Carita Lindstedt

Maintenance of Variation in Warning Signals under Opposing Selection Pressures

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ABSTRACT

Aposematic animals have evolved warning signals e.g. bright colour patterns to inform predators of their unprofitability as prey. Predation is assumed to select for large pattern elements, conspicuousness and uniformity of warning signals as this enhances avoidance learning of predators. In accordance with this classical expectation, I show that predators indeed learn to avoid the aposematic moth larvae (*Parasemia plantaginis*, Arctiidae) with more conspicuous colouration (i.e. large orange patch expressed on a black body) faster than larvae with a smaller, less conspicuous signal. Because of the high heritability of this signal size, selection by predators should favour larger signals and consequently also decrease the variation in the warning colouration of *P. plantaginis* larvae. However, the opposite is true and the colouration of the larvae and adult *P. plantaginis* varies widely. I tested whether thermoregulation, diet or defence against macroparasites and pathogens constrain maximal warning signal expression in this species. If allocation to antipredator traits trades off with other important traits, it could offer one explanation as to why warning signal efficacy is not always maximized. My results demonstrate that the signal size of *P. plantaginis* larvae is constrained by thermoregulation and efficient defence against pathogenic bacteria by giving the benefit for individuals with smaller signal and larger black eumelanin based patterns. I also found that the chemical composition of the diet can be critical to *P. plantaginis* fitness, as it can have effects on the insect's colouration via diet-derived pigments (i.e. flavonoids) that are used to produce the orange signal. Diet is also important because dealing with defence chemicals of the host plant trades off with the number of offspring and induces variation in the colouration of an adult moth. My results underline the fact that it is crucial to study the prey's selective environment as a whole, beyond the predator-prey interactions, to elucidate the selective forces maintaining the diversity in warning signal expression.

Keywords: Aposematism; chemical defence; thermoregulation; host immunity; tritrophic interactions; *Parasemia plantaginis*; costly pigmentation

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-V. I am the first author of papers I-IV, and I carried out a large part of the planning, writing and data collecting for each paper.

I   Lindstedt, C., Lindström, L. & Mappes, J. 2008. Hairiness and warning colours as components of antipredator defence: additive or interactive benefits? Animal Behaviour 75: 1703-1713.

II   Lindstedt, C., Morehouse, N. I., Pakkanen, H., Casas, J., Christides, J. P., Kemppainen, K., Lindström, L. & Mappes, J. Pigment composition of a variable warning signal of *Parasemia plantaginis* larvae (Arctiidae). Submitted manuscript.

III   Lindstedt, C., Lindström, L. & Mappes, J. Thermoregulation constrains effective warning signal expression. Evolution. In press.

IV   Lindstedt, C., Reudler Talsma, J. H., Ihalainen, E., Lindström, L. & Mappes, J. Costs induced by the plant's defence chemicals can maintain variation in aposematic colouration. Submitted manuscript.

V   Friman, V., Lindstedt C., Hiltunen T., Laakso, J. & Mappes, J. Predation on multiple trophic levels shapes the evolution of pathogen virulence. Submitted manuscript.

1 INTRODUCTION

Animals communicate their fitness, social status or defence mechanisms via signals. According to the definition by Maynard Smith and Harper (2003) a signal is: "*any act or structure which alters the behaviour of other organisms, which evolved because of that effect, and which is effective because the receiver's response has also evolved*". For example, most of us get alerted when flying insects with yellow and black stripes buzz around us. This is probably because we are familiar with the painful sting that the yellow and black stripes of wasps and bees indicate. The yellow-black colour pattern is an example of a warning signal that animals have evolved to inform their predators of behavioural, physical or chemical defence mechanisms (Poulton 1890, Guilford 1990, Maynard-Smith & Harper 2003). In addition to visual signals such as colouration (Edmunds 1974) or bioluminescence (De Cock & Matthysen 1999), also, conspicuous sounds (Dunning & Krüger 1995, Rowe 2001), odours (Rowe & Guilford 1999a, Lindström et al. 2001a) and vibrational or electric signals can have a warning function (Guilford 1990).

The efficacy of an aposematic antipredator strategy (i.e. warning signal combined with unprofitability of prey) is based on predator learning (Roper & Redston 1987, Alatalo & Mappes 1996, Rowe & Guilford 1996, Riipi et al. 2001). Predators learn to associate unprofitability (e.g. chemical defence) with conspicuous colouration (e.g. red, yellow or white combined to black patterns) and avoid attacking prey animals that carry that signal in future (e.g. Guilford 1990). The information the warning signal provides is in the mutual interest of both the signaller (prey) and the receiver (predator), making its expression beneficial for both of the recipients (e.g. Maynard-Smith & Harper 2003, Ruxton et al. 2004). Thus, maximal efficacy of a warning signal is expected to be advantageous for both predator and prey as it ensures that the message is properly delivered from signaller to receiver (e.g. Guilford & Dawkins 1991, Sherratt & Beatty 2003, Caro 2005, Ruxton et al. 2004).

1.1 Conspicuousness

Based on receiver psychology, aposematic prey can maximise their defence against predators by presenting warning displays that enhance the unlearnt wariness of predators, increase the avoidance learning rate of predators and are memorable and easy to recognize (reviewed in Guilford & Dawkins 1991, Ruxton et al. 2004). In general, predators learn to avoid unpalatable prey more rapidly if they are conspicuous rather than cryptic (Gittleman & Harvey 1980, Roper & Redston 1987, Alatalo & Mappes 1996). This can result from increased detection efficiency or more rapid decision making (Endler 1988, Guilford 1990, Endler 1991). In addition, conspicuousness may allow fewer recognition errors. Thus, a conspicuous colour pattern may be detected from a longer distance than a cryptic one, giving the predator more time to decide whether to attack or not and thereby leading to fewer mistakes (Wallace 1889, Guilford 1986, Gamberale-Stille 2000). As camouflaged prey are more common than conspicuous ones (Endler 1991), the novelty of the conspicuousness may accelerate the avoidance learning, as the new prey types are learned more rapidly (e.g. Edmunds 1974). New prey items are also examined more closely, which may enhance avoidance learning (Guilford 1990).

Conspicuousness can be achieved in several ways. It can be a result of striking colouration (Wiklund & Sillén-Tullberg 1985, Sillèn-Tullberg 1985, Gamberale-Stille & Tullberg 1999, Gamberale-Stille & Guilford 2003, Exnerová et al. 2006). For instance, particular colour patterns such as yellow, orange and red accelerate predator learning (Sillen-Tullberg 1985, Mappes & Alatalo 1997a, Gamberale-Stille & Tullberg 1999, Exnerová et al. 2006), and also enhance the unlearnt reluctance to attack (Schuler & Hesse 1985, Sillen-Tullberg 1985, Lindström et al. 1999a). It is also possible that predators associate unpalatability with a specific colour patterns more readily, and that these colour patterns happen to be conspicuous as well (Guilford 1990). Alternatively, increased contrast against the background can make prey more conspicuous (Roper & Redstone 1987, Lindström et al. 1999a, Gamberale-Stille 2001). In addition, signal strength and efficacy is also affected by the size of the stimulus, such as large pattern elements (Forsman & Merilaita 1999, Lindström et al. 1999b), large size of the aposematic prey (Gamberale & Tullberg 1996, see also Hagman & Forsman 2003, Nilsson & Forsman 2003) or prey aggregations (Alatalo & Mappes 1996, Mappes & Alatalo 1997a, Gamberale & Tullberg 1998, Tullberg et al. 2000, Gamberale-Stille 2000, Riipi et al. 2001, Lindström et al. 2001a, Beatty et al. 2005, but also see Skelhorn & Ruxton 2006).

1.2 Multimodality

The efficacy of information transfer is also expected to increase with multiple signals stimulating several sensory modalities of the predator (Rowe 1999,

Rowe & Guilford 1999b). For example, predators can use taste as an additional signal in combination with visual stimuli and change their foraging behaviour according to the strength of these two stimuli (Gamberale-Stille & Guilford 2004, Rowe & Skelhorn 2005, Skelhorn & Rowe 2006a). Therefore, unpalatability, and variation in its strength and quality can also affect warning signal efficacy (Marples et al. 1997, Rowe & Skelhorn 2005, Lindström et al. 2006, Skelhorn & Rowe 2006a, Skelhorn & Rowe 2006b, but see Ihalainen et al. 2007).

Prey animals can also increase their conspicuousness by using multiple signals in addition to colouration (e.g. Rowe 1999, Rowe & Guilford 1999a). Such signals that have more than one simultaneously displayed component in more than one sensory modality are called multimodal warning signals (e.g. Rowe 1999, Partan & Marler 2005). For example, the warning coloured and unpalatable seven-spot ladybird (*Coccinella septempunctata*) produces a strong pyrazine odour by reflex bleeding (Marples et al. 1994), which can increase a predator's bias against typical warning colours (Rowe & Guilford 1996). Recently, it has been also suggested that physical defences such as spines or hairs may act as visual cues to a prey's unprofitability and improve both the detectability of prey and the avoidance learning by predators (Inbar & Lev-Yadun 2005, Speed & Ruxton 2005). However, there are few studies testing this hypothesis (Lindstedt et al. 2008).

These signal components for different sensory modalities can work additively, increasing the avoidance learning of a single predator (Marples et al. 1994, Rowe 1999, Rowe & Guilford 1999a, Rowe & Guilford 1999b, Rowe & Guilford 2000, Lindström et al. 2001b, Rowe 2001, but see Vallin et al. 2005, Hauglund et al. 2006, Lindstedt et al. 2008 for non-additive benefits). Alternatively, the signal components can increase the defence of the prey against different predators with dissimilar search behaviour and perception (Pearson 1989, Ratcliffe & Nydam 2008), or different components can be targeted against different phases of predation (Endler 1988).

1.3 Signal uniformity

Predation can result in frequency dependent selection on prey species which can be either negative, favouring rare morphs, or positive, favouring more common forms (e.g. Endler 1991, Ruxton et al. 2004). In general, aposematic animals are expected to be under positive frequency dependent selection, thus, the more common the aposematic individuals are in frequency and density, the more beneficial aposematism should be (Greenwood et al. 1989, Endler 1991, Lindström et al. 2001b). However, for the initial evolution of aposematism positive frequency dependent selection imposes a problem, since rare aposematic prey may be subject to very high predation risk by uneducated predators (e.g. Guilford 1990, Mappes & Alatalo 1997b, Lindström et al. 2001b, Speed 2001). As frequency and density of aposematic individuals exceeds some

threshold abundance, aposematism becomes more beneficial as *per capita* predation risk decreases (Lindström et al. 2001b, see also Riipi et al. 2001). This is probably best illustrated by the defence strategy of desert locusts (*Schistocerca gregaria*). Desert locusts rely on crypsis at low population densities but when the density increases they develop an aposematic colouration (Sword 1999). In addition, locusts also change their feeding behaviour along with the change from cryptic and solitary to gregarious and aposematic strategy by starting to prefer plants containing toxic compounds that the locusts can exploit in their secondary defence (Despland & Simmons 2005).

Warning pattern similarity both within and among species is assumed to increase the efficacy of warning signals (Müller 1879, Beatty et al. 2004). Thus, the costs of educating naïve predators to avoid aposematic prey are shared between the individuals with similar signals leading to increased *per capita* survival. Because of this positive frequency dependent selection for signal design, effective predator learning should select against dissimilar patterns and favour signal uniformity i.e. monomorphism in warning signal expression (see e.g. Müller 1879, Endler 1988, Mallet & Barton 1989, Endler 1991, Mappes & Alatalo 1997b, Joron & Mallet 1998, Kapan 2001, Beatty et al 2004, Rowland et al. 2007). However, even though the benefits of pattern similarity against educated predators are convincing (Alatalo & Mappes 1996, Kapan 2001, Rowland et al. 2007 Ihalainen et al. 2008), laboratory studies with naïve predators suggest that predators can learn and remember variable signals equally well in comparison to monomorphic ones (Rowe et al. 2004, Ihalainen et al. 2007). Thus, it is possible that monomorphism in signal design is more effective against educated predators than naïve ones (Rowe et al. 2004, Ihalainen et al. 2008). Also, variation in warning signal design and conspicuousness may not be as important as long as the signal is distinguishable from the cryptic prey (e.g. Holloway et al. 2001, Sherratt & Beatty 2003, Rowe et al. 2004, Merilaita & Ruxton 2007). Moreover, variation in predator species' perception and susceptibility to prey's defences can weaken the selection toward monomorphism (Endler 1988, Endler & Mappes 2004).

1.4 Variation in warning signal expression among defended species

Assumed adaptive advantage of maximal conspicuousness and selection to monomorphism of warning coloured species should imply a reduction in genetic and phenotypic variation in signal design through stabilising selection. In spite of these expectations, not all chemically defended species are highly conspicuous (e.g. Poulton 1890, Endler 1988, Rowell-Rahier et al. 1995). For example, pine sawfly larvae (Diprionidae) are chemically defended, exhibit defensive movements and aggregate. However, the colouration is cryptic (e.g. Björkman & Larsson 1991, Codella & Raffa 1995, Lindstedt et al. 2006). Also,

some moths like *Utetheisa galapagensis* (Lepidoptera, Arctiidae), impose chemical defences but their colouration can be dull (Roque-Albelo et al. 2002). Conspicuousness can also depend on the viewing distance; larvae of the swallowtail butterfly *Papilio machaon* appear cryptic from a distance but become clearly warningly-coloured and more conspicuous when the distance is shortened (Tullberg et al. 2005).

In addition, considerable phenotypic variation exists in warning signal patterns within species (Brakefield 1985 and Grill & Moore 1998 for Coccinellidae –ladybirds; Ojala et al. 2007 for *Parasemia plantaginis* (Arctiidae), Williams 2007 for bumblebees). For example, Grill & Moore (1998) showed that when reared on a poor quality diet, aposematic ladybirds produced duller colouration than on a better quality diet. Their study suggests that variation in environmental conditions; such as diet quality, could cause phenotypic variation in warning colouration (see also Ojala et al. 2007). Also, ontogenic colour change among different developmental stages of aposematic insects can induce temporal variation in aposematic colouration within species (Grant 2007 for panic moth caterpillars, Tullberg et al. 2008 for *Graphosoma lineatum* (Heteroptera)).

There are also several studies demonstrating genetic variation in the colour patterns of aposematic species (e.g. Holloway et al. 1995 for Coccinellidae –ladybirds; Joron et al. 1999 for *Heliconius numata*; Fisher & Ford 1947, Brakefield & Liebert 1985 and Ojala et al. 2007 for Arctiid moths). For example, in aposematic two spot ladybirds (*Coccinelli bipunctata*) the size of the black spots is strongly heritable (Holloway et al. 1995). The spot size shows variation in the wild, which can partly be explained by varying environmental conditions, but it can also indicate the lack of strong selection on the size of the black spots (Holloway et al. 1995). Holloway et al. (1995) also suggest that balancing selection between melanin production and antipredator function of the colour pattern could be involved in maintaining the observed genetic variation.

Genetic and phenotypic variation in warning colouration may indicate that there are opposing selection pressures on warning signal expression which constrain the conspicuousness and signal uniformity, and thereby maintain variation. Therefore, it is reasonable to test how beneficial conspicuousness really is as an antipredator defence for a defended species and whether there are some ecological and physiological constraints for warning signal expression.

1.5 Risk of being conspicuous

Even though conspicuousness is favourable by reducing attacks by experienced predators (e.g. Alatalo & Mappes 1996, Lindström et al. 1999c), conspicuousness may also incur costs as it makes the prey individuals more vulnerable to attacks by naïve (e.g. Lindström et al. 2001c, Riipi et al. 2001, Lindstedt et al. 2008) or

specialised (Yosef & Whitman 1992) predators. In addition, predators' visual, cognitive and learning abilities, as well as searching behaviour and resistance to prey defences, can vary both within and among species, exposing aposematic prey to a variable predation risk (Calvert 1979, Fink & Brower 1981, Yosef & Whitman 1992, Pinheiro 1996, Mappes & Alatalo 1997a, Marples et al. 1998, Exenerova et al. 2003, 2007). Vulnerability of aposematic prey to predation may also depend on the amount of alternative prey available (Merilaita & Kaitala 2002, Lindström et al. 2004), and the hunger level of predators (Barnett et al. 2004). The variation among predators described above could relax the selection pressure toward conspicuous signalling (Endler & Mappes 2004, Mappes et al. 2005) and allow variation in warning signal conspicuousness.

Alternatively, predators are often wary of novel food and prefer familiar food instead (Marples et al. 2005). If this "conservatism" in diet choice is common among predators, conspicuousness is not necessarily that costly (e.g. Thomas et al. 2003, Marples et al. 2005). Many aposematic colours (black, yellow, red) are also innately aversive in comparison to common cryptic colours such as green and brown, which can also decrease the risk of being warning coloured (Smith 1977, Schuler & Hesse 1985). Still, it is important to remember that the most common strategy for prey animals is to hide from predators' eyes and thus it seems that conspicuousness in general is a risky strategy (Poulton 1890, Endler 1991, Cott 1940, Ojala 2006). It is likely that the cost:benefit ratio of being conspicuous vary in space and time depending on multiple factors, among which the community structure of predators is one of the most important (Mappes et al. 2005).

1.6 Ecological and physiological constraints for signal expression

If effective signals are costly to produce and maintain, the costs can have an impact on signal expression and life-history traits of an aposematic individual (see Endler 1991, Speed & Ruxton 2007). This may be one explanation for the occurrence of weakly conspicuous i.e. less effective warning signals (e.g. references in Endler & Mappes 2004). To date, few studies have addressed the effect of environmental conditions on warning signal expression (but see Grill & Moore 1998, Sword 2002, Sandre et al. 2007, Ojala et al. 2007). Moreover, allocation to antipredator strategy may trade off with other important traits such as defence against pathogens or parasitoids, which also may explain the level of variation observed in warning signals (Losey et al. 1997, delCampo et al. 2005, Lindsay & Altizer 2008). Taking into account the multiple species interactions aposematic animals encounter, can be important in order to understand the variation in aposematic traits (see also e.g. DeWitt & Langerhans 2003, Frey 2004, Foggo et al. 2007).

1.6.1 Costly pigmentation

In sexual signals, the quantity and quality of the colour pigment, (i.e. how it is acquired/absorbed and whether it is involved in other physiological processes such as immunity) affects the overall production and maintenance costs of signals (Hill & Montgomerie 1994, Hooper et al. 1999, Griffith et al. 2006). All animal colours are based on the same pigment repertoires (e.g. Fox 1976). Therefore, it is intriguing to ask how the chemical composition of the pigments deposited to warning signal patterns affects their production and maintenance costs. If there are costs involved, e.g. pigment production is condition dependent; we can expect that the costs to produce bright and large warning signals vary. That is, antipredator efficacy of the warning signal may be traded off with its production costs promoting variation in warning signal expression.

Pigment deposition may be costly in two ways. First, if the pigments are directly obtained from the diet, like carotenoids or flavonoids are, their scarcity in the diet or the availability of this diet can constrain signal expression (Carroll et al. 1997, Hooper et al. 1999). Studies with aposematic *Harmonia* ladybirds suggest that the intensity of the carotenoid-based orange-to-red colour in the ladybirds' elytra varies, depending on the amount of aphids in the diet (Grill & Moore 1998, Bezzerides et al. 2007). Since carotenoids are known to be present in aphids (Britton et al. 1977), this variation is at least partly due to availability of carotenoids in diet (Bezzerides et al. 2007). Thus, the dietary constraints for condition-dependent pigments could induce variation in warning signal intensity. However, Sandre et al. (2007) found the opposite: production of carotenoid-based yellow patterns on different plant diets did not induce any variation in colouration or life-history traits of the aposematic and polymorphic *Orqyia antiqua* larvae. As the amount of carotenoids is rather high in many plant species (Ojala et al. 2005, Sandre et al. 2007), carotenoid scarcity in food is not necessarily such a problem for herbivorous species. Therefore, it seems that condition dependence in pigment deposition may vary among aposematic species depending on their specific ecology, such as, dietary needs and importance of other selection pressures.

Secondly, signalling may be costly if pigments are synthesized *de novo*. This requires access to pools of basic precursors and therefore, pigment synthesis is likely to compete directly with other physiological processes (Stoehr 2006). This in turn may result in tradeoffs with other traits (Griffith et al. 2006). For instance, pterins, ommochromes, and melanins are pigments that must be synthesized from basic precursors (Kayser 1985). Melanins are especially common pigments in the animal kingdom. They form black, brown, yellow and orange patterns (Fox 1976, Riley 1997) and are therefore likely pigments at least in the black patterns of aposematic insects (Koch et al. 1998, Bezzerides et al. 2007). Melanins are synthesized from tyrosine, an essential amino acid that is required for dopamine and serotonin production (Sugumaran 2002). Ommochromes are derived from tryptophan, another essential amino acid linked to many basic physiological processes (Linzen 1974). Interestingly, as plants consist mainly of carbohydrates, it follows that

the diet of herbivores is poor in amino acids and purine-derivatives (review in Mattson 1980, Chown & Nicholson 2004). Therefore, production of melanin based patterns can be expected to be costly for herbivores and trade off with life history traits (Safranek & Riddiford 1975, Windig 1999, Talloen et al. 2004). Because many aposematic insects are herbivorous, it seems likely that pigment production is costly for them, however, this hypothesis is still largely untested.

1.6.2 Signalling and non-signalling functions of colouration

Evolution of conspicuous warning signals can be constrained if animal colouration has non-signalling functions as well (Endler 1980, 1983). One of the most important non-signalling functions of colouration is thermoregulation (Majerus 1998). Thermal melanism is shown to maintain adaptive colour polymorphism in many species (Brakefield 1985, Fields & McNeil 1988, Goulson 1994, Holloway et al. 1997, Windig 1999, Bittner et al. 2002, Hazel 2002, Davis et al. 2005, Trullas et al. 2007). As darker colours absorb heat more effectively, darker (i.e. more melanic) individuals should have an advantage in colder climates (Forsman 1997, Berenbaum & Biesmeijer 2003, Majerus 1998). In their study with *Colias* butterflies, Ellers and Boggs (2003) showed that if animal colouration has multiple functions such as sexual signalling and thermoregulation, opposing selection pressures maintains variation in colouration (see also Chunco et al. 2007). Since aposematic signals often consist of black patterns combined with bright colours such as yellow, red or orange, developing a conspicuous and bright warning signal may decrease the size of melanised areas below the levels that maximizes thermoregulatory efficacy. Therefore, selection by predators on large and bright patterns (e.g. Lindström et al. 1999b, Lindstedt et al. 2008) may be in conflict with the thermoregulatory function of colouration. It is worthwhile noting that dark colouration can also protect individuals from UV-radiation (Hessen 1996, but see Gunn 1998). Even though thermal variation in colouration is generally assumed (see e.g. Majerus 1998, Ojala et al. 2007, Speed & Ruxton 2007) and observed in aposematic species (e.g. Hazel 2002, Davis et al. 2005), its direct consequences to the fitness of aposematic prey have rarely been tested.

1.6.3 Costs of chemical defences of host plant

Many aposematic species are associated with toxic host plants (Brown & Trigo 1994, Nishida 2002). Chemical compounds of plants have harmful effects on herbivores' growth, survival and fecundity (e.g. Larsson et al. 1986, Després et al. 2007, but see delCampo et al. 2005). Consequently, herbivores have evolved behavioural and metabolic adaptations to tolerate these plant defences. Insects can simply avoid eating toxic plants (Chapman 2003), manipulate the plant's chemical defences e.g. by cutting veins (Helmus & Dussourd 2005), suppress induced plant defences with oral secretions (Musser et al. 2002) or biotransform plant toxins into a harmless form (Berenbaum 2002). However, maintenance of tolerance mechanisms against the plants' defence compounds can be costly for

herbivores in terms of energy and time used (Després et al. 2007). Despite these costs, many herbivorous insects also benefit from the plant's defence chemicals. Plant compounds can be sequestered and subsequently used as a defensive substance against predators, parasites or pathogens (e.g. Bowers 1990, Björkman & Larsson 1991, Dyer & Bowers 1996, Björkman et al. 1997, Bowers & Stamp 1997, Camara 1997a, Karban & English-Loeb 1997, Ali et al. 1998, Nieminen et al. 2003, Bernays & Singer 2005, Castella et al. 2008). Moreover, plant chemicals can also protect insects against UV radiation and photoactivated phytotoxins (Berenbaum & Zangerl 1993, Carroll et al. 1997). Therefore, the outcome of plant-herbivore interactions depends strongly on the environmental constraints, such as availability of suitable plants, predation pressure and costs associated to these traits (Brown & Trigo 1994, Björkman et al. 1997, Bowers & Stamp 1997a, Longson & Joss 2006, Després et al. 2007).

1.6.4 Production and maintenance costs of chemical defence

Aposematic insects can derive their unpalatability by biosynthesizing defence chemicals *de novo* or sequestering them from their host plants (e.g. Bowers 1990, Brückman et al. 2000, Sime et al. 2000, Nishida 2002). There is a considerable body of literature suggesting that production and maintenance of defence chemicals imposes costs on herbivores' life history (e.g. Bowers 1990, Brjörkman & Larsson 1991, Berenbaum & Zangerl 1993, Grill & Moore 1998, Dobler & Rowell-Rahier 1996, Fordyce & Nice 2008, but see Holloway et al. 1991, Holloway et al. 1993, Camara 1997b). Mechanisms behind these costs can be diverse (see e.g. Ruxton et al. 2004) but most revolve around the energetic requirements of toxin synthesis, toxin sequestration, and protection of the insect's own tissues from autotoxicity (e.g. Berenbaum & Zangerl 1993). In addition, the defence efficiency of many aposematic herbivores is dependent on the availability of suitable plants for toxin sequestration (Dyer & Bowers 1996, Tullberg et al. 2000, see also Saporito et al. 2006). Therefore, the time spent finding food plants with sufficient nutrient, defence chemical and colour pigment reserves, can trade off with other activities such as mating behaviour. Availability of suitable host plants can also expose individuals to competition for food within species. The costs associated with sequestering and synthesising the insect's chemical defences (Bowers 1990, Brjörkman & Larsson 1991, Dobler & Rowell-Rahier 1996, Grill & Moore 1998, Longson & Joss 2006) could partly explain why intraspecific variation in the strength of chemical defences is common (Bowers & Farley 1990, Dyer & Bowers 1996, Camara 1997a, Klitzke & Brown 2000, Willinger & Dobler 2001, Despland & Simpson 2005).

There is also some evidence that colouration and chemical defence cannot evolve separately in aposematic animals (but see Grill & Moore 1998). For example, in their phylogenetic study, Darst et al. (2006) found that in three Dendrobatidae species, the chemical defence efficacy and the brightness of warning colouration were negatively correlated, suggesting a tradeoff between allocation to efficient chemical defence and conspicuousness. Alternatively, chemical defence efficacy and conspicuousness can be positively correlated

(Summers & Clough 2001, Dendrobatidae; Bezzerides et al. 2007, *Harmonia* lady beetles) indicating that individuals who allocate to conspicuousness also allocate more to their chemical defence efficiency. If these interactions between defence traits exist, it is difficult to separate which of the defence traits is more affected by ecological factors such as dietary constraints and predation pressure. Therefore, it is important to study the possible fitness costs of production and maintenance of warning signals, how they interact with chemical defence and whether there are tradeoffs within the defence traits (see e.g. Speed & Ruxton 2007).

1.6.5 Immunological defence

Many colour pigments found in animal colour patterns are known to be linked with immunological mechanisms. The amount of carotenoids has been shown to correlate positively with better immunity in birds (e.g. Lozano 1994, Camplani et al. 1999, Hõrak et al. 2001); allocation of these pigments to bright patterns may trade off with immunocompetence (Perez-Rodriquez et al. 2008). Melanin is also known to be linked to several metabolic processes affecting general cell physiology and immune functions, especially in insects (e.g. Gillespie et al. 1997). For example, phenoloxidase enzyme activity, which is part of the humoral immune response cascade in insects is shown to be positively correlated with the amount of cuticular melanin (Siva-Jothy 2000, Wilson et al. 2001, Cotter et al. 2004, Armitage & Siva-Jothy 2005). Furthermore, melanism has been shown to correlate with higher encapsulation activity (Rantala et al. 2000, Wilson et al. 2001, Cotter et al. 2004) and thus better protection against macroparasites (Gillespie et al. 1997, Cotter et al. 2004). Because warningly coloured insects often have melanin-based black patterns combined with bright, possibly carotenoid-based patterns (Bezzerides et al. 2007, Sandre et al. 2008, see also Feltwell & Rothschild 1974) it is intriguing to ask how the allocation to these pigments interacts with the immune defence capacity (see also Lindsey & Alitzer 2008). Given that immune response is costly (Moret & Schmid-Hempel 2000, Cotter et al. 2003, Freitak et al. 2003) allocation to effective defence against predators may cause physiological costs and decrease immunity.

1.7   Aims of the study

In this thesis I study warning signals of aposematic animals, the benefits of these signals as a defence against predators, and also the signal production and maintenance. I also examine the costs involved in warning signalling strategy. I determine the selection pressures on the signals from both predator and prey perspective. By studying these multiple selection pressures, I sought to recognize possible selection pressures maintaining variation in warning signals, and the evolutionary forces that could maintain genetic variation in nature.

The first study of the thesis uses a classic predator-based approach by testing how variation in colouration of prey affects its defence against predators. I study how variation in the signal size affects its detectability risk (i.e. conspicuousness) and the avoidance learning rate of predators (Paper I). If a large signal increases the avoidance learning rate, predation pressure should favour larger signals and decrease the variation observed in larval colouration. However, the variation in signal size may not be as important as long as a signal is distinguishable from the cryptic prey (e.g. Wallace 1889, Holloway et al. 2001, Sherratt 2002, Sherratt & Beatty 2003, Rowe et al. 2004). In that case, variation in colouration could then be solely due to selective factors other than predation (see also Ojala et al. 2007). In the first study, I also test the relative importance of different defence components (colouration and hairiness) and whether these multimodal components could work additively, increasing the defence of the prey or, alternatively, whether there is a tradeoff in these defence components which would explain variation in warning signal expression.

In Papers II-V I study possible constraints for signal production. If a conspicuous signal is beneficial against predators but costly to produce and maintain, then the costs could constrain the efficacy of signal expression, and hence relax the selection for maximal conspicuousness. Paper II investigates the chemical structure of the pigments needed to produce the orange-black warning colouration. Knowledge of the colour pigments is crucial in understanding possible physiological and ecological tradeoffs involved in aposematic traits. In Paper III I test whether a thermoregulatory function of colouration could constrain effective warning signalling by favouring the more melanic colouration with smaller warning signals. The focus in Paper IV and also in Paper V is on the ecological interactions affecting the antipredator defence of individuals, and on the tradeoffs between defence and other traits. In Paper IV I test how dealing with the defence chemical content of the host plant affects life history traits, warning colour expression and level of chemical defence of the polyphagous herbivorous *P. plantaginis* moth. I also test whether the amount of chemical defence components in the prey correlate with its colouration. If allocation to conspicuous colouration trades off with the amount of defence chemicals *per se*, then variation in the range of costs and benefits associated with these defence traits could promote variation. In Paper V I test whether allocation to an effective warning signal trades off with defence against parasites and pathogens. I also study how selection on the pathogen's virulence by its own predators affects the extent of the defence tradeoffs of the warning coloured host.

2   MATERIAL AND METHODS

All the rearing experiments with larvae were performed in the Department of Bio- and Environmental Sciences in Jyväskylä between the years 2004 - 2007. The behavioural experiments with birds were conducted in Konnevesi Research stations between the years 2004 – 2006.

2.1   Study species and their maintenance

To study the benefits of conspicuousness as an antipredator strategy against predators (I) and environmental constraints for signal expression (II-V), I used aposematic wood tiger moth larvae (*Parasemia plantaginis*, Arctiidae) as a study species. The larvae of *P. plantaginis* are polyphagous and feed on numerous herbaceous and arborescent plant species (e.g. Chinery 1993, Ojala et al. 2005). The colouration of both larvae (Fig.1) and adult (Fig. 2a and b) varies, which is typical for Arctiid moths (Fisher & Ford 1947, Brakefield & Liebert 1985, Fields & McNeill 1988). Arctiid moths are also generally known to sequester plant's defence chemicals and use them for their own defence (Weller et al. 1999).

The larvae are hairy and have moderately conspicuous colouration comprising an orange patch on an otherwise black body (Fig. 1). The size of this orange patch varies continuously (Ojala et al. 2007). The larvae have 5-7 instars, the first two of which are cryptically coloured; orange-black colouration develops at the third instar (Ojala et al. 2007). Arctiid moths are capital breeders, i.e. the adults do not feed, making the larval diet critical for the fitness of adults. The adults are diurnal and also conspicuously coloured: males are either black and white or black and yellow (Fig. 2a) and females are usually black and white with red-to-orange body and hind wings. The colouration of the body and hind wings of females varies continuously from orange to red (Fig. 2b) (Chinery 1993). In Finland, this species usually has only one generation per year and typically *P. plantaginis* overwinters as 3rd – 4th instar larva.

This species was selected because its larvae show continuous variation in their moderately conspicuous colouration. As this variation has both genetical

and environmental components (Ojala et al. 2007) it enables studies to assess the genetical and environmental constraints for colour pattern formation. As *P. plantaginis* larvae are also polyphagous, it enables manipulation of the diet. In contrast to specialist herbivores, polyphagous herbivores often have to maintain several detoxification mechanisms simultaneously. This is probably costly, and therefore the costs of defence might be easier to detect from a generalist herbivore that has not adapted to use any specific host plant. Moreover, the *P. plantaginis* moth is relatively easy to rear and, in laboratory conditions, the *P. plantaginis* moth can produce two to three generations per year.

FIGURE 1   Variable colouration of the *P. plantaginis* larvae.

FIGURE 2A   Variation in the colouration (yellow or white) and black patterns of adult *P. plantaginis* males.

FIGURE 2B   Continuous variation in the colouration (orange to red) of *P. plantaginis* females.

2.1.1 Selection for warning signal size

P. plantaginis larvae have been reared as a permanent stock established in 2003. The lab stock was established from wild caught females collected from different localities in Central Finland (N=15) and Åland (N=5). During following two generations 30- 50 breeding adults were reared in each generation and effective population size were kept as large as possible to maintain genetic variation. The selection was started in 2004 from 51 families; upward and downward selection lines for divergent phenotypes (i.e. the large and small orange signals) were produced by applying a truncated family selection protocol to the stock (Lynch & Walls 1998). Therefore, we first selected both the individuals with large (number of segments including orange hairs 6 or more) and small signals (number of segments including orange hairs smaller 4 or less) within the family, and after that we crossed the individuals exceeding the threshold value of selected signal sizes within the selection lines in the following generations. The selection lines were reared in laboratory conditions (25 ºC) in a greenhouse at University of Jyväskylä in Central Finland. During the rearing, larvae were fed with lettuce and dandelion (*Taraxacum* sp.) leaves. For the hibernating generation willow leaves (*Salix* sp.) were offered as an additional food before the wintering period. In spite of the variation in the diet during the rearing, the composition of the diet was always the same for all individuals within and among the families, and selection lines in that particular generation.

Signal sizes responded to selection rather strongly (Fig. 3). The genotypic and phenotypic variances for signal size and heritability were estimated by using REML-animal model implemented in ASReml 2.0 -software (VSN international Ltc, Hemel Hempstead, UK). Model, where $y = \mu + a + e$, in which μ is the overall mean, a is the additive genetic effect and e is the residual, was

used. In complex pedigrees this method is powerful for estimating additive genetic variance (e.g. Lynch & Walsh 1998) (III).
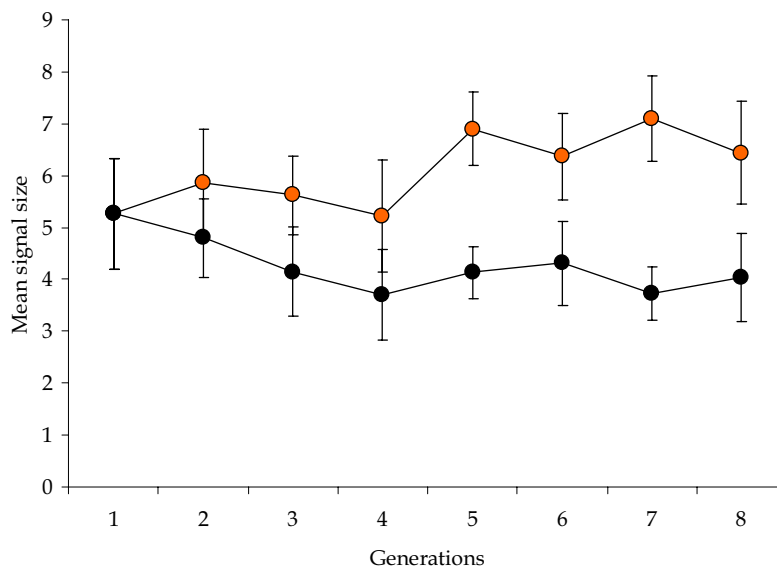

FIGURE 3   The mean signal sizes of small (black spots) and large (orange spots) signal lines. F4 and F6 generation's values show only the means for selected individuals.

2.1.2 Predators

The amount of variation among predator species in their wariness to attack aposematic prey is crucial for assumptions of costly signalling hypothesis. To test how effective the defence strategy of *P. plantaginis* larvae were for different types of predators, I used great tits (*Parus major*), blue tits (*Parus caeruleus*), pied flycatchers (*Ficedula hypoleuca*), domestic chicks (*Gallus gallus domesticus*) and ants (*Formica rufa* –group) as predators. Blue and great tits used for the experiments were trapped from feeding sites and pied flycatchers from their nest boxes around Konnevesi Research Station (Central Finland), and were subsequently ringed for identification. Each bird was kept individually in an illuminated and air-conditioned plywood cage (65 cm x 65 cm x 80 cm, w x d x h) with a daily light period of 11.5 h and at 15 °C. Each cage contained three perches offering roosting and prey- handling sites for the birds. Sunflower seeds, tallow and fresh water were available *ad libitum* for great and blue tits and mealworms were available *ad libitum* for pied flycatchers. All experiments with great tits and blue tits were performed between October and December to avoid disturbing the birds' breeding. The experiments with pied flycatchers

were carried out during July 2002. The birds were released at their capture sites after the experiments. All the birds remained in good health throughout their captivity.

Domestic chicks were obtained from a commercial hatchery (Mänttä, Finland) and transported to the laboratory on the day they hatched. The birds were thus accustomed to the presence of humans from birth. The chicks were housed in two plywood cages (40 cm x 50 cm x 50 cm, w x d x h). The bottom of each cage was made from metal netting and half of it was covered with paper, hay and sawdust which was changed daily. All of the birds were marked with non-toxic coloured marker pens. Water and chick's starter crumbs (Pikku-punaheltta produced by Suomen Rehu, Finland) were provided *ad libitum*. Chicks were kept at approximately 25 º C using heat lamps, and on a 16:8 h light:dark cycle with fluorescent tubes which did not emit UV-light. After the experiment, the chicks were relocated to a small free-range farm.

2.2   Benefits of conspicuousness against predators

To study the benefits of conspicuousness against predators, several predation experiments were conducted. The effectiveness of the defence strategy of *P. plantaginis* larvae against various predator species, the effect of colouration on detection risk and avoidance learning efficiency, and the relative importance of hairiness and colouration as defence components were tested.

2.2.1 Defence against variable predators

I compared how aversive *P. plantaginis* larvae were for ants, chicks, pied flycatchers and blue tits. In the first predation test, I offered larvae with different signal sizes to ants. As a palatable control I used meal worm larvae of same size as *P. plantaginis* larvae. The experiment was performed between 29th June and 10th July 2004. Predation experiments were conducted in the vicinity of 19 ant nests which belonged to the Formica rufa -group. The fourth instar *P. plantaginis* larvae, either with the large or small warning signal, and same sized meal worm were offered to the ants simultaneously. Prey items were placed on petri dishes which were placed on the ant trail where the traffic was about 2-4 ant workers per minute, at least 4 m away from the nest. I observed the behaviour of ants and calculated all attacks and mortality of the prey.

Blue tit experiments were conducted in experimental cages (plywood, 50 - 50 - 70 cm) in Konnevesi research station (see e.g. Ham et al. 2006). Birds were first familiarized to forage on sunflower seeds from a white dish in the cage for 2 h. The cages were lit with energy-saving bulbs (Osram Dulux el longlife 7W) which emitted a minimum of UV light. Thus, any possible differences in the UV reflectance of the larval colour patterns were not visible to the birds. The behaviour of the birds was observed through a small mesh-covered window in one side of the box and experiments took place in a dark room so that the birds

were unaware of the presence of the observer. The cages contained a perch and a water bowl. Prey items were offered through a hatch behind a visual barrier during both training and experiments. The visual barrier enabled us to measure the exact time of prey detection as the bird had to come from behind the barrier or fly to the top of it to observe the prey.

During the testing, I offered blue tits six meal worm larvae and six 4th instar *P. plantaginis* larvae in sequential trials, beginning with the meal worm larvae. To ensure that birds did not reject the moth larvae because they were not hungry, mealworms were used to control the foraging motivation and hunger level of the birds. Both prey items were the same size and alive. The attack rate and mortality of the prey were recorded. Every second *P. plantaginis* larva had a large signal and every second had a small signal. The experiment was performed with the permission of the Central Finland Regional Environment Centre (KSU-2004-L-238/254) and the Experimental Animal Committee of the University of Jyväskylä (29/31.5.2004).

Acceptability tests for pied flycatchers were conducted in Konnevesi research stations in a small aviary. The mealworms were offered first to control the bird's motivation to forage, after which, the living *P. plantaginis* larvae with different sized signals (1 per bird) were offered to the birds presented on the dandelion leaves. The petri dishes containing the prey items were on wooden poles in the aviary (set up and experimental conditions for birds described in Lyytinen et al. 2003). The attack risk (whether bird attacked or not) and mortality (bird ate the prey) were recorded. (Permission numbers were 10/23.4.2002, 29/4.6.2002 and KSU-2002-L-258/254).

The palatability of *P. plantaginis* larvae to naïve domestic chicks and great tits was tested in the experiments described below (I).

2.2.2 Detectability risk (I)

I tested how the orange patch size (small or large) and background colour affected the detectability risk of *P. plantaginis* larvae when the predators were naïve chicks or experienced wild-caught great tits. Two study arena made of plywood were used, with either a green or brown background. In the chicken experiment, five final instar *P. plantaginis* larvae with a large patch, and five larvae with a small patch (total 10 larvae) were placed in the arena. The size of the large orange patch was seven segments (covering approximately 80% of the body) and the size of the small patch was four segments (covering approximately 30% of the body). To increase motivation to forage, 12 mealworms were also placed in the arena. Moth larvae were placed alternately on the arena in the shape of a square with *ca.* 20 cm separating each from the next. Two mealworms were placed in the middle of the square. The experiment was then initiated by placing a chick in the middle of the 'prey square' near the two mealworms. I measured the order in which the prey were detected, attacked and killed.

I chose great tits (*Parus major*) as the wild and experienced predator species, because great tits are bold enough to attack aposematic prey to the

extent of causing mortality (Table 1). Furthermore, great tits are common in Finland and well suited to the experiment because insect larvae compose the largest part of their diet during the breeding season (Royama 1970). The great tit experiment was done in aviaries with similar backgrounds to the chicken experiment. During the experiment the one *P. plantaginis* larvae, either with small or large signal size, was placed in the middle of the background square. I measured the detectability of prey as the time it took for bird to notice the prey, the initial avoidance of prey as the latency to attack (the time between detection and attack) and handling time as the time it took for bird to eat the prey. Each bird's motivation to forage was controlled by offering them mealworms before and after the experiment. The Central Finland Regional Environment Centre gave us permission to capture and keep the great tits (KSU-2006-L-249) and experiments were run under licence from the Experimental Animal Committee of the University of Jyväskylä (18/22.5.2006).

2.2.3 Signal value of warning colour and hairiness (I)

I tested whether the presence of an orange patch, the hairiness of the larvae or a combination of both, were most effective in the defence against predators. Experiments were conducted in similar conditions to the blue tit experiment. Killed-final-instar *P. plantaginis* larvae were used in the experiments. I first ran a two by two factorial design (hair/no hair, signal/no signal) experiment with four treatments: (1) *P. plantaginis* larvae with hair but no orange patch (patch was covered with black paint), (2) larvae with hair and an orange patch (size five to seven segments) (control larvae), (3) hairless and patchless larvae (completely black larvae) and (4) bald larvae with an orange patch (the patch was created with orange paint). In treatments 2 and 4, I added the same amount of black paint on the ventral side of the larvae to control any possible taste effects of the paint. Birds (n = 42) were randomly assigned to one of the treatments. Prey was offered to the predators on a white dish, making all *P. plantaginis* larvae equally conspicuous irrespective of their hairiness or patch sizes. Before the experiment, the birds' motivation to feed was tested by offering a mealworm.

   P. plantaginis larvae were offered to birds in three consecutive trials. I measured the bird's latency to attack as the time from observation to the time it made contact with the prey using its beak (attack) in each trial. Latency to attack has been used previously as a variable in testing the effectiveness of avoidance learning (Shettleworth 1972, Terrick et al. 1995, Marples & Roper 1997, Roper & Marples 1997, Gamberale-Stille 2000, 2001). I also measured the attack and killing risk of the larvae. Since hunger level can affect a predator's readiness to attack defended prey (Sherratt et al. 2004, Barnett et al. 2007), I also measured the hunger level of birds quantitatively by giving them 5 min to eat as many mealworms on a petri dish as they desired after the experiment. I weighed the mealworms before and after offering them to the birds and used the difference as a covariate to test whether hunger level affected the attack latency.

The second experiment was conducted in the same way as a first, except that I manipulated the size of the orange patch along with the hairiness of the larvae. There were four treatments: (1) larvae with hair and a small orange patch (three or four segments), (2) larvae with hair and a large orange patch (six or seven segments), (3) bald larvae with a small orange patch and (4) bald larvae with a large orange patch. The sizes of the patches in treatments 3 and 4, added with orange paint, matched the sizes of those in treatments 1 and 2, respectively. As in the first experiment, the same amount of paint as used for treatments 3 and 4 was painted on the ventral side of the larvae of treatments 1 and 2. Permission numbers for the first experiment were KSU-2004-L-238/254 and 29/31.5.2004 for the first experiment, and KSU-2005-L-309/254 and 39/30.5.2005 for the second experiment.

2.3 Constraints for warning signal expression

To understand the physiological mechanisms underlying warning signal variation and chemical defence in *P. plantaginis*, chemical analyses on pigment composition and defence chemical content of the larvae and adults were performed. In order to measure the environmental and genetical variation in signal production and expression, I also conducted rearing experiments, where signal production was studied in different kinds of environmental conditions.

2.3.1 Analyses for colour pigments and chemical defence (II, IV)

The orange and black pigments composing the signal of *P. plantaginis* larvae were determined by using high-performance liquid chromatography (HPLC), solubility to strong acids and bases and fluorescence analyses, as well as characterization of the pigments *in vivo* via reflectance spectrometry (USB2000, Ocean Optics, Dunedin, Florida). Orange hairs were tested for presence of 7 candidate pigment groups (carotenoids, melanins, flavins, flavonoids, pteridines and purine derivatives, and ommochromes, including papiliochromes), and the black hairs for two melanin pigments (eumelanin and pheomelanin) (II).

In order to test if the defence chemicals of the *Plantago lanceolata* diet (iridoid glycosides) were accumulated in the herbivorous *P. plantaginis* moth, the IG content of larvae and adults were analysed. Analyses were conducted from 10 larva and 138 adults (wings and body separately). For the extraction and HPLC analyses, we followed the protocol described in Reudler Talsma et al. 2008 (IV).

2.3.2 Warning signal size and colour analyses

I measured the signal size as a number of segments including orange hairs. Since larvae always have 13 segments in the body, this measure estimates the proportional size of the orange patch in the larval body.

In order to analyse the female colouration in Paper IV, specimens were photographed with an ultraviolet sensitive Fujifilm Finepix S3 Pro UVIR camera with a UV transmitting lens (Coastal Optical systems) under a light bulb emitting both visible and UV wavelengths (Arcadia Reptile D3). UV reflectance was included in the initial analysis as avian predators are sensitive to UV wavelengths (Cuthill 2006). The response of the camera's RGB (red, green and blue) channels to increasing light intensity (radiance) was measured as non-linear, and so the response of each of these channels was first linearised (see Stevens et al. 2007). A grey standard (Labsphere Spectralon diffuse reflectance standard, which reflected 50% of all light across the avian visual spectrum), was included in every photo, allowing the digital images to be converted to reflectance data using a custom MATLAB programme (see Stevens et al. 2007). From the photos, mean values of reflectance in the long, medium, short and ultraviolet reflectance images were recorded.

2.3.3 Tradeoffs in warning signal expression

To test the possible tradeoffs in signal expression, I conducted factorial rearing experiments, where I studied the signal production as reaction norms in high and low quality environments. Environments differed in their a) thermal radiation intensity (III) and b) the defence chemical content (iridoid glycosides) of the diet (IV). In addition to warning signal, I have measured the performance through development time, growth rate, weights, fertility and survival, in order to find out possible life history costs that signal production in different quality environments may cause. Individuals for these experiments originated from the low and high signal selection lines, thus I have been able to compare the environmental and genetical variation in signal expression simultaneously.

To study the relationship between signal size and defence against pathogens (V), *P. plantaginis* larvae from small and large signal lines were infected with different strains of the ubiquitous bacterial pathogen, *Serratia marcescens*, with contrasting evolutionary histories with protozoan predator (*Tetrahymena thermophila*) (Friman et al. 2008). The infection was initiated by injecting the bacterial cells into the larvae. Also, the water control was used to control the effect of injection. As the *Serratia* strains were either grown with or without their predator, this allowed me to test the effect of co-evolution of bacteria and their predator on the virulence of the bacteria. The defence against pathogens was measured as the survival rate of infected larvae. In addition, to test if host defence against pathogens correlates with defence against macroparasites (e.g. Hymenoptera, Diptera) the encapsulation response of all larvae was measured before the bacterial infection. The method for

encapsulation measurements in *P. plantaginis* is described in more detail in Ojala et al. (2005).

3 RESULTS AND DISCUSSION

A prey individual is defined aposematic if it has a conspicuous warning signal combined with unprofitability, such as physical defence or unpalatability. Even though aposematism does not require complete avoidance by predators, predators should find aposematic prey aversive, and learn to avoid it when repeatedly encountered (e.g. Ruxton et al. 2004). In the case of *P. plantaginis* larvae, prey preference experiments with different predator species showed that larvae were not eaten by ants, blue tits or pied flycatchers (Table 1). In addition, naive domestic chicks caused zero mortality for *P. plantaginis* larvae (I). Great tits ate *P. plantaginis*, although the species was not preferred food for the birds and clearly more aversive prey than palatable mealworms (I). As great tits learned to associate the orange-black colouration to unprofitability of the larvae (I), *P. plantaginis* species can be determined aposematic. This is further supported by the finding that *P. plantaginis* larvae were able to sequester iridoid glycosides into their body. Iridoid glycosides are known to be aversive for many species (Dyer & Bowers 1996, Camara 1997a, Willinger & Dobler 2001, Nieminen et al. 2003) and could have a role as a secondary defence mechanism of *P. plantaginis* larvae.

3.1 Colouration

The evolution of colour patterns in prey insects is considered to result from the selective pressures imposed by visually hunting predators (Fisher 1930, Cott, 1940, Edmunds, 1974, Endler 1991). Therefore, the importance of colouration of *P. plantaginis* and the significance of the variation in the size of the orange patch on larval body to the predators was first investigated. The size of the orange patch increased the conspicuousness of *P. plantaginis* larvae, since predators detected larvae with a large orange patch more quickly than larvae with a small orange patch on both green and brown backgrounds. Increased conspicuousness was also costly as large patched larvae were attacked more

eagerly than larvae with a small patch. However, the detectability risk varied between the predator species. Against naïve chicks, the detectability risk of the larvae was small because chicks attacked only 16 % of larvae offered and they did not eat any of the larvae after an attack. In contrast, great tits attacked nearly all prey offered.

TABLE 1   The proportion (%) of *P. plantaginis* larvae with small or large signal size attacked and killed by different predator species. Values in brackets show the total number of prey tested.

Predator species	Attack %		Killing %	
	Small signal	Large signal	Small signal	Large signal
Ants (*Formica* spp.) (N = 19 nests)	100 (20)	100 (18)	0	0
Chicks (*Gallus domesticus*) (N = 21)	12 (105)	23 (105)	0	0
Great tits (*Parus major*) (N = 64)	86 (99)	90 (93)	57	45
Blue tits (*Parus caeruleus*) (N = 10)	12 (28)	18.5 (32)	0	6
Pied flycatchers (*Ficedula hypoleuca*) (N = 7)	0 (5)	0 (2)	0	0

The effect of background colour on detection risk also varied between the predator species. The background did not affect the order in which chicks detected the larvae, but great tits detected both prey types faster on the green background than on the brown background, suggesting that contrast with the background increased the conspicuousness of both small and large patched larvae. This suggests that the detection risk is not only dependent on the larval colouration but also on the colouration of their background. However, increased detection risk trades off with enhanced avoidance learning; a large orange patch was learned faster than a small patch. Also, the difference in total survival of the larvae against great tit predation was 12 % between the two signal size extremes (Table 1) giving the overall benefit for larvae with larger signals.

It is possible that conspicuousness can only be sustained by well-defended prey (Sherratt 2002). If the predator's susceptibility to aposematic prey's chemical defences varies (Calvert et al. 1979, Fink & Brower 1981, Yosef & Whitman 1992), the conspicuous advertisement of the warningly coloured

animals can be very risky. Therefore, intermediate or weak warning signals may be selected for, instead of the extremely conspicuous warning signals with a too high detection risk (Endler & Mappes 2004). Although larger orange patches increased the avoidance learning rate of great tits, causing selection for increasing conspicuousness (see also Lindström et al. 1999b, Gamberale-Stille & Tullberg 1999, Riipi et al. 2001), the cost of educating predators to avoid a large orange signal seems to be high, due to the high detection risk. Thus, the benefit of a large orange signal is not extremely high, which could relax the selection pressure toward larger signals when combined with the other costs of large signal (or benefits of melanin).

3.1.1 Colouration and physical defences

Because multimodal defences have been shown to work additively, increasing the learning efficiency of predators (Marples et al. 1994, Rowe & Guilford 1999b, Lindström et al. 2001a), it was surprising to find that when comparing the signal value of hairiness and colouration, hairiness did not increase the learning rate of the predators in combinationn with colouration (see also Speed & Ruxton 2005). Instead, it seems that hairiness benefits, only slightly, the individuals with inconspicuous colouration. However, by increasing the defence capacity of inconspicuously coloured prey, hairiness could allow some variation in the warning colour pattern. The size of the warning signal does not necessarily improve the individual's fitness significantly when combined with physical defences.

Lack of additive benefits between the different defence components has been found before. For instance, Vallin et al. (2005) did not find additive benefits between visual signal (eye-spots) and sound, in the peacock butterfly (*Inachis io*). Also Hauglund et al. (2006) found that sound did not enhance other aversive signals such as colouration and taste. However, this does not necessarily mean that these non-additive signal components do not have a function in the defence repertoires of prey species. Components of warning displays can also work independently with each part having its own target audience (Pearson 1989, Endler1991, Partan & Marler 2005, Ratcliffe & Nydam 2008). Thus, hairiness may be a more effective defence mechanism against more wary bird predators (e.g. naïve birds like chicks or wary predators such as blue tits) (see also Heinrich 1979) as well as insect predators such as ants (Dyer 1995, Osborn & Jaffe 1998) whose predation behaviour and sensory capabilities differ markedly from birds. Different forms of physical defences could also differ in efficacy: while sharp spines are known to increase survival against predators (Barnhisel 1991, Mikolajewski & Rolf 2004), hairiness is not necessarily as effective. However, according to the optimal diet model, predators should prefer prey with lower handling costs when they have the possibility to choose (Krebs & Davies 1993). Therefore, hairiness could increase the survival of the prey through increased handling costs (see also Rowell-Rahier et al. 1995) if the amount of energy obtained from them (per time unit) is lower compared to prey with a shorter handling time. It is also possible that hairiness is solely

maintained due to constraints imposed by the animal's phylogenetic history, as many Arctiidae have hairy larvae (Chinery 1993). Hairiness can also have other important functions such as thermoregulation and protection against physical injuries or parasites, which make it a beneficial trait to maintain.

3.2 Constraints for warning signal production

Heritability of the signal size of *P. plantaginis* larvae is reasonably high (III) and the signal size responds to selection quite strongly (Fig. 3). Moreover, even though there is some phenotypic plasticity in the signal size of the larvae (III, Ojala et al. 2007) it is not very extensive (IV) and signal sizes always differ more between the signal selection lines than between the temperature (III) or diet (IV) treatments. Therefore, selection by predators should favour expression of larger signals and decrease variation (I). However, larvae of this species have considerable variation in the size of the warning signals which suggest that selective forces, other than predation, constrain the conspicuousness of the warning signal.

Quality and availability of diet suitable for obtaining and producing pigments could constrain the warning signal expression (II). Pigment analyses for the orange colour of *P. plantaginis* larvae suggest that the larvae produce the colour of their orange warning signal by depositing flavonoids and trace elements of eumelanin into the hairs. The black pattern elements are hairs containing high levels of eumelanin. Therefore, the amount of precursors needed for melanin synthesis, and on the other hand the amount of flavonoids in diet, can both constrain the expression of either orange or black colour patterns. The results of the pigment analyses are consistent with the study of Ojala et al. (2007). According to their results, the *P. plantaginis* larvae reared on high quality diet (*Rumex* sp.), that also had a high flavonoid concentration (Ojala et al. 2005), expressed larger signals than larvae reared on an artificial diet including only traces of flavonoids. Although a large signal is beneficial against predators (Lindstedt et al. 2008), findings from pigment analyses combined with the results of Ojala et al. (2007) offer indirect evidence that flavonoid concentration of the diet could constrain the size of the orange warning signal. Interestingly, the study of Ojala et al. (2007) also indicates that traces of eumelanin found in the orange hairs of the larvae can play a significant role in the formation of the signal pattern, since some *P. plantaginis* larvae could express fairly large but less bright orange signals also on an artificial diet with very low flavonoid content. This suggests that the warning signal sizes of *P. plantaginis* larvae are not purely dependent on the flavonoids in diet, and that the larvae can probably compensate the variation in diet quality by using two kinds of pigments to produce their warning signal pattern (see also Grether et al. 2001).

In addition to protection from predators, animal colouration has also other important functions such as sexual signalling (Endler 1987) or especially

in ectothermic species thermoregulation (Majerus 1998). Therefore, increased melanism can be selected for if expression of smaller warning signals enhances thermoregulation (discussed in Ojala et al. 2007, Speed & Ruxton 2007). Study III shows that the orange warning signals of *P. plantaginis* were always smaller and darker (i.e. larvae were more melanic and less conspicuous) when the larvae were reared at a low temperature. Although detection risk is smaller for larvae with a small warning signal, this increased melanism can hamper defence against predators (I) since smaller signals also decrease avoidance learning rate of predators. The benefit of expressing a small and dark warning signal is an enhanced growth rate and a shorter development time in colder environments (III). Thus, spatial and temporal variation in temperature can maintain both genotypic and phenotypic variation in warning colouration by giving fitness benefits for more melanic individuals in colder environments and years.

I show that a high level of defence chemical in the host plant can affect fitness and warning colouration of aposematic prey. When *P. plantaginis* larvae from small and large signal lines were reared on diets differing in their iridoid glycoside (IG content), the IG content of individuals was rather constant irrespective of the differences in the IG content of the diets. This suggests that *P. plantaginis* larvae efficiently excrete most of the IGs from their body. Increased excretion of IGs was costly for the larvae as high IG content of the diet decreased the moth's fitness (shown as a lower number of offspring).

The amount of IGs in the diet did not directly affect larval colouration but induced variation in adult female colouration. Females from the large signal line were lighter orange (as opposed to dark red) than females from the small signal line on the high IG diet. Thus, having to deal with host plant defences can have more drastic effects on adult colouration if an individual has allocated to efficient warning colouration as a larva. Furthermore, since the red colouration is more efficient against predators in females (Eager, Ihalainen, Kahilainen, Lindstedt, Mappes unpublished), the high IG concentration of the diet may hamper the efficient defence against predators of adult females. Toxin content of the diet did not have any effects on male colouration. However, the results suggest that in males, the larval and adult colouration can be linked. If a male allocates to an efficient warning signal as a larva, it is more likely to have white (vs. yellow) hind wings as an adult. This could indicate a possible tradeoff in pigment allocation between larval and adult colouration, or the genetic linkage between male colouration and larval colouration. Thus, selection pressures maintaining variation in the frequencies of larval signal sizes and their interaction with adult colouration could partly explain the occurrence of different colour morphs of males. Finally, I did not find any support for tradeoffs between allocation to warning colouration and allocation to chemical defence. Neither was there a positive interaction between these two defence traits. This suggests that in the aposematic *P. plantaginis* moth, the costs of maintaining chemical defence and producing a warning signal are separate, thus these traits could evolve independently.

Conspicuousness can also be constrained by physiological costs such as decreased immunity (V). When infected with the most virulent (ancestral) *S. marcescens* bacterial strain, *P. plantaginis* larvae with small orange warning signals (more melanic individuals) survived better than those with large signals (V). The higher amount of melanin in the body could give this advantage for individuals with a small warning signal, as higher cuticular melanin has shown to indicate better immunity in insects (Wilson et al. 2001, Cotter et al. 2004, Armitage & Siva-Jothy 2005). However, it may be that only more virulent pathogens constrain warning colouration: the large signal larvae infected with the least virulent *S. marcescens* strain tended to survive better than those infected with the ancestral strain. The survival of larvae from the small signal line was rather similar irrespective of the virulence of bacterial treatment. Thus, selection for the warning signal size in a host like *P. plantaginis* could also affect the fitness of the pathogens through the differential immune response of the host. This suggests that a pathogen's ability to cause infections does not only depend on its own past evolutionary history, but is also affected by the phenotypic and genetic background of its host. In addition, signal size did not affect the defence capacity against artificial parasites (nylon implants) mimicking natural parasitoids. This is consistent with other studies showing that the strength of encapsulation response does not necessarily correlate with resistance against pathogens (Cotter et al. 2004, Kocks et al. 2005, Rantala & Roff 2005). It seems that in *P. plantaginis*, allocation to an effective warning signal could be constrained by defence against pathogenic bacteria, but not by the defence against macroparasites. The result that allocation to efficient defence against bird predators (I) trades off with defence against pathogens instead of defence against macroparasites could indicate that pathogenic bacteria are a less important source of mortality for *P. plantaginis* larvae in the wild. Since conspicuousness is also likely to attract attention of macroparasites (Zuk et al. 2006) the encapsulation response could be a very important component of the immune system for warning coloured individuals.

4 CONCLUSIONS

Great efforts in the studies of warning colour evolution have been made to explain the initial evolution of aposematism. Consequently, predator psychology, and the efficacy of warning signals (signal quality) as an antipredator strategy have gained a lot of attention (recent review in Ruxton et al. 2004). At present, much is known about how predators select for certain signal traits such as conspicuousness, or the level of chemical defence. At the same time, a number of new questions have emerged (e.g. Endler & Mappes 2004, Mappes et al. 2005, Ojala et al. 2007, Sandre et al. 2007, Speed & Ruxton 2007); with the focus shifting to the costs and benefits of warning displays, and how variation in the cost:benefit ratio could explain the diversity in aposematic strategies observed in the wild. By studying the selective environment of prey animals as a whole, and acknowledging that aposematic individuals have to cope with a range of selection pressures that affect the optimisation of their defences (e.g. Grill & Moore 1998, Speed & Ruxton 2007, Ojala et al. 2007), we take a new perspective to the evolution of aposematism. This thesis provides further empirical evidence for these ecological and evolutionary tradeoffs associated with warning signal expression (I, II, III, IV and V).

The first part of the thesis demonstrates the classical tradeoff between detection risk and higher predator avoidance learning rate (I). Therefore, although warning coloured *P. plantaginis* larvae with both small and large signal size were protected against several predators (Table 1, I), the detection risk of prey with a large orange signal was higher than prey with a small orange signal (I). In contrast, when the prey items were encountered repeatedly, predators learned to avoid larvae with large signals faster than the larvae with small signals, giving the benefit to the more conspicuous prey type (I). In total, the survival of larvae with large signals was 12% higher, giving them a significant selective advantage. Therefore, depending on the predator community structure (e.g. number of inexperienced or bold predators) and seasonal and spatial variation within it (Mappes et al. 2005), the selection pressures on signal size may vary, relaxing selection for the larger signal size. Also, since hairiness only increased the survival of inconspicuous larvae, but

did not affect the defence of larvae with an orange patch (I), I conclude that multicomponent defences are therefore not necessarily additive. Thus, the variation in the warning colouration of aposematic animals may be partly explained by variation in the relative benefits of different components of a warning signal to different predators.

Results from the pigment analyses further strengthens the view that selection pressures other than predator-prey interactions (I) can have a strong impact on the warning display design (see also III, IV). Since both flavonoids and melanins deposited to warning colouration of *P. plantaginis* larvae are known to have important roles in ecological and physiological traits of insects (e.g. McGraw 2005). My results suggest two alternative routes to how pigmentation costs may emerge in *P. plantaginis* larvae. According to the 'costly flavonoid' hypothesis, the production costs of a large warning signal could arise via ecological constraints; variation in the quality (flavonoid concentration) and availability of a diet could constrain the amount of pigments sequestered (e.g. Burghardt et al. 2000) and reduce signal size. Alternatively, as flavonoids are known to have a role in free radical scavenging and immune defence (Simmonds 2003, McGraw 2005), allocation of flavonoid pigments to these physiological functions could trade off with colour expression and constrain the size and brightness of the orange signal. However, in light of the study by Ojala et al. (2007), the fitness costs associated with flavonoid pigmentation seem to be relatively low in comparison with production of black melanin based patterns; darker *P. plantaginis* larvae with higher amounts of melanin had lower survival and growth rates compared to larvae with a large orange pattern element, irrespective of the diet quality. This offers indirect evidence for the 'costly melanin' hypothesis i.e. the coverage of black hairs on the body could be more expensive to produce than the orange patch (see also Safranek & Riddiford 1975, Windig 1999, Talloen et al. 2004, Ma et al. 2008). This would further support the idea that melanin-based black colour patterns can be expensive to produce, even though in more challenging conditions where the dark colouration provides, for example, thermoregulatory benefit (III, see also Otaki 2007)), these costs can be balanced. The goal of future studies is to test condition dependence of pigment deposition in aposematic colour patterns by directly manipulating the pigment reservoirs such as flavonoid or nitrogen content (i.e. amino acid content) of a diet.

It has been shown that environmental constraints such as diet quality (Grill & Moore 1998, Ojala et al. 2007) and population densities (Sword 1999, 2002), in addition to predation, can affect warning signal expression. My results further suggest that allocation for effective thermoregulation (III) can constrain the conspicuousness of the warning signals, if thermoregulation selects for increased melanism in an aposematic insects. This tradeoff between signalling and non-signalling functions of colouration can be applied to maintenance of variation in cryptic colouration as well. Many cryptic species have dark melanised patterns (e.g. True 2003, Ruxton et al. 2004) and variation in their size may have effects on the protective value of the colour pattern if individuals with larger black pattern elements are more conspicuous.

The last part of the thesis deals with the ecological interactions between different trophic levels. I show that these interactions can create opposing selection pressures on fitness and defence traits in aposematic animals that could induce variation in these traits (IV, V). Paper V demonstrates that allocation to conspicuous warning signal trades off with effective defence against highly virulent pathogens. However, the magnitude of this tradeoff depends on the virulence of bacteria. Also, the tritrophic-interactions between host plant, prey and predator can affect warning colour expression (IV). The defence chemical content of the host plant can have several consequences on the fitness and defence traits of aposematic insects and therefore, variation in the host plant's toxin content can maintain signal variation directly (as shown in adult female colouration), but also indirectly through fitness costs and via more complex interactions between larval colouration, host plant toxicity and adult colouration. The results do not offer evidence for tradeoffs between warning colour production and chemical defence *per se*, although there was some evidence that increased chemical defence efficiency may trade off with the overall performance (IV).

To conclude, although aposematic individuals benefit from the conspicuousness of their warning signal to avoid predation (I), fitness costs associated with signal expression can constrain the efficacy of signalling. Variation in the costs and benefits associated with warning signalling could offer one explanation for the occurrence of weak and intermediate warning signals, and maintain the diversity in aposematic strategies observed in the wild. Therefore, it is important to study how different ecological interactions can shape the evolution of warning colouration. By studying the warning signal expression both from the predator and prey perspective, we can construct a more comprehensive, and also a more realistic view, about the selection pressures affecting the dynamics of warning colour expression. Simultaneously, we attain a better understanding of how natural selection affects the maintenance of phenotypic and genetic diversity in wild populations.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Vastakkaiset evolutiiviset valintapaineet ylläpitävät vaihtelua varoitussignaloinnissa

Aposemaattiset eläimet viestittävät saalistajille syömäkelvottomuudestaan (esimerkiksi piikeistä, myrkyllisyydestä tai pahanmakuisuudesta) varoitussignaaleilla, kuten huomiota herättävällä värityksellä. Saalistajat oppivat yhdistämään näyttävän varoitussignaalin saaliin epämiellyttävään ominaisuuteen eivätkä mielellään koeta pyydystää samannäköisiä saaliita enää tulevissa kohtaamisissa. Näin ollen varoitussignaalit hyödyttävät myös saalistajaa, joka signaalin opittuaan välttyy pyydystämiseen liittyvistä riskeistä (esimerkiksi haavoittuminen tai myrkytys) ja kustannuksista (esimerkiksi turha energian kulutus). Meille monelle tuttu esimerkki aposemaattisesta eläimestä ovat ampiaiset, joiden keltamusta väritys toimii varoitussignaalina niiden puolustusmekanisminaan käyttämästä kivuliaasta pistosta.

Saalistajan oppimistehokkuuden kannalta ja siten myös saaliin hengissä selviytymisen kannalta varoitussignaalien tulisi olla mahdollisimman samankaltaisia, koska saalistajille yhden signaalin oppiminen ja muistaminen on helpompaa kuin usean erilaisen. Samoin signaalin näkyvyyteen, kuten kirkkauteen tai kokoon, pitäisi kohdistua suuntaavaa valintaa, sillä mitä suurempi ja näkyvämpi signaali on, sitä tehokkaammin saalistajat oppivat, tunnistavat ja muistavat sen. Tämän pedon oppimistehokkuuden maksimoinnin vuoksi aposematismiteoria ennustaakin vähäistä vaihtelua varoitussignaaleissa.

Luonnossa puolustautumisen vahvuus ja varoitussignaalien näkyvyys kuitenkin usein vaihtelevat. Aposematismi on myös harvinaisempi puolustautumisstrategia verrattuna suojaväritykseen. Näin ollen on todennäköistä, että hyötyjen lisäksi aposemaattisesta puolustautumisesta on myös kustannuksia, jotka selittävät miksi aposematismi ei ole tämän yleisempää. Koska näkyvämmin värittyneet yksilöt ovat saalistajille helpompia havaita, esimerkiksi kokemattomat linnunpoikaset, jotka eivät ole vielä oppineet varoitussignaalin merkitystä, löytävät näkyvät saaliit helpommin kuin taustaan sulautuvat. Tämä näkyvyyskustannus voisi osittain selittää havaitun vaihtelun varoitussignaloinnin voimakkuudessa. Vaihtelua varoitussignaloinnissa voidaan selittää myös signaalin tuottamisen kustannuksilla. Näkyvän varoitussignaalin tuottaminen voi vaatia energiaa ja resursseja, joita ei voida siten hyödyntää muihin elintärkeisiin toimintoihin, kuten lisääntymiseen tai kasvuun. Nämä mahdolliset signaalin tuottamisesta johtuvat kustannukset voivat johtaa signaalin näkyvyyden ja elinkiertopiirteiden välillä kompromissiratkaisuihin, mikä ilmenee vaihteluna joko signaalin näkyvyydessä tai yksilöiden elinkiertopiirteissä.

Aposemaattisuuden evoluution tutkimuksen pääpaino on ollut vahvasti signaloinnin tehokkuudessa saalistusta vastaan eli siinä miten petojen aiheuttamat valintapaineet muokkaavat signaalien evoluutiota. Samaan aikaan sig-

naalissa esiintyvän vaihtelun tutkiminen ja sitä ylläpitävien mekanismien sel-
vittäminen saaliin näkökulmasta on jäänyt taka-alalle. Tämän vuoksi väitöskir-
jatutkimukseni lähtökohtana oli tutkia varoitussignaalin vaihtelua aiheuttavia
valintapaineita sekä saalistajan että saaliin näkökulmasta. Olen erityisesti pyr-
kinyt selvittämään signaalin näkyvyyteen, tuottamiseen ja myrkyllisyyden yl-
läpitoon liittyviä kustannuksia ja sitä kautta selittämään luonnossa puolustau-
tuvilla lajeilla havaittavaa vaihtelua. Tutkimuslajina olen käyttänyt aposemaat-
tista täpläsiilikästä (*Parasemia plantaginis*). Sen toukat ovat karvaisia (fysikaali-
nen puolustus) ja niissä on varoitussignaalina mustalla pohjalla oranssi täplä
(varoitusväri), jonka koossa on ympäristön ja perimän aiheuttamaa vaihtelua.
Toukkien tapaan myös aikuiset yksilöt ovat aposemaattisia ja niiden väritys
vaihtelee yksilöiden ja sukupuolten välillä.

Väitöskirjani tulosten mukaan varoitussignaalin näkyvyydestä on sekä
ekologisia että fysiologisia kustannuksia, jotka voivat ylläpitää vaihtelua va-
roitussignaalin näkyvyydessä. Vaikka pedot oppivatkin näkyvän signaalin no-
peammin (suuri oranssi täplä), näkyvä signaali lisää riskiä joutua kokematto-
mien tai rohkeiden petojen saaliiksi heikentäen valintaa näkyvyyttä kohtaan.
Näkyvä signaali (paljon oranssia, vähän mustaa) heikentää myös yksilön läm-
mönsäätelykykyä, koska tummien lämpösäteilyä tehokkaammin sitovien aluei-
den osuus värityksessä pienenee kirkkaiden värikuvioiden kasvaessa. Lisäksi
näkyvän signaalin tuottaminen voi heikentää puolustuskykyä taudinaiheuttajia
vastaan. Tämä selittynee melaniinipitoisten tummien alueiden pienenemisellä,
sillä melaniinin on aiemmin todettu lisäävän hyönteisten puolustuskykyä bak-
teereja ja loisia vastaan. Näin ollen kylmemmässä ympäristössä tai oloissa, jossa
bakteeri-infektion riski on suuri, tummemmat yksilöt (pieni oranssi täplä) pär-
jäävät paremmin, ja niiden osuuden populaatiossa voidaan olettaa kasvavan.
Väitöskirjani tulokset osoittavat myös, että ravinnon kemiallinen koostumus ja
sen saatavuus voi vaikuttaa aposemaattisen yksilön väritykseen, koska *P. plan-
taginis* toukat käyttävät oranssin varoitussignaalin tuottamiseen ravinnosta
saatavia flavonoideja. Myös ravinnon puolustuskemikaalien erityksestä ja kä-
sittelystä aiheutuvat kustannukset aikaansaavat vaihtelua varoitusvärityksessä
sekä vaikuttavat aposemaattisten yksilöiden lisääntymismenestykseen.

Väitöskirjani tulokset auttavat osaltaan rakentamaan entistä realistisem-
man teorian varoitusväreistä, jossa yhden valintapaineen sijaan pystytään tar-
kastelemaan useamman samaan aikaan toimivan valintatekijän vaikutuksia va-
roitussignaalin näkyvyyden ja koon vaihteluun. Vaihtelua synnyttäviä ja yllä-
pitäviä mekanismeja tutkimalla pystytään lisäksi paremmin kaventamaan kui-
lua aposematismin teorioiden ennusteiden (valinta signaalien yhdenmukai-
suutta ja näkyvyyttä kohtaan) ja luonnossa havaittavan todellisen vaihtelun vä-
lillä. Samalla tulokseni lisäävät tietoa mekanismeista, jotka ylläpitävät feno-
tyyppistä ja geneettistä monimuotoisuutta luonnossa, joka on perusedellytys
eliöiden sopeutumiselle ympäristö olosuhteiden muutoksiin.

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