, n.s. p>0.05).

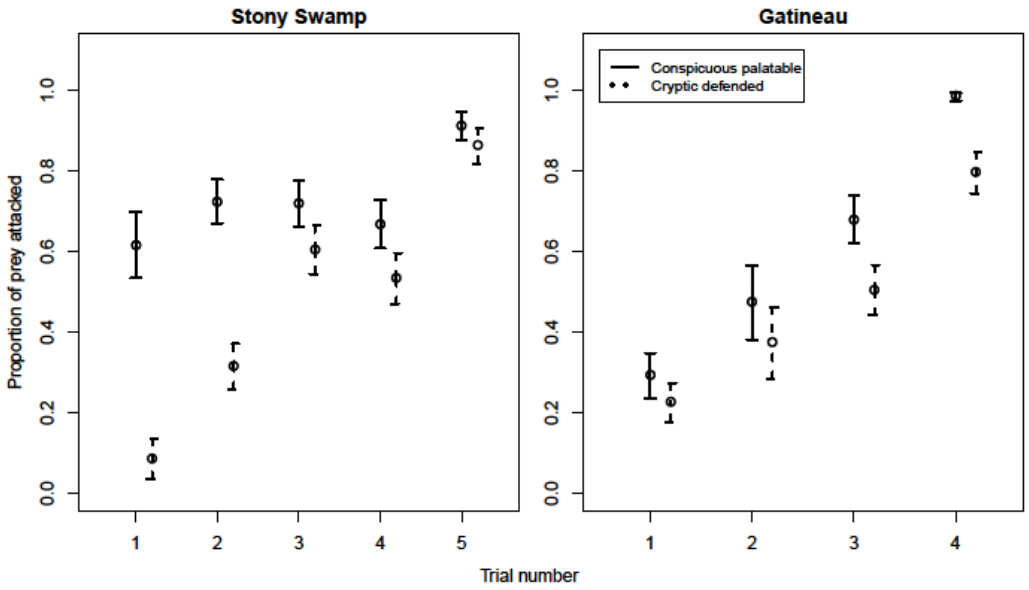
Model Factors	Stony Swamp	Gatineau
Defense	41.951(1, 507)***	10.739(1, 396)**
Group Size	0.280(2, 507)n.s.	0.887(2, 396)n.s.
Trial Number	21.059(4, 507)***	52.104(3, 396)***
Defense*Group Size	0.410(2, 507)n.s.	0.146(2, 396)n.s.
Defense*Trial	4.967(4, 507)**	0.452(3, 396)n.s.
Group Size*Trial	1.149(8, 507)n.s.	1.616(6, 396)n.s.
Defense*Group Size*Trial	0.543(8, 507)n.s.	2.186(6, 396)*



C-3 Cumulative survival probability for groups of artificial prey. Prey were in groups of 1, 2 or 5, and either conspicuous and palatable (CP) or cryptic and defended (CD). Differences are non-significant in both sites.



C-4 Proportion of prey attacked ± standard error. In both sites, a significantly higher proportion of conspicuous palatable prey were attacked compared to cryptic defended prey. There was no significant difference between different sized prey groups.



C-5 Proportion of prey attacked ± standard error. In both sites, a significantly higher proportion of conspicuous palatable prey were attacked compared to cryptic defended prey. There was also a significant increase in the proportion of prey attacked in later trials compared to earlier ones, for both types of prey.

Appendix D – Supplementary figures for chapter 3

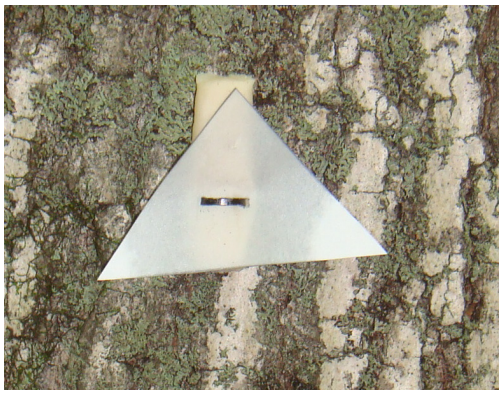
D-1 Map of experimental sites in Gatineau Park, Gatineau, QC.

A 

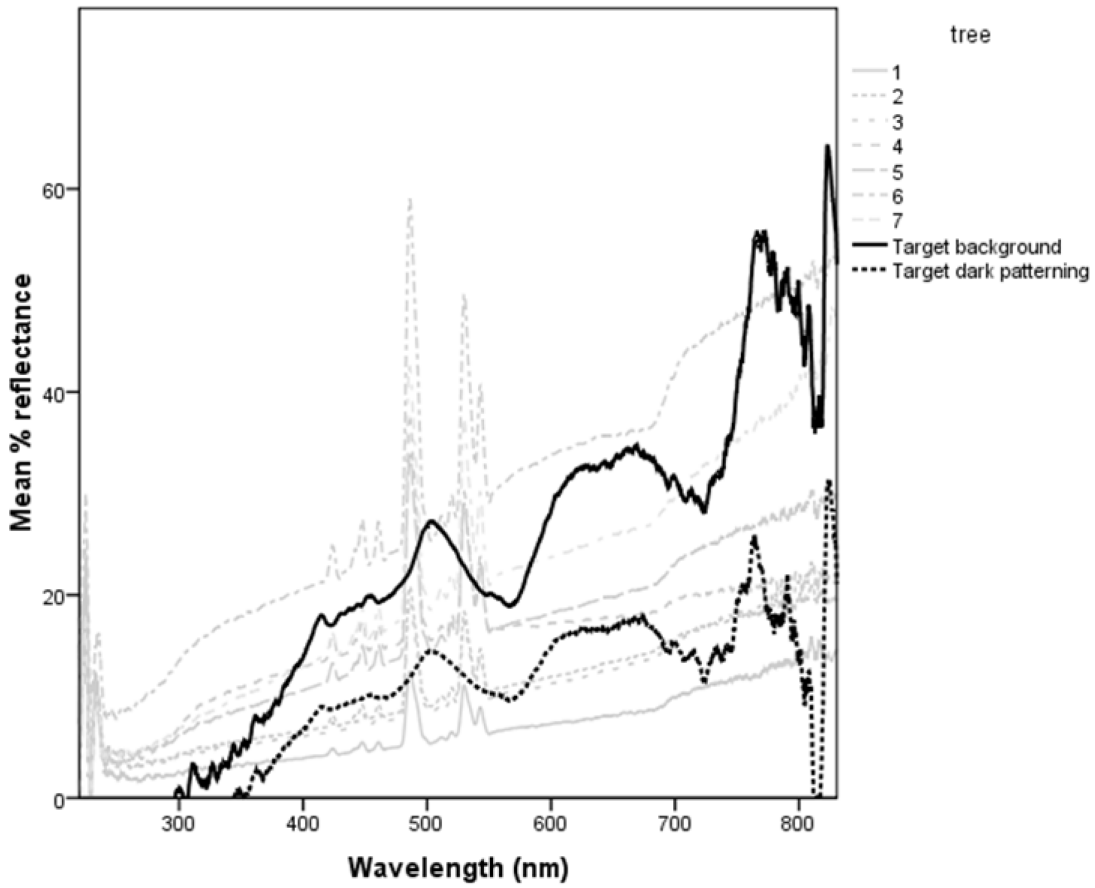
B 

C 

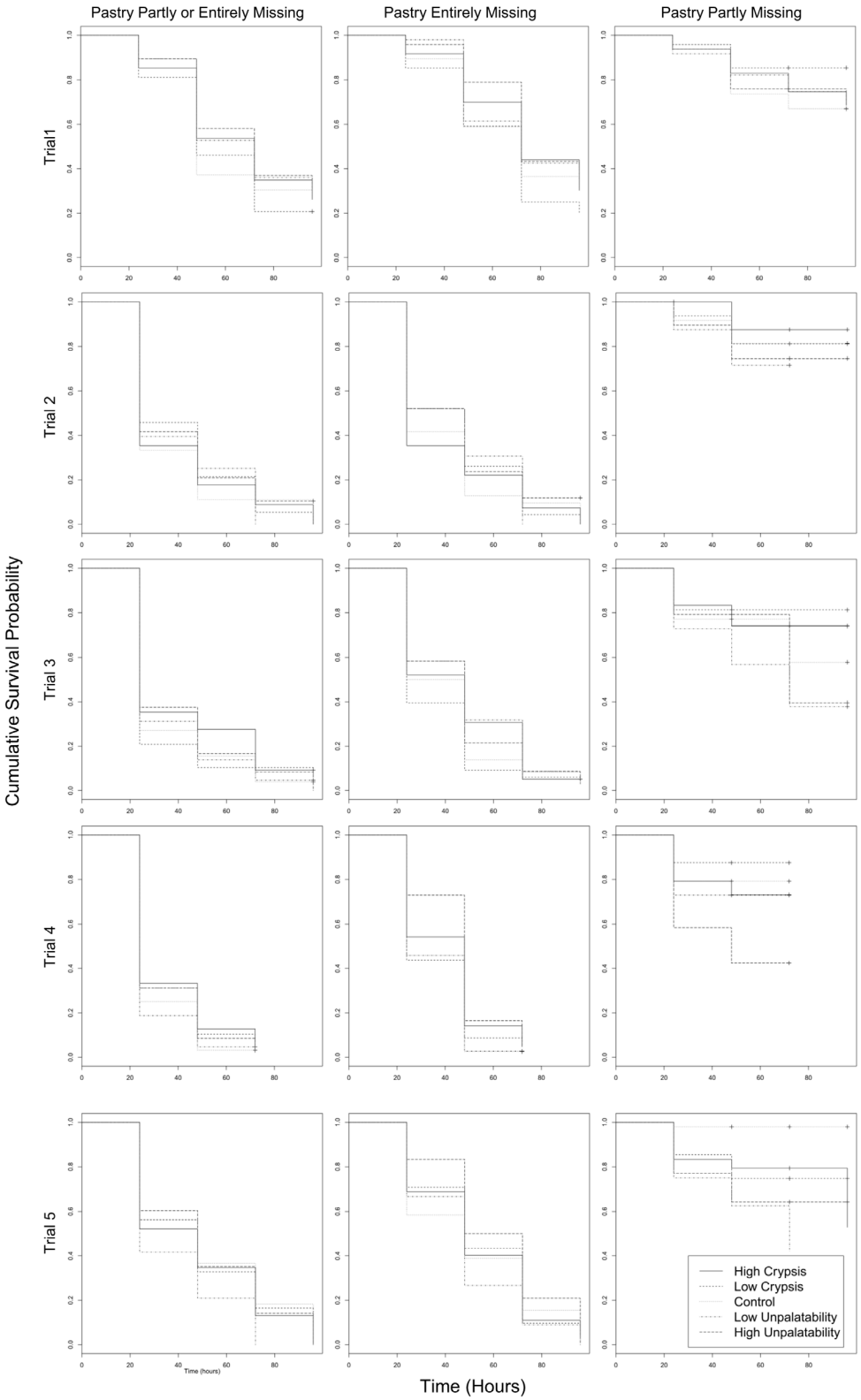
D 

E 

D-2 Artificial prey targets used in the experiment. A: high crypsis, B: low crypsis, C: high unpalatability, D: low unpalatability, E: control. In sites 3 and 4, the colours of the low and high unpalatablility targets were reversed.

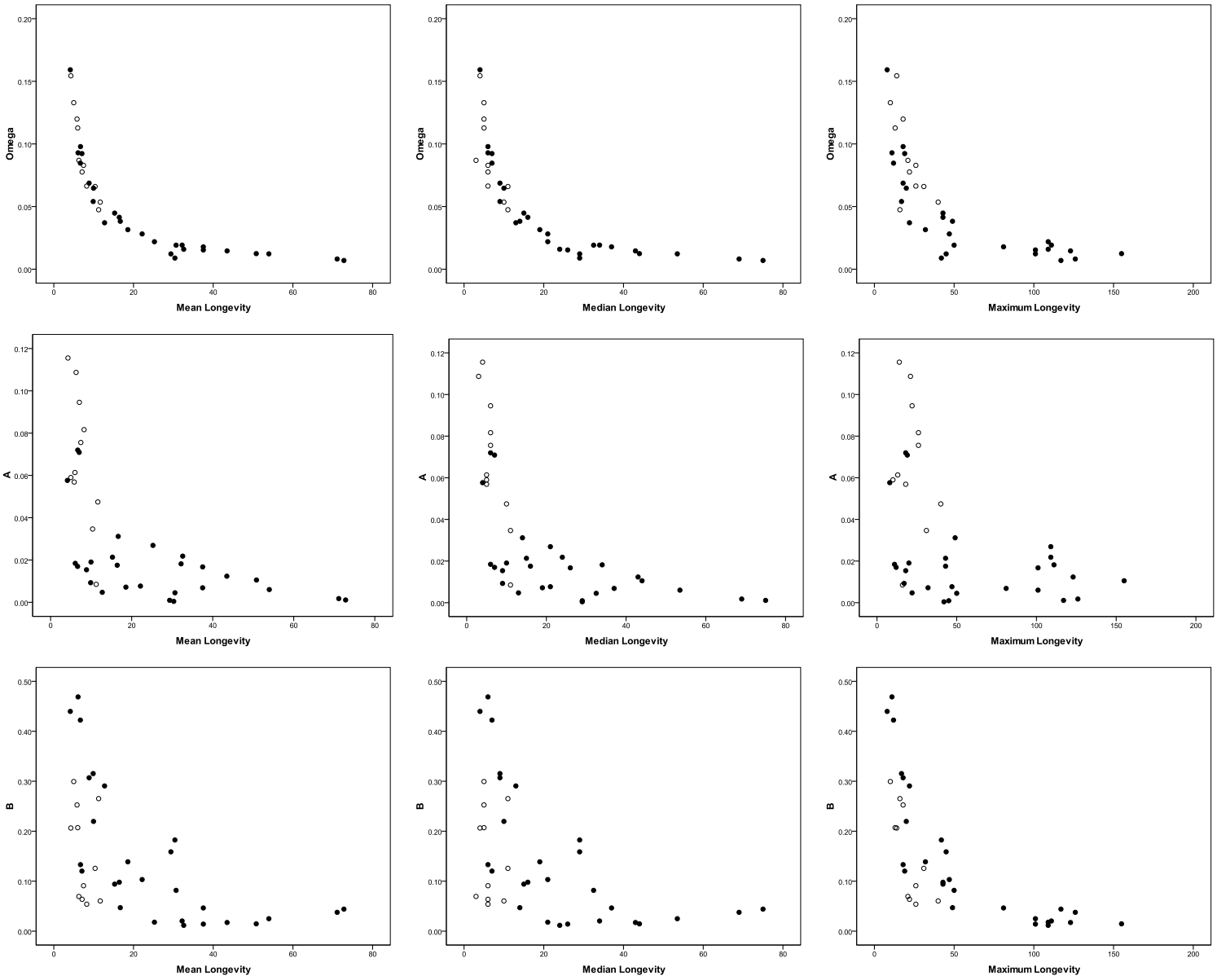


D-3 Mean % reflectance by wavelength from the bark of 7 maple trees (*Acer saccharum*), as well as the two cryptic prey colours. Reflectance curves were obtained by averaging 10 measurements from each sample.



D-4 Cumulative survival probability for each prey type, separated by trial (rows) and predation measure (columns). There were significant differences between trials for all three predation measures.

Appendix E – Supplementary figures and tables for chapter 4



E-1 Parameters in the fitted Gompertz senescence model (ω, a and b) vs. mean, median and maximum longevity for 22 species of Lepidoptera. Open circles indicate field survivorship, while closed circles indicate laboratory survivorship.

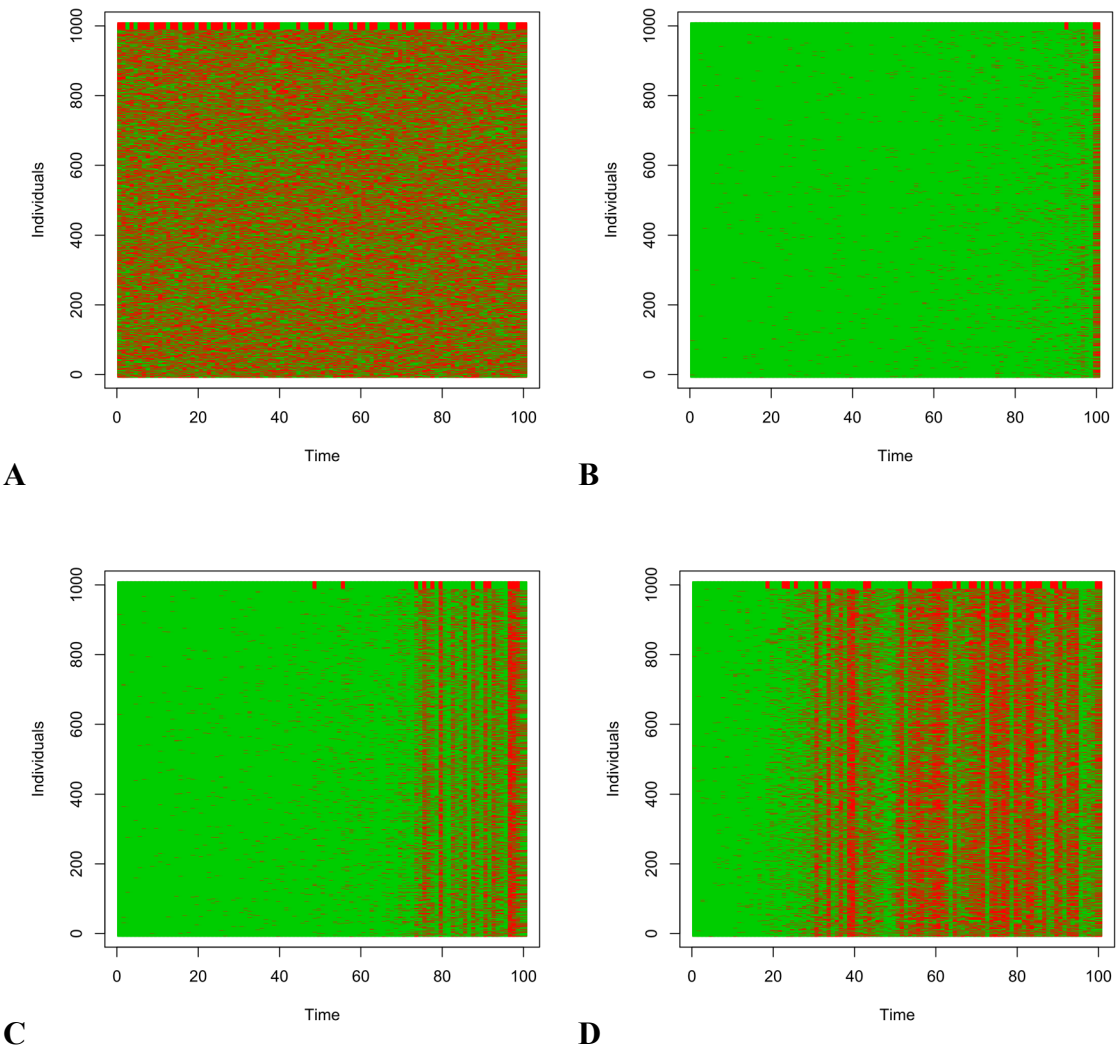
E-2 Results from nine general linear models with senescence parameters (ω, a and b) as dependent variables and mean, median and maximum longevity as covariates. Relationships between senescence parameters and longevity measures were significant in all models.

Dependent Variable	Covariate	*r*	*F*	d.f.	Sig.
ω	**Mean Longevity**	0.554	46.963	1, 36	P < 0.0001
	Median Longevity	0.521	41.275	1, 36	P < 0.0001
	Maximum Longevity	0.519	40.921	1, 36	P < 0.0001
a	**Mean Longevity**	0.320	18.418	1, 36	P < 0.0001
	Median Longevity	0.338	19.923	1, 36	P < 0.0001
	Maximum Longevity	0.194	9.891	1, 36	P = 0.003
b	**Mean Longevity**	0.300	16.840	1, 36	P < 0.0001
	Median Longevity	0.248	13.230	1, 36	P < 0.001
	Maximum Longevity	0.464	33.094	1, 36	P < 0.0001

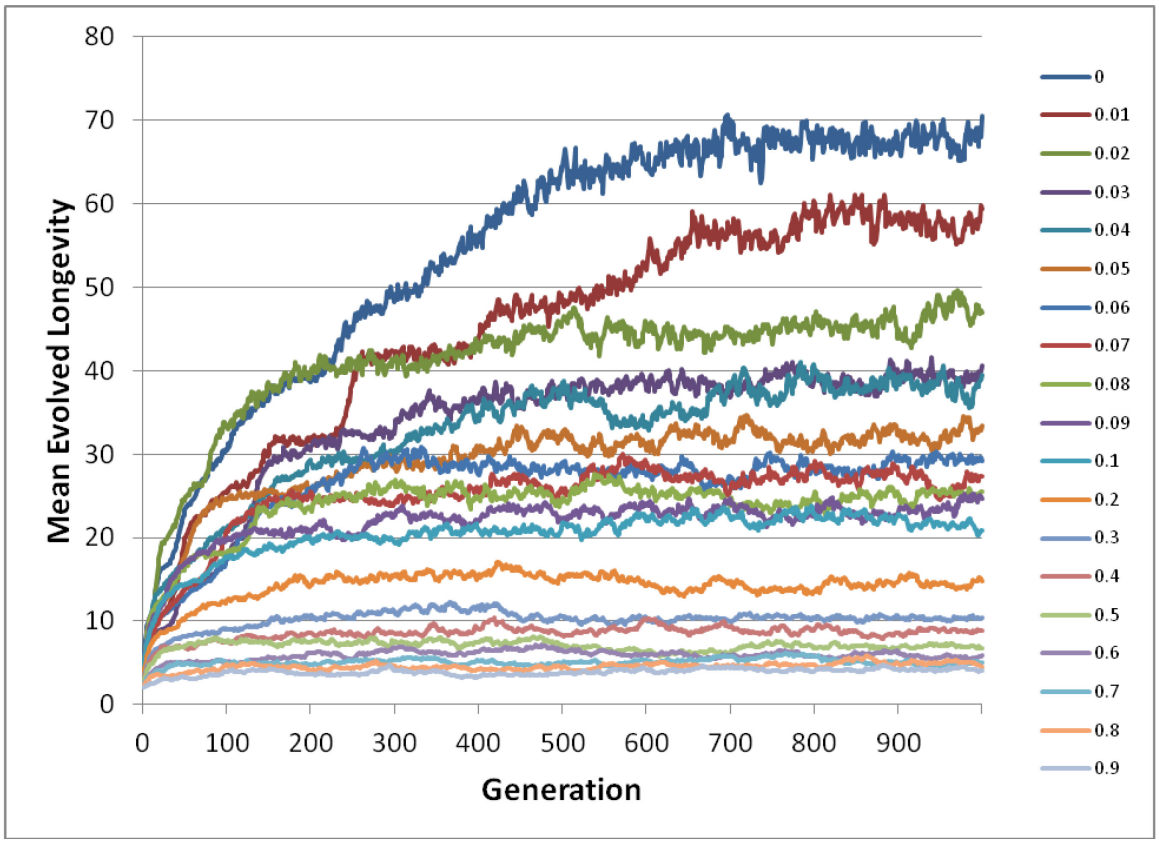
E-3 Results from four general linear models with median longevity, maximum longevity, a and b as dependent variables. In all models, there were two factors (defence and sex) as well as an interaction term. Aposematic species had significantly higher median and maximum longevity than non-aposematic species, but there were no significant differences in either Gompertz parameter (a or b). There were no significant differences between males and females in any of the models, and no interactions between aposematism and sex.

Dependent Variable	Model Factors	*F*	d.f.	Sig.
Median Longevity	**Aposematism**	11.130	1, 32	P = 0.002
	Sex	0.936	1, 32	P = 0.341
	Aposematism * Sex	0.198	1, 32	P = 0.659
Maximum Longevity	**Aposematism**	5.190	1, 32	P = 0.030
	Sex	2.061	1, 32	P = 0.161
	Aposematism * Sex	1.423	1, 32	P = 0.242
a	**Aposematism**	3.429	1, 32	P = 0.073
	Sex	0.014	1, 32	P = 0.908
	Aposematism * Sex	0.042	1, 32	P = 0.839
b	**Aposematism**	0.684	1, 32	P = 0.414
	Sex	2.216	1, 32	P = 0.146
	Aposematism * Sex	0.799	1, 32	P = 0.378

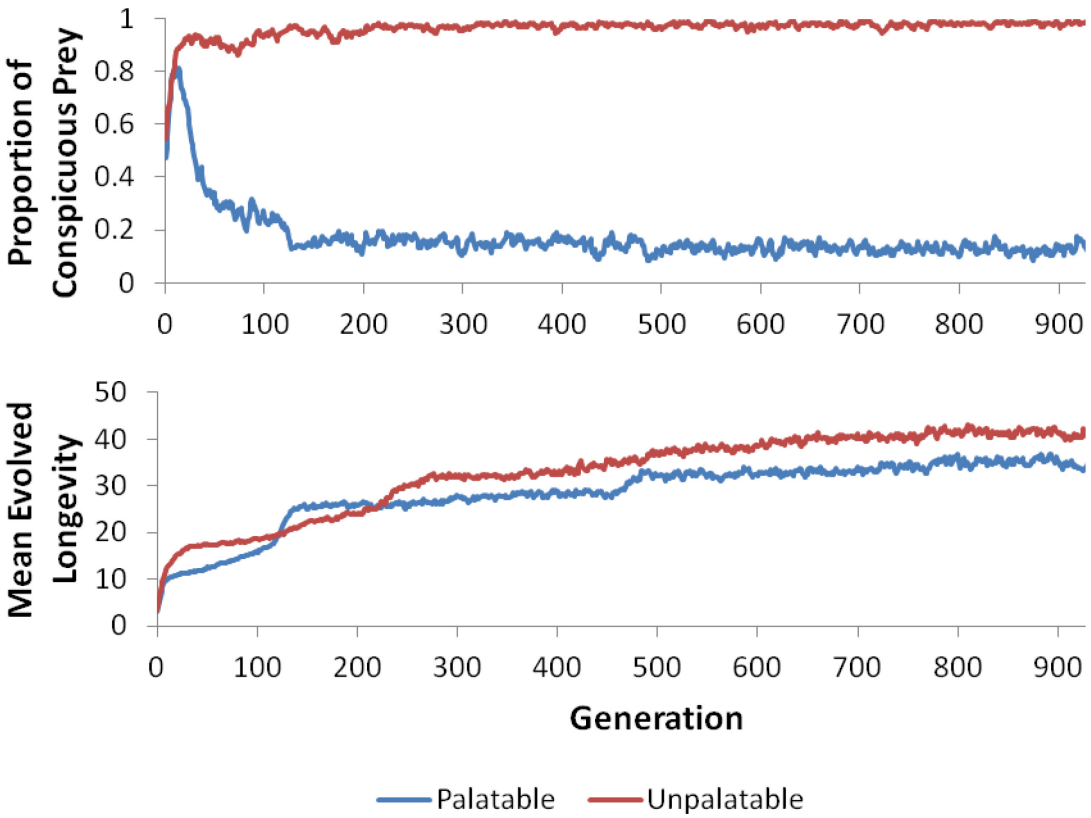
Appendix F – Supplementary figures for chapter 5



F-1 Graphical representation of a simulated population of 1000 individuals (rows) with a genome of length 100 (columns). Life and death genes (green and red, respectively) are randomly distributed in the initial population (A), and after 1000 generations longevities converge, either on the maximum value determined by the length of the genome when the rate of extrinsic mortality is 0 (B), or on intermediate values when the rate of extrinsic mortality is 0.01 (C) and 0.1 (D). Number of individuals = 1000, maximum genome length = 100, forward and backward mutation rates = 0.005.



F-2 Mean longevity vs. generation number for populations with a range of extrinsic mortality values. Each line represents a different fixed rate of extrinsic mortality. Populations contain 1000 individuals with a maximum longevity of 100 time units and a forward and backward mutation rate of 0.005. Number of individuals = 1000, maximum genome length = 100, forward and backward mutation rates = 0.005.



F-3 Proportion of conspicuous prey and mean evolved longevity vs. generation for a single palatable and unpalatable population. Number of individuals = 1000, maximum genome length = 100, forward and backward mutation rates = 0.005, number of generations = 1000, $\lambda_l = 1$ for unpalatable populations, $\lambda_l = 0$ for palatable populations, conspicuous prey visibility = 1, cryptic prey visibility = 0.3, neophobia = 2.

Appendix G – R code for genetic algorithm with evolving longevity trait, Pavlovian predator and two competing prey morphs

```
N <- 1000                  #number of individuals
maxt <- 100                #maximum possible lifespan
ngen <- 1000               #number of generations
mut <- 0.01                #mutation rate of life/death genes
mutvis <- 0.01             #mutation rate of visibility genes

npred <- 100               #number of predators
lambdaL <- 1               #prey palatability
lambdaF <- 1               #default predator attack rate

alphaLcryp <- 0.1
#rate of predator learning for cryptic prey
alphaLapo <- 0.7
#rate of predator learning for aposematic prey
alphaFcryp <- 0.005
#rate of predator forgetting for cryptic prey
alphaFapo <- 0.005
#rate of predator forgetting for aposematic prey
crypvis <- 0.1
#chance of a predator seeing a cryptic prey indivudal
during an encounter
apovis <- 1
#chance of a predator seeing an aposematic prey individual
during an encounter
neo <- 0
#number of neophobic avoidance events before predators
start to attack aposematic prey

crypval <- 0
neoval <- 0
numcryp <- 0
numapo <- 0
numpredcryp <- 0
numpredapo <- 0
avglong <- 0
avglongcryp <- 0
avglongapo <- 0

for(d in (1:10)){
for(e in seq(0, 1, 0.1)){
crypvis <- e
for(f in seq(0, 20, 2)){
neo <- f
```

```
pop <- cbind(matrix(sample(x = c(0,1), size = maxt*N,
replace = TRUE, prob = c(0.5, 0.5)), nrow = N), rep(0,N))
#creates population as a matrix of individuals (rows) with
death genes at different time steps (columns) (0=dead,
1=alive)
popvis <- sample(x = c(0,1), size = N, replace = TRUE, prob
= c(0.5, 0.5))
#creates vector of visibility genes for each prey
individual (0=cryptic, 1=aposematic)

for(k in 1:ngen){
#runs the simulation for ngen generations

surv <- apply(pop, 1, which.min)
#calculates intrinsic survival time for each individual

Predcryp <- matrix(rep(c(lambdaF, rep(0, maxt)), npred),
nrow = npred, byrow = TRUE)
#creates matrix of predators (rows) with starting predation
probabilities for cryptic prey, and 0 for other time steps
(columns)
Predapo <- matrix(rep(c(lambdaF, rep(0, maxt)), npred),
nrow = npred, byrow = TRUE)
#creates matrix of predators (rows) with starting predation
probabilities for aposematic prey, and 0 for other time
steps (columns)
predneo <- rep(neo, npred)
#creates vector of neophobic avoidance events for each
predator
encounter <- matrix(cbind(replicate(maxt, sample(seq(1,N),
size = npred, replace    = FALSE))), nrow = npred)
#creates matrix of prey individuals encountered by each
predator for each time step, without replacement
rpred <- matrix(runif(npred*maxt), nrow = npred)
#creates matrix of attack probabilites for each encounter

h <- 1
countpredcryp <- 0
countpredapo <- 0
while((h < max(surv)) & (h <= maxt)){
#runs through each time step until all prey are dead

alive <- as.numeric(surv > rep(h, N))
#is each prey individual alive at the given time step?
```

```
learncryp <- alive[encounter[,h]]*(-popvis[encounter[,h]] +
1)*as.numeric(rpred[,h] < Predcryp[,h]*crypvis)
#reorders alive based on encounter, screens for cryptic
prey, gives vector of successful predation events modified
by crypvis
forgetcryp <- -learncryp + 1
#gives vector of unsuccessful predation events
Predcryp[,h+1] <- Predcryp[,h] +
learncryp*alphaLcryp*(lambdaL - Predcryp[,h]) +
forgetcryp*alphaFcryp*(lambdaF - Predcryp[,h])
#each predator's attack variable moves towards either
lambdaL ("learning") or lambdaF ("forgetting")

learnapo <-
alive[encounter[,h]]*popvis[encounter[,h]]*as.numeric(rpred
[,h] < Predapo[,h]*apovis)
#reorders alive based on encounter, screens for aposematic
prey, gives vector of successful predation events modified
by apovis
avoid <- which(predneo > 0 & learnapo > 0)
#gives vector of predation events with neophobic predators
predneo <- predneo - learnapo
#reduces neophobia counters for avoided predation events
predneo <- replace(predneo, predneo < 0, 0)
#resets negative neophobia counters to 0
learnapo[avoid] <- 0
#removes avoided predation events
forgetapo <- -learnapo + 1
#gives vector of unsuccessful predation events
Predapo[,h+1] <- Predapo[,h] + learnapo*alphaLapo*(lambdaL
- Predapo[,h]) + forgetapo*alphaFapo*(lambdaF -
Predapo[,h])
#each predator's attack variable moves towards either
lambdaL ("learning") or lambdaF ("forgetting")

surv[encounter[which(learncryp == 1 | learnapo ==1), h]] <-
h
#survival time is updated for each prey individual that was
consumed by a  predator
h <- h + 1
countpredcryp <- countpredcryp + sum(learncryp)
#number of attacks by predators against cryptic prey
countpredapo <- countpredapo + sum(learnapo)
#number of attacks by predators against aposematic prey

}
```

```
lotto <- rep(c(1:length(surv)), surv)
#creates one lottery ticket for every year that each
individual was alive
oldpop <- pop
#preserves a copy of the prey population
oldpopvis <- popvis
#preserves a copy of prey visibility genes
reproduce <- sample(lotto, N, replace = TRUE)
#draws lottery tickets
pop <- rbind(oldpop[reproduce,])
#creates new population based on draw
popvis <- oldpopvis[reproduce]
#creates new vector of visibility genes based on draw

mtime <- cbind(matrix(sample(x = c(1, -1, 0), size =
maxt*N, replace = TRUE, prob = c(0.5*mut, 0.5*mut, 1-mut)),
nrow = N), rep(0,N))
#creates matrix of possible mutation events for each
individual at each time step
pop <- replace(pop, mtime > 0, 1)
#performs a round of forward mutation on the new population
pop <- replace(pop, mtime < 0, 0)
#performs a round of backward mutation on the new
population

mvistime <- sample(x = c(1, -1, 0), size = N, replace =
TRUE, prob = c(0.5*mutvis, 0.5*mutvis, 1-mutvis))
#creates vector of possible mutation events for each prey
individual
popvis <- replace(popvis, mvistime > 0, 1)
#performs a round of forward mutation on the new population
popvis <- replace(popvis, mvistime < 0, 0)
#performs a round of backward mutation on the new
population

}

#Output code:
crypval <- c(crypval, crypvis)
neoval <- c(neoval, neo)
surv <- apply(pop, 1, which.min)
numcryp <- c(numcryp, length(surv[which(popvis == 0)]))
numapo <- c(numapo, length(surv[which(popvis == 1)]))
numpredcryp <- c(numpredcryp, countpredcryp)
numpredapo <- c(numpredapo, countpredapo)
avglong <- c(avglong, mean(surv))
```

```
if(length(surv[which(popvis == 0)]) == 0){
     avglongcryp <- c(avglongcryp, 0)
}else{
     avglongcryp <- c(avglongcryp, mean(surv[which(popvis
== 0)]))
}
if(length(surv[which(popvis == 1)]) == 0){
     avglongapo <- c(avglongapo, 0)
}else{
     avglongapo <- c(avglongapo, mean(surv[which(popvis ==
1)]))
}

}
}
}

output <- cbind(crypval, neoval, numcryp, numapo,
numpredcryp, numpredapo, avglong, avglongcryp, avglongapo)
write.csv(output, file = "__10 reps unequal learning mixed
population lambdaL1.csv"
```

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