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Developmental, morphological, and behavioural plasticity in the reproductive strategies of stink bugs and their egg parasitoids

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Résumé

L’environnement façonne la physiologie, la morphologie et le comportement des organismes par l’entremise de processus écologiques et évolutifs complexes et multidimensionnels. Le succès reproducteur des animaux est déterminé par la valeur adaptative d’un phénotype dans un environnement en modification constante selon une échelle temporelle d’une à plusieurs générations. De plus, les phénotypes sont façonnés par l’environnement, ce qui entraine des modifications adaptatives des stratégies de reproduction tout en imposant des contraintes. Dans cette thèse, considérant des punaises et leurs parasitoïdes comme organismes modèles, j’ai investigué comment plusieurs types de plasticité peuvent interagir pour influencer la valeur adaptative, et comment la plasticité des stratégies de reproduction répond à plusieurs composantes des changements environnementaux (qualité de l’hôte, radiation ultraviolette, température, invasion biologique). Premièrement, j’ai comparé la réponse comportementale et de traits d’histoire de vie à la variation de taille corporelle chez le parasitoïde *Telenomus podisi* Ashmead (Hymenoptera : Platygastridae), démontrant que les normes de réaction des comportements étaient plus souvent positives que celles des traits d’histoires de vie. Ensuite, j’ai démontré que la punaise prédatrice *Podisus maculiventris* Say (Hemiptera : Pentatomidae) peut contrôler la couleur de ses œufs, et que la pigmentation des œufs protège les embryons du rayonnement ultraviolet; une composante d’une stratégie complexe de ponte qui a évoluée en réponse à une multitude de facteurs environnementaux. Puis, j’ai testé comment le stress thermique affectait la dynamique de la mémoire du parasitoïde *Trissolcus basalis* (Wollaston) (Hymenoptera : Platygastridae) lors de l’apprentissage de la fiabilité des traces chimiques laissées par son hôte. Ces expériences ont révélé que des températures hautes et basses prévenaient l’oubli, affectant ainsi l’allocation du temps passé par les parasitoïdes dans des agrégats d’hôtes contenant des traces chimiques. J’ai aussi développé un cadre théorique général pour classifier les effets de la température sur l’ensemble des aspects comportementaux des ectothermes, distinguant les contraintes des adaptations. Finalement, j’ai testé l’habileté d’un parasitoïde indigène (*T. podisi*) à exploiter les œufs d’un nouveau ravageur invasif en agriculture, *Halyomorpha halys* Stål (Hemiptera : Pentatomidae). Les résultats ont montré que *T. podisi* attaque les œufs de *H. halys*, mais qu’il ne peut s’y développer, indiquant que le ravageur invasif s’avère un « piège évolutif » pour ce parasitoïde.

Cela pourrait indirectement bénéficier aux espèces indigènes de punaises en agissant comme un puits écologique de ressources (œufs) et de temps pour le parasitoïde. Ces résultats ont des implications importantes sur la réponse des insectes, incluant ceux impliqués dans les programmes de lutte biologique, face aux changements environnementaux.

Mots clés : Plasticité phénotypique, comportement animal, biologie de la reproduction, entomologie, Pentatomidae, Platygastridae

Abstract

The environment shapes the physiology, morphology, and behaviour of organisms through complex, multidimensional ecological and evolutionary processes. The reproductive success of individual animals is determined by how well their phenotype is suited to an environment that is constantly changing over single and multi-generational time scales. At the same time, phenotypes are shaped by the environment, which triggers adaptive modifications of animal reproductive strategies while also imposing important constraints. In this thesis, using stink bugs and their parasitoids as model organisms, I considered how several types of plasticity can interact to influence biological fitness, and how plasticity in reproductive strategies responds to several important components of environmental change (host quality, ultraviolet radiation, temperature, biological invasions). Firstly, I compared the response of behavioural and life history traits to body size variation in the parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae), finding that reaction norms of behavioural traits more often had positive slopes than life history traits. Next, I found that the predatory stink bug *Podisus maculiventris* Say (Hemiptera: Pentatomidae) can selectively control the colouration of its eggs. Egg pigmentation in this species protects embryos against ultraviolet radiation as part of a complex oviposition strategy that evolved in response to a suite of environmental factors. Then, I tested how thermal stress affects the memory dynamics of the parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae) learning the reliability of chemical traces left by its host. These experiments revealed that both high and low stressful temperatures prevented forgetting, affecting the time allocation of parasitoids on patches of host chemical traces. I also developed a general framework to classify temperature's effects on all aspects of ectotherm behaviour, distinguishing constraints from adaptive behavioural adjustments. Finally, I tested the ability of an indigenous parasitoid (*T. podisi*) to attack the eggs of a new invasive pest of agriculture, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae). The results showed that *T. podisi* attacks the eggs of *H. halys* but cannot develop, demonstrating that the invasive pest is an "evolutionary trap" for indigenous parasitoids, which could indirectly benefit native stink bug species by acting as an egg and time sink for the parasitoid. These findings have important implications for how insects, including those involved in biological control programs, respond to environmental change.

Key words**:** phenotypic plasticity, animal behaviour, reproductive biology, entomology, Pentatomidae, Platygastridae

Table of contents

List of tables

List of figures

List of symbols and abbreviations

AIC – Aikaike’s Information Criterion

ANOVA – Analysis of Variance

ASM – Anaesthesia-Sensitive Memory

CI – Confidence Interval

CNS – Central Nervous System

CPG – Central Pattern Generator

dd – degree days

df – degrees of freedom

DNA – Deoxyribonucleic acid

et al. – and colleagues

GLM – Generalized Linear Model

GLMM – Generalized Linear Mixed Model

HOL – Hymenoptera Online

ID – Identification

LRT – Likelihood Ratio Test

LSD – Least Significant Differences

LT – Leaf Top

LU – Leaf Underside

MTE – Metabolic Theory of Ecology

n – sample size

PCR – Polymerase Chain Reaction

PI –Pigmentation Index

PRT – Patch Residence Time

RH – Relative Humidity

RHT – Right Hind Tibia

SE – Standard Error

TRP – Transient Receptor Protein

UTD – Universal Temperature Dependence

UV – Ultraviolet

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Thesis overview

Chapter 1. This chapter, the General Introduction, gives the general context for the thesis research, and presents the main objectives.

Chapter 2. In order to provide the reader with the baseline information needed to appreciate the sections that follow, this chapter reviews the scientific concepts and literature relevant to the main themes covered in the thesis, and also presents the biological study system.

Chapter 3. Here, I list the contributions of myself and my co-authors to the scientific articles contained in the thesis.

Chapter 4. The first scientific article, entitled "Size-induced phenotypic reaction norms in a parasitoid wasp: an examination of life history and behavioural traits". In this study I characterize developmental and behavioural plasticity in an egg parasitoid in response to body size variation.

Chapter 5. The second scientific article, entitled "An insect with selective control of egg colouration". Here, I describe a novel example of within-individual morphological plasticity in the egg laying strategy of a predatory stink bug.

Chapter 6. The third scientific article, entitled "A scenario for the evolution of selective egg colouration". In this study, I considered how morphological and behavioural plasticity could have co-evolved in response to biotic and abiotic environmental factors, to give rise to the adaptation discovered in the previous chapter.

Chapter 7. The fourth scientific article, entitled "Thermal stress affects patch time allocation by preventing forgetting in a parasitoid wasp". In this chapter, I consider how behavioural plasticity (learning and memory) of a parasitoid is affected by ecologically realistic temperature stress.

Chapter 8. In this fifth scientific article, entitled “The effects of temperature on ectotherm behaviour”, I synthesize existing literature on behavioural plasticity of animals in response to temperature, construct a framework to classify these responses, and make suggestions to direct future research.

Chapter 9. The sixth scientific article, entitled “An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid”. In this chapter, I describe how a biological invasion could render a parasitoid’s normally adaptive behavioural plasticity maladaptive.

Chapter 10. This chapter, the General Discussion, summarizes the main results of the thesis, comments on their general implications, and suggests directions for future research.

Chapter 1: General Introduction

Phenotypic plasticity is a concept encompassing the dependence of organisms' physiology, behaviour, and morphology on their biotic and abiotic environment (West-Eberhard 1989). Phenotypic plasticity can shield organisms from selective pressures, slowing rates of evolutionary change, but can also expose them to novel environments that encourage the evolution of new, genetically-determined traits (Huey 2003; Price et al. 2003). However, the phenotypic plasticity of animal traits is not always an adaptive response: constraints play a major role in shaping phenotypes, especially in a world in flux where, for example, biological invasions are becoming more commonplace (Simberloff et al. 2013) and the climate is changing (Stocker et al. 2013). Investigations of adaptive components of phenotypic plasticity as well as the influence of potentially maladaptive constraints are important to understand how individuals and ecosystems respond to environmental change, and the mechanisms underlying animal behaviour and ecology. However, it is relatively uncommon for studies of phenotypic plasticity to explicitly consider both adaptive and constraining components of multiple forms of plasticity, and how they interact to shape animal reproductive strategies (but see Berger et al. 2012).

In this thesis, I will present several novel aspects of plasticity involved in the reproductive strategies of stink bugs (Hemiptera: Pentatomidae) and their egg parasitoids (Hymenoptera: Platygastridae). These insects include important pests, as well as beneficial biological control agents, in natural and agricultural ecosystems worldwide. I focus on adaptive responses and constraints associated with developmental resource availability, temperature, egg colouration, and new host/prey associations resulting from a biological invasion. I adopted a combination of behavioural and evolutionary ecology approaches, both in the lab and field, using several different biological systems. My general objectives were to:

(1) Test the effects of body size on both life history and behavioural reaction norms of egg parasitoids, and how they would collectively contribute to shaping the relative fitness of different-sized individuals.

(2) Investigate the possibility of within-individual selective plasticity in egg colouration of a predatory stink bug, and the ecological factors that could have spurred its evolution.

(3) Investigate the effect of realistic temperature stress on the learning and memory (behavioural plasticity) of egg parasitoids learning the reliability of chemical cues left by their hosts.
(4) Develop a unified framework for the effects of temperature on all facets of ectotherm behavioural plasticity, distinguishing constraints from adaptive behavioural adjustments to temperature.
(5) Evaluate the behavioural capacity of a parasitoid native to North America to accept the eggs of an invasive species, and predict the consequences for invaded ecosystems.

Detailed hypotheses and predictions relating to each of the objectives above are outlined in subsequent chapters.

Chapter 2: Literature Review

2.1 Phenotypic plasticity

Definition and role in evolution

A single genotype can produce a range of phenotypes, depending on environmental context. This environmental dependency of phenotype, whether expressed as variation in biochemistry, physiology, morphology, life history, or behaviour, is collectively termed phenotypic plasticity (West-Eberhard 1989; Whitman and Agrawal 2009). Phenotypic plasticity includes irreversible phenotypic variation resulting from the effect of environmental conditions on development (e.g., developmental plasticity), as well as reversible, short-term phenotypic shifts in real time (e.g., behavioural plasticity). The dependence of phenotype on environment for a given genotype is modeled with "reaction norms", which describe the response of discrete or continuous character states to either discrete or continuous environmental variables (Roff 1992; Via et al. 1995) (Figure 2-1).

Environmental variation causing single genotypes to produce multiple phenotypes was historically considered to be due to "unfortunate defects in the delicate genetic apparatus" (reviewed by West-Eberhard 1989), however it is now clear that phenotypic plasticity plays a major, even dominant, role in ecology and evolution. Indeed, natural selection acts on the genotype via the phenotype, and environmentally-induced phenotypic variation provides a greater variety of phenotypes through which selection can act on underlying genetic structures. Thus, the inclusion of phenotypic plasticity in evolutionary thinking creates an even more important role for the biotic and abiotic environment: the environment not only selects among different genotypes via their associated phenotypes, but it also *creates* much of the phenotypic variation needed for selection to occur (West-Eberhard 1989; Whitman and Agrawal 2009). Plasticity can allow organisms to persist in changing and heterogeneous environments, thus facilitating the evolution of genetically determined traits (Price et al. 2003; Zuk et al. 2014). Conversely, plasticity can function to shield genotypes from selection and slow the pace of evolution (Huey et al. 2003). Although there has been long debate over how much of phenotypic plasticity is adaptive, as well as if and how plasticity itself is the target of natural selection (Via et al. 1995; Pigliucci 1996), it is now generally accepted that, for a given environmental influence, the resulting phenotypic plasticity comprises both active (controlled by the organism, adaptive) and passive (environmental constraints, sometimes maladaptive) components (Whitman and Agrawal 2009). Furthermore, phenotypic plasticity can be costly (e.g., in terms of trait maintenance or production), and so it should only be maintained under certain conditions when the benefits outweigh these costs; for example, when the environment is sufficiently variable and this variability is sufficiently predictable (DeWitt et al. 1998). When studying the effect of abiotic and biotic environmental factors on organismal phenotypes, separating adaptive components from the effects of constraints, and taking potential costs into account, are key challenges for behavioural and evolutionary ecologists.

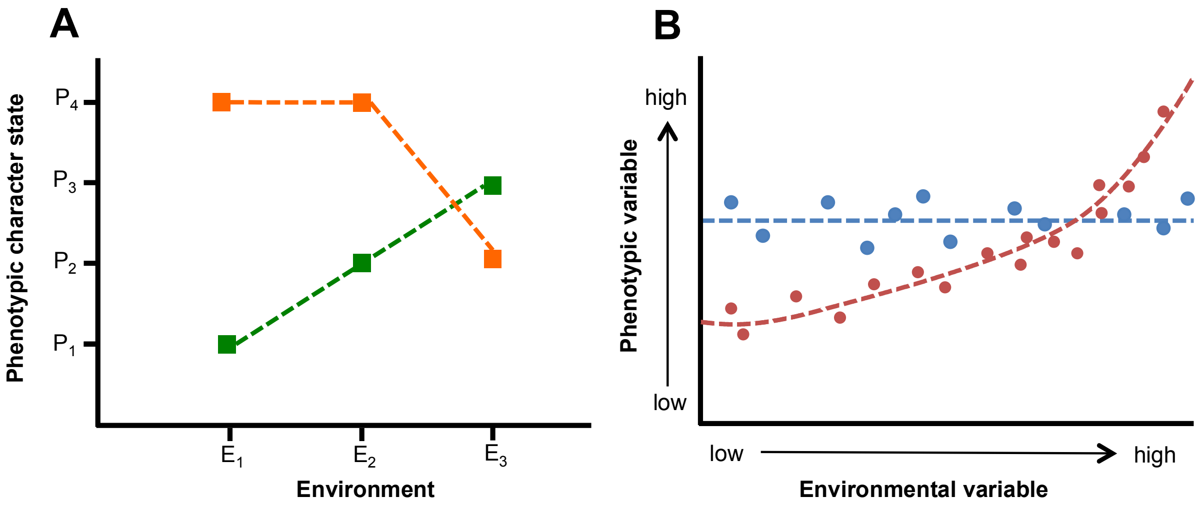

Figure 2-1. Examples of phenotypic reaction norms for discrete and continuous phenotypic traits. (A) The response of discrete character states of two genotypes to three different discrete environments. (B) The response of a continuous phenotypic trait to a continuous environmental variable by two genotypes. There is no phenotypic response of the "blue" genotype to the environment; i.e., it is "canalized". Based on figures in Via et al. (1995).

Developmental plasticity

For organisms with determinate growth trajectories (e.g., most insects), the environmental conditions experienced during development can irreversibly influence the phenotype (e.g., morphology, behaviour, physiology, sex) of the reproductively mature individual. The environmental conditions inducing these phenotypic changes could include abiotic (e.g. temperature, exposure to toxins) and/or biotic (e.g. food intake, competition) factors (Whitman and Agrawal 2009). All of these effects are grouped under the term “developmental plasticity”. Smith-Gill (1983) distinguished two general classes of developmental plasticity: developmental conversion and phenotypic modulation. Developmental conversion encompasses changes in developmental programming that (i) have a specific genetic basis, (ii) respond to a specific set of environmental stimuli, and (iii) produce discrete phenotypes or states (e.g., Figure 2-2A). Phenotypic modulations, on the other hand, represent nonspecific (typically continuous) phenotypic variation due to physiological constraints of the environment acting on “developmental rates or degrees of expression” (Smith-Gill 1983) (e.g., Figure 2-2B). Developmental conversions are invariably adaptive (relative to a passive response), since they require coadaptation of sensory and developmental physiology (Smith-Gill 1983). Phenotypic modulations, although probably more common, are not necessarily adaptive, although they can be subject to selection that alters the environmental sensitivity of developmental pathways. Adaptive developmental plasticity is most likely to evolve when environmental conditions during development are predictive of the selective regime that reproductively mature individuals will be faced with (in the case of developmental conversions), or when a completely passive response to phenotypic modulations would seriously affect reproductive fitness (in the case of phenotypic modulations) (Smith-Gill 1983; West-Eberhard 1989).

Morphological plasticity

The morphological phenotype (e.g., colouration, shape, structure) of organisms is subject to both developmental and within-life stage plasticity. There are many examples of developmental conditions triggering irreversible, alternative adult phenotypes (polyphenisms), with classic examples including different body forms of *Nemoria arizonaria* Grote (Lepidoptera: Geometridae) caterpillars feeding on different plants (Greene 1989), horn size in *Onthophagus* dung beetles that vary bimodally with body size (Emlen 1994; Figure 2-2A), and seasonal colour morphs of *Precis octavia* (Cramer) (Lepidoptera: Nymphalidae) butterflies that are triggered by photoperiod and temperature (Rountree and Nijhout 1995). Continuous, irreversible adult size variation in many species of ectotherms can be produced by different developmental temperatures, according to the “temperature-size rule” (Atkinson 1994). There are also many cases of reversible morphological plasticity within life stages, including colour changes on short timescales in taxa such as chameleons, octopuses, parrotfishes, cuttlefishes (Figure 2-3A), and grasshoppers, with functions including camouflage, communication, and thermoregulation (Stuart-Fox and Moussalli 2009).

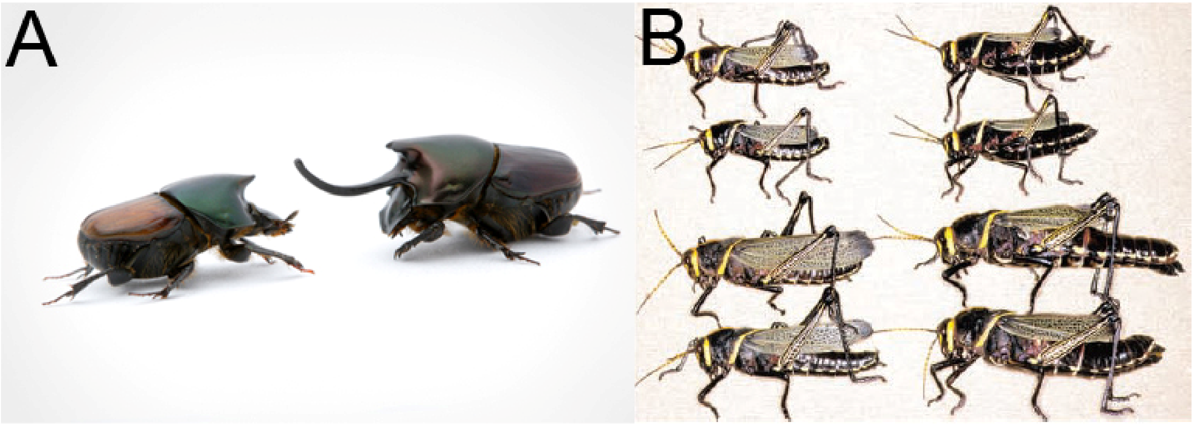

Figure 2-2. Examples of developmental plasticity. (A) Small- and large-horned *Onthophagus* dung beetles, an adaptive polyphenism triggered by developmental resource availability (Photo: Alex Wild, used with permission); (B) Body size variation in *Taeniopoda* grasshoppers in response to rainfall and vegetation abundance (Photo: Douglas Whitman; from Whitman and Agrawal 2009).

Phenotypic plasticity of reproductive adults can influence the fitness of their offspring. This is well illustrated by the intra-specific variation sometimes observed in animal egg colouration and patterning, which can have consequences for camouflage, thermoregulation, brood parasitism, and sexual selection (reviewed in Kilner 2006; Stevens 2015). For example, there are polyphenisms in several species of birds wherein different members of the same population laying different colours of eggs (Kilner 2006). These adaptive polyphenisms function, for example, to allow brood parasites to exploit several different host species (Figure 2-3B) or allow ground-nesting birds to exploit a variety of nesting surfaces while maintaining background matching for camouflage (Lovell et al. 2013). Within individual animals, variation in the colouration of the eggs they lay is typically due to constraints, with egg pigmentation co-varying with age or nutritional stress (Wickman and Karlsson 1987; Siefferman et al. 2006). These constraint-induced variations in egg colour could act as sexual signals (Moreno et al. 2003) or honest indicators of egg toxicity to predators (Winters et al. 2014). To date, however, there are no examples of within-individual selective plasticity in egg pigmentation, where egg colour determination occurs adaptively in real time in response to current environmental conditions. This implies that responses of egg pigmentation to changing environmental conditions occur mostly over multigenerational timescales, possibly narrowing the range of environments available to deposit eggs for individual animals.

Figure 2-3. Examples of morphological plasticity. (A) Active colour changes by cuttlefish (Mollusca: Sepiida) have camouflage and signaling functions (Photo: Nick Hobgood, licensed under Creative Commons); (B) The eggs of seven different species of British birds (top row) and the associated morph (polyphenism) of the common cuckoo, a brood parasite (bottom row) [Photos: Mary Caswell Stoddard; modified from Stoddard and Stevens (2011)].

Behavioural plasticity

Behaviour can be defined as the coordinated actions or inaction of an organism in response to its external and/or internal environment (*sensu* Levitis et al. 2009). Here, "behavioural plasticity" will be used interchangeably with "behaviour", to distinguish it from other forms of plasticity. Behavioural plasticity acts as the interface between organisms and their environment, mediating how they "…determine where to live and reproduce, which resources to use, which mates to select, and how to respond to competitors and predators" (Duckworth 2009). Behaviour is considered to be among the most labile of organismal attributes – in general contrast to developmental or morphological phenotypes, behavioural phenotypes can react to environmental variation in real-time (West-Eberhard 1989; Duckworth 2009). Learning and memory add an additional layer to the flexible nature of behaviour, since they allow organisms to modify their behavioural phenotype based on past experience (Dukas 2013). The facts that behaviour (i) is so reactive to the environment and (ii) can actually change an organism's environment (and thus what selective pressures it is exposed to), have led to the inevitable conclusion that behaviour plays a pivotal role in evolution (Wyles 1983; Duckworth 2009; Zuk et al. 2014).

One view of behaviour's role in evolution is that it buffers organisms against environmental heterogeneity by allowing them to move to favourable environments, thus slowing the pace of evolutionary change ("behavioural inertia"; Huey et al. 2003). The classic case of behavioural inertia is behavioural thermoregulation. Behavioural thermoregulation permits ectotherm animals (which typically cannot internally regulate their body temperature) to maintain a relatively stable body temperature by moving throughout thermally heterogeneous environments, thus reducing the selective pressure on thermal physiology (Bogert 1949). The concept of "behavioural drive", in contrast, espouses the capacity of behaviour to act as an instigator for the evolution of genetically-determined traits, by exposing organisms to new environments or changing the way that they interact with their current environment (Losos et al. 2004; Duckworth 2009; Zuk et al. 2014). Good evidence of behavioural drive has been found in *Anolis* lizards whose anti-predator behaviour led to changes in limb length (Losos et al. 2004) and field crickets whose pre-existing behavioural response to environments with reduced acoustic signaling (non-calling males increasing their sensitivity to calling males songs and adopting a "satellite male" strategy) may have led to the spread and maintenance of a wingless, non-calling mutant male genotype (reviewed in Zuk et al. 2014). Sol et al. (2005) found that taxa of passerine birds with larger brains (relative to body size), a trait associated with greater behavioural plasticity, show increased levels of evolutionary diversification. Examples of behavioural drive that include plausible mechanistic explanations, however, are still relatively rare in the literature.

Like all forms of plasticity, behaviour is also affected by constraints: it would be naïve to assume that every behavioural change observed in response to the environment is an adaptive response that increases fitness. The physiological processes underlying behaviours are susceptible to factors such as temperature (Hance et al. 2007) and environmental contaminants (Montiglio and Royauté 2014), which can produce maladaptive behavioural phenotypes. Learning and memory are also sensitive to disruption by various forms of environmental stress, including temperature, crowding, predation risk, and calcium levels (Xia et al. 1998; Lukowiak et al. 2010; Teskey et al. 2012). Biotic environments also change frequently, and adaptive behaviours that mediate trophic interactions, for example, may become maladaptive when a new interacting species is introduced to the system (Schlaepfer et al. 2005). In the next two sections, I will focus on how behavioural plasticity interacts with two components of environmental change – temperature and biological invasions.

2.2 The role of behavioural plasticity in thermal adaptation

The relationship between temperature and behaviour typifies the reciprocal dynamic between phenotypic plasticity and environmental factors – temperature influences behaviour (e.g., via metabolic effects), and behaviour can influence the temperature experienced by an organism (via behavioural thermoregulation). Temperature has constraining effects on the incidence and rate at which behaviours are performed, as a consequence of its accelerating (increasing temperature) or decelerating (decreasing temperature) influence on metabolic rate (Angilletta 2009; Dell et al. 2011). Temperature can also influence plastic "decision-making" behaviour, both directly (through sensory perception of temperature changes) and indirectly (through its constraining effects on behaviourally relevant aspects of physiological state) (Chapter 8 of this thesis). Aside from thermoregulatory behaviours, behavioural ecologists still have a relatively poor grasp of how temperature influences adaptive decision-making. However, a few key findings from reptiles and fishes suggest that this question is certainly worth exploring further in other groups of ectotherms. For example, the antipredator response of lizards switches from fleeing at high temperatures to aggression (biting) at low temperature, presumably because running performance is very sensitive to temperature whereas bite strength is not (Hertz et al. 1982; Herrel et al. 2007). A recent study showed that the attack rates of pike (*Esox lucius*) on its prey (*Salmo trutta*) dropped sharply below a threshold temperature of 11°C, even though the predator was able to swim just as fast as their prey at the lower temperatures. This result suggested that the predators were "aware" of some kind of temperature-induced sensory constraint or that their "motivation" to attack was temperature-dependent (Ohlund et al. 2015).

In both of these examples, the relative role of direct temperature perception and the influence of thermal constraints remains somewhat unclear – teasing out these two components of behavioural responses to temperature is an important target for future investigations that will be further elaborated in this thesis.

2.3 The role of behavioural plasticity in species interactions and ecology

The ecological significance of behavioural plasticity

Behavioural plasticity mediates how species interact with each other and their environment, with a host of ecological consequences (Agrawal 2001; Miner et al. 2005). For example, behavioural plasticity has been implicated in promoting the coexistence of prey species with a shared natural enemy (Ishii and Shimada 2012), mediating indirect non-consumptive ecological effects (Werner and Peacor 2003), stabilizing population dynamics (reviewed in Miner et al. 2005), and allowing organisms to "construct" ecological niches (Odling-Smee et al. 1996). Here, I will briefly discuss the role of behavioural plasticity in determining the response of communities to new biotic interactions brought on by biological invasions.

Case study: biological invasions and evolutionary traps

Although the geographic redistribution of species is not new, the relatively recent expansion of human activity across the globe has resulted in an unprecedented shuffling of species among biotic communities over vast distances. These so-called "biological invasions" have resulted in interactions between species with no recent shared evolutionary history, with a wide variety of ecological consequences (Mooney and Cleland 2001; Strauss et al. 2006). Existing behavioural plasticity in members of invaded communities can allow the native species to respond adaptively to these new interactions; for example, by using existing anti-predator responses against an invasive predator, or employing pre-existing behavioural plasticity to avoid consuming an invasive toxic prey species (reviewed by Berthon 2015). In some cases, native consumers may be able to benefit from a biological invasion by adopting the new invader into their resource repertoire (Carroll et al. 1998). However, behavioural responses that are normally adaptive for the native species can be rendered maladaptive if the cues used to initiate the behaviour are unreliable in the context of the interaction with the new, invasive species. In this case, the invasive species acts as an "evolutionary trap" for native species (Schlaepfer et al. 2002; Schlaepfer et al. 2005). For example, the cane toad (*Bufo marinus*) was introduced to Australia and subsequently adopted into the prey repertoire of native predators. However, cane toads are toxic when ingested and caused sickness, death, and population declines in several species of Australian lizards, snakes, and marsupials that were unable to detect the toad's toxicity because of their lack of shared evolutionary history (Schlaepfer et al. 2005; Shine 2010). Interestingly, some species of Australian predators appear to be escaping from this evolutionary trap through either rapid physiological/morphological adaptation (Phillips and Shine 2004; Phillips and Shine 2006), or behavioural plasticity, including learning (Greenlees et al. 2010; O'Donnell et al. 2010). Thus, behavioural plasticity can lead organisms into evolutionary traps, as well as to help them escape.

2.4 Study system

Stink bugs

Stink bugs are heteropteran insects in the family Pentatomidae with piercing-sucking mouth parts, a broad, shield-shaped body, hemelytral forewings, and membranous hindwings (Figure 2-4). The common name "stink bug" refers to their capacity to release a foul-smelling secretion if disturbed, that can serve as an alarm pheromone (Lockwood and Story 1987) and/or a defense to deter predators (Krall et al. 1999). Stink bugs have a hemimetabolous life cycle, including an egg, nymph, and adult stage. Eggs are typically laid in tight masses on plant leaves or stems, with the number of eggs in each mass varying within and among species. Stink bugs have a wide variety of lifestyles, ranging from plant feeding (phytophagous) to primarily predatory (zoophytophagous) species (reviewed in McPherson 1982; McPherson and McPherson 2000).

Phytophagous stink bugs tend to be generalists, with several species known to feed on many different plant species, and move between habitats as the growing season progresses (e.g., Todd 1989). It is also common for several different species to co-occur on the same host plants or within the same habitat. Several phytophagous species are important agricultural pests; for example the southern green stink bug, *Nezara viridula* (L.) (Figure 2-4A) and the brown marmorated stink bug, *Halyomorpha halys* Stål (Figure 2-4B). Others are occasional/minor pests (e.g., *Euschistus servus*; Figure 2-4C) or are considered economically unimportant (e.g. *Cosmopepla linteriana*, Figure 2-4D).

Predatory stink bugs (subfamily Asopinae) also tend to be generalists, preying on several life stages of many insect species in a wide range of habitats, both natural and agricultural. Both nymph and adult stages are predatory. A few species are important biological control agents against lepidopteran and coleopteran pests in agroecosystems. For example, *Podisus maculiventris* Say (Figure 2-4E) is mass-produced by the biological control industry for field and greenhouse applications. Because of its economic importance, the physiology, behaviour, and ecology of *P. maculiventris* have been the subject of extensive study (e.g., Evans 1982; Ruberson et al. 1986; Wiedenmann and O'Neil 1990; Baek et al. 2014).

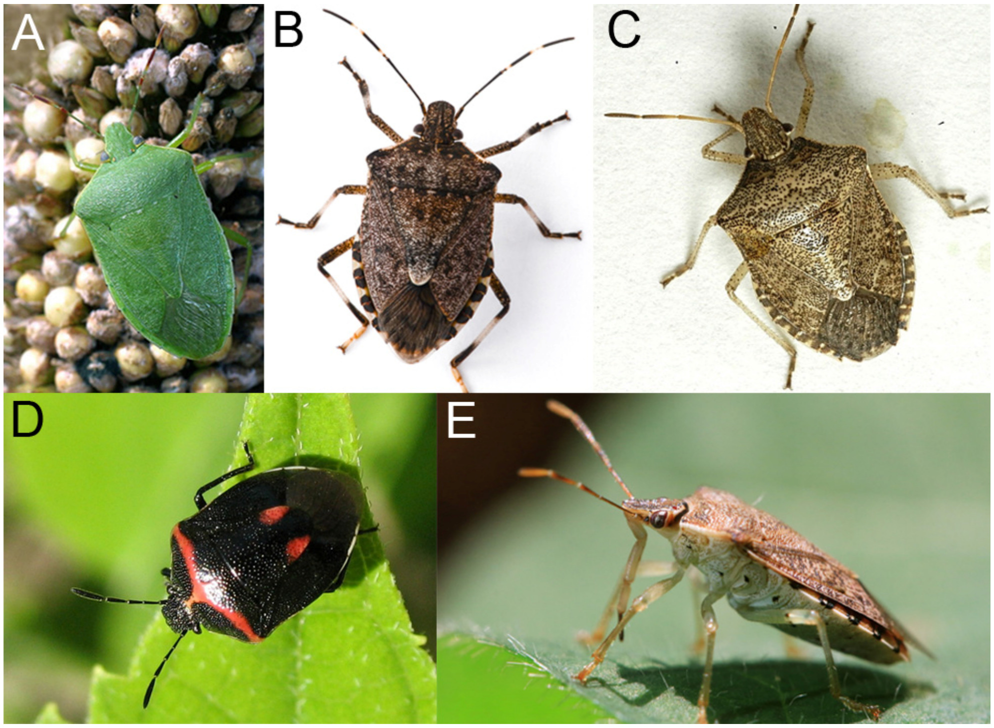

Figure 2-4. Stink bugs (Hemiptera: Pentatomidae) studied in this thesis. (A) *Nezara viridula*, the southern green stink bug (Photo: Russ Otters, licensed under Creative Commons); (B) *Halyomorpha halys*, the brown marmorated stink bug (Photo: Tim Haye, used with permission); (C) *Euschistus servus*, the brown stink bug (photo: Herb Pilcher, licensed under Creative Commons) (D) *Cosmopepla lintneriana*, the twice-stabbed stink bug (Photo: Gilles Gonthier, licensed under Creative Commons); (E) *Podisus maculiventris*, the spined soldier bug (Photo: Leslie Abram, used with permission).

Hymenopteran egg parasitoids

Hymenopteran egg parasitoids lay their eggs inside the eggs of other insects which contain the entirety of the developmental resources available to parasitoid offspring. The parasitoid offspring's development invariably coincides with the host's death. The adult females of these small (typically ~0.5-2 mm long), often short-lived wasps have evolved a variety of sophisticated strategies to find and exploit their hosts (reviewed in Fatourous et al. 2008; Vinson 2010). Many species, notably in the families Trichogrammatidae and Platygastridae, are used in biological control programmes against important insect pests of agriculture (Orr 1988; Wajnberg and Hassan 1994). Egg parasitoids are also important model organisms in behavioural and evolutionary ecology, since plasticity in their reproductive and life history traits (e.g. fecundity, offspring sex ratios, longevity, dispersal ability) due to variation in ecological factors (e.g. temperature, host quality) can be easily linked to differences in realized fitness gains by testing how they affect the ability of adults to locate and parasitize hosts (Boivin 2010).

Scelionid parasitoids of stink bug eggs

The most common parasitoids attacking stink bugs are egg parasitoids in the family Platygastridae (formerly Scelionidae, known colloquially as "scelionid parasitoids") (McPherson 1982; Austin et al. 2005; Figure 2-5). In order to locate host eggs, these parasitoids utilize cues such as substrate-borne host cuticular kairomones, volatile synomones released by plants following host feeding or oviposition, or "eavesdropping" on host sexual pheromones and acoustic signaling (reviewed by Conti and Colazza 2012). At the host acceptance phase, the most important cues are the shape of the host egg and the adhesive substance used by host females to fix their eggs to the laying substrate. Female scelionids aggressively defend their developing offspring from intruding conspecific or heterospecific females and sometimes remain on the host patch for hours after it is completely parasitized (Waage 1982; Field 1998). Although host eggs can be parasitized multiple times by conspecific or heterospecific females, it is typical for only one offspring to develop per host egg. As in many parasitoids of aggregated, immobile hosts, sex ratios tend to be heavily biased towards females. Upon offspring emergence from the host patch, males compete for possession of the patch and the opportunity to mate with subsequently emerging females (Waage 1982). In contrast to several other groups of egg parasitoids, scelionids are relatively long-lived (up to several months) in the laboratory (Yeargan 1982; Arakawa et al. 2004). Females tend to emerge with a large proportion of their lifetime egg production already mature (Yeargan 1982; Arakawa et al. 2004), although some geographic strains of certain species mature nearly all of their lifetime egg production after having emerged (Zhou et al. 2014).

The two species of scelionid parasitoids studied in this thesis are *Telenomus podisi* Ashmead (Figure 2-5A) and *Trissolcus basalis* (Wollaston) (Figure 2-5B). Both species have relatively broad host ranges that are mostly limited to stink bugs (HOL 2015; Salerno 2000). *Telenomus podisi* is one of the most common parasitoid of several species of stink bugs in North America (Yeargan 1979; McPherson 1982; Orr et al. 1986; Koppel et al. 2009). *Trissolcus basalis* is distributed across many areas of the world (e.g., North and South America, Europe, Africa, Australia) and is most closely associated with *N. viridula* throughout its range (Jones 1988). Both *T. basalis* and *T. podisi* have been reported to attack both predatory and phytophagous stink bug species (HOL 2015).

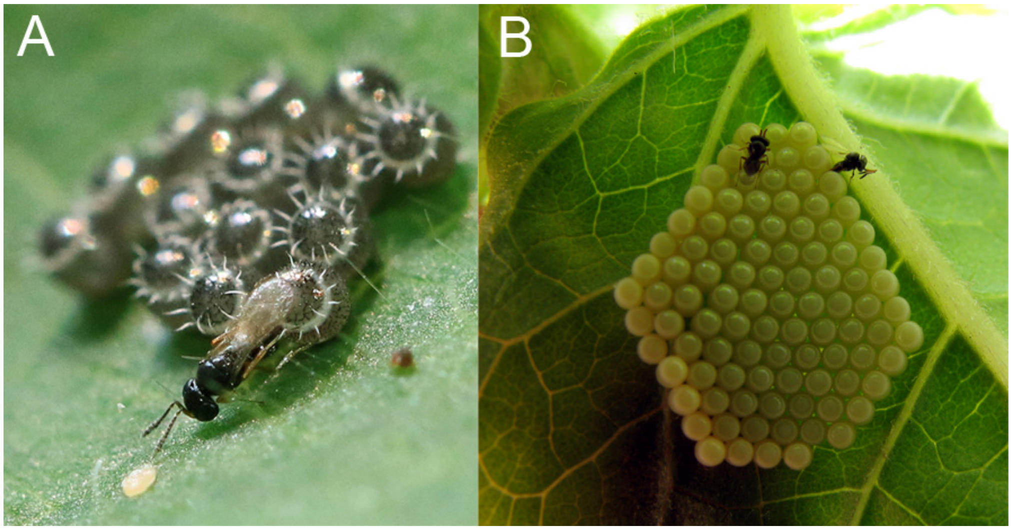

Figure 2-5. The scelionid (Hymenoptera: Platygastridae) parasitoids of stink bug eggs studied in this thesis. (A) *Telenomus podisi* parasitizing *Podisus maculiventris* eggs (Photo: Leslie Abram, used with permission). (B) Two *Trissolcus basalis* females exploiting the same *Nezara viridula* egg mass (Photo: Antonino Cusumano, used with permisson).

Chapter 3: Contribution of the author and co-authors

I originally presented the idea of studying the behavioural and evolutionary ecology of stink bugs and their parasitoids to my co-directors, Dr. Jacques Brodeur and Dr. Guy Boivin, in 2012 when we were discussing potential ideas for my Ph.D. research. Although the general ideas behind each of the subprojects was originally conceived by me with help from my co-supervisors, they grew in to collaborations with researchers at other universities and research institutes, postdoctoral fellows, fellow graduate students, and undergraduate students. These collaborators made vital contributions to each of the articles in this thesis and increased their comprehensiveness, scope, and quality far beyond what I would have been able to accomplish myself.

Being the first author of five of these articles and co-first author on one of them, I took a leading role in conceptualization, experimental design, data collection, statistical analyses, writing, revisions, and responding to peer review (in the case of already published works).

In every case, my co-supervisors Dr. Jacques Brodeur and Dr. Guy Boivin played key roles in conceptualizing ideas, designing experiments, interpreting results, revising manuscripts, and responding to reviewers, as well as providing infrastructure, technical help, and financial support to myself and my student co-authors. Accordingly, they are co-authors on every article.

Below I will outline how each of my other co-authors contributed to each article.

Chapter 4. Abram PK, Parent J-P, Brodeur J, and Boivin G (in press) Size-induced phenotypic reaction norms in a parasitoid wasp: an examination of life history and behavioural traits. Biological Journal of the Linnaean Society doi: 10.1111/bij.12658

Jean-Philippe Parent, a fellow Ph.D. student in the Boivin/Brodeur lab, performed a subset of the experiments and programmed the automated video analysis that was necessary to analyze some of the results. He also contributed to data analysis and presentation, some manuscript writing, and revisions both before and after peer review.

Chapter 5. Abram PK, Guerra-Grenier E, Després-Einspenner M-L, Ito S, Wakamatsu K, Boivin G, and Brodeur J (2015) An insect with selective control of egg coloration. Current Biology 25:2007-2011

Eric Guerra-Grenier and Marie-Lyne Després Einspenner, two exceptional undergraduate summer students, contributed largely to this project. Eric took the lead in designing and performing two of the major experiments in the paper, was integral in initiating the international collaboration that resulted in the biochemical analyses, and also made major conceptual contributions. Marie-Lyne helped design one of the major experiments in the paper, carried out nearly all of the experimental work for this experiment, and took the lead on developing the initial methodology to indirectly quantify egg pigmentation using digital photography. Dr. Shosuke Ito and Dr. Kazumasa Wakamatsu (Fujita Health University, Japan), the world's leading experts on melanin biochemistry, performed the biochemical analyses contained in this article and provided the interpretation of its significance.

Chapter 6. Abram PK*, Torres-Campos I*, Guerra-Grenier E, Boivin G, and Brodeur J (in review) A scenario for the evolution of selective egg colouration. Submitted to: Behavioral Ecology (*=co-first authorship)

Inma Torres-Campos (Ph.D. student, Instituto de Hortofruticultura Subtropical y Mediterránea, Spain) did a four-month research internship at our laboratory in 2014. She performed nearly all of the experimental work for the field study component of the paper, and contributed integrally to its planning and conceptualization. Inma also contributed to the statistical analyses for the field experiments and co-wrote the first draft of the manuscript with myself. This was a truly collaborative undertaking between the two of us, and I believe that this is one of the rare cases where co-first authorship status is really merited. Eric Guerra-Grenier contributed largely to the conceptualization, experimental design, and data collection for the two laboratory experiments included in the article. Despite my lack of sole-first author status, I think the inclusion of this article in my thesis is justifiable for two reasons: (i) It is strongly linked to the previous chapter of the thesis and (ii) because of the multiple experiments and enormous amount of data collected for this work, the effort I contributed was equivalent to or greater than any of the other papers in this thesis.

Chapter 7: Abram PK, Cusumano A, Peri E, Brodeur J, Boivin G, and Colazza S (2015) Thermal stress affects patch time allocation by preventing forgetting in a parasitoid wasp. Behavioral Ecology 26:1326-1334.

This article was the product of a four-month research internship that I did at the University of Palermo, Italy, during the fall of 2014. Dr. Antonino Cusumano, a postdoctoral fellow at the University of Palermo, trained me in the specialized experimental methods I needed to know, helped me design the experiments, engaged me in discussions that were key to interpreting and analyzing results, and contributed important article revisions. Dr. Ezio Peri (Researcher) and Dr. Stefano Colazza (Full Professor) provided infrastructure, supervision, and financial support for the research, and also contributed important ideas and article revisions throughout the process.

Chapter 8: Abram PK, Boivin G, Moiroux J, and Brodeur J (2015) The effects of temperature on ectotherm behaviour. In preparation for submission to: Biological Reviews.

Dr. Jacques Brodeur had the original idea to write a review article about the effects of temperature on insect behaviour. Myself and Dr. Joffrey Moiroux, a postdoctoral fellow in our lab, set about compiling a database of relevant studies, and identifying patterns in their results. Dr. Moiroux also prepared some of the tables at the end of the manuscript, and contributed considerable revisions to the first draft.

Chapter 9: Abram PK, Gariépy TD, Boivin G, and Brodeur J (2014) An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. Biological Invasions 16:1387-1395

This article resulted from a research visit I made to the laboratory of Dr. Tara Gariépy (Agriculture and Agri-Food Canada, London, Ontario) in 2013. Dr. Gariépy provided invaluable guidance during the design and data collection phases the experiment, as well as infrastructure, biological material, and technical support. She also helped revise the manuscript and respond to reviewer comments.

Chapter 4: Size-induced phenotypic reaction norms in a parasitoid wasp: an examination of life history and behavioural traits

Paul K. Abram, Jean-Philippe Parent, Jacques Brodeur, and Guy Boivin

Published as: Abram et al. (in press) *Biological Journal of the Linnaean Society* doi: 10.1111/bij.12658

4.1 Abstract

The amount of resources available during development often affects body size, causing phenotypic variation in life history traits and reproductive behaviours. However, past studies have seldom examined the reaction norms of both life history and behavioural traits versus body size. We measured the phenotypic plasticity of several life history (age-specific egg load, egg size, longevity) and behavioural (oviposition rate, host marking rate, walking speed) traits of the egg parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae) in response to body size variation. We predicted that life history traits would show more evidence of size compensation than behavioural traits, resulting in fewer positively-sloped size versus trait reaction norms among the former. As predicted by life history models, smaller wasps appear to shift resource allocation towards early-life reproduction, having a similar egg load to large individuals nine days after emergence. Surprisingly, longevity was unaffected by body size. However, egg size, the number of offspring produced during oviposition bouts, and the rate of subsequent egg synthesis were greater for larger individuals. In addition, as predicted, the reaction norms of behavioural traits versus body size were all positively sloped. Thus, despite possible adaptive compensatory plasticity of life history traits by small individuals, behavioural constraints directly related to body size would contribute to maintaining a positive size-fitness relationship.

<u>Key words:</u> behavioural constraints, body size, egg load, egg size, host handling rate, longevity, parasitoid fitness, reproductive traits, resource allocation, *Telenomus podisi*

4.2 Introduction

During immature development, organisms can experience varying degrees of resource availability. Under a given nutritional regime, phenotypic plasticity should partition resources between different traits in a way that will maximize the fitness of the resulting phenotype when it interacts with the environment (Sultan and Bazzaz 1993), subject to limits and constraints (DeWitt et al. 1998). So, although individuals that develop with access to fewer resources will often have low fitness relative to those that develop with access to plentiful resources, phenotypic plasticity can allow them to 'make the best of a bad situation'. However, one of the most common and unavoidable consequences of developing with few resources is having a small body size, especially when the amount of available developmental resources is fixed and the organism's size at maturity is determinate (Nylin and Gotthard 1998; Teder et al. 2014). Body size itself can have direct effects on fitness since it influences traits such as competitive ability (Arnott and Elwood 2009), mating success (Jennions and Petrie 1997), and predation risk (Wahle 1992; Alcock 1995). For a given genotype, the phenotypic expression of each trait across the possible range of body sizes can be described by trait versus size 'reaction norms' (Roff 1992). The slopes of these reaction norms are indicative of the plasticity of each trait in response to developmental resource limitation (Stearns and Koella 1986; Teder et al. 2014).

Insect parasitoids are an ideal group of organisms to study reaction norms of reproductive traits and size. Parasitoids are limited to a single host for development, which can vary in the amount of resources it provides depending on factors such as the host's life stage (Nicol and Mackauer 1999; Henry et al. 2009), nutritional status (Harvey et al. 1995), or species (Brodeur et al. 1998; Nicol and Mackauer 1999; Arakawa et al. 2004; Martel et al. 2011). For koinobiont parasitoid species, whose hosts continue to grow after being parasitized, the influence of host size is not straightforward (Cloutier et al. 2000; Harvey et al. 2004). However, the size of idiobiont parasitoids (where the amount of resources is fixed at oviposition) such as those that parasitize the eggs of other insects, generally increases with the size of the host in which they develop (Godfray 1994), especially for solitary species, where only one parasitoid develops per host (Charnov and Stephens 1988).

Several parasitoid reproductive traits tend to be positively related to adult size (Roitberg et al. 2001). Small female parasitoids often have lower lifetime fecundity and age-specific egg loads (King 1987; Honěk 1993; Jervis et al. 2008; Durocher-Granger et al. 2011; Boivin and Martel 2012), smaller eggs (O'Neill and Skinner 1990; Durocher-Granger et al. 2011; Martel et al. 2011), and reduced longevity (Visser 1994; Ellers et al. 1998; Boivin and Lagacé 1999; Jervis et al. 2008; Boivin and Gauvin 2009). However, there is some evidence that parasitoids, depending on resource availability during development, can adjust relative resource allocation between somatic maintenance (e.g. lipid stores) and reproduction (egg production) to suit the life history imposed by their body size. The model of Ellers and Jervis (2003) for synovigenic parasitoids (species that continue to develop eggs during the adult stage), supported by a few laboratory studies (Olson and Andow 1998; Thorne et al. 2006; but see Fischbein et al. 2013), predicts that small individuals can compensate for resource limitation during development (and the life history it imposes) by investing proportionally more resources into egg production early in their lives. In addition to the effects of the environmental and nutritional conditions under which the fitness of differently-sized parasitoids is assessed (Kazmer and Luck 1995; Bezemer et al. 2005), this type of shift in resource allocation by small parasitoids may be responsible for the fact that weakly positive or zero-sloped reaction norms are observed when examining the relationship between parasitoid body size and some reproductive traits (e.g., King 1987, King 1988; Visser 1994; West et al. 1996; Thorne et al. 2006). Compensatory adaptive plasticity might be especially likely in generalist parasitoid species that parasitize hosts of a wide range of sizes, thus commonly exposing small phenotypes to selection (*sensu* Berger et al. 2012).

While the aforementioned life history traits in parasitoids of different body sizes are the most commonly measured and considered in theoretical models, 'behavioural' traits are also likely to have a large impact on the relative fitness of parasitoids of different sizes. Body size can affect parasitoids' ability to subdue larger hosts that possess behavioural defenses (Lauzière et al. 2000; Henry et al. 2009; Wu et al. 2011), fight with competitors for access to hosts (Petersen and Hardy 1996), drill through a host's protective outer layer (Morris and Fellowes 2002), or move within or between patches of hosts (Visser 1994; Bennett and Hoffmann 1998; Wang and Messing 2004; Boivin and Gauvin 2009). Since these limitations are directly related to adult body size (a fixed, inevitable consequence of resource limitation), compensation by small individuals via differential resource allocation during development is unlikely, and one would expect trait versus size reaction norms with uniformly positive slopes. However, it is difficult to test the validity of this idea from existing literature, since the reaction norms of size and multiple life history and behavioural traits are seldom measured within the same species.

In this study we examined, under laboratory conditions, the slopes of trait versus size reaction norms for several life history (age-specific egg load, egg size, longevity) and behavioural (walking speed, oviposition rate, host marking rate) traits, using females of the egg parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae). While we generally anticipated positively sloped reaction norms, we predicted that higher proportional early-life investment in reproduction by small individuals could weaken the relationship between size and early-life egg load (a weak positive or zero-sloped reaction norm) (Ellers and Jervis 2003), with a corresponding tradeoff in terms of lifespan (a strong positively-sloped reaction norm) (Ellers et al. 2000a; Jervis et al. 2008). Furthermore, the measured behavioural traits should be constrained by body size, resulting in consistently positively sloped size-trait reaction norms. By taking the approach of simultaneously measuring a diversity of reaction norms of both life history and behavioural traits within the same parasitoid species, we expected to gain novel insights into size-fitness relationships.

4.3 Materials and Methods

Study system and insect colonies

Telenomus podisi is the most common and widespread egg parasitoid of several stink bugs (Hemiptera: Pentatomidae) in North America, including herbivorous and predatory species (Yeargan 1979; McPherson 1982; Koppel et al. 2009). Host eggs are laid in patches of varying sizes, with average egg mass sizes ranging between 13-40 eggs depending on species (Yeargan 1979; Orr et al. 1986). This parasitoid species is solitary and females from the strain cultured in our laboratory are strongly synovigenic (*sensu* Jervis et al. 2001; ovigeny index < 0.05), typically emerging with very few mature oocytes (average ~2.0; Zhou et al. 2014), but capable of parasitizing >100 hosts during their lives (P.K.A., unpublished data). If deprived of hosts, females reach their maximum egg load of up to 30 eggs ~8-12 days after emergence, and start resorbing eggs after 15-21 days (P.K.A.. unpublished data). *Telenomus podisi* does not feed on host eggs (P.K.A., personal observations). This parasitoid species is long-lived, surviving up to 100 days under laboratory conditions (see below).

Telenomus podisi were originally collected from the London and Ottawa (Ontario, Canada) areas in the summers of 2011 and 2012, and reared continuously thereafter on eggs of *Podisus maculiventris* Say (Hemiptera: Pentatomidae). Identifications of colony foundresses were confirmed by Dr. Lubomir Masner (National Collection of Insects, Arachnids, and Nematodes, Ottawa, Ontario, Canada). We consider our laboratory population to be a mixed genotypic sample from the area of collection; the phenotypic responses to host size measured in this study are thus measured in the broad sense discussed by Gianoli and Valladares (2012), rather than that of an individual genotype. *Telenomus podisi* of different sizes to be used for experiments were obtained by exposing 30-100 eggs (24-48h after being laid) of *Cosmopepla lintneriana* Kirkaldy (egg volume: 0.18 ± 0.02 mm^3; mean ± SE; n = 10), *P. maculiventris* (0.33 ± 0.01 mm^3), or *Euschistus servus* Say (0.66 ± 0.03 mm^3) (all Hemiptera: Pentatomidae) to groups of 10-20 female parasitoids for 24 h in a Petri dish (diameter: 8.5 cm; height: 1.5 cm). Eggs were fixed to white filter paper with white, non-toxic glue (LePage©, Canada). Negative fitness effects of intraspecific superparasitism on offspring were unlikely, since (i) patch guarding behaviour of this species reduces superparasitism levels (Field 1998), and (ii) fitness effects of intraspecific superparasitism in this superfamily of parasitoids are negligible (Abram et al. 2012; Cusumano et al. 2015). After exposure to parasitoids, eggs were incubated under standard rearing conditions (24 ± 1°C at ~50% RH, 16L:8D light cycle) until parasitoid emergence. Female parasitoids, which emerged in the presence of males for mating, were isolated within 24 h of emergence and kept individually in 1.5 ml Eppendorf tubes with a drop of honey/water mixture (~50:50) until used for dissections or experiments (the age of females depended on the experiment; see below).

Podisus maculiventris adults were originally collected from several locations in the London and Ottawa regions in 2011 and 2012, and reared continuously thereafter, renewed with individuals ordered from a greenhouse supply company (Biobest, Canada). Adults and nymphs were fed with live mealworm (*Tenebrio molitor* L.) larvae and fresh green beans in muslin cages (30cm x 30 cm). Eggs were collected daily from the sides of cages.

Post-overwintering *E. servus* and *C. lintneriana* adults were collected in June and July of 2013 from mullein plants and fallow fields in the general vicinity of Montréal and Ottawa (Canada). *Euschistus servus* were fed with fresh green beans, sweet corn, carrots, and pumpkin seeds in muslin cages (30 cm^3). *Cosmopepla lintneriana* were fed with mullein leaves, green beans, and pumpkin seeds in plastic cylinders (height: 15 cm, diameter: 11 cm). Eggs of both species were collected daily from the sides of cages (*E. servus*) or crumpled paper towel provided as oviposition substrate (*C. lintneriana*).

Parasitoid size vs. host egg size

First, the relationship between host egg volume and resulting parasitoid offspring size was characterized. The volume of the eggs of the three different stink bug species (n = 10 individual eggs from 10 different egg masses laid by different stink bug females, <48 h after laying) were estimated using the parabolic barrel volume formula $V = \pi H(3r^2 + 4Rr + 8R^2)/15$, where r is the radius of the top of the egg, R is the radius at the center of the egg, and H is the height of the egg. Measurements were taken using a PixeLINK camera model PL-B681CF (PixeLINK, Ottawa, ON, Canada). As an index of parasitoid body size, the right hind tibia (RHT) length of 40 females that had emerged from each of the host species' eggs was measured using the same equipment (to an accuracy of 0.01µm). Due to the small size of *T. podisi*, accurately measuring the mass of individual wasps was not feasible with available equipment, although weighing pooled groups of wasps indicated that, on average, a 32% increase in tibia length (299.69 – 397.09 µm; mean ± SE) corresponds to a 264% increase in wet body mass (2.2 – 8.0 mg; means of pooled groups) (P.K.A.; unpublished data). Because RHT length is a linear measurement whereas host egg volume is three-dimensional, we linearized measurements of host egg volume by taking their cubed-root (as was done for weight measurements in Teder et al 2014) before plotting them against RHT length (Figure 4-1).

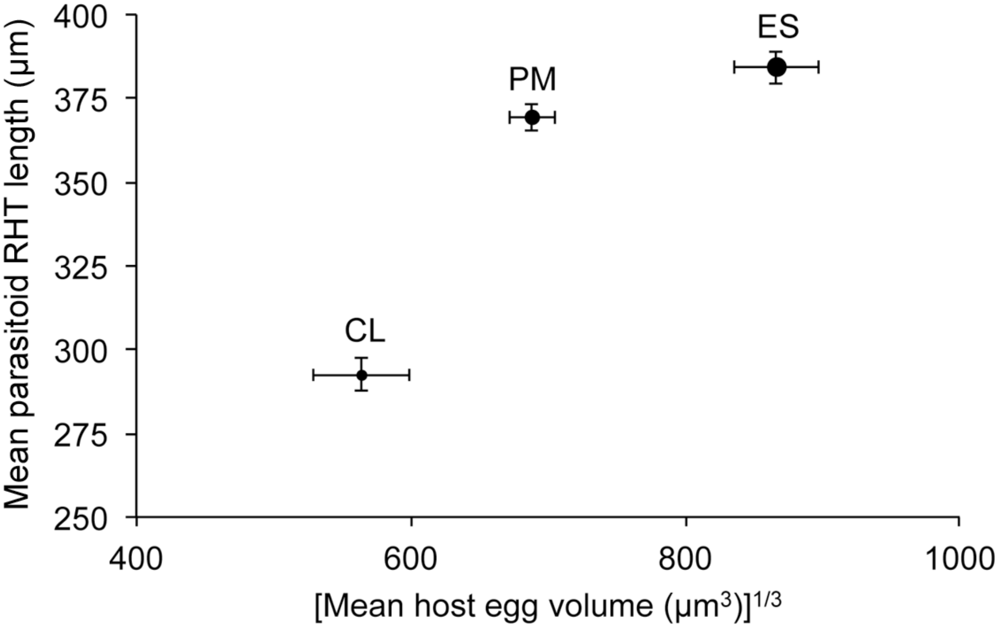

Figure 4-1. Mean right hind tibia (RHT) length (± 95% CI) of *T. podisi* in relation to the linearized estimated relative volume of the eggs of three host species (± 95% CI). CL = *C. lintneriana*; PM = *P. maculiventris*; ES = *E. servus*).

Early-life egg load and size of mature oocytes

Given that our strain *T. podisi* is almost completely synovigenic (Zhou et al. 2014) and thus emerges with almost no mature eggs, we did not use egg load upon emergence ('initial egg load') as our measure of early-life reproductive investment, as is standard for parasitoids that show moderate pro-ovigeny (Jervis and Ferns 2004; Jervis et al. 2008). Rather, as a measure of 'early-life' egg load, similar to the Reproductive Concentration Index in Wajnberg et al. (2012), we counted the number of mature oocytes present following emergence but before any egg laying in hosts. We dissected 20 mated females that had emerged from each of the three host species' eggs at two points along the trajectory of oocyte maturation: three days after emergence (before all oocytes had matured) and nine days after emergence (when females were approaching their maximum egg load and nearly all oocytes had matured). Dissections took place in a drop of water under 40 X magnification. Mature oocytes were opaque with a well-defined chorion whereas immature oocytes were transparent without a well-defined shape. Additionally, we estimated the size of oocytes in 9 day-old females by tracing their perimeter and calculating the contained area using digital measurement software (Image-Pro Plus version 5.1.0.20, Media Cybernetics, Rockville, USA). To avoid pseudoreplication, we calculated the mean size of oocytes for each female and conducted the analysis with per-female averages. The RHT length of parasitoids was measured as described above.

Longevity

We next measured the life expectancy of *T. podisi* when the parasitoids had unlimited access to carbohydrates, but no access to hosts. With access to only water, parasitoids would have died too soon (1-5 days, medians of 2-3 days; P.K.A.; unpublished data) for us to be able to detect potential differences among differently sized individuals. Female *T. podisi* reared from the three host species (total n = 75) were isolated within 24 h of emergence and mating, and placed in Eppendorf tubes with a drop of honey water (renewed every 10 days) under standard rearing conditions. Parasitoids were observed every 1-3 days until death, recorded as the midpoint between the last two observations. The RHT length of parasitoids was measured following their death.

Walking speed

As an indirect measurement of the parasitoids' dispersal capacity, we measured the walking speed of 5-day-old females reared from the three host species (total n = 33) and given unlimited access to honey water prior to testing. Between six to ten hours after the start of photophase, parasitoids were filmed walking under the lid of an empty Petri dish (diameter: 4.5 cm; height: 0.5 cm) for 5 minutes under ambient laboratory conditions (23 ± 1°C, 30-50% RH). We used Dino-Lite cameras (model AM-4012NZT, Taiwan), recording on a Mini Video Recording System (Model KL92). The videos resolution was 640x480 at 15 frames per second. To monitor the insect's position, we used the Bio-tracking software (Georgia tech; www.bio-tracking.org), which tracks an organism's position for each frame, giving it an x,y coordinate based on pixels. Walking speed was calculated by dividing the total distance traveled by the total time in motion. Total traveled distance was the sum of all differences in position in pixel transformed in length. All differences in position of a single pixel (horizontally, vertically or diagonally) were excluded in order to minimize noise and reduce false movements (1 pixel = 0.065 mm). Total time in motion corresponds to the sum of frames where the difference in position was greater than a single pixel (1 frame = 0.067 s). After the experiment, the RHT length of all females was measured.

Number of offspring produced and subsequent egg load renewal

We next tested whether the parasitoids reared from the smallest and largest host species' eggs differed in the number of offspring they could produce (when hosts are provided *ad libitum*), their capacity to refill their stock of eggs after exhausting their initial egg supply, and the size of the resulting oocytes. *Telenomus podisi* females reared from *C. lintneriana* (n = 12) or *E. servus* (n = 11), mated and nine days old, were released in a Petri dish arena (diameter: 4.5 cm, height: 0.5 cm) with a mass of 35 *P. maculiventris* eggs (enough to exhaust their egg supply; see egg loads in Figure 4-2) under standard rearing conditions starting 6-8 h after the onset of photophase. Eggs were glued in clustered masses with white non-toxic glue, upright on the substrate (filter paper circle; 2 cm diameter) to mimic the structure of naturally-laid egg masses. Parasitoids were left in arenas with hosts for 24 h and placed in a 1.5 ml Eppendorf tube with honey water for 8 days to refill their egg load. They were then dissected to determine the number of mature eggs, the mean size of mature oocytes; and the RHT length of each female. Host eggs from the 24 h parasitism period were incubated under standard rearing conditions during ~18-22 days and the number of parasitoid offspring successfully emerging from each egg mass were scored. Remaining eggs were then dissected to count the number of parasitoid offspring that fully developed but failed to emerge (<2%); these offspring were included in the measurement of the number of offspring produced by each female.

Oviposition and host marking rate

To measure the relationship between the size of *T. podisi* and the rate of host handling (oviposition and marking), we filmed the first 5 hours of the above experiment in which parasitoids were offered host egg masses. Videos were played back and the duration of each bout of oviposition (insertion of the ovipositor into the host egg which was followed by marking; see Abram et al. 2014) and host marking (rubbing the ovipositor across the surface of the host egg following oviposition) was measured using event recording software (CowLog version 2.11; Hänninen and Pastell 2009). The mean duration of oviposition and marking was calculated for each individual, and was converted to a rate by calculating the reciprocal value of per-individual means. Thus, these two measurements did not include movement between hosts on the patch, but rather the rates at which hosts were handled when encountered.

Statistical analyses

To test the effect of the size of *T. podisi* on the various traits measured, we originally included host species and parasitoid size (RHT length) as factors in the analysis, also testing for their interaction (i.e., whether there were significant size-trait value relationships within host species). In almost all cases, adding 'host species' as a factor to a model containing 'size' did not increase explanatory power (and vice-versa), and their interaction was never significant. In other words, the effects of parasitoid size and host species were inextricably linked, and the effect of parasitoid size on the measured parameters was independent of host identity. However, comparisons of non-nested models (i.e. with 'size' as the independent variable vs. 'host' as the independent variable) with Aikake's Information Criterion (AIC) revealed that size tended to be better than host species at explaining the observed trait variation (for 9/10 of the measured traits) (see Table 4-1). We proceeded by testing only the effect of parasitoid size, while considering it to be biologically synonymous with the host species from which they emerged.

To characterize the relationship between parasitoid size and the various traits measured, we used linear regressions for normally distributed/homoscedastic data (oocyte size) and Generalized Linear Models (GLMs) or survival models for data that did not fit these assumptions. We fitted GLMs with poisson error distributions (log-link) for count data (egg load, number of offspring produced), and gamma error distributions (reciprocal link) for data where the variance changed faster than linearly with the mean (walking speed, oviposition and host marking rate). We used quasi-likelihood when a given model showed evidence of overdispersion (Crawley 2012). The effect of parasitoid size on longevity (time until death) was tested using parametric survival analysis assuming a Weibull distribution ('survreg') (Crawley 2012; Therneau 2014). In all cases, adequacy of model fit was assessed with residual plots. Statistical significance of parasitoid size in the GLMs and survival analysis was

Table 4-1. Comparison of female size (right hind tibia length) versus host species as predictors of reproductive traits in *Telenomus podisi*. Models containing ‘size’ or ‘host’ alone followed the same pattern of statistical significance for each trait (first two columns of p-values from the left), although size was a better predictor in all but one case. Additionally, in all but one case, adding ‘host’ or the ‘host x size’ interaction as predictors to a nested model already containing ‘size’ did not add any additional explanatory power. See text of Methods section for the type of statistical models used for each trait.

	Trait ~ size[a]			Trait ~ host[a]			Trait ~ size + host[c]		Trait ~ size x host[c]	
Trait	test statistic[d]	P	AIC[b]	test statistic[d]	P	AIC[b]	test statistic[d]	P	test statistic[d]	P
Egg load (3 days)	$\chi^2_1 = 10.04$	0.0015	311.38	$\chi^2_1 = 17.89$	0.00013	305.52	$\chi^2_2 = 8.16$	0.017	$\chi^2_3 = 8.34$	0.08
Egg load (9 days)	$\chi^2_1 = 2.02$	0.15	337.76	$\chi^2_1 = 1.82$	0.402	339.96	$\chi^2_2 = 1.09$	0.58	$\chi^2_3 = 2.74$	0.6
Egg load (regenerated)	$\chi^2_1 = 8.71$	0.00316	112.31	$\chi^2_1 = 8.62$	0.0033	112.397	$\chi^2_1 = 0.11$	0.74	$\chi^2_2 = 1.62$	0.44
Egg size (9 days)	$F_1 = 5.26$	0.025	189.38	$F_1 = 3.63$	0.033	189.4	$F_2 = 0.96$	0.39	$F_3 = 1.14$	0.34
Egg size (regenerated)	$F_1 = 1.203$	0.29	66.32	$F_1 = 0.52$	0.48	67.05	$F_1 = 1.48$	0.24	$F_2 = 0.69$	0.52
Longevity	$\chi^2_1 = 0.67$	0.41	664.28	$\chi^2_1 = 3.15$	0.21	664.81	$\chi^2_2 = 2.97$	0.23	$\chi^2_3 = 4.09$	0.39
Walking speed	$F_1 = 10.44$	0.0029	131.82	$F_1 = 5.26$	0.011	133.315	$F_2 = 0.40$	0.67	$F_3 = 0.19$	0.94
Drilling duration	$F_1 = 27.00$	<0.0001	210.22	$F_1 = 20.98$	0.0002	212.83	$F_1 = 0.16$	0.69	$F_2 = 0.35$	0.71
Marking duration	$F_1 = 45.02$	<0.0001	159.53	$F_1 = 37.44$	<0.0001	161.57	$F_1 = 0.11$	0.75	$F_2 = 0.25$	0.78
Offspring produced in 24h	$F_1 = 12.81$	0.0019	141.42	$F_1 = 8.80$	0.0076	145.61	$F_1 = 1.85$	0.19	$F_2 = 0.88$	0.43

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[a] Test statistics and p-values are from likelihood ratio tests (LRTs) comparing the model to a nested model with only the intercept

[b] AIC values permit the comparison (within rows) between the non-nested models; the lower value (underlined) indicates the preferred model (lowest AIC) in each case.

[c] Test statistics and p-values are from LRTs comparing the model to a nested model with only size as a predictor (values of $P < 0.05$ are in italics).

[d] Subscripts denote test degrees of freedom.

determined using likelihood ratio tests (LRTs) unless quasi-likelihood or a gamma error distribution was used, in which case F-tests were employed instead (Crawley 2012). All statistical analyses were carried out with R software, version 2.15.1 (R Core Team 2013)

4.4 Results

Parasitoid size vs. host egg size

Telenomus podisi females emerging from eggs of the three host species differed widely with respect to size (Figure 4-1), with the largest wasps (which emerged from *E. servus* eggs) having on average a 31% longer tibia than the smallest individuals (which emerged from *C. lintneriana* eggs). Mean parasitoid size followed the same rank order as the volume of the host species' eggs from which they emerged. However, the difference in parasitoid size between *C. lintneriana* and *P. maculiventris* eggs was greater than that between parasitoids emerging from *P. maculiventris* and *E. servus* eggs, despite similar differences in relative egg volume of the host species (Figure 4-1).

Early-life egg load and size of mature oocytes

The egg load of *T. podisi* females increased with increasing parasitoid size (by 58.4% over the range tested) when measured three days after emergence (GLM with poisson errors, $\chi^2_1 = 10.04$, $P = 0.0015$) (Figure 4-2). Nine days after emergence, however, there was no association between egg load and parasitoid size (GLM with poisson errors, $\chi^2_1 = 2.02$, $P = 0.15$) (Figure 4-2). There was a weak but significant positive relationship (19.6% increase in model predictions over the range measured) between parasitoid size and the size of mature oocytes nine days after emergence (linear regression, $F_{1,58} = 5.26$, $R^2 = 0.083$, $P = 0.025$) (Figure 4-3).

Longevity

Telenomus podisi females lived up to 94 days when deprived of hosts and supplied with honey water, with 87% of individuals surviving at least 30 days. The time until death of *T. podisi* females was not related to their size (survival model, $\chi^2_1 = 0.67$, $P = 0.41$) (Figure 4-4).

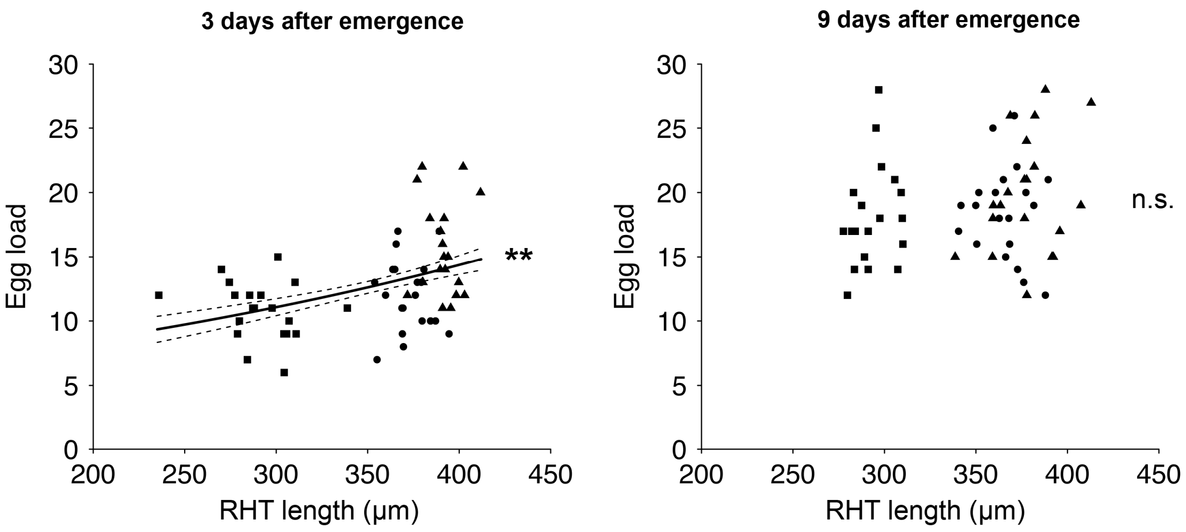

Figure 4-2. Early-life egg load of *T. podisi* of different sizes (right hind tibia length), 3 and 9 days after emerging from eggs of *C. lintneriana* (square points), *P. maculiventris* (circular points), or *E. servus* (triangular points). Trendline on the left shows predictions (±SE) of a GLM with poisson errors fitted to the data; ** indicates $p < 0.01$. On the right, n.s. indicates $p > 0.05$ from a GLM with poisson errors.

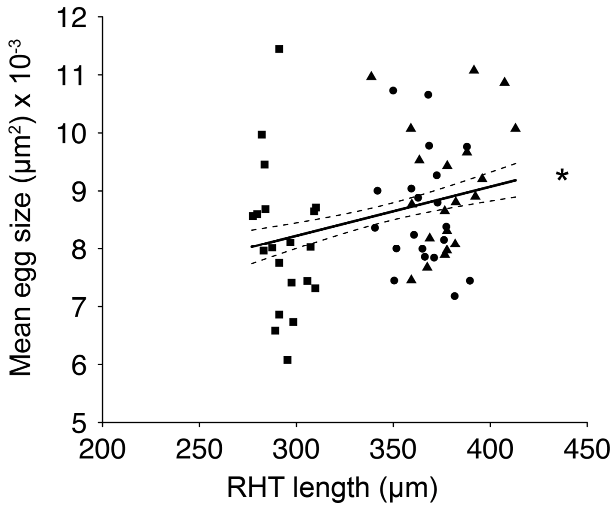

Figure 4-3. Mean size of mature oocytes of *T. podisi* of different sizes (right hind tibia length), 9 days after emerging from eggs of *C. lintneriana* (square points), *P. maculiventris* (circular points), or *E. servus* (triangular points). Trendline shows predictions (±SE) of a linear regression fitted to the data; * indicates $p < 0.05$; $R^2 = 0.083$.

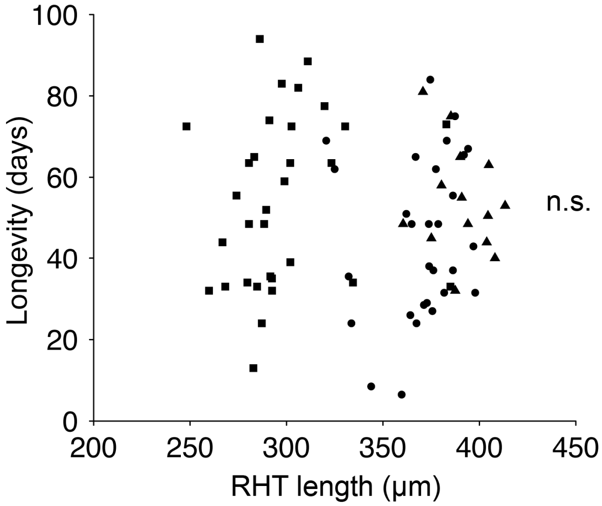

Figure 4-4. Longevity of *T. podisi* of different sizes (right hind tibia length), having emerged from eggs of *C. lintneriana* (square points), *P. maculiventris* (circular points), or *E. servus* (triangular points), and provided with honey water *ad libitum*. n.s. indicates $p > 0.05$ from a survival model with a Weibull distribution fitted to the data.

Walking speed

The walking speed of *T. podisi* females increased with parasitoid size (GLM with gamma errors, $F_{1,31} = 10.44$, $P = 0.0029$), by on average 30.7% over the range of parasitoid sizes tested (Figure 4-5).

Number of offspring produced and subsequent egg load renewal

When *T. podisi* females with their full egg complement were offered a patch of *P. maculiventris* eggs for 24 h, larger wasps produced more offspring (GLM with quasi-poisson errors, $F_{1,19} = 12.81$, $P = 0.0019$) than smaller females; there was a 74.5% increase in offspring production over the range of parasitoid sizes tested (Figure 4-6). Nine days afterwards, larger females had produced significantly more (+48.9%) eggs (GLM with poisson errors, $\chi^2_1 = 8.71$, $P = 0.0032$), although there was no relationship between parasitoid size and oocyte size in this case (linear regression, $F_{1,17} = 1.20$, $R^2 = 0.031$, $P = 0.29$) (Figure 4-7).

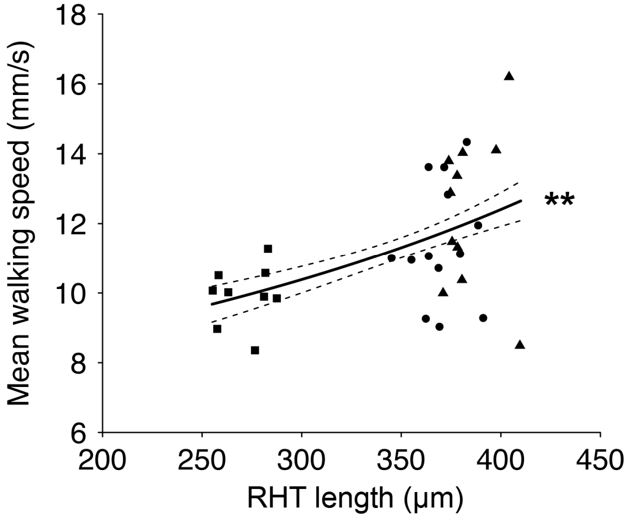

Figure 4-5. Mean walking speed of *T. podisi* of different sizes (right hind tibia length), having emerged from eggs of *C. lintneriana* (square points), *P. maculiventris* (circular points), or *E. servus* (triangular points). Trendline shows the predictions of a GLM with gamma errors (±SE) fitted to the data; ** indicates p < 0.01.

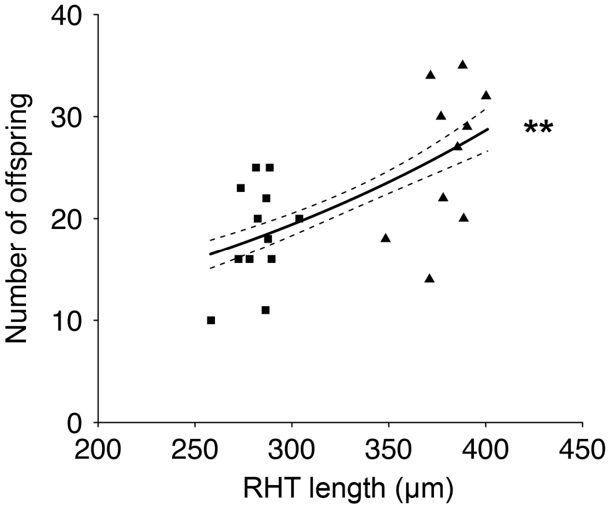

Figure 4-6. Number of offspring produced by *T. podisi* of different sizes (right hind tibia length), having emerged from eggs of *C. lintneriana* (square points) or *E. servus* (triangular points) during a 24h oviposition bout. Trendline shows the predictions of a GLM with quasipoisson errors (±SE) fitted to the data; ** indicates p < 0.01.

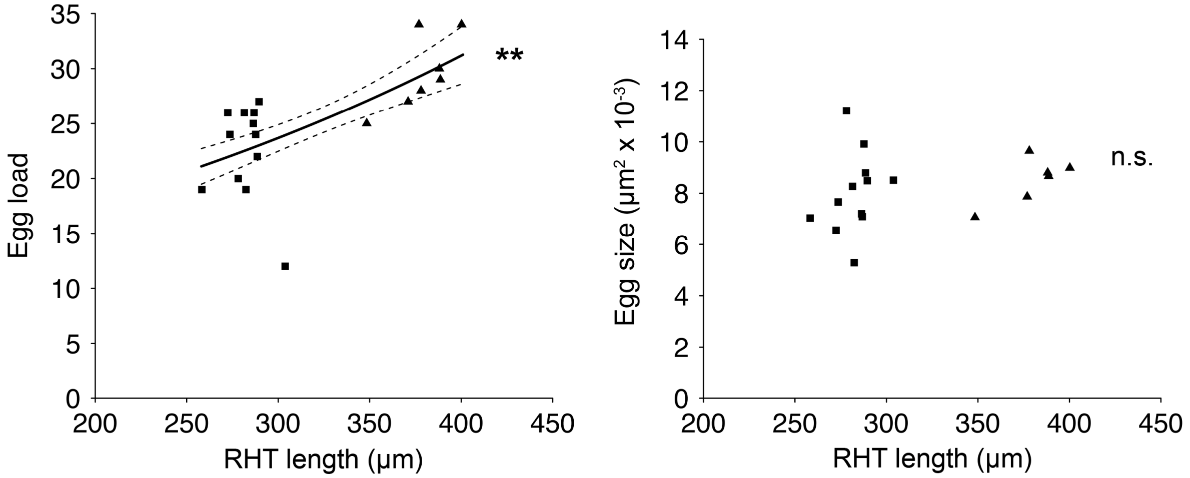

Figure 4-7. Mean egg load and mature oocyte size of *T. podisi* of different sizes (right hind tibia length) having emerged from eggs of *C. lintneriana* (square points) or *E. servus* (triangular points), eight days after an oviposition bout that exhausted their initial egg supply. Trendline on the left shows the predictions of a GLM with quasi-poisson errors (±SE) fitted to the data; ** indicates $p < 0.01$. On the right, n.s. indicates $p > 0.05$ and $R^2 = 0.031$ from a linear regression fitted to the data.

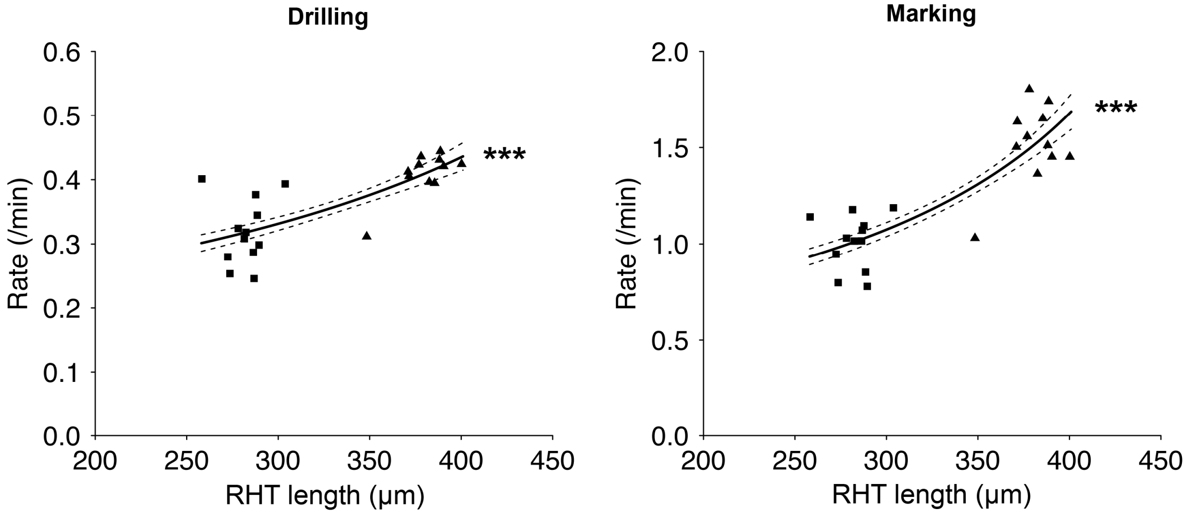

Figure 4-8. Mean rate of drilling and host marking for *T. podisi* of different sizes (right hind tibia length), having emerged from eggs of *C. lintneriana* (square points) or *E. servus* (triangular points). Trendlines show the predictions of GLMs with gamma errors (±SE) fitted to the data; *** indicates $p < 0.0001$.

Oviposition and host marking rate

Oviposition (GLM with gamma errors, $F_{1,21} = 24.211$, $P < 0.0001$) and host marking ($F_{1,21} = 54.70$, $P < 0.0001$) rates increased with increasing parasitoid size (Figure 4-8). The increases in rate of oviposition and marking predicted by the fitted models were 44.9% and 80.6%, respectively, over the range of parasitoid sizes tested.

4.5 Discussion

Most reaction norms of size and reproductive traits measured in *T. podisi* were positively sloped. Unexpectedly, however, the lack of relationship between parasitoid size and initial egg load nine days after emergence was not accompanied by a tradeoff in terms of decreased longevity for small females. On the other hand, as predicted, the reactions norms of size and 'behavioural' traits (walking speed, oviposition and host marking rate) were all positively sloped. Larger females also produced more eggs after exhausting their initial egg supply, and parasitized more hosts during a 24 h oviposition bout. Thus, despite apparent physiological compensation by small individuals via differential allocation of resources, a determinate adult body size and its consequences for behavioural fitness proxies would definitively tip the scales in favour of larger *T. podisi*, and the generally accepted positive size-fitness relationship for female parasitoids probably holds true.

There were diminishing returns in parasitoid size as the size of host eggs increased (Figure 4-1). Other studies have also observed non-linear relationships with diminishing returns between host volume and parasitoid size (Bigler et al. 1987; van den Assem 1989; Wang and Messing 2004), although directly proportional relationships have also been observed (Martel et al. 2011). There are at least two explanations for diminishing returns in parasitoid size in response to host volume: (i) a physiological constraint on resource uptake or (ii) differences in nutritional quality between the different host species. A physiological constraint on resource uptake due to, for example, an upper genetic limit on the body size of the wasps, would prevent an excess of resources to be allocated to a further increase in parasitoid body size (Wang and Messing 2004). Indeed, *E. servus* is among the largest species of pentatomids attacked by *T. podisi* in North America (McPherson 1982), so it is plausible that its egg size is near the limit of resource uptake capacity by *T. podisi* during development.

While we cannot exclude the possibility that there may have been differences in nutritional quality among the eggs of the three host species, this explanation seems unlikely. If the three host species' eggs varied significantly with regard to nutritional quality independent of volume, we would have expected host species to explain variation in the various reproductive traits measured better than parasitoid size, which was generally not the case (Table 4-1). In addition, for another egg parasitoid (*Trichogramma euproctidis*), phenotypic differences among differently sized parasitoids developing in three host species were related to host size and not host nutritional composition (Martel et al. 2011).

Models incorporating life history theory predict that individuals with access to fewer resources during development (i.e., smaller parasitoids) should allocate proportionally more to early-life reproduction than large individuals (Ellers et al. 2000; Ellers and Jervis 2003; Jervis et al. 2008), since they are more affected by environmental stochasticity, and sample fewer host patches during their lives. This prediction is supported by some (Olson and Andow 1998; Thorne et al. 2006), but not all (Fischbein et al. 2013), experimental evidence. In our study, when we examined the first batch of eggs synthesized following emergence in *T. podisi*, there was a positive relationship between size and fecundity three days after emergence, but not after nine days when the parasitoids were approaching their maximum egg load. This may indicate that large individuals synthesize eggs faster after emergence (also see below), but that small individuals can eventually 'catch up' and shift resource allocation to allow for a similar maximum number of mature oocytes. This would be expected if smaller individuals were to concentrate reproduction in early life to compensate for their higher level of susceptibility to environmental stochasticity, and corresponding shorter longevity (Ellers et al. 2000b; Ellers and Jervis 2003). However, contrary to our expectations and the majority of studies on insect parasitoids (e.g., Boldt and Marston 1974; van den Assem et al. 1989; Bai et al. 1992; Tillman and Cate 1993; Harvey et al. 1994; Visser 1994), the reaction norm of size and longevity in the laboratory was zero-sloped for *T. podisi*. This result begs the question of how small parasitoids can produce just as many eggs as large parasitoids in early life (i.e. in the days following emergence but before host encounter), but still live just as long, given that there should be a tradeoff between resource allocation to reproduction and somatic maintenance (Ellers et al. 2000a). A few other studies have found a lack of relationship (*Trichogramma maidis*: Bigler et al. 1987; *Spalangia cameroni*: King 1988), a domed relationship (*Pteromalus* *puparum;* Takagi 1985), or even a negative relationship between the size and longevity of parasitoids in the absence of hosts (*Goniozus nephantidis*; Hardy et al. 1992). In the current study, a zero-sloped size versus longevity reaction norm could be explained by (i) unlimited access to carbohydrates (honey) allowing smaller individuals to fuel somatic maintenance and compensate for lower resource (lipid) stores (Strand and Casas 2008), or (ii) somatic maintenance and other physiological processes such as egg generation being more metabolically costly in larger individuals (Ellers et al. 2000b).

One possible metabolic cost for larger individuals could be the production of larger oocytes. As observed in several other studies (O'Neill and Skinner 1990; Durocher-Granger et al. 2011; Martel et al. 2011), the oocytes synthesized by larger *T. podisi* individuals tended to be bigger nine days after emergence. So, while the number of eggs synthesized was similar across parasitoid sizes, larger parasitoids invested overall more resources in egg production. It is possible that larger eggs could be advantageous by providing more resources for developing offspring following oviposition. In support of this idea, Giron and Casas (2003) found that oocyte size was associated with higher larval survival; however, other studies in parasitoids have found no clear advantage to larger oocytes (Durocher-Granger 2011; Ameri et al. 2014).

Despite the fact that there were zero-sloped size versus longevity and size versus egg load reaction norms in *T. podisi*, we found evidence that the fitness gap between large and small individuals would likely widen with increasing time following emergence. Larger wasps produced more offspring when offered hosts for 24 h, and subsequently synthesized more eggs than smaller parasitoids. Since the egg load of the differently-sized parasitoids to which we offered hosts was initially similar (Figure 4-2), it can be inferred that large females synthesized more eggs during the course of patch exploitation, as observed by Wang and Messing (2004) in the parasitoid *Dirhinus giffardi*. A superior egg synthesis rate would provide a considerable advantage to larger individuals, particularly in host-rich environments where transient egg limitation is more frequent than time limitation (Rosenheim et al. 2000).

Egg load, longevity, and realized fecundity are the three most commonly measured traits in parasitoids of different sizes. However, other, less commonly measured traits that are a result of adult size – a consequence of fixed resource availability during development – can translate into behavioural differences that have consequences for fitness (e.g., Petersen and Hardy 1996; Morris and Fellowes 2002; Henry et al. 2009). Indeed, we found strong positively-sloped reaction norms of size and walking speed, oviposition rate, and host marking rate of *T. podisi* (Figure 4-5; Figure 4-7). Walking speed can be used as a proxy for the dispersal ability of parasitoids (reviewed in Visser 1994), and probably best represents the rate at which parasitoids search and move between host patches on the same plant, rather than between habitats (which would be determined by flying capacity; Fahrner et al. 2014). Although not examined in this study, walking speed (and body size itself) could also be important for the capacity of female *T. podisi* to defend host patches after ovipositing (P.K. Abram, in preparation). The higher rate (i.e., decreased duration) of oviposition by larger individuals may be due to greater strength of protractor muscles and hydrostatic pressure in the abdomen – the two probable mechanisms behind ovipositor extension in scelionids with the *Ceratobaeus*-type ovipositor (Austin 1983). In terms of host marking rate, larger size may expedite the process by allowing the abdomen to rotate over a larger area of the host egg, increasing the muscular power and therefore the speed of the oscillatory marking movements, or by being associated with greater availability or synthesis rate of marking pheromone. Together, increased walking speed, oviposition rate, and marking rate – which are all most likely directly related to body size (i.e., the dimensions of the physical structures involved in each behaviour) – would allow larger parasitoids to exploit host patches more efficiently and move between them more quickly. Furthermore, these results indicate that there could be less compensatory plasticity shaping reaction norms of size and behavioural traits, compared to other life history traits that are subject to flexible resource allocation during development. However, the possibility remains that small individuals of other species could show behavioural compensation for some ‘size-mandated’ traits. For example, an increase in stride frequency with decreasing body size could maintain a zero-sloped reaction norm of body size versus walking speed (Parent, Brodeur and Boivin, in prep.).

In several life history models, the prediction that smaller individuals should shift resource allocation towards early-life reproduction depends, in part, on smaller individuals having a shorter lifespan and being more affected by environmental stochasticity (Ellers et al. 2000b; Ellers and Jervis 2003; Berger et al. 2012). We did not observe the shorter lifespan expected of smaller *T. podisi* females. However, taken together, our results suggest that it could be advantageous for small parasitoids to concentrate reproduction in early life, even if they have a similar physiological longevity. Small wasps could suffer more mortality due to greater susceptibility to stochastic environmental events (e.g. wind or rain storms, drought-induced desiccation), and may be overall less likely to find host patches due to a poorer dispersal capacity (Fahrner et al. 2014; walking speed results above). Under this scenario, living long enough to find a first host patch and having enough mature eggs to exploit it would be critical for smaller females, given that opportunities for future reproduction would be limited compared to larger females (which can disperse faster, synthesize eggs faster, and exploit patches more efficiently). Alternatively, concentrating reproduction in early life could still be advantageous for small individuals even if females of all sizes experience the same high level of environmental stochasticity. Following the ‘Relative Fitness Rule’ (Giraldeau and Boivin 2008; Moiroux et al. 2015), being able to maximally exploit the first patch of hosts encountered could increase the fitness of small individuals relative to larger individuals, in the event that there is a stochastic mortality event that affects the entire population. Small individuals are advantaged by such stochastic mortality, since their loss of future fitness gain (proportional offspring contribution to the next generation) is lower relative to that of large individuals (Giraldeau and Boivin 2008).

Among parasitoid species, the distribution and availability of hosts are considered important drivers of the temporal distribution of egg production during the lives of parasitoids (i.e., reproductive schedules), as well as their per-capita egg investment (i.e., the size and number of eggs produced). For example, parasitoid species that attack aggregated hosts are more likely to invest relatively more in early-life reproduction, and produce smaller and less yolk-rich eggs, compared to species that attack solitary hosts (Harvey 2008). Availability of hosts throughout the lifetime of a single parasitoid species can also affect to what extent reproduction is concentrated in early life (Harvey et al. 2001). While these factors undoubtedly influence the broad patterns of parasitoid life history strategies, variation in parasitoid size can serve to ‘fine-tune’ reproductive schedules and per-capita egg investment, as we have demonstrated in our study. Thus, for *T. podisi*, reproductive schedules and per-capita reproductive investments likely reflect a combination of its evolutionary relationship with its hosts’ ecology, the timing of host encounters during parasitoids’ lives, and size-related phenotypic plasticity, the latter of which depends on the host species in which the female has developed.

In nature, reaction norms of size and reproductive traits interact with each other, and with environmental characteristics (e.g., stochasticity, host density), to determine the realized fitness of different phenotypes. The relationship between size and fitness of parasitoids can differ considerably between the field and laboratory (Visser 1994; West et al. 1996; Ellers et al. 1998); this may be due to the fact that reproductive traits most commonly measured in the laboratory (egg load and longevity) are, in reality, interacting with size-related behavioural constraints. Considering a wider range of reaction norms, including behavioural traits, as we have done in this study, could allow laboratory studies to better estimate how size affects realized fitness in the field, especially when integrated with dynamic modelling approaches (e.g., Berger et al. 2012).

4.6 Acknowledgements

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Chapter 5: An insect with selective control of egg colouration

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5.1 Summary

The colour and patterning of animal eggs has important consequences for offspring survival. There are examples of between-species and polymorphic differences in egg colouration in birds and amphibians (Licht 2003; Kilner 2006; Yang et al. 2010), as well as cases of birds and insects whose nutritional status or age can cause within-individual variation in egg pigmentation (Moreno et al. 2005; Siefferman et al. 2006; Winters et al. 2014). However, no studies to date have demonstrated that individual animals can selectively control the colour of their eggs. Here, we show that individual females of the predatory stink bug *Podisus maculiventris* Say (Hempitera: Pentatomidae) can control the pigmentation of their eggs during oviposition, as a response to environmental conditions. The colour of egg masses produced by individual females can range from pale yellow to dark black/brown. Females tend to lay darker eggs, which are more resistant to ultraviolet (UV) radiation, on the upper surface of leaves where UV exposure is highest in nature. Conversely, they lay lighter eggs on the undersides of leaves. However, egg colour is not determined by the intensity of UV radiation falling on the surface where they are laid. Rather, female stink bugs appear to use a visual assessment of oviposition substrate reflectance to determine egg colour. Unexpectedly, biochemical analyses revealed that the egg pigment is not melanin, the most ubiquitous light-absorbing pigment in animals. Our study offers the first example of an animal able to selectively control the colour of its eggs.

Key words: plasticity, ovipary, pigmentation, sunscreen, ultraviolet radiation, Pentatomidae

5.2 Results and Discussion

Description and quantification of egg pigmentation

First, we undertook a descriptive evaluation of *P. maculiventris* egg pigmentation (Figure 5-1). The eggs' chorion (shell) was always pale white immediately after laying, reaching its final pigmentation level within an hour. The dark pigment, when present, was contained in the outermost layer of the chorion, especially concentrated in chorionic spines (which were also present in eggs with little or no pigment). In most cases, pigmentation was homogenous on individual eggs and within the same egg mass. In contrast to some other descriptions of stink bug eggs (Lambdin and Lu 1984; Javahery 1994; Dimaté et al. 2014), the variation in egg colour described here is not due to the age of eggs, although egg contents do darken slightly in the later stages of embryonic development (Figure 5-1).

Next, we developed a 'Pigmentation Index' (PI) to quantify variation in egg pigmentation. First, we took standardized photos (white balance-corrected) of egg brightness under controlled lighting conditions. We then plotted the average brightness measurements of pooled groups of differently pigmented eggs against their spectral absorbance when solubilized in Soluene-350 (Ozeki et al. 1996), subtracting out the absorbance of the unpigmented egg shell (Figure 5-2). This calibration curve corrected for the non-linearity of the photographic measurements with regards to light intensity (Stevens et al. 2007), and allowed us to approximate the relative amount of pigment in eggs in subsequent experiments simply by taking photographs and converting the resulting brightness measurements to PI.

Demonstration of within-individual conditional plasticity in egg colouration

We then tested whether individual *P. maculiventris* are able to lay eggs of different pigmentation levels, and whether females modify egg pigmentation in response to the colour (reflectance) of the substrate on which they are laying. Individual females were monitored, over the course of their lives, in Petri dishes painted black, white, or half black/half white. Females were supplied with a mate, insect prey, and plant material. Eggs were collected from the dishes every 2-3 days to measure their PI. Individual females were able to lay eggs spanning the full range of pigmentation levels; the average difference in PI between the

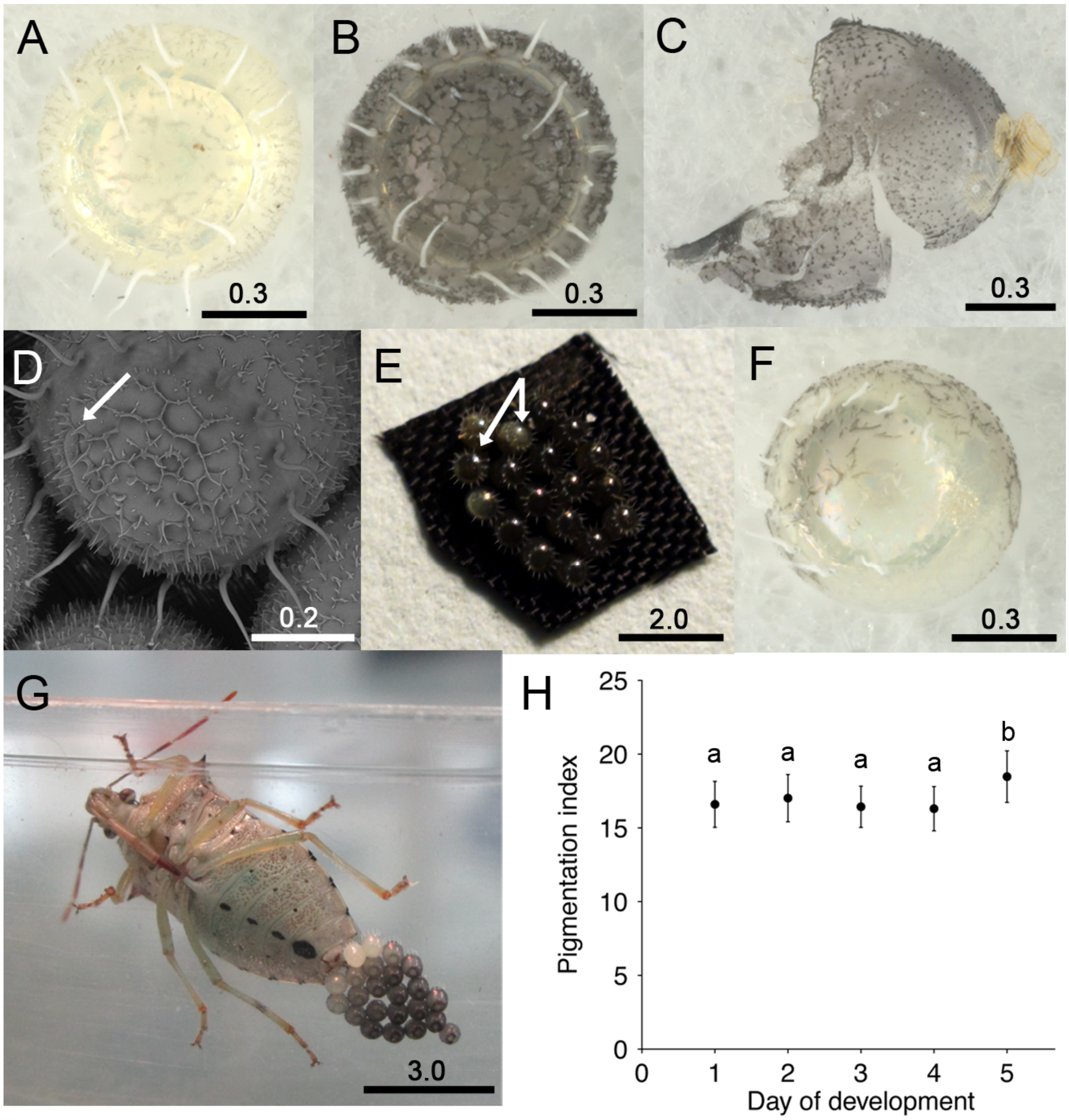

Figure 5-1. Description of *Podisus maculiventris* egg pigmentation. Overhead photos of (A) lightly (Pigmentation Index = 4.3) and (B) heavily (Pigmentation Index = 21.5) pigmented eggs are shown. Pigment is contained within the exochorion, seen removed in (C), particularly concentrated in exochorionic spines (D), which are also present on lightly pigmented eggs. Pigmentation rarely shows noticeable variability (E) within the same egg mass or (F) on the same egg. (G) The chorion is pale white immediately after laying, reaching its final pigmentation within an hour. (H) The apparent darkness of the egg varies slightly over a 5-day period due to embryonic development (linear mixed model with individual egg identity as a random factor; day of development $\chi^2 = 22.58$, $p < 0.001$, total $n = 25$ eggs), but not significantly so until the 5th day, soon before emergence of the nymph (different letters indicate significant differences between categories; Tukey contrasts, $p < 0.05$). All scale bar measurements are in millimeters.

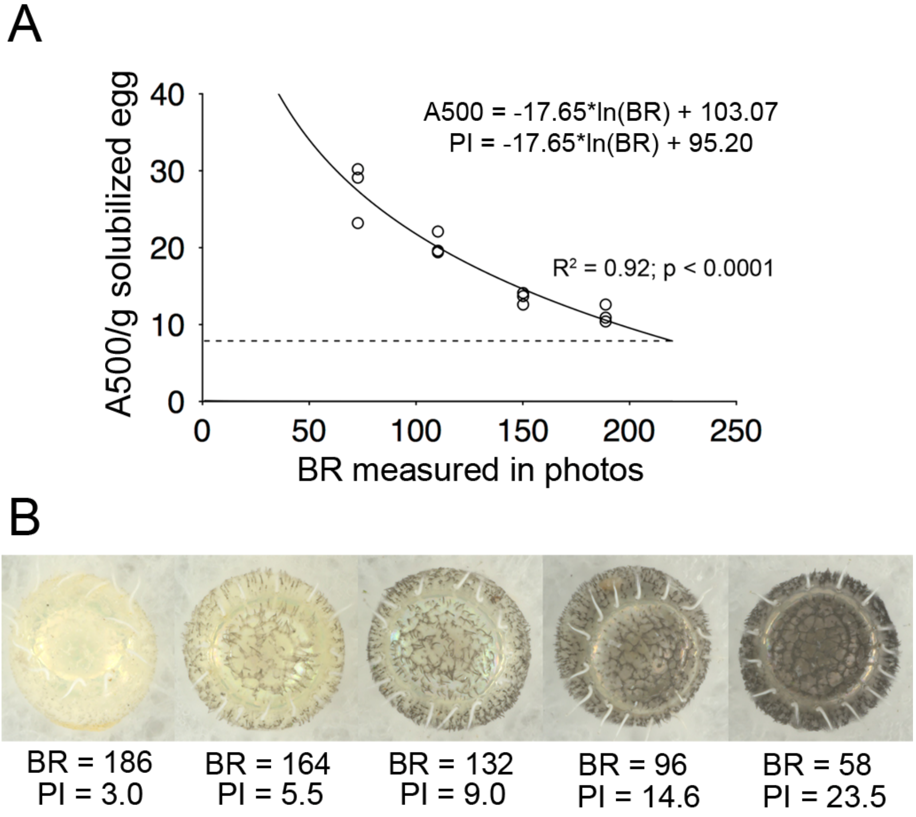

Figure 5-2. Quantification of *Podisus maculiventris* egg pigmentation**.** (A) A calibration curve of the absorbance of 500 nm light (A500) by solubilized *Podisus maculiventris* eggs versus their brightness (BR) measured in photographs, after subtracting out the background A500 of unpigmented eggs (7.87/g), was used to assign a Pigmentation Index (PI) to eggs of different pigmentation levels; examples shown in (B).

lightest and darkest egg laid was 15.97 ± 1.93 (mean ± 95% CI) and 20/35 individuals laid both heavily pigmented (PI > 15) and lightly pigmented (PI < 5) eggs during their lives. Eggs tended to be more pigmented in black petri dishes than white dishes, and more pigmented when laid on the bottom of dishes than when laid on the side of the dish or the underside of the lid (Figure 5-3). In half black/half white dishes, there was a tendency towards less pigmented eggs on the white half of the dish, although it was not significant when controlling for the more important effect of laying position (Figure 5-3). These results provided evidence of context-dependent plasticity in egg pigmentation in *P. maculiventris*, supported the hypothesis that substrate reflectance plays a role in determining egg pigmentation, and revealed the previously unexpected importance of laying position.

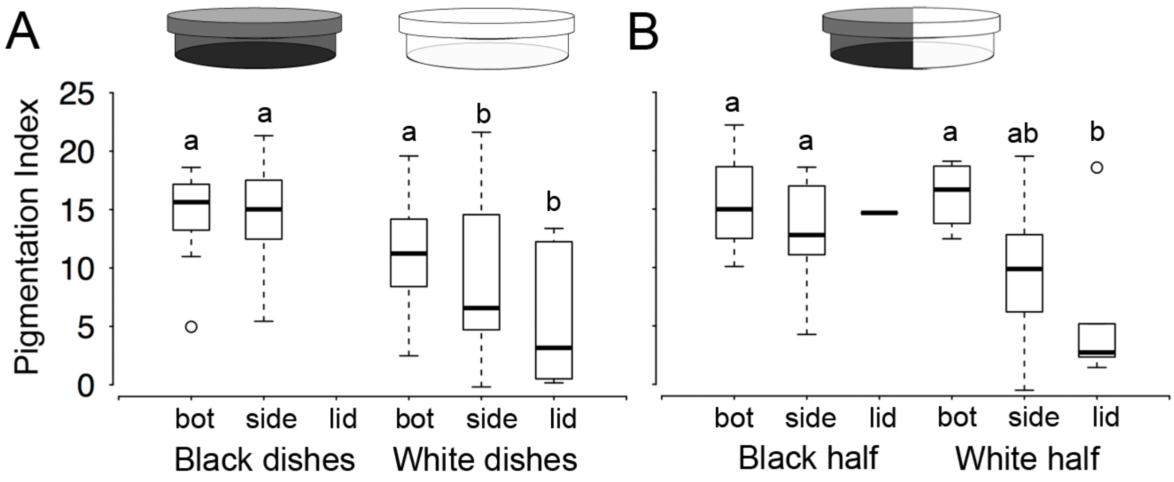

Figure 5-3. The pigmentation of eggs laid by *Podisus maculiventris* in painted Petri dishes, depending on whether eggs were laid on the bottom (bot), side, or inside lid of the dish. (A) Egg pigmentation index differed significantly between black and white petri dishes (linear mixed model with female ID as random factor; $\chi^2 = 11.58$, $p < 0.001$), and varied among laying positions ($\chi^2 = 11.37$, $p = 0.0034$). (B) Egg pigmentation was only marginally different between the two sides of half black and half white petri dishes ($\chi^2 = 3.39$, $p = 0.066$), but varied among positions ($\chi^2 = 9.09$, $p = 0.011$). Different letters indicate differences among categories ($p < 0.05$; Tukey contrasts following linear mixed model analysis). Categories missing or with single data points were excluded from analysis. Total N in (A)/(B): = 25/14 females; 128/58 egg masses.

Egg pigmentation is correlated with plant structure and luminosity levels

We reasoned that the response of egg pigmentation to laying location in the previous experiment could be the expression of an evolved response to plant structure, which can modulate exposure to biotic and abiotic mortality factors (Brodeur and McNeil 1992; Gutschick 1999; Onzo et al. 2010; Sudo and Osakabe 2013). Furthermore, although black and white oviposition substrates are not present in nature, the direction and intensity of light could change the apparent reflectance of plant leaf surfaces and elicit differences in egg pigmentation. For example, sunlight passing through leaves from above illuminates their lower surfaces and increases their reflectance levels relative to leaf tops. Thus, one would expect lighter eggs to be laid on leaf undersides if egg pigmentation level is negatively correlated with substrate reflectance. Furthermore, reduced lighting levels could cause the reflectance of all leaf surfaces to be lower overall, increasing egg pigmentation levels. To test these predictions, we placed groups of female *P. maculiventris* in cages containing soybean (*Glycine max* [L.]) plants, and measured the PI of egg masses laid on leaf tops and undersides. Cages were either exposed to full ambient lighting conditions or shaded to reduce luminosity levels more than fifty-fold. We found that *P. maculiventris* laid eggs that were on average 2.1 times more pigmented on the upper surface of leaves compared to those laid on leaf undersides (Figure 5-4). Bugs laid slightly more pigmented eggs (+17%) in the shaded cages, but the large difference between the pigmentation of eggs on leaf tops versus leaf undersides was maintained (Figure 5-4). Overall, 47.3% of eggs were laid on leaf tops in the fully lit cages, compared to 44% in the shaded cages; these proportions did not differ significantly between treatments (Fisher exact test; p = 0.84). We reasoned that the strong correspondence between laying position on leaves and egg pigmentation could be the key to understanding the adaptive significance of *P. maculiventris* egg colouration.

Egg pigmentation protects developing embryos against ultraviolet radiation

The peculiar tendency of some predatory stink bugs, including *P. maculiventris*, to lay many of their egg masses on the upper surface of leaves has been previously noted (Esselbaugh 1946). In contrast, most plant-dwelling arthropods, including many species of stink bugs, tend to lay their eggs on the undersides of plant leaves (Chew 1977; Colazza and Bin 1995; Sudo and Osakabe 2011; Potter and Woods 2013). Laying eggs on the undersides of leaves is generally considered to provide a sheltered microclimate for developing embryos, offering protection against wind, rain, overheating, and desiccation. Perhaps most significantly, leaves block the passage of UV radiation (Gutschick 1999), which could otherwise cause embryonic mortality by damaging cellular machinery and causing DNA replication errors (Cockell and Knowland 1999; Ohtsuka and Osakabe 2009). In other animals, including humans, pigments such as melanin can act as sunscreens by absorbing UV radiation (Cockell and Knowland 1999; Ortonne 2002). Thus, applying pigment to eggs laid on the tops of leaves could protect developing embryos from exposure to UV radiation. To test this hypothesis, we exposed egg masses of different pigmentation levels to four different doses of UV radiation during the 16h light period for each day of their development. The different doses were administered by varying the distance of egg masses from a UVA/B lamp, and for the lowest-intensity treatment, attenuating UV wavelengths (< 390 nm) using a filter. The probability of *P. maculiventris* embryonic survival decreased as the dose rate of UVA/B radiation administered to eggs increased (Figure 5-5). Furthermore, embryos were much more likely to survive at a given dose rate of UVA/B radiation when eggs were more pigmented (Figure 5-5). To our knowledge, this is the first convincing evidence of a pigment protecting insect eggs from UV radiation damage.

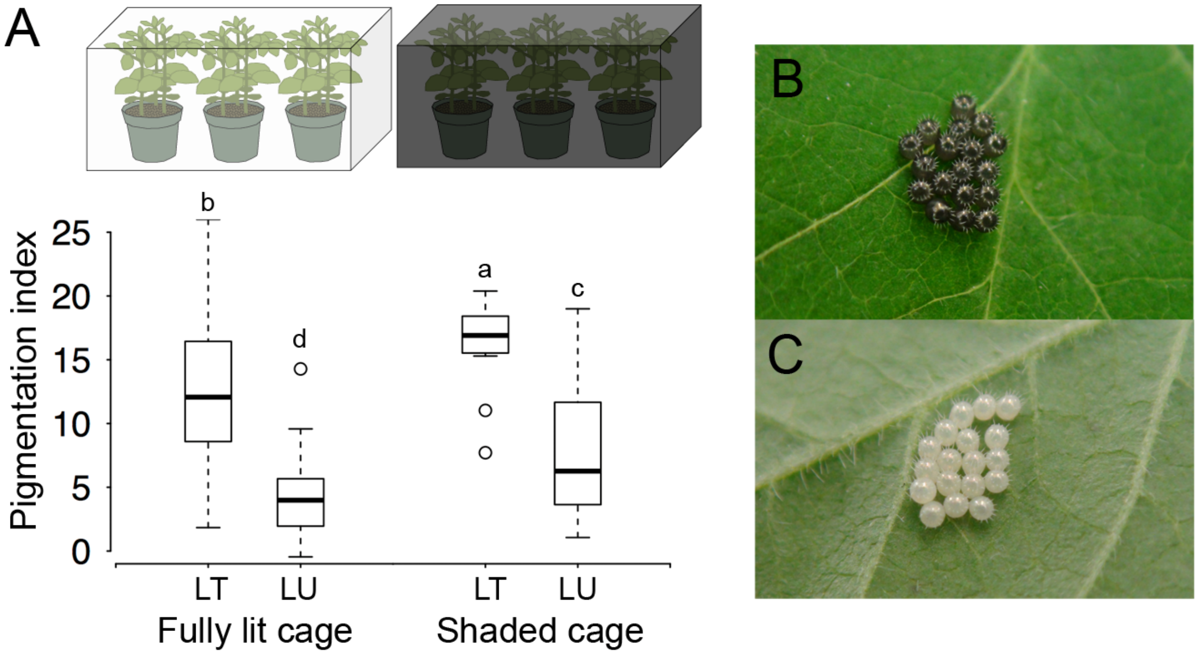

Figure 5-4. The effect of leaf position and luminosity level on egg pigmentation. (A) The pigmentation of eggs laid by *Podisus maculiventris* on soybean leaf tops (LT) or leaf undersides (LU) in cages that were either fully lit (12000-13000 lux) or shaded (200-300 lux). Different letters indicate statistically significant differences (adjusted $p < 0.05$; Tukey contrasts following linear mixed model analysis). Eggs were more pigmented on leaf tops (linear mixed model with experiment block as random factor; $\chi^2 = 72.04$, $p < 0.0001$), and in the shaded cage treatment ($\chi^2 = 14.18$, $p < 0.001$); there was not a significant interaction between leaf position and luminosity ($\chi^2 = 0.066$ $p = 0.80$). Total n = 110 egg masses. (B) a heavily pigmented egg mass (PI > 20) laid on the top of a leaf. (C) a lightly pigmented egg mass (PI < 5) laid on the underside of a leaf.

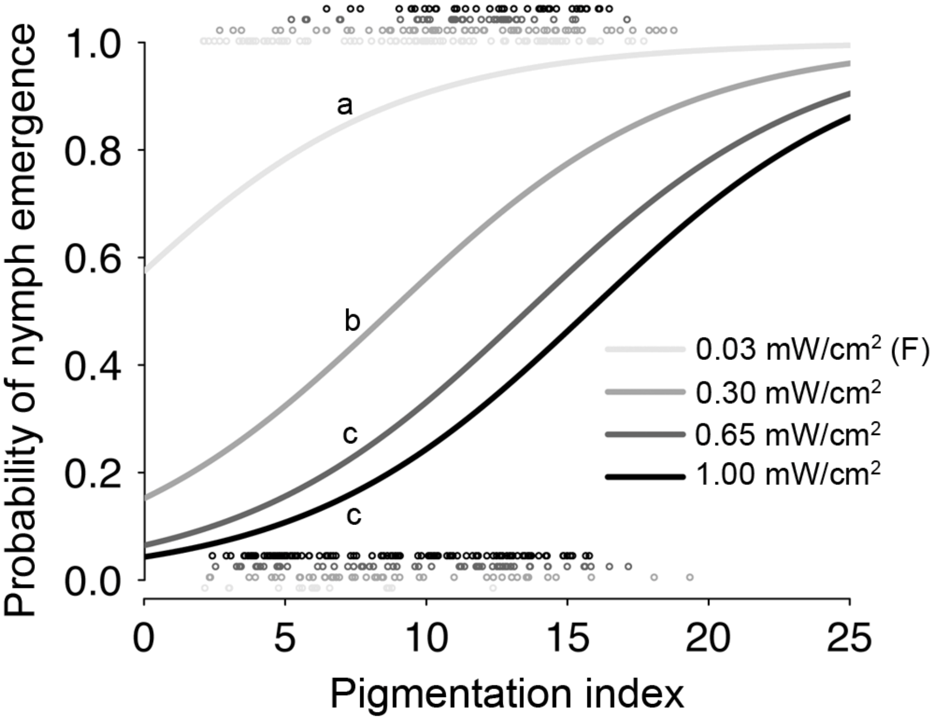

Figure 5-5. The probability of *Podisus maculiventris* nymphs successfully developing and emerging when developing in eggs of different pigmentation levels, and exposed to four different constant intensities of UV radiation emanating from a UVA/B lamp (300-390 nm; applied during 16 h light period of each day during development). 'F' indicates that a UV-filtering lens was placed over the eggs to achieve the given intensity. Points show the successes and failures of individual eggs (displaced vertically for clarity). Lines show predictions from a logistic regression model fitted to the data; both UV treatment ($\chi^2 = 129.07$, $p < 0.0001$) and pigmentation index ($\chi^2 = 46.61$, $p < 0.0001$) were significant predictors of emergence probability. Different letters indicate significant differences between UV treatments (Tukey contrasts; $p < 0.05$). Total n = 460 eggs.

Determination of egg pigmentation by females during oviposition

We next asked how egg pigmentation is controlled, and what cues could be involved. Eggs themselves could accumulate pigment in response to ambient levels of UV radiation, as has been observed in various life stages of other animals (Ortonne 2002; Debecker et al. 2015). Alternatively, female stink bugs may be able to detect the intensity of incident UV radiation (or visual wavelengths of light correlated with the presence of UV) and use this information to adjust the application of pigment to eggs. Another possibility is that *P. maculiventris* utilizes indirect gravitational or visual information to adjust pigment application to eggs. We attempted to distinguish between these hypotheses by conducting an experiment where individual stink bugs, contained in petri dishes, laid inside on the underside of white fabric illuminated from above, where we knew that they would tend to lay lightly pigmented eggs. To test whether egg pigmentation is influenced by the presence of UV light falling on the oviposition surface, we applied UVA/B radiation, filtered UVA/B radiation (wavelengths below 390 nm attenuated), or no light from below. In a fourth treatment, petri dishes were kept in complete darkness inside a closed box. Female *P. maculiventris* laid lightly pigmented eggs regardless of the type of radiation falling on the oviposition substrate (Figure 5-6). Furthermore, in the complete absence of any light, females tended to lay dark eggs on the underside of the white surface (Figure 5-6).

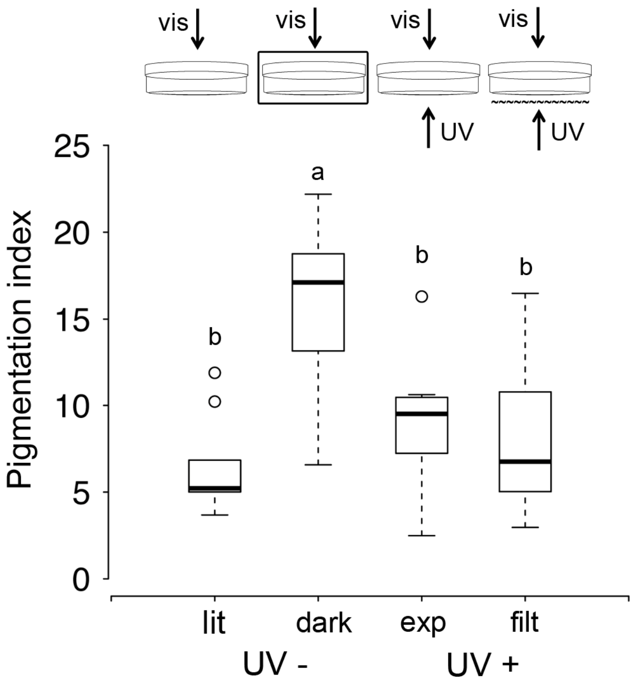

Figure 5-6. The pigmentation of eggs laid by *Podisus maculiventris* females on the underside of a white substrate, inside Petri dishes. Treatments: *lit* – visual light from above, no UV from below; *dark*: visual light from all directions blocked, no UV from below; *exp* – visual light from above, UV from below; *filt* – visual light from above, filtered UV from below. Pigmentation index varied significantly among treatments (linear mixed model with individual as random factor; $\chi^2 = 23.90$, $p < 0.0001$). Different letters indicate significant differences between treatments (Tukey contrasts; $p < 0.05$). Total n = 50 egg masses.

These results demonstrated that egg pigmentation (i) is not determined by the intensity of ultraviolet or visual light falling on the oviposition substrate, (ii) is not due to pigment accumulation by the egg in response to UV radiation, and (iii) is not determined by gravity. Rather, they support the idea that females evaluate visual characteristics of the substrate to determine egg pigmentation. Integrating the results of the previous experiment conducted on soybean plants (Figure 5-4), slightly more pigmented eggs may be laid in shaded environments because leaf surfaces appear overall darker, although extreme differences in luminosity were needed to produce such an effect. The fact that leaf position was a much more important determinant of egg pigmentation than luminosity suggests that ovipositing females may partially overcome this constraint by using a relative, rather than absolute, visual assessment of the substrate. The mechanism for this assessment could be an evaluation of the ratio of incident light (hitting the oviposition substrate) to reflected light (coming from the oviposition substrate). This ratio would be lower on the undersides of leaves, whose surface reflectance is increased by light passing through them from above. This proposed mechanism is similar to that suggested for crabs, prawns, and flatfish that dynamically modify their own pigmentation to match background brightness (Stevens et al. 2013). Given that temporal patterns of luminosity and UV radiation vary widely in nature (due to cloud cover, time of day, etc.), this kind of indirect, relative visual assessment could actually be a more reliable indicator of eggs' future cumulative UV exposure than direct measurement of light levels at the time of oviposition. The visual assessment of oviposition surfaces by *P. maculiventris* deserves further investigation, as it could reflect a general mechanism by which insects, including those that do not pigment their eggs, select oviposition sites.

The egg pigment is not melanin

Most dark pigmentation in insects is attributable to melanin (True 2003), a pigment composed of monomer units connected by strong carbon-carbon bonds, conferring a strong capacity to absorb UV radiation (Cockell and Knowland 1999). Expecting to confirm our suspicion that the *P. maculiventris* egg pigment is melanin, we conducted standard biochemical analyses to detect markers of the two known groups of animal melanins – eumelanins and pheomelanins (Ozeki et al. 1996; Wakamatsu et al. 2002; Ito et al. 2011; Wakamatsu et al. 2012).

Surprisingly, the amount of pigment in eggs (i.e., their spectral absorbance) was not correlated with the concentration of markers for either type of melanin, and the concentration of markers in the samples analyzed was extremely low overall (Figure 5-7). However, the spectral absorbance of heavily pigmented eggs was similar to that of sepia melanin (Figure 5-7). Thus, the egg pigment is not melanin, but appears to have a similar biological activity. Future work will focus on identifying the chemical composition and structure of this potentially novel pigment.

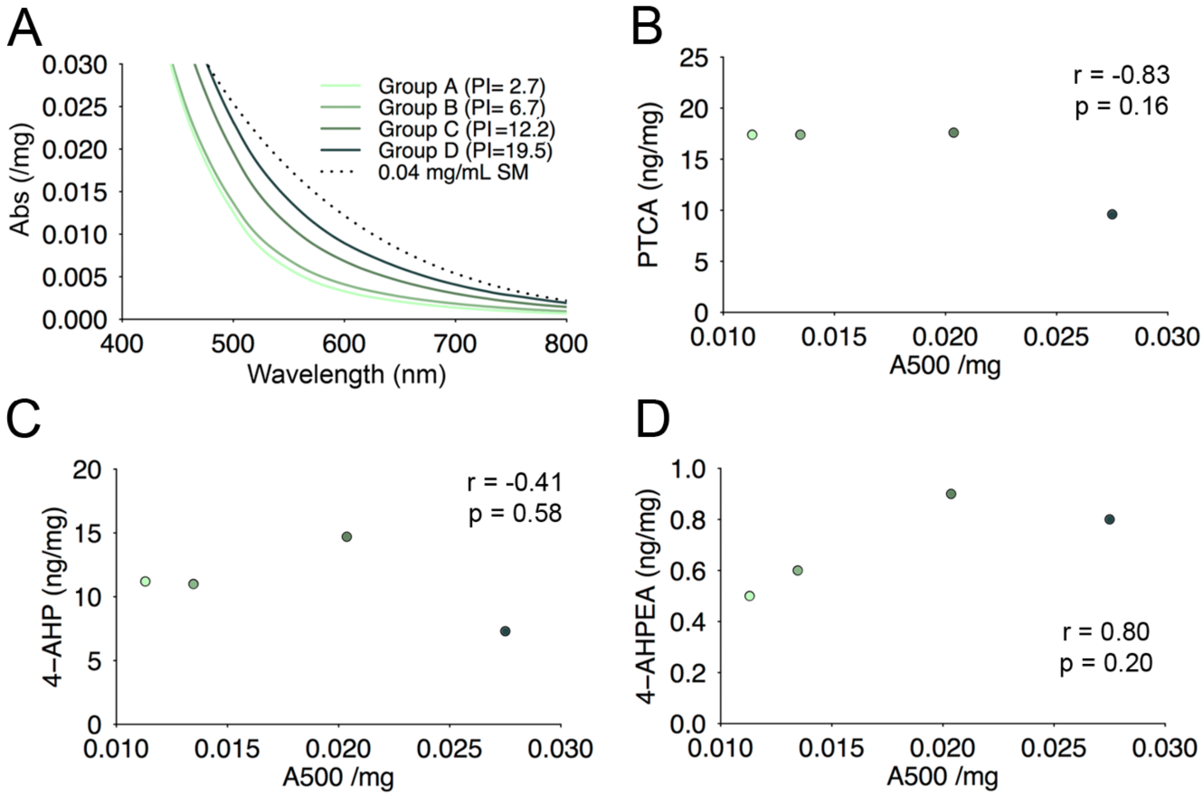

Figure 5-7. Spectral absorbance and biochemical analysis of *Podisus maculiventris* eggs. (A) Absorbance spectra of pooled groups of eggs with different pigmentation levels compared to 0.04 mg/mL sepia melanin (SM); Relationships between spectral absorbance at 500 nm (A500, marker for total melanin) and (B) PTCA (marker for eumelanin), (C) 4-AHP (marker for pheomelanin), and (D) 4-AHPEA (marker for cysteinyl dopamine-derived units). In (B)-(D) each point is the average of a duplicate assay.

The evolution of selective egg pigmentation

For oviparous animals, being able to selectively apply pigment to eggs would presumably widen the range of potential environments available for oviposition, while minimizing the costs of pigment production. Our study raises the question of why the egg colour of most animals is fixed, and by extension what set of conditions would be needed to favor the evolution of selective egg pigmentation. Firstly, a physiological mechanism would have to evolve by which mothers can selectively apply pigment to eggs; this mechanism is as yet unknown for *P. maculiventris* (or any other organism). Additionally, for selective egg pigmentation to be evolutionarily stable, at least two conditions would have to be met: (i) depositing eggs in locations where pigmentation is needed would have to result in a net increase in offspring survival, and (ii) laying non-pigmented eggs in locations where pigment is unneeded being advantageous in some situations. For *P. maculiventris*, the first condition could be met if predation pressure is higher on the undersides of leaves, as has been observed in other plant-dwelling arthropod systems (Brodeur and McNeil 1992; Sudo and Osakabe 2013). The upper surface of leaves would then represent ‘enemy free space’ (Jeffries and Lawton 1984; Fukaya et al. 2013), and applying the pigment when eggs are laid there would minimize the cost of the resulting tradeoff in terms of higher UV radiation exposure. The second condition could be met if there is a significant physiological cost of pigment production, as demonstrated in many other systems (Roff and Fairbairn 2013; Debecker et al. 2015; Roulin 2015). Laying eggs on the underside of leaves without having to pay the cost for applying pigment – while accepting the risk that eggs could be killed by predators – could be adaptive under some situations, especially if predators are uncommon or females are nutritionally stressed and have less resources to allocate to pigment production.

The possibility remains that the *P. maculiventris* egg pigment could provide additional, secondary benefits not explored in the current study. For example, plasticity in egg pigmentation could camouflage eggs, if the matching of egg pigmentation with substrate reflectance decreases the contrast between eggs and leaf surfaces with respect to the visual systems of predators and parasitoids. Indeed, some egg parasitoids of stink bugs have visual biases towards certain colours (de Aquino et al. 2012), although the extent to which these visual biases are important for short-range host localization is unknown. Additionally, dark egg pigmentation could potentially allow eggs to collect more radiative heat and develop more rapidly (Clusella-Trullas et al. 2008), though we suspect that temperature differences between leaf tops and undersides may not be enough to select for this adaptation. The possibility that egg pigmentation could have one or more secondary functions for *P. maculiventris* is currently under evaluation (Torres-Campos and Abram, in prep).

Conclusions

Although seldom studied to date, the pigmentation of insect eggs could have a wide variety of ecological roles, and may explain much of the variation in oviposition site selection and habitat use by insects in natural settings. Even though *P. maculiventris* is the first animal found to have selective control of egg pigmentation, we suspect that it is far from the only species with this adaptation. Indeed, the example described here occurs in an extremely well studied and economically important insect species that is reared in laboratories around the world, and was thus ‘hiding in plain sight’. A diverse array of similar adaptations, and a multitude of evolutionary variations on this theme, could be waiting to be discovered.

5.3 Materials and Methods

Study system

Podisus maculiventris is a predatory stink bug indigenous to North America that has also been introduced to other regions of the world as a part of classical biological control programs. Nymphs and adults feed on a wide variety of arthropods in diverse habitat types (McPherson 1982). The general biology and ecology of *P. maculiventris* has been extensively studied (e.g., Evans 1982; Ruberson et al. 1986; Weidenmann and O’Neil 1990), in part due to its frequent use as a biological control agent of arthropod pests in agroecosystems and mass production by the biological control industry. Eggs are barrel-shaped and metallic, typically laid in tight-fitting clusters of up to 50 (but typically between 8 and 20) eggs on the upper and lower surfaces of the leaves of many different plant species (Esselbaugh 1946; McPherson 1982).

Insect colonies

Colonies of *P. maculiventris* were established from individuals (~200) collected from several locations in the London and Ottawa (Ontario, Canada) regions in 2011 and 2012. Colonies were maintained continuously thereafter in ventilated cages 30 cm^3 (BugDorm, Taiwan) for late-instar (IV-V) nymphs, and ventilated plastic cylinders (diameter: 11.0 cm, height: 15.5 cm) for early-instar (I-III) nymphs. Nymphs and adults were fed with the larvae and pupae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and fresh green beans. Upon molting, adults were transferred to plastic bins (l: 29.0 cm, w: 17.0 cm, h: 10 cm) lined with green polyester fabric (Fabricville, Montréal, Canada) for oviposition substrate, from which egg masses were collected daily for experiments. When cohorts of adults of known ages were needed, newly-molted (<48 h) adults were separated into plastic cylinders in groups of 10-20 individuals until they were used for experiments, following sexual maturation and mating (ca. 7-10 days after molting). Unless stated otherwise, insects were kept at 24 ± 1°C, 50 ± 5% RH, and a 16L:8D photoperiod, at an illumination of 9000 ± 1000 lux, produced by linear fluorescent lights (Philips 86W F96T8/TL841/H0/Plus).

Quantification of egg pigmentation

To measure their pigmentation, eggs were first removed from the oviposition substrate and glued upright on white filter paper with non-toxic white glue (LePage©, Canada). Photographs of the eggs were then taken under standardized lighting conditions (9000 ± 1000 lux) under 14x magnification with a digital microscope camera (Dino-Lite AM-4012NZT, London, Canada) connected to a digital recording device (MSD09, Dino-Lite, London, Canada). Each photograph included a square of filter paper painted white on one side and black on the other, to subsequently allow the correction of white balance using ImageJ software version 1.48 (Rasband 2008) and its “Chart White Balance” macro. Next, in ImageJ, the circular area on the top of each egg, bounded by the egg’s chorionic processes, was selected and its brightness (=[image values of red channels + green channels + blue channels]/3) was measured as a proxy for egg pigmentation. These measurements corresponded well to qualitative visual assessments of egg pigmentation (see Figure 5-2), and were highly repeatable when the same eggs were photographed and analyzed separately by two different experimenters (Pearson correlation, r = 0.97, n = 50 eggs). Whenever eggs were photographed on green fabric instead of white filter paper, a correction (filter paper brightness = 0.9401*fabric brightness + 28.27) obtained by measuring the brightness of the same eggs on fabric and filter paper ($R^2 = 0.91$, n = 47), was applied. Measurements of egg brightness were always taken between 2-72 hours after eggs were laid.

Next, we developed a calibration curve to relate the brightness measurements taken in photographs to the actual quantity of pigment in eggs (measured via spectral absorbance). This was necessary to correct for the fact that measurements of brightness by imaging devices are non-linear with regards to light intensity, which can cause over-estimation of low reflectance values and under-estimation of high values (Stevens et al. 2007). We collected pooled groups of 300-400 eggs that belonged to four brightness categories (i) 60-80 (mean = 72), (ii) 100-120 (mean = 110), (iii) 140-160 (mean = 150), (iv) 180-200 (mean = 189). Samples of eggs (11-12 mg) from each category were directly subjected to Soluene-350 solubilization (Ozeki et al. 1996), with three replicates performed for each brightness category. The absorbance spectrum of each sample was measured in the visual light range (400-800 nm) and compared to a standard sample of 1 mg/mL sepia-melanin (compressed by a factor of 0.04). Spectral measurements below 400 nm were not possible because of strong absorption by Soluene-350 at lower wavelengths, although absorbance of melanin is known to increase exponentially into the UV range (Cockell and Knowland 1999). To calculate pigmentation index (PI), the absorbance of 500 nm light for each egg category was plotted against its average brightness, a regression curve was fitted to the data, and the predicted background absorbance of eggs with the highest possible brightness (220; i.e., the absorbance of background egg constituents in the absence of pigment) was subtracted out (see Figure 5-2).

Embryonic development and egg colour

To characterize how embryonic development (i.e., the darkening of egg contents) affected measurements of egg pigmentation, we measured the brightness of the same 25 eggs on each of five consecutive days after they were laid (nymphs emerge after 6 days). Brightness measurements were then converted to PIs for analysis. Eggs spanned a wide range of PIs (min: 6.4, max: 30.75, median: 16.6) on the first day of measurement (photos taken between 2 and

16 h after laying). To test whether egg pigmentation measurements varied significantly over the development period, we ran linear mixed models with PI as the dependent variable, day of development as a categorical fixed factor, and egg ID as a random factor.

Oviposition substrate reflectance

The goal of the first experiment was to determine the effect of substrate reflectance on the pigmentation of eggs laid by *P. maculiventris*, and to confirm that a single individual can produce eggs of different levels of pigmentation. Three types of Petri dish arenas (d: 9.0 cm, h: 1.8 cm) were prepared, differing with respect to the water-based acrylic paint (DecoArt, United Kingdom, DCA47-black and DCA01-white) applied to their entire outside surface: (i) B: black (n = 14), (ii) W: white (n = 11), and (iii) BW (n = 14): painted black on one side and white on the other (i.e., half of the dishes' lid and half of the bottom were painted with each shade of paint). The paint did not completely block the transmission of light through the lids of the dishes; lids were somewhat illuminated from light passing through them from above. Mating couples of *P. maculiventris* (7-10 days old) were placed in the arenas and provided with two *T. molitor* larvae and a small (4-6 cm) piece of green bean. Food was replaced and eggs were collected every 2-3 days, until the death of the female. Upon collection of eggs, their location within the Petri dish (top, side, or bottom; white side or black side in the BW treatment) was noted, and their brightness was measured, converted to PI and averaged for each egg mass. To compare the PI of eggs laid in B and W dishes, we fitted linear mixed models with PI as the dependent variable, dish colour and laying position as fixed categorical factors, and female ID as a random factor. The same analysis was run to compare the PI of eggs laid on the white and black half of the BW dishes. Exploratory data inspection clearly revealed no temporal trend in PI over the course of females' lives, so this factor was not considered.

Laying position on leaves and luminosity level

Next, an experiment was conducted to evaluate the effect of lighting level on the pigmentation of eggs laid by *P. maculiventris*, and to investigate how plasticity in egg pigmentation manifests itself when a natural oviposition substrate (i.e., plant leaves) is available. Ten female and five male *P. maculiventris* (10-20 days old) were placed in ventilated plexiglass cages (51.0 x 35.5 x 30.5 cm) containing three pots (d: 15.2 cm, h: 10.7 cm) with five soybean (*Glycine max* (L.)) cultivar BeSweet, 2001-11C, Stokes, Canada) plants (stage V2) each for 72h. Each of five full blocks contained two cages set up at the same time, one exposed to ambient lighting ("Fully lit": 12000-13000 lux) and the other completely covered with a single sheet of black polyester fabric (Fabricville, Montréal, Canada), which reduced luminosity levels inside the cage more than 50-fold ("Shaded": 200-300 lux). Eggs were collected, noting their position (leaf top, leaf underside). Egg masses laid on the plexiglass cage or on the pot were excluded from the analysis. For each egg mass collected, the brightness of a subset of five randomly selected eggs was measured, converted to PI, and averaged. With egg mass PI as the dependent variable, we then fitted linear mixed models with shading treatment and laying position as fixed categorical factors, and block as a random factor.

Effects of UV radiation and egg pigmentation on embryonic developmental success

To test whether egg pigmentation affects the developmental success of eggs under different doses of UV radiation, egg masses were first collected from the *P. maculiventris* colony, left on small (~1-2 cm^2) pieces of the green polyester fabric, and photographed to measure and correct the brightness of each egg (later converted to PI). They were then placed at one of four different UV intensities by varying their distances from a UV lamp (Exo-Terra Repti Glo 15W linear desert bulb, 41.5 cm long), which was resting on the top of an open glass frame (40.8 x 20.3 x 25.9 cm), under standard lighting conditions, producing an overall illumination of 8000 ± 400 lux inside the frame. The UV lamp simulated natural sunlight by emitting both visual and UVA/B-spectrum light, with UV emission ranging between 290 and 400 nm, peaking in the UV-A at 330-370 nm. Although other studies sometimes administer UV-A and UV-B radiation to arthropods separately (Onzo and Sabelis 2010; Potter and Woods 2013), we considered a full-spectrum treatment more representative of natural conditions, especially since there could be an interactive effect of UV-A and UV-B wavelengths (Krutmann 2006). UV intensity (mW/cm^2) was measured with a UVA/B light meter (model 850009, Sper Scientific, Scottsdale, AZ), whose responsivity curve ranged between 290 and 375 nm, peaking between 310-360 nm, and thus closely matched the output of the UV lamp. Egg masses assigned to the lowest intensity-treatment, 0.03 mW/cm^2, were at the same distance (22.1 cm) from the lamp as the 0.30 mW/cm^2 treatment, but a UV lens filter (Polaroid Pro Series 86mm Super Slim L39 MC UV Filter) was suspended 2 cm above the egg masses to attenuate the passage of UV light below 390 nm. The two remaining treatments of 0.65 mW/cm^2 and 1.0 mW/cm^2 were 14.1 and 9.3 cm from the UV lamp, respectively, and did not have a UV filter placed over them. The four intensities of UV radiation resulted in cumulative doses that are within the range present in exposed locations outdoors, based on measurements performed in Montréal, Canada in the summer of 2014 with the same UV-meter (P.K.A.; unpublished data). A household fan blowing under the UV lamp was used to equalize temperature at 25 ± 1°C throughout the vertical column of the setup. When the emergence of nymphs commenced, egg masses were removed from the setup and placed under standard rearing conditions inside glass tubes (d: 2.2 cm, h: 5.2 cm) noting the number of individuals emerging twice daily thereafter. Egg masses with no emergence were removed after 8 days, ~24-48 h after expected emergence (there was no subsequent emergence from these masses). The egg masses were inspected under a dissection microscope (40 x magnification) and the state of each egg (emerged or not) was recorded. Eggs that did not emerge contained embryos along the spectrum of maturation, although they typically died in the early stages of development. With emergence probability as the dependent variable, we then fitted a logistic regression with egg PI as a continuous factor and UV treatment as a categorical factor.

Oviposition under different UV exposure and lighting conditions

We next tested whether pigmentation of eggs is directly related to the presence of UV radiation and/or visible light. Adults were placed in transparent plastic Petri dishes (d: 9.0 cm, h: 1.3 cm) with white polyester fabric on inside of the upper surface, to create conditions where females would normally lay relatively lightly-coloured eggs, as determined by previous experiments (i.e., mimicking 'leaf underside conditions'). They were provided with two *T. molitor* larvae and three to four green bean seeds (instead of pods) to minimize the amount of light blocked from below. These arenas were placed under standard rearing conditions (9000 ± 1000 lux of illumination from above) at one of four conditions with respect to UV exposure and illumination: (i) *lit* – suspended 16.0 cm over a plastic tray, with no UV from below; (ii) *dark*: inside a black metal box (18.8 x 21.2 x 7.5 cm) lined with black foam, with 0 mW/cm^2 of UV radiation and 0 lux of illumination; (iii) *exp* – suspended 16.0 cm above a UV lamp (see above), with UV intensity from below ranging from 0.30 to 0.70 mW/cm^2, depending on location within the Petri dish; (iv) *filt* – placed 16.0 cm above the UV lamp and fitted with a UV filter (see above), with the resulting attenuated UV intensity from below ranging from 0.01 to 0.03 mW/cm^2. All aforementioned UV intensities were measured through the surfaces of the Petri dishes to account for the fact that the plastic attenuated UV intensity by ~3-5%. The experiment lasted for 48 h, after which eggs were collected and their position in the Petri dish was noted. Any eggs not laid on the white fabric were excluded from the analysis. The brightness of all eggs was measured, converted to PI, and averaged for each egg mass. We then fitted a linear mixed model to the data, with egg mass PI as the dependent variable, light treatment and laying position as fixed factors, and female ID as a random factor.

Biochemical analyses of the egg pigment

We conducted biochemical analyses to test for markers of eumelanin (pyrrole-2,3,5-tricarboxylic acid; PTCA), pheomelanin (4-amino-3-hydroxyphenylalanine; 4-AHP), and cysteinyl-dopamine derived units (4-amino-3-hydroxyphenylethylamine; 4-AHPEA), the last of which is a chemical precursor to pheomelanin. Egg samples of a known brightness (see above) were homogenized with Ten-Broeck glass homogenizer at a concentration of 10 mg/mL H_2O, and 100 µL (1 mg) aliquots were subjected to alkaline hydrogen peroxide oxidation (Ito et al. 2011) and hydroiodic acid hydrolysis (Wakamatsu et al. 2002). 4-AHPEA was analyzed as described previously (Wakamatsu et al. 2012). In these previous studies, the concentrations of the aforementioned markers correlate well with the amount of melanin (and thus spectral absorbance) in a sample. We conducted Pearson's correlation analyses to test whether the mean pigmentation index of a sample (i.e., its absorbance of 500 nm light) was correlated with the concentration of each marker after chemical analysis.

Statistical analyses

All statistical analyses were conducted with the R software package, version 2.15.1 (R Core Team 2013). For linear mixed models, assumptions of error normality and homoscedasticity were verified via evaluation of residual and quantile-quantile plots. We also made sure that logistic regression fits showed no signs of overdispersion (Crawley 2012). Tukey contrasts among levels of categorical factors were performed with the “glht” function in the “multcomp” package of R software.

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Chapter 6: A scenario for the evolution of selective egg colouration

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6.1 Abstract

Behavioural plasticity can drive the evolution of new traits in animals. In oviparous species, plasticity in oviposition behaviour could promote the evolution of new physiological or morphological traits of eggs by exposing them to different selective pressures in novel oviposition sites. In the predatory stink bug *Podisus maculiventris* Say (Hemiptera: Pentatomidae), egg pigmentation is associated with oviposition site selection. Females are able to selectively apply a pigment on their eggs depending on leaf side; laying lightly pigmented eggs on leaf undersides, and more pigmented eggs, which are more resistant to ultraviolet (UV) radiation damage, on leaf tops. In this article, we propose an evolutionary scenario to understand the evolution *of P. maculiventris* egg pigmentation and its selective application. In field and laboratory experiments, we tested the influence of a suite of ecological factors that: (i) could have favoured a behavioural shift toward using leaf tops as oviposition site and, thus, the evolution of a UV- protective egg pigment (i.e. a thermoregulatory benefit or exploitation of enemy-reduced space), and (ii) subsequently, could have led to the evolution of selectivity in egg pigmentation (i.e. camouflage or costly pigment). We found evidence supporting the hypothesis that a higher predation pressure on leaf undersides encouraged females to shift part of their oviposition effort to leaf tops. We also found that egg pigmentation shortened development time in exposed environments; this is the first evidence of an insect egg pigment providing a thermoregulatory advantage. Our results only partially supported the hypothesis that a constraint on pigment production could have favoured the evolution of selective egg pigmentation. Our study contributes to an understanding of how plasticity in oviposition behaviour could shape the responses of organisms to ecological factors affecting their reproductive success, and spur the evolution of new morphological traits.

Key words: behavioural plasticity, oviposition behaviour, enemy-free space, camouflage, thermoregulation, *Podisus maculiventris*

6.2 Introduction

Behaviour plays an important role in evolutionary processes. On one hand, behavioural plasticity can buffer the rate of evolutionary change by allowing organisms to avoid environmental selective pressures by moving away from them (Huey et al. 2003). However, an alternative view has also been advocated, wherein behaviour can set the pace at which evolution occurs (Wcislo 1989; Duckworth 2009). That is, behavioural plasticity can drive the evolution of novel genetically-determined traits by exposing individuals to different selective forces, when they change their way of interacting with the current environment or move to a new environment ('behavioural drive') (Zuk et al. 2014). Additionally, highly plastic behaviours, such as those that play an important role in life history, are more likely to favour the evolution of new traits, since individuals must be able to respond to environmental changes directly affecting their survival and reproduction (Zuk et al. 2014).

One set of behaviours that is expected to be subject to strong selective pressures is an organism's oviposition strategy, which determines when, where and how their eggs are laid. In oviparous species without maternal care, eggs are vulnerable to attack by natural enemies and exposure to the elements. Since eggs are immobile, they cannot respond to changes in mortality risk over time. This set of circumstances has shaped the evolution of "be prepared" strategies (Hamdoun and Epel 2007), involving the coevolution of maternal oviposition behaviour (e.g., selection of protected sites) with egg morphology and physiology (e.g., colour, patterning, temperature/dessication tolerance). For example, eggs of amphibian species that lay in concealed locations tend to have less melanin pigmentation than those of species that lay in open water where there are high levels of damaging ultraviolet (UV) radiation (Licht 2003). In biological systems such as these, the evolution of new physiological or morphological traits of eggs may have originally been favoured by plasticity in oviposition behaviour, exposing them to novel selective pressures.

Most plant-dwelling arthropods tend to lay their eggs on the undersides of plant leaves (Esselbaugh 1946; Ohtsuka and Osakabe 2009; Potter et al. 2009; Sudo and Osakabe 2011), where offspring are protected against abiotic risks such as wind, rain, desiccation, and overheating. Leaves also act as shelters against UV radiation since they contain compounds that absorb these damaging wavelengths (Gutschick 1999; Izaguirre et al. 2007). In the predatory stink bug *Podisus maculiventris* Say (Hemiptera: Pentatomidae), about half of egg masses are laid on the tops of leaves (Esselbaugh 1946), and egg pigmentation is associated with oviposition site selection (Abram et al. 2015). In fact, *P. maculiventris* has the ability to selectively control the pigmentation of its eggs, which vary from pale yellow to dark black/brown (Abram et al. 2015). Female *P. maculiventris* lay more pigmented eggs, which are more resistant to UV radiation, on the upper surface of leaves, whereas their eggs are lightly pigmented on the undersides of leaves (Abram et al. 2015). Other pentatomid species studied to date have non-pigmented eggs that are mostly (but not all) laid on leaf undersides (Esselbaugh 1946; Basnet et al. 2015), suggesting that the egg pigmentation strategy of *P. maculiventris* could have evolved from this ancestral state, operating with the existing behavioural plasticity in oviposition site selection. Thus, *P. maculiventris* egg pigmentation, and subsequently its selective application, could be the product of 'behavioural drive'. Building on this idea, we hypothesize that: (1) there are some ecological factors that have favoured a behavioural shift towards laying a higher proportion of eggs on the tops of leaves, (2) this behavioural shift subsequently created the selective pressure favouring the evolution of UV-protective egg pigmentation, and (3) an additional factor or set of factors have subsequently favoured the evolution of selective pigment application. These hypotheses are discussed in the 'Evolutionary scenario' below and mapped onto Figure 6-1.

In the present article, we tested each of the hypotheses proposed in the evolutionary scenario and their corresponding predictions. First, we evaluated the enemy free space, camouflage, and thermoregulation hypotheses under field conditions. We tested for differences in predation/parasitism levels and embryonic development times among egg masses with different levels of pigmentation, depending on leaf side (top or underside) and whether they were placed in an exposed or shaded environment. Additionally, in laboratory experiments, we evaluated (i) whether *P. maculiventris* egg pigmentation selectively responds to temperature, and (ii) whether the amount (modified by starvation) and rate of consumption (modified by temperature) of metabolic resources limits the amount of pigment available for eggs.

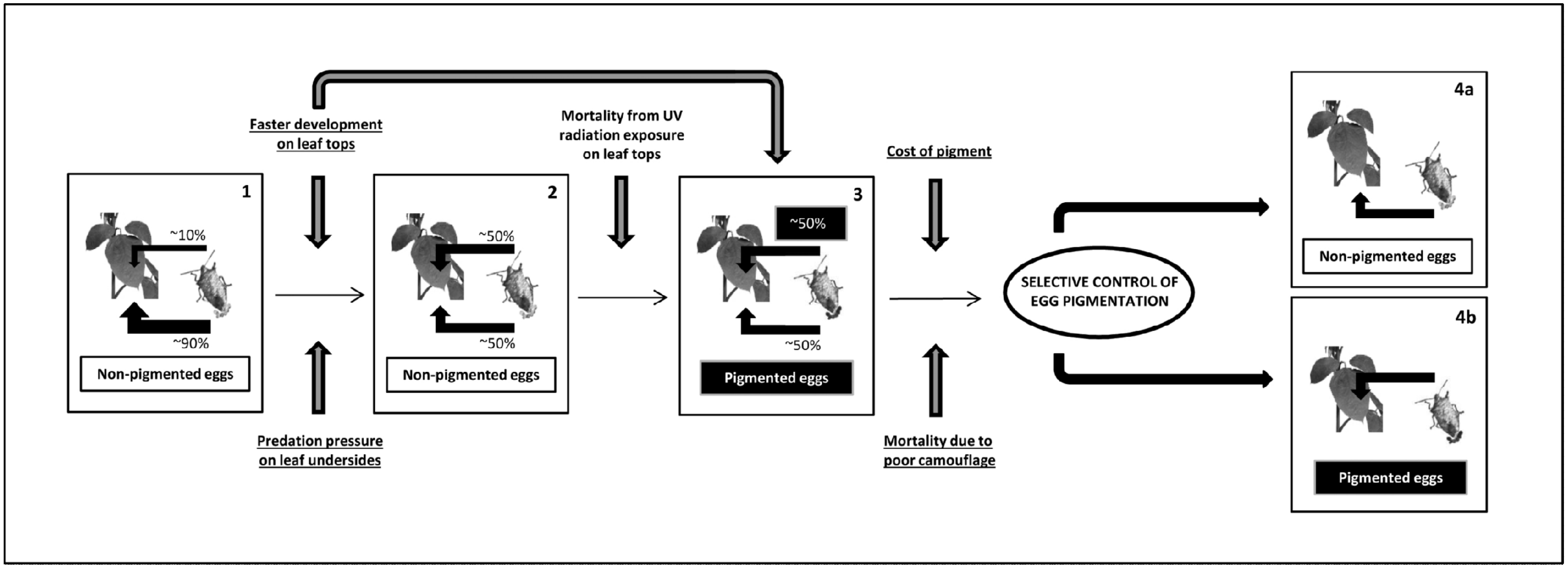

Figure 6-1. Diagram showing a hypothetical scenario for the evolution of selective control of egg pigmentation by *Podisus maculiventris*. In an ancestral state, *P. maculiventris* females would lay non-pigmented eggs preferentially on leaf undersides (Box 1). Ecological factors such as higher predation pressure on leaf undersides and/or faster egg development on leaf tops could have led stink bug females to a behavioural shift towards laying a higher proportion of egg masses on leaf tops (Box 2), exposing the eggs to a new selective pressure (UV radiation damage), which then favoured the evolution of egg pigmentation (Box 3). The evolution of selective control of pigment application could subsequently arise in response to selective pressures such as mortality due to poor camouflage and/or a constraint related to a physiological cost of pigment production (path from Box 3 to 4a-4b). Underlined text separates hypotheses tested in the present article from those explored in other publications (non-underlined text) (Abram et al. 2015).

6.3 Evolutionary scenario

Why would it be adaptive to shift a higher proportion of oviposition effort to leaf tops?

Enemy free space hypothesis– The upper surface of leaves could represent ‘enemy-free’ or ‘enemy-reduced’ space (Jeffries and Lawton 1984). Indeed, lower predation and/or parasitism pressure on the tops of leaves have been reported in other plant-dwelling arthropod systems. For example, Brodeur and McNeil (1989, 1992) observed that parasitoids modified their host’s behaviour, inducing its movement from underside to upper surface of leaves where levels of hyperparasitism and predation were lower. Similarly, some herbivorous mites species reside on the tops of leaves to mitigate predation risk (Sudo and Osakabe 2013). Sunlight levels could also interact with leaf side to influence on predation and parasitism pressures in a particular habitat. For example, Stoepler and Lill (2013) observed higher parasitism of two species of moth larvae in sunny habitats, although whether there was an interactive effect of plant structure was not evaluated. Thus, if the exploitation of enemy-free space provided the impetus for the ancestors of *P. maculiventris* to shift oviposition to leaf tops, one would expect to observe overall higher rates of predation and/or parasitism of eggs on the undersides of leaves, compared to leaf tops, possibly depending on sunlight levels.

Thermoregulation hypothesis– Laying eggs on leaf tops might also confer a thermoregulatory advantage. Previous studies have indeed reported that leaf surfaces can show thermal heterogeneity (Jones 1999; Caillon et al. 2014) and that development times on the tops of leaves tend to be shorter (Brodeur and McNeil 1992), reducing the duration of time during which individuals are susceptible to abiotic mortality factors and attack by natural enemies (i.e. the “slow- growth-high-mortality” hypothesis) (Benrey and Denno 1997). Thus, if surface temperatures are higher on leaf tops than on leaf undersides, this oviposition strategy could allow *P. maculiventris* eggs to accumulate more radiative heat and develop more quickly. Additionally, darker egg pigmentation could confer faster heating rates than light colouration at a given level of solar radiation, which is particularly important at low air temperatures (Kingsolver 1987; Jong et al. 1996). If this is true, one might expect *P. maculiventris* females to fine-tune selective egg pigmentation depending on temperature, laying more pigmented eggs under cooler conditions to maximize absorption of radiative heat (*sensu* the 'thermal melanism hypothesis'). Finally, this dynamic could also be affected by sunlight levels, which would reduce the overall amount of radiative heat available to eggs. One would thus expect higher rates of development on leaf tops and for darker-coloured eggs, but more so in exposed environments characterised by higher levels of solar radiation.

Why would selective egg pigmentation evolve?

In our hypothesized evolutionary scenario (Figure 6-1), a shift to laying eggs on leaf tops would subsequently impose a selective pressure to evolve pigment application to protect eggs from UV radiation. We next asked why selective egg pigment application evolved, whereby *P. maculiventris* females continue to lay lightly coloured eggs on the undersides of leaves.

Camouflage hypothesis– One possibility is that selective egg pigmentation camouflages eggs, resulting in lower parasitism and predation rates than would be achieved by pigmenting all eggs. In oviparous animals, especially birds, a widespread adaptive strategy to prevent egg predation is the application of pigment on eggs that matches background patterning (e.g., nesting sites) with the purpose of camouflaging the eggs against predators (Kilner 2006; Lovell et al. 2013). In our evolutionary scenario (Figure 6-1), evolving an egg pigment that protects eggs on leaf tops against UV radiation could cause eggs laid on leaf undersides to be poorly matched to substrate reflectance and suffer higher rates of attack by natural enemies. Leaf undersides have a high apparent surface reflectance due to sunlight passing through them from above, whereas the surface reflectance of leaf tops is relatively low (Abram et al. 2015). During the evolution of *P. maculiventris'* oviposition strategy, there could have been selective pressure to match the 'brightness' of eggs to the reflectance of oviposition substrates, reducing the contrast between eggs and leaf surfaces and providing camouflage against natural enemies. The validity of this hypothesis depends on whether the main predators and parasitoids of *P. maculiventris* eggs utilize visual cues, such as visual biases towards specific colours or the contrast between eggs and the oviposition substrate, to locate their prey/hosts. Although not much is known in this area, *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae), the most common parasitoid of *P. maculiventris* eggs, has a visual bias toward light colours

(Aquino et al. 2012). To provide support to the camouflage hypothesis, parasitism and predation rates would need to be higher when the colour of eggs is poorly matched to their oviposition substrate: light eggs would be attacked at a higher rate than dark eggs on leaf tops, and the reverse would be true on leaf undersides.

Pigment cost hypothesis– An alternative explanation for the evolution of selectivity in egg pigmentation is the influence of the physiological cost of pigment production, as has been demonstrated in several other species (Stoehr 2006; Roff and Fairbairn 2013). That is, female *P. maculiventris* may avoid applying the UV-protective pigment to eggs when it is unnecessary (i.e. on leaf undersides where eggs are already protected from UV radiation), to avoid having to pay the cost of pigment production – especially when external factors further constrain pigment synthesis. In previous studies, factors constraining pigment production such as nutrient limitation and temperature have received special attention (McGraw et al. 2002; Talloen et al. 2004). Starvation reduces the overall amount of resources available to allocate to different functions, while increasing temperatures increase the rate of metabolic resource utilization. When starved or experiencing higher temperatures, metabolic resources should thus be shifted away from reproduction (including pigment production) and towards functions more critical for survival. Here, one would thus expect less pigment to be applied to eggs when *P. maculiventris* females are starved, and when they are kept at higher temperatures.

6.4 Materials and Methods

Study system

Podisus maculiventris is a predatory stink bug native to North America. Nymphs and adults feed on many different arthropod species in a variety of habitats (McPherson 1982). Eggs, ranging from pale to dark in colour, are barrel-shaped, have a metallic appearance, and are laid in tight-fitting clusters of 4-50 eggs on the upper and lower sides of the leaves of many different plant species (Esselbaugh 1946; McPherson 1982). *Podisus maculiventris* eggs are vulnerable to attack by a variety of egg predators and parasitoids (Yeargan 1979).

Insect colonies

Podisus maculiventris colonies were established from individuals (~200) collected from several locations in the London and Ottawa (Ontario, Canada) regions in 2011 and 2012. Colonies were maintained continuously thereafter, fed with the larvae and pupae of mealworms (*Tenebrio molitor* L.; Coleoptera: Tenebrionidae) and fresh green beans. Green polyester fabric (Fabricville, Montréal, Canada) was used as oviposition substrate. Unless stated otherwise, insects were kept at 24 ± 1°C, 50 ± 5% RH, and a 16L: 8D photoperiod, at an illumination of 9000 ± 1000 lux, produced by linear fluorescent lights (Philips 86W F96T8/TL841/H0/Plus).

Quantification of egg pigmentation

In all experiments, we quantified the degree of egg pigmentation following methods detailed elsewhere (Abram et al. 2015). Briefly, egg brightness was measured digitally from photographs of eggs. We then used a calibration curve, obtained by measuring the spectral absorbance of solubilized eggs, to convert brightness measurements to Pigmentation Index (PI), correcting for the non-linearity of the digital photographs with regards to light intensity (Stevens et al. 2007; Abram et al. 2015). Resulting PI values for *P. maculiventris* can range from 0 (eggs with no pigment) to 30 (very dark eggs).

Parasitism, predation, and development time of eggs under field conditions

The field experiment tested for differences in predation/parasitism levels and embryonic development time among egg masses with different levels of pigmentation, depending on both leaf side (top or underside) and environment (exposed or shaded). This experiment took place on soybean (cultivar BeSweet, 2001 11C, Stokes, Canada) plants planted in peatmoss/perlite/vermiculite mix (Berger growing mix, Québec, Canada) in two large plastic bins (l: 1.27 m, w: 0.85 m, h: 1.07 m) spaced 5.5 m apart (in the N-S direction) in a mixed field of grasses and wildflowers located in a semi-urban environment next to the Montréal Botanical Gardens (N 45 33.717, W 73 34.302). The soybean was planted in late May (2014) at a density of 50 plants per bin, and was in the vegetative stage (V5+, omafra.gov.on.ca) at the beginning of the experiment in mid-July, and at the full seed stage (R6) at the end of the experiment in late August. Soybean was chosen because *P. maculiventris* and its natural enemies are often present in soybean fields in North America (Yeargan 1979). To create shaded conditions in one plot, one bin was covered by three layers (1.5 x 1.0 m) of black nylon mesh (mesh size: 2 x 4 mm) suspended 0.6-0.7 m above the top of the bin with wooden posts. The sides were left open and accessible to parasitoids and predators. The other bin ('exposed') was left unshaded.

Egg masses containing between 8 and 16 eggs were collected from the colony, on small pieces of green polyester fabric (1-2 cm^2), within 24 h of being laid. Their PI was then measured as described above. Egg masses spanning the full range of pigmentation were used, and were randomly assigned to one of four treatments on a haphazardly selected plant: (i) leaf top, shaded plot (n = 57); (ii) leaf underside, shaded plot (n = 57); (iii) leaf top, exposed plot (n = 70); (iv) leaf underside, exposed plot (n = 70). The pieces of fabric holding the eggs were affixed with a thin layer of waterproof, non-toxic adhesive (Gorilla Glue, Cincinnati, USA) to the uppermost set of mature trifolate leaves on each plant. After collections early in the season revealed that a large proportion of egg masses were lost (probably due to predation), we applied a ring (diameter: ~3 cm) of Tanglefoot glue (Grand Rapids, USA) on the leaf around a subset (n = 142/254) of egg masses from late July to late August, distributed among all leaf side/plot treatments: (i) leaf top, shaded plot (n = 29); (ii) leaf underside, shaded plot (n = 29); (iii) leaf top, exposed plot (n = 42); (iv) leaf underside, exposed plot (n = 42). We reasoned that the glue would exclude biotic (i.e., walking natural enemies during host/prey localization), but not abiotic (e.g., wind, rain) factors potentially responsible for missing eggs. Indeed, we commonly saw parasitoids (*T. podisi*) and predators (e.g. ladybird beetle adults and larvae, *Orius* sp.) trapped in the glue.

Egg masses were monitored for *P. maculiventris* nymph emergence twice daily, and nymphs were removed as they were counted. Eggs were left on the plants until 100 degree days (base 10.7°C; using Environment Canada Weather data for Montréal) had elapsed since they were placed in the field. This is 21.8 more degree days more than required for the egg-nymph development of *P. maculiventris* (De Clercq and DeGheele 1992), ensuring that all nymphs had emerged before eggs were removed from plants. Upon collection, eggs were brought back to the laboratory and placed in small, ventilated plastic cups (diameter: 4.2 cm, height: 1.5 cm) under standard rearing conditions to allow parasitoids to develop and emerge (~10-30 days after collection). Following parasitoid emergence, all egg masses were dissected to score the developmental state of each egg: unemerged parasitoid, unemerged nymph, incomplete nymph development (aborted), attacked by a predator, unknown.

To obtain estimates of the relative differences in abiotic conditions between plots and leaf sides, measurements of temperature, visual light intensity, and UVA/B radiation intensity were taken during the morning (10:00-11:30) and afternoon (16:00-17:30) of 12 different dates, temporally distributed throughout the experimental period (from mid-July to late August). The three variables were measured on the top and underside of one of the uppermost mature trifoliate leaves of ten randomly selected plants in the two treatments (shaded and exposed). Temperature was measured by placing a thermocouple (HH23 Microprocessor Thermometer, Omega, Laval, Canada) on top and underside of leaves surfaces. The intensities of visual light and UVA/B radiation were measured using, respectively, a digital lux meter (model LX101B, 550nm peak spectral sensitivity) and a UVA/B light meter (model 850009, SPER Scientific, Scottsdale, AZ, 310-360 nm peak spectral sensitivity). At each side, visual light and UV radiation were measured from above and from below, then pooled to calculate an estimate of the total incident radiation.

Temperature and the pigmentation of eggs laid under laboratory conditions

The first laboratory experiment tested whether temperature affects the pigmentation of eggs laid by stink bug females. Five females and three males *P. maculiventris* (11-25 days after moulting; mean = 18.36 days), were confined to five soybean plants, stages V1-V2, in a plastic pot (diameter: 15.2 cm, height: 10.7 cm) using a perforated plastic bread bag (27.5 x 45.0 cm) fixed to the rim of the plant pot with an elastic band. Five *T. molitor* larvae were provided as prey, confined to prevent them from escaping predation. Three replicates (i.e. plant pots) were performed at each of the three temperatures tested: 20, 25 or 30 ± 1 °C. These temperatures span the linear range of the species' developmental rate curve (De Clercq and DeGheele 1992). After 72 h, egg masses were collected and their position (leaf top, leaf underside, or on the plastic bag) was noted. Egg masses laid on the plastic bag were excluded from analysis. For each egg mass collected (n = 74), the brightness of a subset of five eggs was measured, averaged, and converted to PI.

Starvation and the pigmentation of eggs laid under laboratory conditions

We next tested whether pigment production by *P. maculiventris* females is constrained by nutrient limitation by both varying the quantity of available resources (either starving females of insect prey or feeding them *ad libitum*) and the rate of consumption of metabolic resources (temperature). Female stink bugs were collected while mating for the first time (7-10 days after moulting into adults). Following mating, females were isolated in Petri dishes (diameter: 9 cm, height: 1.5 cm) with a piece of green bean and two *T. molitor* larvae. Petri dishes were used instead of plants so that the arena size and type of plant resources could be standardized. The bottom interior surface of these Petri dishes was covered with black polyester fabric to create an oviposition substrate that would encourage females to lay more heavily pigmented eggs (see Abram et al. 2015) and create conditions where pigment stores could become limited. Females were then placed at either 25.0 ± 1 °C or 30.0 ± 1 °C, at a photoperiod of 16L: 8D and 50 ± 10 % RH. After the first 24 h, *T. molitor* larvae were taken away from half of the females (starved) and re-supplied daily for the other half (fed). Both feeding regimes had access to green beans, replaced every 24 h. Eggs were collected at least twice daily, noting their position in the arena and the PI of all eggs was measured (instead of a subset, in case there was more variability in egg mass colour caused by pigment limitation when females were starved) in order to calculate an average brightness for each egg mass, which was then converted to PI. Experiments lasted 7 days in total, and were replicated 10 times for each of the four feeding regime/temperature combinations. A total of 135 egg masses (1,457 eggs) were laid during the experiment. Cannibalism was recorded when it occurred (eggs were emptied of their contents).

Statistical analyses – Field experiments

To determine the statistical significance of associations between plot (exposed or shaded), leaf side (top or underside), and their interaction on temperature, lighting, and UV radiation intensity, we fitted linear mixed models, including sampling date and time of day as random factors. Lighting and UV intensity data were log (y + 0.5)- and log (y)-transformed, respectively, prior to analysis in order to meet model assumptions of error normality and homoscedasticity.

Next, we performed a set of analyses to determine the effects of plot, leaf side and egg pigmentation on the proportion of eggs that (i) successfully emerged, (ii) were not recovered, (iii) were parasitized, (iv) showed direct evidence of predation, or (v) were neither parasitized nor predated, but did not emerge. The first two proportions were calculated with all eggs in the mass as the denominator, while the latter three proportions were calculated with only the number of recovered eggs as the denominator. These analyses were performed over the entire experimental period, and only for egg masses that did not have glue applied around them (total n =112). Statistical significance was assessed with generalized linear mixed models (GLMMs) with a binomial error distribution. Setup date (the day egg masses were placed in the field) was included as a categorical random factor to account for temporal variability (we assumed that the measured factors were not necessarily linearly related to the amount of time elapsed since the beginning of the experiment) and to account for a lack of balance in the experimental design (i.e., different numbers of replicates performed for each setup day and some egg masses were not recovered). To account for over-dispersion, we also included observation-level random effects in these models (Elston et al. 2001; Harrison 2014). The significance of fixed factors in each model was determined using likelihood ratio tests (LRTs) comparing simplified models with and without the factor in question (Crawley 2012), also testing for interactions between fixed factors.

To examine the effect of glue application on the various mortality factors, similar analyses were performed, but using only data for the time period during which the glue was applied to a subset of egg masses (total n = 142), and including glue application (present or absent) as an additional factor in the analyses. The effect of glue application on parasitism was not tested, since the incidence of parasitism was too low during this period to ascertain significance (only two egg masses were parasitized).

The effect of plot, leaf side, and egg pigmentation on the mean time of embryonic development of each egg mass (in degree days; see above) was tested using a linear mixed model with the same random factors listed above, after verifying assumptions of model error normality and homoscedasticity. Egg masses with and without glue application were pooled for this analysis, after verifying that the glue did not have a significant effect on development time (LRT, $\chi^2_1 = 0.032$, $p = 0.86$).

Statistical analyses – Laboratory experiments

For the first laboratory experiment testing the effect of temperature on egg mass PI, we ran a linear mixed model with temperature and laying position as fixed factors and replicate (plant pot) as a random factor.

For the starvation experiment, with egg mass PI as the dependent factor, we again used a linear mixed model including starvation treatment, and temperature treatment as fixed factors, and replicate (stink bug individual), the number of days since the start of the experiment, and laying position (top, side, or bottom of inner surface of petri dish) as random factors. Using a GLMM (Gamma error distribution) with the same fixed and random factors, we also tested whether the within-egg mass coefficient of variation of PI varied significantly among treatments (i.e., whether high temperature or starvation caused higher variability in within-egg mass pigmentation). Finally, for each stink bug female, we tested the dependence of the total number of egg masses laid (GLM with Poisson error structure) the number of total eggs laid (GLM with Poisson error structure), and the proportion of eggs cannibalized (GLM with quasi-binomial error structure) on starvation and temperature treatment.

For all analyses, significance of each factor was determined using LRTs, as described above (except in the case of linear models and GLMs using quasi-likelihood, in which case F-tests were used). Residual plots were used to confirm adequate model fit. Predictions and 95 % confidence intervals (CI) for each model were obtained using the lsmeans package in R version 2.15.1 (R Core Team 2013), which was used to conduct all other statistical analyses as well.

6.5 Results

Parasitism, predation, and development time of eggs under field conditions

Temperature was higher in the exposed plot than the shaded plot, but was not affected by leaf side (Table 6-1; Figure 6-2A). Both the amount of lighting and UV radiation were affected by a significant interaction between plot and leaf side (Table 6-1; Figure 6-2B,C). Luminosity and UV radiation levels were overall higher in the exposed plot (compared to the shaded plot) and on leaf tops (compared to leaf undersides), and the relative differences between the two plots were more pronounced on leaf tops than on leaf undersides (Figure 6-2B,C).

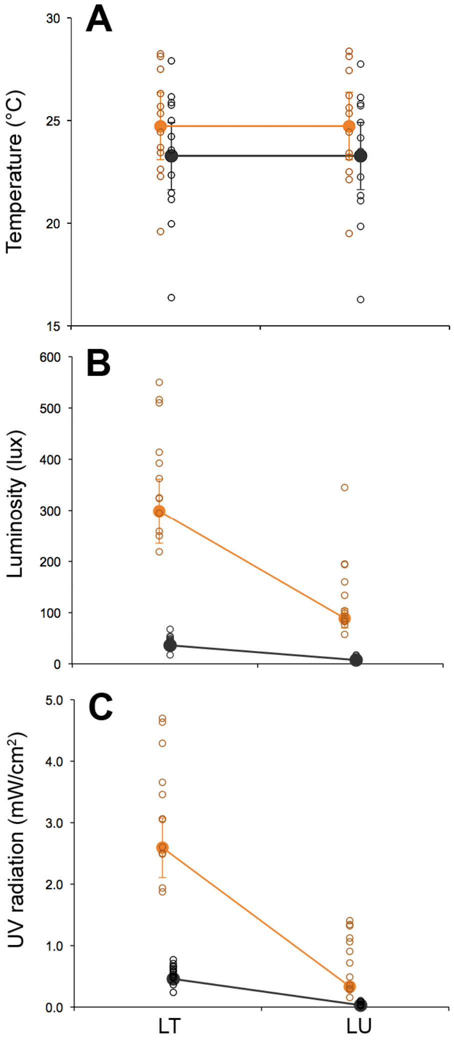

Figure 6-2. Abiotic conditions in the field experiment depending on plot (exposed – empty orange points; shaded – empty black points) and leaf side (LT – leaf top, LU – leaf underside). Each data point shows the average of ten measurements per leaf side, repeated twice a day (morning and afternoon), on a given sampling date. Fixed-effect estimates (large filled points) are back-transformed predictions (± 95% CI) from linear mixed models with plot and leaf side as fixed effects, and sampling date and time of day as random effects. See Table 6-1 and text of Results for statistical significance.

The overall percent emergence and the loss of *P. maculiventris* eggs to the various mortality factors measured in the field experiment are shown in Figure 6-3. Overall percent emergence was significantly higher in the shaded plot than in the exposed plot (Figure 6-3; Table 6-2), and although this parameter was not significantly affected by leaf side or egg mass PI, there was a tendency ($p = 0.051$) towards a higher percent emergence on leaf tops (Table 6-2). Similarly, the percentage of remaining eggs showing direct evidence of predation was significantly higher in the exposed plot, but was not affected by leaf side or egg mass PI (Figure 6-3; Table 6-2). The percentage of recovered eggs that were parasitized was not significantly influenced by plot, leaf side, or egg mass PI (Figure 6-3; Table 6-2). The percentage of eggs that were unrecovered was not affected by egg mass PI, but was influenced by a significant interaction between plot and leaf side (Figure 6-4; Table 6-2) – in the exposed plot, significantly more eggs were unrecovered when they were placed on the undersides of leaves (LRT, $\chi^2_1 = 12.42$, $p < 0.001$), whereas there was no significant difference in egg recovery between leaf sides in the shaded plot ($\chi^2_1 = 0.10$, $p = 0.75$).

Table 6-1. The statistical significance[a] of the influence of experimental plot (exposed vs. shaded), leaf side (top vs. underside), and their interaction on abiotic conditions in the field experiment. Significance of the main effects is not presented when the interaction between them was significant. See Figure 6-2 for effect sizes.

Variable	**Factor**	χ^2_{df}	**P**
Temperature (°C)	Plot	287.80	<0.0001*
	Leaf side	1.20	0.27
	Plot*Leaf side	0.0012	0.97
Lighting (lux)	Plot*Leaf side	24.07	<0.0001*
UV radiation (mW/cm^2)	Plot*Leaf side	846.81	<0.0001*

[a] Likelihood ratio tests comparing linear mixed models with sampling date and time of day as random effects.

Table 6-2. Statistical significance of plot (exposed vs. shaded), leaf side (top vs. underside), and egg mass pigmentation index (PI) for various mortality factors and development time of *Podisus maculiventris* eggs. Statistical information is not presented for random effects of mixed models, non-significant interaction effects, or main effects whenever their interactions were significant. See text of Results and Figures 6-4, 6-5, and 6-6 for effect sizes.

Measurement	**Factor**	χ^2_{df}	**P**
% overall emergence [a]	Plot	40.73	<0.0001
	Leaf side	3.80	0.051
	Egg mass PI	1.82	0.18
% unrecovered eggs [a]	Plot * Leaf side	4.46	0.035
	Egg mass PI	1.20	0.55
% emergence of recovered eggs [a]	Plot	4.60	0.032
	Leaf side	0.92	0.34
	Egg mass PI	0.19	0.66
% parasitism [a]	Plot	0.01	0.99
	Leaf side	0.00	1.00
	Egg mass PI	0.00	1.00
% predation [a]	Plot	483.54	<0.0001
	Leaf side	0.10	0.75
	Egg mass PI	1.75	0.19
development time [b]	Plot * Egg mass PI	8.01	0.019
	Leaf side	0.55	0.46

[a] Likelihood ratio tests (LRTs) comparing nested generalized linear mixed models (binomial error distribution).

[b] LRTs comparing nested linear mixed models.

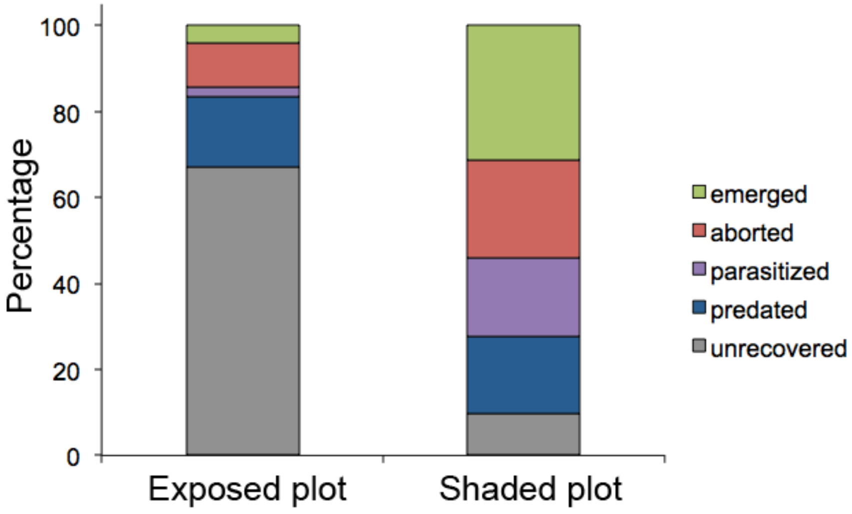

Figure 6-3. Percentages of *Podisus maculiventris* eggs that emerged, aborted, were parasitized, were predated, or were not recovered, in the exposed and shaded soybean field plots (only for egg masses without glue applied around them; total n = 56 egg masses/719 eggs in the exposed plot, 56 egg masses/704 eggs in the shaded plot). Data are pooled with respect to leaf side and egg mass pigmentation.

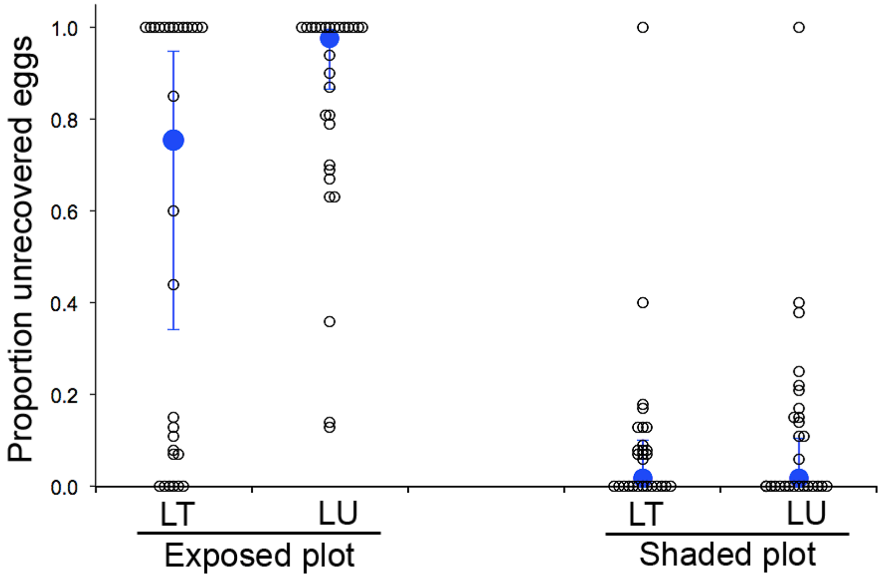

Figure 6-4. Proportion of *Podisus maculiventris* eggs that were not recovered in the field experiment, depending on plot (shaded or exposed) and leaf side (LT – leaf tops, LU – leaf undersides). Each data point represents a single egg mass; points with the same value are displaced horizontally for clarity. Large, filled blue points are predictions (± 95% CI; fixed effects only) from a generalized linear mixed model with sampling date and egg mass ID as random effects. See Table 6-2 and text of Results for statistical information.

Applying a ring of glue around egg masses resulted in an almost complete reduction in the proportion of eggs showing direct evidence of predation ($\chi^2_1 = 7.64$, $p = 0.0057$), causing a 97.7% reduction in the exposed plot and a 100% reduction in the shaded plot. The effect of glue application on the proportion of unrecovered egg masses depended on plot and leaf side (significant three-way interaction; $\chi^2_3 = 25.91$, $p < 0.0001$) (Figure 6-5). Applying glue around eggs in the exposed plot reduced the proportion of unrecovered eggs more on the underside than the top of leaves ($\chi^2_1 = 7.66$, $p = 0.0056$), whereas the proportion of unrecovered eggs in the shaded plot was not affected by glue application ($\chi^2_1 = 3.52$, $p = 0.061$), leaf side ($\chi^2_1 = 0.42$, $p = 0.52$), or their interaction ($\chi^2_1 = 0.76$, $p = 0.38$) (Figure 6-5).

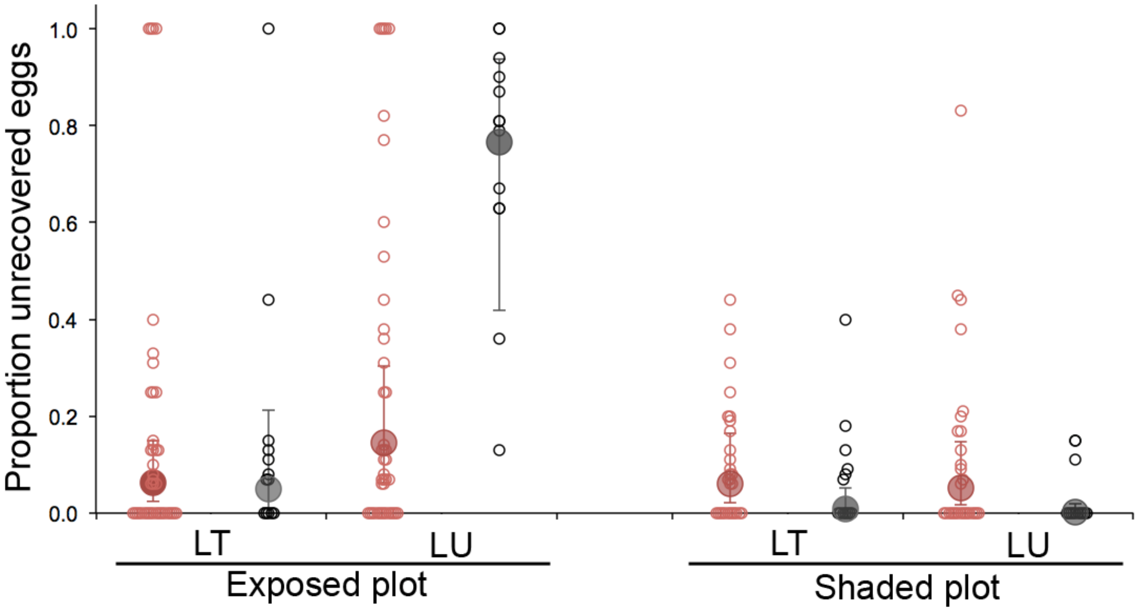

Figure 6-5. Proportion of *Podisus maculiventris* eggs that were not recovered in the field experiment (during the period of time where glue was applied around a subset of egg masses to partially exclude natural enemies), depending on plot (exposed or shaded), leaf side (LT – leaf top; LU – leaf underside), and whether or not glue was applied (red points – glue applied; black points – glue not applied). Each data point represents a single egg mass; points with the same value are displaced horizontally for clarity. Large filled points are predictions (± 95% CI; fixed effects only) from a generalized linear mixed model with sampling date and egg mass ID as random effects. See text of Results for statistical information.

The mean development time of *P. maculiventris* embryos, expressed as degree-days, was unaffected by leaf side but was influenced by a significant interaction between egg mass PI and plot (Table 6-2; Figure 6-6). Higher egg pigmentation was associated with a faster development in the exposed plot ($\chi^2_1 = 6.91$, $p = 0.0085$), whereas development time was not influenced by egg pigmentation in the shaded plot ($\chi^2_1 = 0.035$, $p = 0.85$) (Figure 6-6). The development time of light-coloured eggs was similar between the two plots, whereas darker-coloured eggs tended to develop more rapidly than light-coloured eggs in the exposed plot.

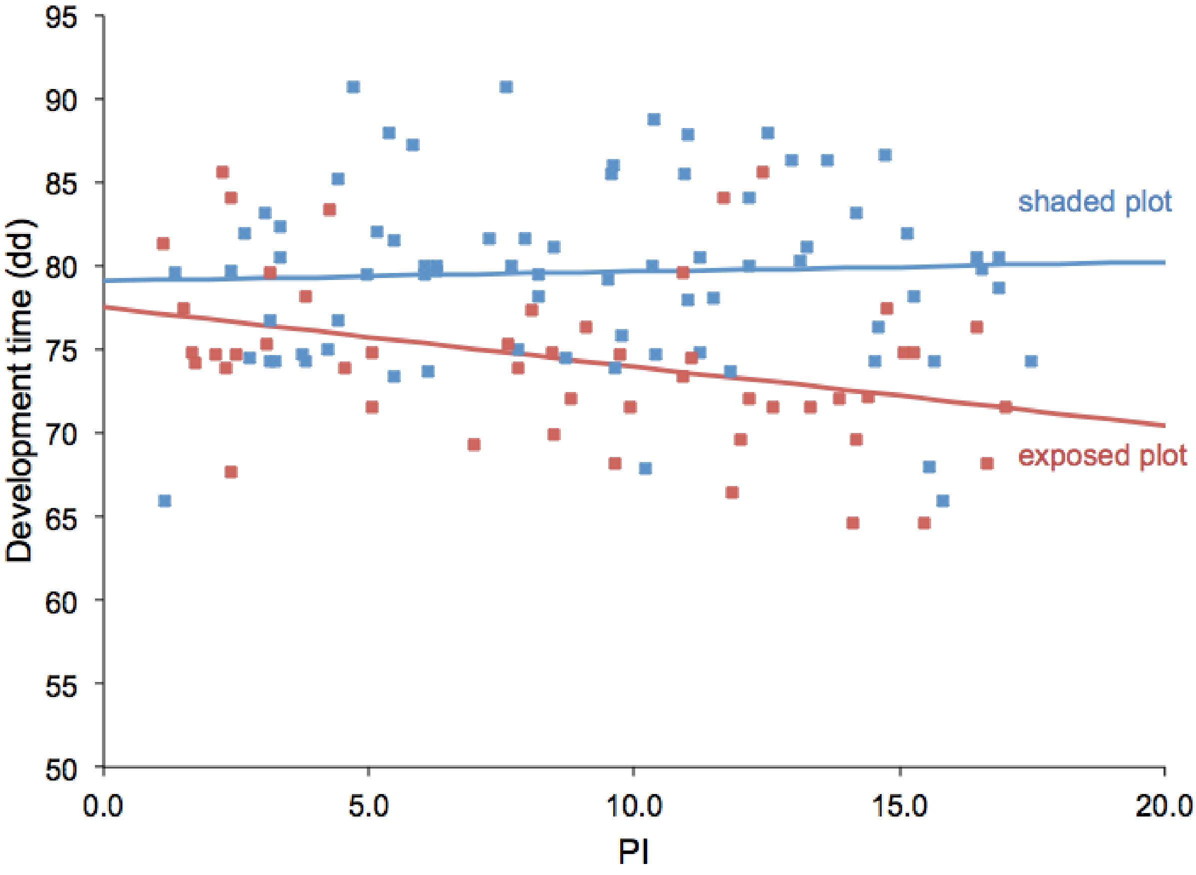

Figure 6-6. Embryonic development time, in degree days (dd), of *Podisus maculiventris* egg masses of different pigmentation levels (pigmentation index; PI) placed on soybean plants in the shaded or exposed plot (data for each plot pooled with respect to leaf side). Regression lines are derived from linear mixed model parameter estimates (fixed effects only; refer to Table 6-2 for statistical significance).

Temperature and the pigmentation of eggs laid under laboratory conditions

The PI of egg masses laid by *P. maculiventris* on soybean plants was affected by an interaction between laying side and temperature (LRT, $\chi^2_2 = 7.15$, $p = 0.028$) (Figure 6-7). Eggs were more pigmented on leaf tops than leaf undersides at all three temperatures, with between-position differences in PI ranging from 175 % at 25°C down to 94 % at 30°C. On leaf tops, females laid less pigmented eggs at 30°C, whereas egg pigmentation was unaffected by temperature on leaf undersides (Figure 6-7).

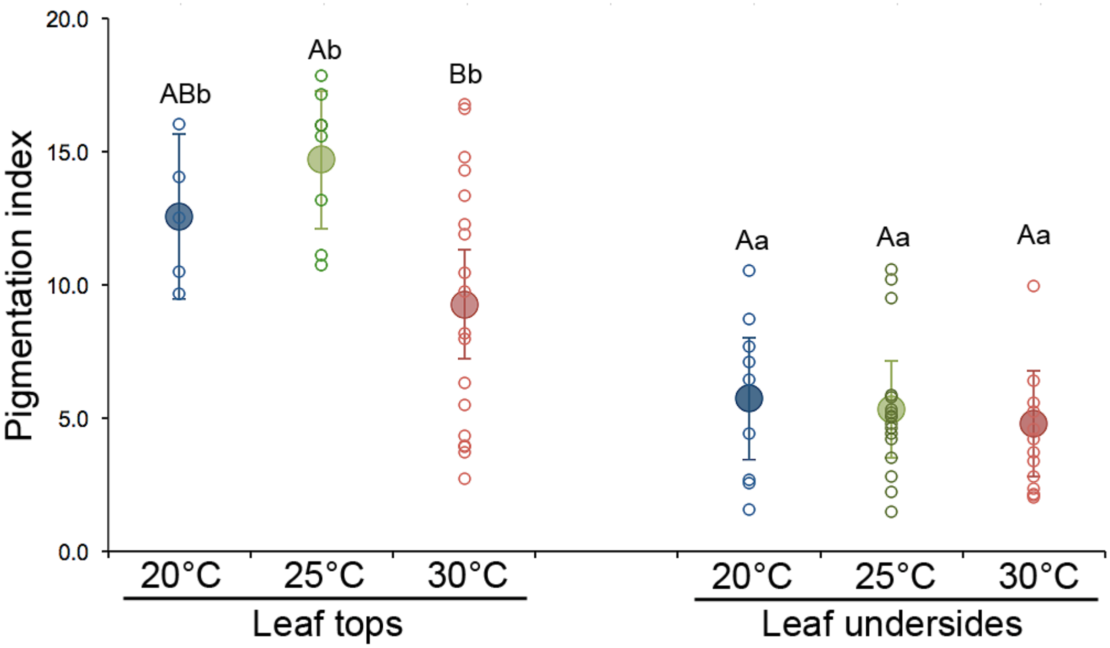

Figure 6-7. Pigmentation index (PI) of eggs laid on the tops and undersides of soybean leaves by *Podisus maculiventris* females in the laboratory, depending on temperature. Large filled points are predictions (± 95% CI; fixed effects only) from a linear mixed model with experimental block as a random effect (see text of Results for statistical significance of each factor). Letters above bars are from Tukey contrasts– Lower case letters: contrasts within temperatures between laying positions; upper case letters: contrasts within positions among temperatures. Model predictions not labelled with the same letter of a given case are significantly different ($p < 0.05$).

Starvation and the pigmentation of eggs laid under laboratory conditions

The pigmentation of egg masses was not different between fed and starved *P. maculiventris* females (Table 6-3). However, egg masses were overall less pigmented at 30°C than at 25°C (Table 6-3), in line with the results from the previous experiment (Figure 6-7). Within-egg mass variation in PI was not affected by starvation treatment, temperature, or the interaction of these two factors (Table 6-3). Females laid similar total numbers of egg masses across treatments, but laid on average 79% more eggs when fed (i.e., there were more eggs in each mass), independent of temperature (Table 6-3). The proportion of eggs cannibalized was increased significantly by starvation, and cannibalism was less prevalent at 30°C than at 25°C (Table 6-3).

6.6 Discussion

The goal of our study was to investigate a plausible evolutionary scenario for the egg colouration strategy of the predatory stink bug *P. maculiventris*, whereby behavioural plasticity in ancestral oviposition site selection would have driven the evolution of selective pigment application to eggs. We addressed two main questions: first, what pressure(s) would have selected for proportionally more eggs being laid on leaf tops (and thus spur the evolution of UV-protective egg pigmentation); and second, what pressures would have favoured the subsequent evolution of selective egg pigmentation. Our results support the idea that behavioural plasticity in oviposition site selection could have allowed a shift towards laying eggs in ‘enemy-reduced’ space on leaf tops, then exposing the eggs to a new selective pressure: UV radiation, which would in turn select for a protective pigment. However, why *P. maculiventris* continues to lay light eggs on the undersides of leaves remains unclear, as our hypotheses to explain the evolution of selectivity in egg received weak (camouflage hypothesis) or indirect (cost of pigment production hypothesis) support.

We suggested that the upper surface of leaves could represent a safer site for eggs with lower levels of predation and/or parasitism (‘enemy reduced space’). Our results indicated that the proportion of eggs that were parasitized, or showed direct evidence of predation, did not depend on the position of the eggs on leaves in both shaded and exposed environments. However, the proportion of unrecovered eggs, the most important mortality factor in our

Table 6-3. Mean (±SE) egg mass pigmentation index (PI), total number of egg masses laid, total number of eggs laid, and the proportion of eggs cannibalized by *P. maculiventris* females either fed *ad libitum* (F) or starved (S) with respect to insect prey, and kept at one of two constant temperatures.

Variable	**Starvation treatment**	**Temperature treatment**		**Statistical significance***		
		25°C	30°C	Starvation	Temperature	Starv ⊥ Temp
Egg mass PI[a]	F	14.73 ± 0.75	8.93 ± 0.72	$\chi^2_1 = 0.01$ $p = 0.99$	$\chi^2_1 = 15.75$ $p < 0.001$	$\chi^2_1 = 0.027$ $p = 0.87$
	S	14.79 ± 0.71	9.25 ± 1.04			
N total egg masses[b]	F	4.80 ± 0.61	2.57 ± 0.86	$\chi^2_1 = 1.74$ $p = 0.19$	$\chi^2_1 = 2.36$ $p = 0.12$	$\chi^2_1 = 0.25$ $p = 0.61$
	S	1.85 ± 0.59	1.14 ± 0.51			
N total eggs[b]	F	54.50 ± 8.77	52.67 ± 12.82	$\chi^2_1 = 121.22$ $p < 0.001$	$\chi^2_1 = 0.17$ $p = 0.68$	$\chi^2_1 = 0.14$ $p = 0.71$
	S	29.10 ± 6.62	29.40 ± 8.08			
% eggs cannabalized[c]	F	0.40 ± 0.28	0.00 ± 0.00	$F_1 = 128.93$ $p < 0.001$	$F_1 = 17.69$ $p < 0.001$	$F_1 = 0.25$ $p = 0.62$
	S	48.0 ± 8.3	15.0 ± 8.4			

[a] Likelihood ratio tests (LRTs) comparing linear mixed model with replicate (individual), number of days since the start of the experiment, and laying position as random factors. Means are pooled with respect to random factors; only significance of fixed factors is presented.

[b] LRTs comparing nested generalized linear models with poisson error structure.

[c] F-tests comparing generalized linear models with quasi-binomial error structure.

*Subscripts on test statistics indicate test degrees of freedom.

study, was overall higher for eggs placed on the undersides of leaves in the exposed plot, and was reduced on the undersides of leaves when glue was applied in a ring around eggs. A probable explanation is that a large proportion of unrecovered eggs were removed by predators without leaving direct evidence of predation. The most likely predator is the ladybird beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), which was commonly observed in the exposed plot. Indeed, in preliminary follow-up laboratory experiments, most *P. maculiventris* eggs attacked by adults and larvae of *H. axyridis* were completely removed from the substrate (P.K.A.; unpublished observations). Furthermore, ladybird beetles are known as important egg predators of other stink bug species (Ragsdale et al. 1981; Stam et al. 1987; Tillman 2008). *Harmonia axyridis* likely focuses the majority of its foraging efforts on leaf undersides because its most common prey (e.g., aphids) typically aggregate there, and thus the eggs of other potential prey such as *P. maculiventris* are at a greater risk of incidental intraguild predation. Greater predation risk on leaf undersides has previously been observed for aphid mummies and plant-feeding mites (Brodeur and McNeil 1992; Sudo and Osakabe 2013), and could be a general phenomenon mediating the distribution of insects throughout plant architecture. Although the identity of predators responsible for this trend remains to be directly demonstrated, the exploitation of ‘enemy reduced space’ (from predators, but apparently not parasitoids) remains a valid hypothesis for why *P. maculiventris* lays a relatively large proportion of eggs on leaf tops.

We hypothesized that laying eggs on leaf tops also has a thermoregulatory advantage, if surface temperatures are higher on the tops of leaves than on the undersides of leaves. Additionally, dark pigmentation could further accelerate development by allowing eggs to collect and retain more radiative heat (Kingsolver 1987; Jong et al. 1996). We found little support for the first hypothesis but did find good evidence for the latter. Temperature was similar between leaf tops and leaf undersides in either of the two plots, and, correspondingly, there was no difference in the development time of eggs between leaf tops and leaf undersides. However, development time decreased with increasing levels of egg pigmentation, but only in the exposed plot, presumably because of higher levels of radiative heat. To our knowledge, this is the first evidence of an insect egg pigment providing a thermoregulatory benefit. Rapid development mediated by dark colouration has, however, been observed in other life stages for several other arthropod species; for example, in the cabbage moth, high densities of larvae induce a switch towards dark phase larvae to develop faster and avoid competition for food (Goulson and Cory 1995). In addition, we found that in the laboratory, stink bugs did tend to lay more pigmented eggs at the two lower temperatures tested, similar to what is predicted by the thermal melanism hypothesis (Clusella-Trullas et al. 2007), in that dark colouration provides faster heating rates than light colouration in cooler environments. Although we cannot quantify whether differences in egg pigmentation among temperatures would be large enough to confer a significant advantage in terms of development time, it seems more likely that this result could have instead been due to a constraint on pigment production at higher temperatures (see below). Overall, our results suggest that laying a greater proportion of eggs on the tops of leaves probably did not evolve as a result of a thermoregulatory selective advantage; however, once the pigment evolved, it may have provided an additional benefit by allowing eggs to develop faster in exposed environments.

Assuming that shifting a greater proportion of oviposition effort on leaf tops spurred the evolution of egg pigmentation in *P. maculiventris*, what factor(s) would subsequently select for discriminating application of the pigment (i.e., ‘choosing’ not to apply it when egg masses are laid on leaf undersides)? Perhaps the most attractive explanation is that matching egg colour to substrate reflectance camouflages eggs from predators and parasitoids. We thus predicted that mortality from natural enemies would increase with increasing egg pigmentation on leaf undersides (which have a high surface reflectance due to sunlight passing through them), and the opposite trend on leaf tops (which have a relatively low surface reflectance). Our results did not match these predictions. Neither the egg mass colouration itself, its interaction with leaf side, or whether it was placed in an exposed/shaded environment affected the proportion of eggs that were predated, parasitized, or unrecovered. It is possible that other cues (e.g. infochemicals) play a more important role than visual cues associated with colour contrast during host/prey localization by natural enemies of stink bug eggs (Obata 1986; Fatouros et al. 2008).

The evolution of reproductive strategies is often shaped by ecological and physiological constraints. Thereby, we hypothesized that *P. maculiventris*’ strategy to preferentially apply pigment to eggs only when there is a high risk of UV exposure (i.e., when eggs are laid on leaf tops) (Abram et al. 2015), would minimize the physiological cost of pigment production. If pigment production is costly (Stoehr 2006; Roff and Fairbairn 2013), we would expect females to lay less pigmented eggs when subjected to metabolically stressful conditions (starvation, high temperatures). Overall, this hypothesis was partially supported by our data. The mean pigmentation level of eggs laid by starved females was not significantly different from those fed *ad libitum*, despite the fact that females deprived of food showed clear evidence of starvation; there were higher levels of filial cannibalism by starved females, and they laid fewer eggs per egg mass. Although starvation did not affect egg pigmentation levels, we cannot rule out the possibility that pigment production is costly – since starved females laid fewer eggs, an effect of nutrient limitation on pigment supply could have been masked by a reduction in the number of eggs laid (i.e., there was less pigment available, but also less eggs on which pigment had to be applied). We did observe that females laid overall less coloured eggs at above-optimal, stressful temperatures (30 °C) in two of our experiments. These results support our hypothesis that stressful temperatures could cause a shift in metabolic resource allocation away from pigment production and towards survival (somatic maintenance); or alternatively, that stressful temperatures negatively affect the efficiency of the biochemical reactions leading to pigment synthesis. However, we still cannot rule out that changes in egg pigmentation at different temperatures are actually adaptive (see above discussion of thermoregulatory benefits).

In addition to varying egg mass pigmentation and leaf side in our experiment, we also investigated how overall levels of solar radiation could interact with these factors to influence biotic and abiotic mortality factors of *P. maculiventris* eggs. There were clear effects of the exposed/shaded treatment on almost all parameters measured, with overall egg mortality being much higher in the exposed plot. Although there were large measured differences in abiotic variables between the two plots, these results should be interpreted with caution, since only one true replicate was performed for each shading treatment. Still, these results raise the question of why *P. maculiventris* would choose to lay in exposed environments, rather than in shaded environments, where a UV-protective pigment would not be needed, and overall egg mortality is lower. These costs could be partially offset by a shorter development time, although it seems unlikely that this would completely compensate for such an elevated mortality risk. Rather, *P. maculiventris* could choose to forage and lay eggs in exposed environments because of a greater abundance of herbivore prey (Lincoln and Mooney 1984; Louda and Rodman 1996; Barber and Marquis 2011), which would also provide a suitable habitat for emerging immature stages to find enough nutrients for growth and development, reducing the length of time that they spend in these more vulnerable stages (Refsnider and Janzen 2010). Additionally, the overall higher temperatures in exposed habitats could confer greater search rates and reduced handling times for foraging adults and immature stages (Sentis et al. 2012). We thus suggest that there may be a trade-off between prey availability/consumption rate and immature mortality (due to predation and UV radiation) in exposed versus shaded environments, but that the costs in exposed environments are offset by (i) laying eggs on the tops of leaves where predation pressure is lower, and (ii) applying a pigment to protect eggs from UV radiation damage and reduce the development time of its eggs. Thus, we hypothesize that the egg pigmentation strategy of *P. maculiventris* may have permitted this species to expand the range of potential environments available for reproduction to include exposed habitats with a greater abundance of prey.

In summary, we have provided some evidence in support of the hypothesis that ancestral plasticity in *P. maculiventris* oviposition site selection behaviour, allowing this species to shift its oviposition efforts from the underside to upper surface of leaves, could have driven the evolution of selective control of egg pigmentation (Figure 6-1). Future work should investigate how females make the choice of where to lay their egg masses under natural conditions, based on factors such as predation risk and nutritional status. For example, *P. maculiventris* may be able to detect cues from potential egg predators (Blaustein 1999; Rudolf and Rödel 2005), and use this information to dynamically shift more oviposition effort to the tops of leaves. This biological model system could prove to be ideal for the study of how animal egg laying strategies evolved in response to biotic and abiotic factors, and how behavioural plasticity can spur the evolution of novel morphological traits, and as a consequence the exploitation of a wider range of habitats. Although these issues remain to be thoroughly explored, they may be relevant to the reproductive strategies of many animal species.

6.7 Acknowledgements

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Chapter 7: Thermal stress affects patch time allocation by preventing forgetting in a parasitoid wasp

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7.1 Abstract

Learning and memory allow animals to adjust their foraging strategies through experience. Despite the known impact of temperature on many aspects of the behavioural ecology of animals, memory retention in the face of realistic thermal stress has seldom been assessed. In the laboratory, we studied the behavioural expression of an egg parasitoid's memory when exposed to thermal stress that could be encountered in nature. We hypothesized that thermal stress would disrupt memory consolidation and/or modify the optimality of memory retention, thus affecting patch time allocation strategies. Memory consolidation was resilient to 1h of thermal stress following an unrewarded experience (learning) on a patch of host-associated infochemicals. Neither high (40°C) nor low (10°C) thermal stress changed the intensity of the experienced wasps' behavioural response relative to those held at a moderate temperature (25°C). Next, we investigated how temperature stress could affect the parasitoids' memory retention ('forgetting'). When kept at a constant moderate temperature after learning, residence times of wasps re-tested on host cues increased relative to controls (naïve wasps) over a period of four days as they presumably 'forgot'. However, both hot and cool daily temperature cycles prevented forgetting; the residence times of re-tested experienced wasps in these treatments did not change relative to controls over time. We discuss to what extent this may be an adaptive response by the parasitoids versus a physiological constraint imposed by temperature. Our findings contribute to an understanding of the impact of thermal stress on foraging strategies that involve learning and memory.

Key words: learning, optimal memory window, temperature, foraging, host associated cues, *Trissolcus basalis*

7.2 Introduction

Learning and memory allow organisms to use information from past experiences to adjust their behaviour. Memories of learned experiences should not be retained indefinitely, however – as individuals move through their environment in space and time, information regarding the past may eventually become unreliable and it may be adaptive to 'forget' after a certain amount of time (Kraemer and Golding 1997). This optimal memory window is thought to be dynamic within individuals, depending on factors such as the direct costs of memory retention, environmental variability, and individual state (e.g., age, energy level) (Dunlap et al. 2009). An unexplored aspect of this framework is that animals could adaptively adjust the length of memory retention in response to abiotic stressors in the environment. Alternatively, abiotic environmental stress could act as a constraint by interfering with the mechanisms underlying memory consolidation or retention (Beck and Rankin 1995; Sangha et al. 2003; Knezevic et al. 2011; Teskey et al. 2012). However, the effect of natural abiotic stress on memory retention remains poorly understood, particularly in an ecological context.

Temperature is one of the most important abiotic factors affecting the ecology of many organisms. For ectotherm animals, which cannot typically regulate their body temperature internally, the rates of metabolic processes are in large part determined by environmental temperature (Gillooly et al. 2001; Clarke 2003), that in turn scale up to affect many aspects of their behaviour and ecology (Brown et al. 2004). For a given population, the functioning of physiological and behavioural processes is typically optimal within a certain thermal range. At 'extreme' or 'stressful' temperatures (i.e., near the lower and upper limits of a given animal's thermal tolerance), performance tends to decrease (Huey and Kingsolver 1989; Angilletta et al. 2002). Because temperature fluctuations are becoming more frequent and extreme as a result of global climate change (Stocker et al. 2013), it is increasingly relevant to study the responses of ectotherm animals to extreme temperatures. Although some studies have examined the effect of temperature stress during immature stages on subsequent adult learning capacity (e.g., Jones et al. 2005; van Baaren et al. 2005), studies on the effects of realistic temperature stress on memory retention following learned experiences are rare and have involved simple behavioural responses (siphon withdrawl reflex of a gastropod; Sangha et al. 2003; Teskey et al. 2012) whose general ecological significance is difficult to ascertain.

Animal memory could respond to stressful temperatures in adaptive and/or non-adaptive ways. On one hand, exposure to stressful temperatures could act as a constraint by eliminating less stable forms of memory that are present soon after learning (while memory is being consolidated), as has been observed in many ectotherm species when applying low-temperature anaesthesia (e.g., Erber 1976; Xia et al. 1998; Sangha et al. 2003; van den Berg et al. 2011). However, the temperatures applied in past studies are typically more extreme than those that would be experienced by the study organisms in natural situations. Once memories are consolidated, temperature stress could alter the rate of active forgetting processes (Berry et al. 2012; Hadziselimovic et al. 2014). A further possibility is that animals could adaptively alter the length of memory retention in response to thermal stress. The model of Dunlap et al. (2009), supported by some empirical investigations (Pravosudov and Clayton 2001; Friedrich et al. 2004; Orsini et al. 2004), predicts that organisms in a poor state (e.g., poor nutritional condition) with a high energetic cost of living should have the longest possible memory window, since employing a learned response to a familiar stimulus maximizes payoffs under these circumstances. It follows that the optimal memory window should increase under stressful thermal regimes when metabolic costs (e.g. lipid and carbohydrate consumption, cost of metabolic adaptations to thermal stress) are high. In order to examine the validity of these ideas, it is most relevant to test temperature regimes that occur in nature, in biological systems where the behaviours related to memories are closely linked to fitness.

Parasitoids are insects whose eggs and larvae develop in or on the body of other arthropods, whereas the adults are free-living (Quicke 1997). These organisms face the problem of finding hosts that are distributed in patches throughout complex and heterogenous environments. In order to focus foraging effort in areas where hosts are most likely to be present, many species of parasitoids make use of infochemical cues that may be associated with, but do not guarantee, the presence of hosts suitable for parasitism (i.e., indirect host-associated cues) (Fatouros et al. 2008). Learning allows foraging parasitoids to dynamically adjust their sensitivity to these infochemicals based on how reliably they signal the presence of hosts and adjust their patch time allocation strategies accordingly (Vet 1999; Fatouros et al. 2008; Vinson 1998).

Trissolcus basalis (Wollaston) (Hymenoptera: Platygastridae), a parasitoid of stink bug (Hemiptera: Pentatomidae) eggs, uses host adult walking traces as an indirect host-associated cue (Colazza et al. 1999). When parasitoids encounter a substrate contaminated with host walking traces, they show an arrestment response characterized by an increased turning rate and reduced walking speed that focuses searching and thus increases their residence time in the kairomone-contaminated area (Colazza et al. 1999, 2014). When parasitoids are not rewarded with an oviposition in host eggs within a certain amount of time, their sensitivity to host walking traces progressively decreases, they eventually leave the contaminated area, and they show a less intense arrestment response (i.e., decreased residence time, faster walking speed, and lower turning tendency) on the next patch they encounter (Peri et al. 2006). However, the arrestment response of wasps with an unrewarded experience can be restored to levels typical of naïve wasps if the host footprints are associated with an oviposition in host eggs or if more than 72 h elapses and they presumably 'forget' (Peri et al. 2006). This foraging strategy, which depends on learning and memory, shows similarities to classic mechanistic models of parasitoid patch time allocation (Waage 1979; Wajnberg 2006) and the underlying processes may generalize across many taxa.

In this study, we tested the effect of short- and long-term temperature stress on the behavioural expression of memory (patch time allocation) in *T. basalis* following an unrewarded experience with indirect host-associated cues under laboratory conditions. Following the above reasoning, we hypothesized that (i) realistic high and low temperature stress applied during memory consolidation would cause memory loss (amnesia), and (ii) the memory window (the length of memory retention) would be extended when wasps are held at stressful temperatures over the period of several days. This is the first study of the response of insect memory to thermal stress within a range that could be realistically experienced in nature and should set the stage for future investigations that examine underlying mechanisms and potential ecological consequences of such stress.

7.3 Methods

Study system

Trissolcus basalis is a minute (~1.5 mm in length), cosmopolitan egg parasitoid of many different species of hemipteran insects worldwide (Loch 2000; Salerno 2000; HOL 2015). One of its most closely-associated host species, *Nezara viridula* (L.) (Hemiptera: Pentatomidae), is extremely polyphagous, having been reported on over 30 families of host plants, and is an important pest of soybean cultivations (Todd 1989; McPherson and McPherson 2000). Adult *N. viridula* often move between habitats during the same season, sometimes using different sites for feeding, mating and oviposition (Todd 1989). *Trissolcus basalis* can use several different host-associated infochemical cues to localize host egg masses, including host-derived volatiles, herbivory- and oviposition-induced plant volatiles, and adult walking traces (reviewed in Fatouros et al. 2008; Conti and Colazza 2012; Colazza et al. 2014).

Insect Colonies

Trissolcus basalis and *N. viridula* colonies were established from individuals collected in Western Sicily, Italy, in the summer of 2014. *Trissolcus basalis* colonies were maintained by exposing *N. viridula* egg masses to groups of 5-7 female wasps in cylindrical glass tubes (volume: 85 mL), and emerging offspring were fed with drops of pure liquid honey. *Nezara viridula* colonies were housed in ventilated wooden cages (47.5 x 34.5 x 34.5 cm) and fed with cabbage (*Brassica oleracea* L.) leaves, sunflower seeds, tomatoes, and bouquets of field-collected *Solanum nigrum* L. White paper towel was provided as an oviposition substrate, and egg masses were collected every 2-3 days to maintain the *T. basalis* and *N. viridula* colonies. Female *T. basalis*, assumed mated, were isolated from the colony and placed in a small PCR tube (0.2 mL) with a drop of pure liquid honey the day before they were used for experiments. Females were 2-6 days old when tested, and had no oviposition experience. All insect colonies were maintained at 26±1°C, 16:8h L:D, and 60±10% RH.

General Bioassay Protocol

To measure the intensity of the behavioural response of parasitoid females to host walking traces, we used a protocol similar to that of Peri et al. (2006). Briefly, bioassays were conducted in open arenas with a 25 x 25cm sheet of filter paper, with a circular area (6 cm diameter) in the middle of the filter paper exposed to a single female *N. viridula* for 30 min, in order to treat it with the stink bug's walking traces (the rest of the arena was left uncontaminated). *Trissolcus basalis* females were then released singly into the center of the arena and observed until the wasp left the open arena (training bouts typically last about 200-250 s for naïve wasps; see Peri et al. 2006). The intensity of arrestment responses of parasitoids in these arenas in the various experimental treatments was assessed with "Xbug", an unpublished video tracking system and motion analysis software package, which allowed the determination of the parasitoid's patch residence time, mean linear velocity (walking speed), and tortuosity index (a measurement of how much the parasitoid's path deviated from a straight line; Peri et al. 2006). All bioassays took place in an isolated room maintained at 25 ± 1°C, with lighting on the arena provided by two 19cm-long fluorescent tubes.

For both experiments described below, parasitoids were exposed to temperature treatments by placing the closed PCR tubes containing the wasp in the heating block of a thermocycler (MasterCycler Personal, Eppendorf, Germany). To ensure that parasitoids were exposed to the temperature at which the thermocycler was set, they were confined to the part of the tube in contact with the heating block with a small piece of cotton inserted in the upper portion of the tube.

Experiment 1: The effect of thermal stress on memory consolidation

The goal of the first experiment was to test whether high or low temperature stress applied after an experience on host kairomones disrupted the parasitoids' memory consolidation. Although we do not know how long the anaesthesia-sensitive memory (ASM) phase lasts for *T. basalis*, this phase, during which memory is sensitive to cold shock, can range from less than an hour to more than two hours in other parasitoids (van den Berg et al. 2011; Kruidhof et al. 2012; Schurmann et al. 2015) and is typically less than one hour in *Drosophila* (Marguiles et al. 2005). We thus chose to apply the temperature stress immediately after learning to maximize the chances of disrupting memory consolidation. The three temperature treatments were: 10°C (low temperature stress), 25°C (control), and 40°C (high temperature stress). The two extreme temperatures are near the limits of the air temperature experienced by *T. basalis* during the period in which it has been recorded parasitizing hosts (May-October; Peri et al. 2014) under field conditions in Western Sicily. They are also near the upper (~42-43°C) and lower (~8-9°C) temperature thresholds that induce heat stupor and anaesthesia, respectively (P.K.A.; unpublished data). 'Experienced' female parasitoids were obtained using the following procedure: wasps were (i) released on a first arena with host cues (hereafter, training), (ii) re-collected into a PCR tube once they left the arena (end of training), and exposed to one of the three temperature treatments for 1h, (iii) acclimated inside the same tube in the bioassay room at 25°C for 15 minutes, and then (iv) released and filmed on a second patch of host cues ('testing') at 25°C, for a total training-testing interval of 1.25 h.

To obtain an estimate of wasps' baseline response to host cues in the absence of experience, and to disentangle the other effects of temperature stress from its effect on memory formation, we also tested 'naïve' females that were exposed to the same temperature treatments and acclimation period but had no previous experience (no training). Replicates were eliminated if the females did not show an arrestment response (i.e., flew away immediately when placed on the patch) on the second patch of host cues (7.6% of trials; non-responders were present in all treatments).

For each of the six treatments (naïve/experienced x 10°C/25°C/40°C), between 38 and 46 successful replicates were performed, for a total of 239 wasps tested. Experiments were performed over a period of nine days, between 8:45 – 15:30 h, with temperature and experience treatments balanced with respect to time of day.

Experiment 2: The effect of thermal stress on memory retention

The second experiment tested whether temperature affected the parasitoids' memory retention over a period of several days. Temperature data were obtained for Contessa Entellina, Italy (one of the collection sites for our culture of *T. basalis*), for 2009-2013. Since *T. basalis* actively forages for hosts between May and October in nature (Peri et al. 2014), we considered temperature data from only these months. The ten days with the highest (all during July and August) and lowest (all during May or October) average temperatures were selected, and the hourly temperatures for each period were averaged to produce a thermal profile of 'typical' cool and hot days that parasitoids could experience while foraging in nature (Figure 7-1). Experimental temperature regimes that could be reproduced in a thermocycler were visually fitted to each of these curves, having approximately the same average daily temperature (hot: ~30°C, cool: ~16°C; Figure 7-1). As a positive control, recreating the conditions of Peri et al. (2006) under which experience was forgotten after 72h, we included a third temperature treatment where wasps were kept at a constant temperature of 25 ± 1°C. Experiments were performed in blocks that included one of the two extreme temperature treatments and the control treatment; all blocks were then pooled for analysis after testing for similarity of the control treatment across blocks.

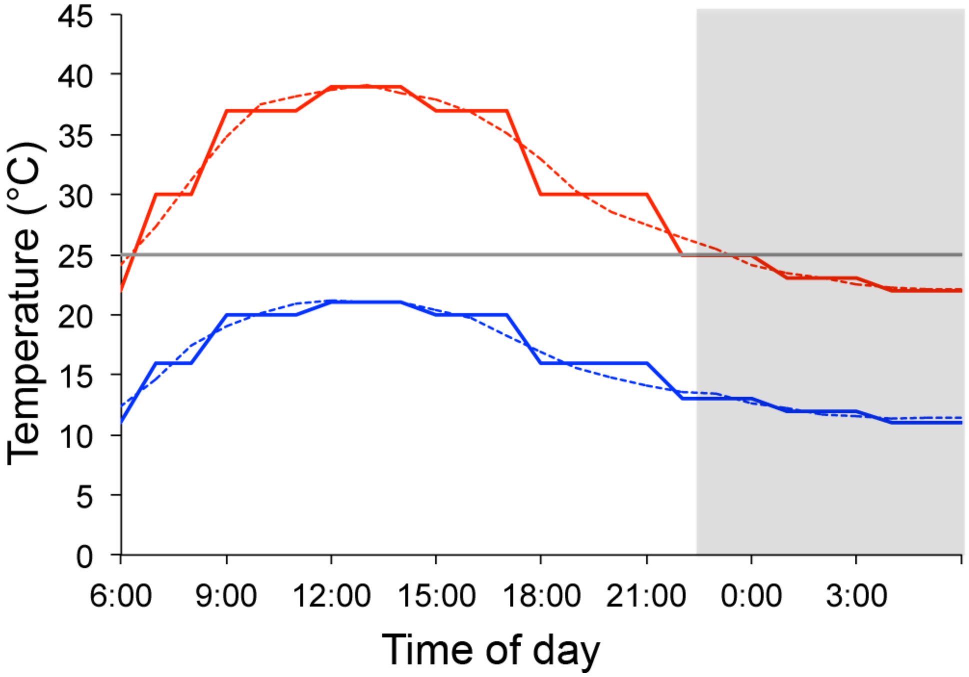

Figure 7-1. Hourly thermal regimes at which wasps were held between trials on host walking traces in Experiment 2 (solid lines; red – 'hot', blue – 'cool', grey – 'control'). For the hot and cool treatments, dotted lines show averaged hourly field temperature data on which the experimental regimes were based (see Methods). White background – photophase; Shaded background – scotophase.

Experienced wasps were trained on a first arena of host walking traces at 25°C, collected into a PCR tube, and immediately assigned to one of the three temperature regimes (hot, cool, or control). In parallel, an equal number of naïve wasps were assigned to each of the temperature treatments without training them on a contaminated arena. On the same day (1-5h after training) and during the three subsequent days (day 1: 18-30 h, day 2: 42-54 h, day

3: 66-78 h after training), subsets of wasps from each temperature/experience treatment combination were removed from the temperature treatments, acclimated at 25°C for 15 minutes, and tested on an arena of host walking traces at 25°C between 8:45 – 15:30 h (each wasp was re-tested only once). The above time intervals (hereafter, 'test intervals') thus represent the duration that wasps were held at the temperature regimes (for experienced wasps, the time since the end of training). After excluding wasps that did not show an arrestment response to host cues (4.1%), were lost (3.7%) or died before they were tested (2.2%), a total of 459 wasps were included in the analysis (241 control, 108 cool, 110 hot).

Statistical Analyses

For Experiment 1, we tested the dependence of the arrestment response (residence time, linear speed, and tortuosity index) on experience status (naïve vs. experienced), temperature treatment, and experience × temperature treatment interaction. We also statistically controlled for the time of day at which the wasp was tested by including it as a factor in the model when it was significant. However, we do not focus on its effects in our interpretation of the results since it was likely due to simple differences in parasitoid activity levels over the course of the day (Colazza and Pompanon 1994).

For Experiment 2, we tested the dependence of the arrestment response on experience, temperature regime, test interval, and time of day. Since our hypotheses include the possibility that the effect of experience over time could depend on temperature, we also tested for all possible two- and three-way interactions between experience, temperature, and test interval. When significant three-way interaction effects were present, we subsequently analyzed each temperature treatment separately to aid in the interpretation of the effects of other factors.
For both experiments, parametric survival models assuming a Weibull distribution ('survreg'; Crawley 2012; Therneau 2014) were fitted to residence time data (which were not normally distributed, typical of time-to event data), and linear models (assuming a normal error distribution) were fitted to velocity and tortuosity index data. Model fit was assessed with residual plots. Significance of each factor in the survival analyses was determined using likelihood ratio tests (LRTs) comparing the full model with and without the factor in question, starting with higher-order interactions (Crawley 2012). Significance levels for factors in the

linear models were derived directly from *F*-tests in the simplified model (containing only significant factors); statistical information given for non-significant factors is from when they are added to the simplified model.

All statistical analyses were carried out with R software, version 2.15.1 (R Core Team, 2013).

7.4 Results

Experiment 1: The effect of thermal stress on memory consolidation

Short-term temperature stress did not affect the wasps' behavioural expression of memory retention, since none of the characteristics of the arrestment response were significantly influenced by an interaction between temperature treatment and experience (Table 7-1). As expected, experienced *T. basalis* females previously trained on host walking traces showed a significantly less pronounced arrestment response than naïve females, spending less time on the arena, walking faster, and having a lower tortuosity index (Figure 7-2; Table 7-1). Temperature treatment had a significant effect on linear velocity independent of experience, with wasps walking slower after having previously been at 40°C than at 10°C, although this effect was not present for residence time or tortuosity index (Figure 7-2; Table 7-1).

Experiment 2: The effect of thermal stress on memory retention

The residence time of *T. basalis* females was influenced by a significant three-way interaction between experience, temperature, and test interval (LRT, df=5, χ^2=11.17, $P = 0.048$). The predictions of the survival model containing the three-way interaction (overall model significance: $\chi^2 = 180.75$, df=11, $P < 0.0001$) are plotted through the observed residence times of *T. basalis* females in Figure 7-3. Under the control temperature regime, the residence time of experienced females increased relative to that of naïve females with increasing time elapsed since their unrewarded experience (i.e., there was a significant experience x test interval interaction; Table 7-2), with the difference between the predicted residence time of naïve vs. experienced wasps decreasing from 133.3% (301.00 ± 22.34 s vs. 129.04 ± 10.68 s; Kaplan-Meier estimates ± SE) at 1h to 47.2% (290.29 ± 20.79 vs. 197.20 ± 15.35) after 72h (Figure 7-

3). Under the cool temperature regime, the residence time of naïve wasps decreased with increasing test interval, while it increased under the hot temperature regime (Table 7-2; Figure 7-3). However, these two temperature treatments differentially affected the residence time of experienced and naïve wasps (relative to the control temperature regime) – the residence time of experienced wasps increased less over time than would be expected if they showed the same response to the temperature treatments as the naïve wasps (Table 7-2; Figure 7-3). The responses to time interval of naïve and experienced wasps thus had similar slopes under the cool and hot regimes (i.e., non-significant time interval × experience interaction; Table 7-2), and meant that the difference between naïve and experienced wasps was just as pronounced three days after the unrewarded experience as the day on which they were trained.

Table 7-1. Statistical comparison of arrestment response characteristics of *T. basalis* females on host walking traces in Experiment 1, depending on whether they had previously been trained on another patch of host walking traces (experienced or naïve), exposure temperature between patches, the interaction of the two factors, and the time of day at which the test took place.

Measurement	**Factor**	**Test statistic$_{df}$**	***P***
Residence time[a]	experience	$\chi^2_1 = 41.13$	< 0.0001
	temperature	$\chi^2_2 = 3.34$	0.19
	experience × temperature	$\chi^2_2 = 1.84$	0.40
	time of day	$\chi^2_1 = 9.16$	0.0025
Linear velocity[b]	experience	$F_{1,235} = 66.17$	< 0.0001
	temperature	$F_{2,235} = 6.45$	0.0019
	experience × temperature	$F_{2,233} = 0.90$	0.41
	time of day	$F_{1,234} = 1.75$	0.19
Tortuosity index[b]	experience	$F_{1,236} = 5.28$	< 0.0001
	temperature	$F_{2,234} = 1.26$	0.29
	experience × temperature	$F_{4,232} = 1.23$	0.30
	time of day	$F_{1,236} = 12.25$	< 0.001

[a] Significance was assessed with LRTs comparing survival models.

[b] Significance was assessed with F-tests comparing nested linear models

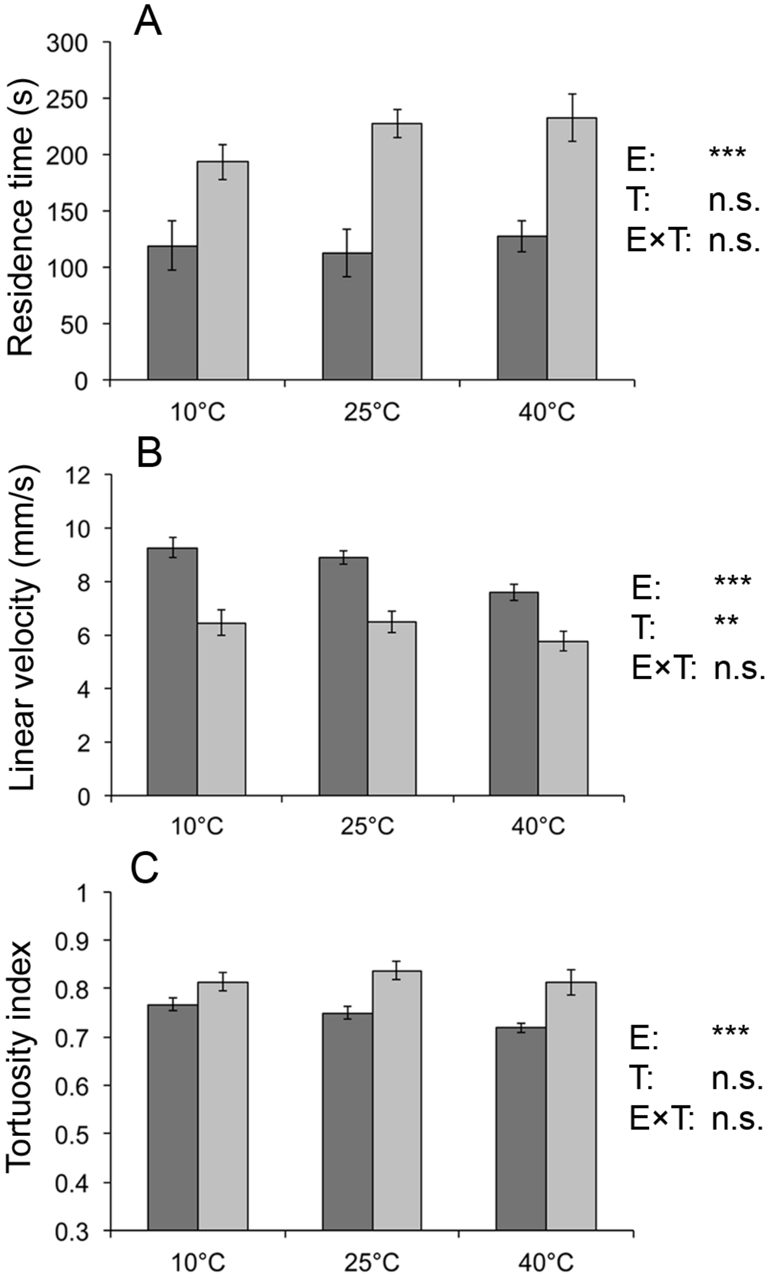

Figure 7-2. The mean (±SE) (A) residence time, (B) linear velocity, and (C) tortuosity index of experienced (dark bars; tested 1.25 h after being trained on another arena of host walking traces) and naïve (light bars; no previous experience) *T. basalis* females tested on host walking traces at 25°C when held at one of three temperatures (10°C, 25°C, or 40°C) during the hour between training and testing (experienced wasps) or for one hour before testing (naïve wasps). In each panel, legends show the statistical significance of experience (E), temperature (T), and their interaction (E×T). n.s. - not significant ($p > 0.05$), ** $p < 0.01$, ***$p < 0.001$. Refer to Table 7-1 for detailed statistical information.

Table 7-2. Statistical comparison of the residence time of *T. basalis* females on host walking traces after being held at three different thermal regimes, depending on whether they had previously been trained on another patch of host walking traces (experienced or naïve), the test interval spent in the temperature regime, the interaction of the two factors, and the time of day at which the test took place.

Temperature	**Factor**	χ^2_{df}	***P***
control	experience		
	test interval		
	experience × test interval	$\chi^2_1 = 7.07$	0.0078
	time of day	$\chi^2_1 = 0.19$	0.66
cool	experience	$\chi^2_1 = 59.50$	< 0.0001
	test interval	$\chi^2_1 = 4.19$	0.041
	experience × test interval	$\chi^2_1 = 0.60$	0.44
	time of day	$\chi^2_1 = 0.45$	0.50
hot	experience	$\chi^2_1 = 31.73$	< 0.0001
	test interval	$\chi^2_1 = 9.27$	0.0023
	experience × test interval	$\chi^2_1 = 0.046$	0.83
	time of day	$\chi^2_1 = 0.73$	0.39

Significance was assessed with LRTs comparing survival models. Significance of the main effects was not tested when the interaction between them was significant.

Linear velocity was significantly higher for experienced *T. basalis* females than for naïve females, but did not change with increasing test interval and was not affected by temperature or any of the interactions between these factors (Table 7-3; Figure 7-4). Tortuosity index was significantly lower for experienced wasps than naïve wasps, and was influenced by a significant interaction between temperature regime and test interval (Table 7-3; Figure 7-5). Modelling each temperature treatment separately revealed a marginally non-significant increase in tortuosity index over time in the control ($F_{1,238} = 1.743$, $P = 0.083$) and hot ($F_{1,107} = 1.92$, $P = 0.058$) temperature regimes, and a marginally non-significant decrease with increasing test interval in the cool temperature regime ($F_{1,105} = 1.86$, $P = 0.066$) (Figure 7-5).

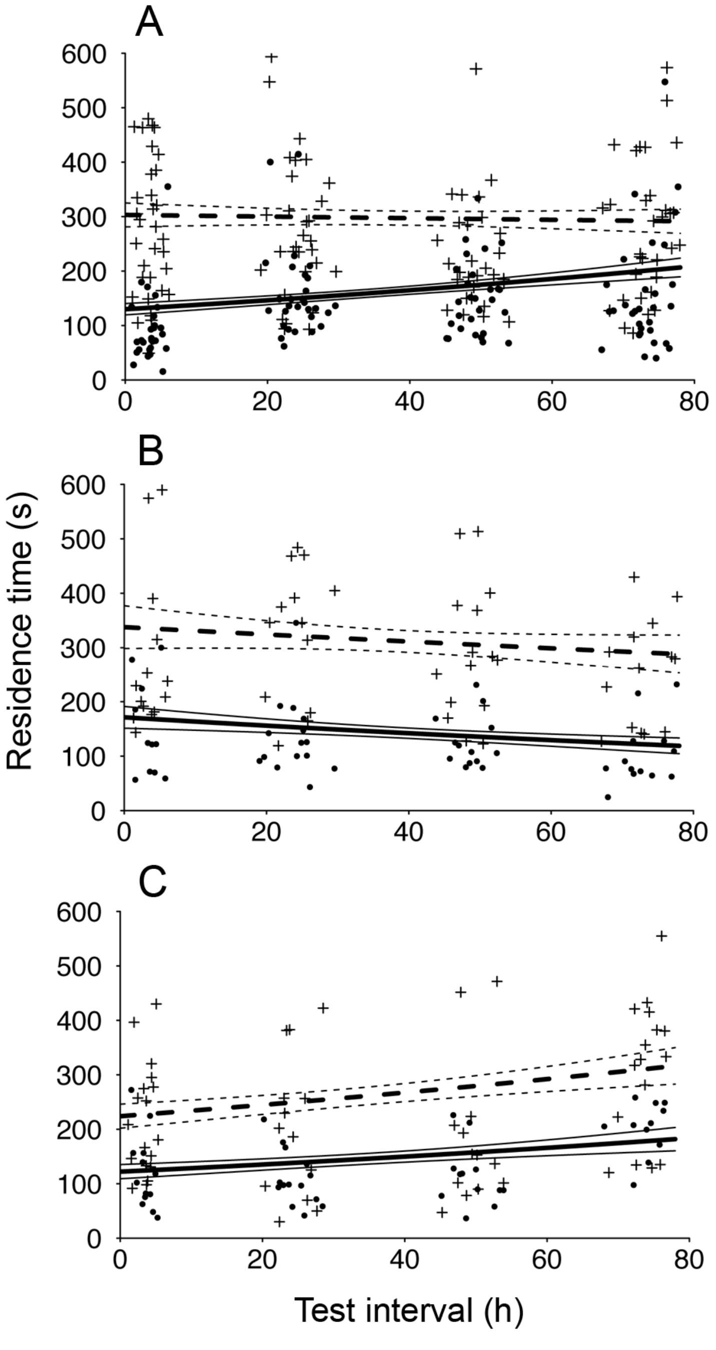

Figure 7-3. The residence times of *T. basalis* females tested on host walking traces after being held at (A) constant temperature of 25°C, (B) a low-temperature regime, or (C) a hot-temperature regime for 0-80 h (see Figure 7-1), depending on whether they were experienced (filled circles; trained on another arena of host walking traces before being exposed to the temperature regime) or naïve (crosses; no training before being exposed to the temperature regime). Lines (solid – experienced; dashed – naïve) show Kaplan-Meier estimates (±SE) of a survival model fitted to the data (see Results).

Table 7-3. Statistical comparison of the linear velocity and tortuosity index of *T. basalis* females on host walking traces, depending on whether they had previously been trained on another patch of host walking traces (experienced or naïve), the temperature regime at which they were held before testing, the test interval spent in the temperature regime, the interactions between these factors, and the time of day at which the tests took place.

Measurement	**Factor**	F_{df}	**P**
Linear velocity	experience	$F_{1,457} = 141.30$	< 0.0001
	temperature	$F_{2,455} = 0.37$	0.69
	test interval	$F_{1,456} = 0.48$	0.49
	experience × temperature	$F_{4,453} = 1.25$	0.29
	experience × test interval	$F_{2,455} = 0.24$	0.79
	temperature × test interval	$F_{3,454} = 0.70$	0.70
	experience × temperature × test interval	$F_{6,451} = 0.77$	0.59
	time of day	$F_{1,456} = 0.87$	0.35
Tortuosity index	experience	$F_{1,452} = 35.08$	< 0.0001
	temperature	$F_{2,452} = 6.06$	0.0025
	test interval	$F_{1,452} = 2.86$	0.091
	experience × temperature	$F_{2,450} = 1.24$	0.29
	experience × test interval	$F_{1,451} = 0.60$	0.44
	temperature × test interval	$F_{2,452} = 4.00$	0.019
	experience × temperature × test interval	$F_{3,449} = 1.03$	0.38
	time of day	$F_{1,451} = 0.67$	0.41

Significance was assessed with F-tests comparing nested linear models

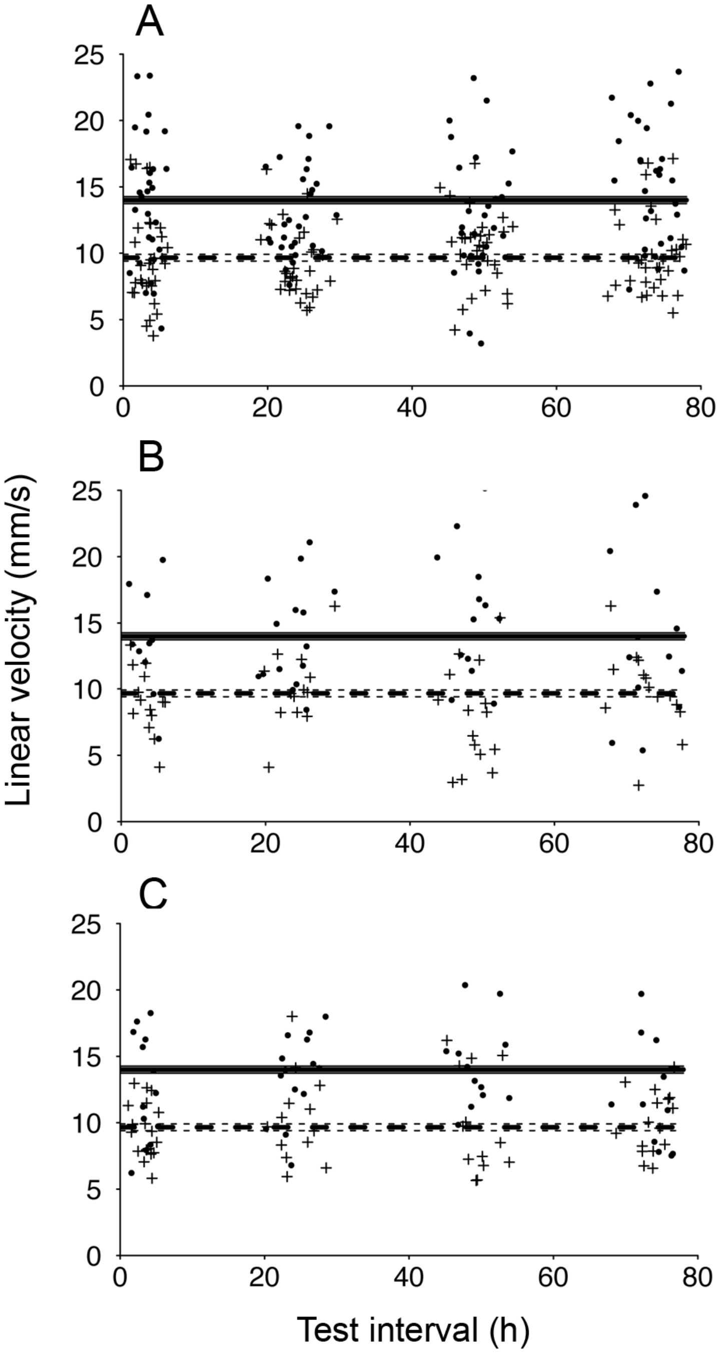

Figure 7-4. The linear velocity of *T. basalis* females tested on host walking traces after being held at (A) constant temperature of 25°C, (B) a low-temperature regime, or (C) a hot-temperature regime for 0-80 h (see Figure 7-1), depending on whether they were experienced (filled circles; trained on another arena of host walking traces before being exposed to the temperature regime) or naïve (crosses; no training before being exposed to the temperature regime). Lines (solid – experienced; dashed – naïve) show predictions (±SE) of a linear model fitted to the data (see Table 7-3).

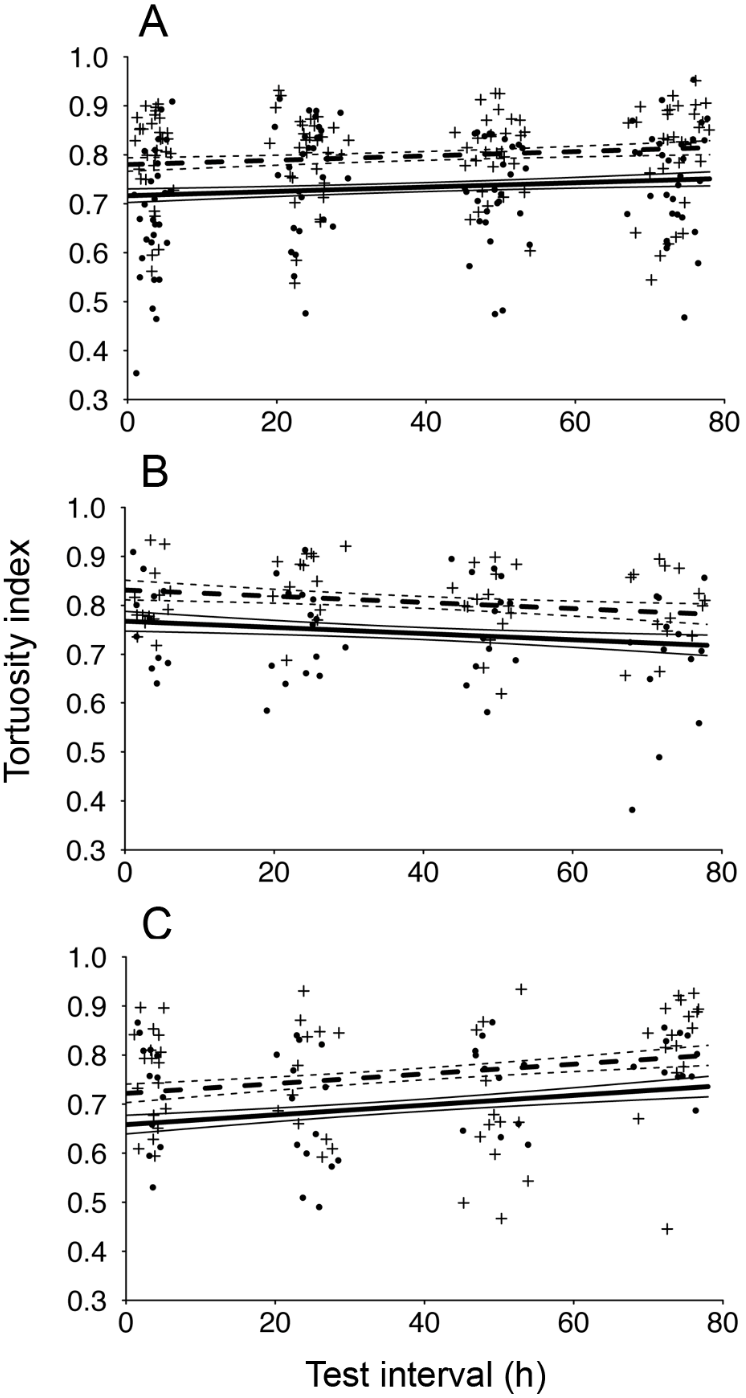

Figure 7-5. The tortuosity index of *T. basalis* females tested on host walking traces after being held at (A) constant temperature of 25°C, (B) a low-temperature regime, or (C) a hot-temperature regime for 0-80 h (see Figure 7-1), depending on whether they were experienced (filled circles; trained on another arena of host walking traces before being exposed to the temperature regime) or naïve (crosses; no training before being exposed to the temperature regime). Lines (solid – experienced; dashed – naïve) show predictions (±SE) of a linear model fitted to the data (see Table 7-3).

7.5 Discussion

Depending on the stability and possible adaptive plasticity of memory retention in the face of thermal stress, stressful temperatures could affect the ability of ectotherms to adjust behaviours such as time allocation as they move through heterogeneous environments. Contrary to our first hypothesis, our results suggest that *T. basalis*'s memory is stable in the face of short-term exposure to stressful temperatures at the limits of what this parasitoid would encounter in nature. However, in line with our second hypothesis, the behavioural expression of memory persisted when wasps were held at high and low temperature regimes over the period of several days, whereas the wasps appeared to forget their unrewarded experience when held at a moderate temperature. To our knowledge, this is the first experimental evidence that realistic temperature stress could modify time allocation strategies via an effect on memory retention.

It is well established that brief exposure to extreme low temperatures soon after learned experiences causes amnesia in several ectotherm organisms, since memories are often the least stable in their early phases (reviewed in Marguiles et al. 2005; Hoedjes et al. 2011). In addition to disrupting early anaesthesia-sensitive memory phases, cold shock can affect the consolidation of later, more stable forms of memory (van den Berg et al. 2011). Although it has not previously been tested in insects, it is plausible that short-term heat stress could also disrupt memory consolidation (Beck and Rankin 1995). If these effects extend to naturally occurring temperatures, a very hot afternoon or a cool night could induce full or partial amnesia in parasitoids and, as a result, a poor estimate of the reliability of host-associated cues. In our study, however, brief (1 h) low-temperature stress at the lower limit of what could actually be experienced during *T. basalis*'s foraging period in nature (10°C) did not affect the intensity of the wasps' subsequent arrestment response when applied directly following an unrewarded experience (Figure 7-2). Similarly, brief high-temperature stress (40°C) did not disrupt the behavioural expression of memory consolidation (Figure 7-2), as was also found by Teskey et al. (2012) in the snail *Lymnaea stagnalis* when testing the effect of temperature stress on its siphon withdrawal reflex. It seems unlikely then, that short-term thermal stress in nature could induce amnesia by acting as a constraint on memory consolidation.

While consolidating memories when facing thermal stress would be important in the short term, it may be equally important for animals to retain, and then eventually forget unrewarded experiences in the longer term. *Nezara viridula*, the most frequent host species of *T. basalis*, is multivoltine, attacks a wide range of host plants in different environments over the course of a season, and uses different plants for mating and oviposition (Todd 1989; McPherson and McPherson 2000). Thus, the reliability of adult walking traces as an indicator of host egg presence may be variable among habitats. Among-habitat variation in the reliability of host-associated cues would make it important for parasitoids to reset their sensitivity to walking traces (i.e., forget an unrewarded experience) when moving between low-quality and high-quality habitats, in order to avoid underinvesting foraging time in a new, potentially profitable habitat (Colazza et al. 2014). As expected, the residence time of *T. basalis* with a previous unrewarded experience on patches of host walking traces tended to increase over the 80 h testing period (relative to naïve controls) when held at a moderate temperature between trials, suggesting that 'forgetting' took place (Figure 7-3A). When held at either hot or cool daily temperature cycles, however, there were differential effects of thermal stress on naïve and experienced individuals over time, when compared to the control temperature treatment. In both the cool and hot treatment, this resulted in consistently lower residence times for experienced wasps compared to naïve wasps across the testing period, indicating that forgetting did not take place under these regimes. This result could indicate an adaptive modification of the parasitoids' memory window, or by a physiological constraint imposed by temperature (*sensu* Moiroux et al. 2014) – two possibilities we will now discuss.

There is some theoretical (Dunlap et al. 2009) and empirical (Pravosudov and Clayton 2001; Friedrich et al. 2004; Orsini et al. 2004) support for the idea that memory duration should be maximized when organisms are in poor state, since the fitness cost of not responding correctly (in this case, leaving the patch sooner) to a familiar stimulus (host walking traces) is higher when the organism is stressed (Dunlap et al. 2009). Hence, a parasitoid that is physiologically stressed during a heat wave or a series of cold nights, and whose ability to reproduce depends on finding hosts before energy reserves are depleted, is better off remembering the correct response to the previously unreliable cue, rather than forgetting and then having to re-learn that the cue is unreliable. An alternative to this adaptive explanation is that temperature simply acts as a constraint on forgetting. If, as recent studies suggest, forgetting is indeed an active physiological process (Berry et al. 2012; Hadziselimovic et al. 2014) and the rates of underlying cellular and neurological mechanisms are temperature-dependent, one might expect forgetting to follow a traditional thermal performance curve (Angilletta et al. 2002). Thus, as observed in our study, the rate of forgetting would be maximal within the parasitoid's 'comfortable' thermal range, and lowest at the extremes. The only other studies of memory retention in the face of thermal stress were conducted in the gastropod *L. stagnalis*, where there was also evidence of longer memory retention when exposed to stressful high or low temperatures after a learned experience (Sangha et al. 2003; Teskey et al. 2012). The authors provided primarily non-adaptive, constraint-based explanations for the observed effects (i.e., 'priming' of neurons at high temperatures, disruption of molecular mechanisms associated with forgetting at low temperatures) and did not consider an adaptive explanation in either case.

Obviously, thermal stress would not affect the time allocation strategies of foraging animals only via its effect on memory retention. Residence time of foraging animals while responding to host-associated cues in nature would depend on current temperature (van Damme et al. 1990) (which, for simplicity, we held constant in this study). Previously experienced temperatures interacting with aspects of physiological state that are unrelated to memory can also affect parasitoid residence time (e.g., van Baaren et al. 2005; Bourdais et al. 2012). For example, we observed an increase in residence time for naïve wasps spending greater amounts of time in the 'hot' temperature regime, and the reverse trend under the 'cool' regime (Table 7-2). This could represent gradual physiological acclimation of the parasitoids to the stressful thermal regimes over time, and supports the idea that heat waves or cool periods could affect the subsequent time allocation strategies of parasitoids in additional ways that do not directly depend on their previous foraging experience (see Hance et al. 2007).

Our results showed varying degrees of correspondence between our three measurements of the behavioural expression of memory (residence time, linear velocity, and tortuosity index), especially when comparing the short-term and long-term experiments. In the short-term experiment (Experiment 1), differences in residence time between treatments generally mirrored changes in linear velocity and tortuosity index. However, in the long-term experiment, the increase in the residence time of experienced wasps relative to naïve controls over time (i.e., 'forgetting') (Figure 7-3A) was not accompanied by the expected decrease in linear velocity and increase in tortuosity index (Figure 7-4, Figure 7-5). The reasons for this discrepancy are unclear and deserve further investigation, although it indicates that the forgetting observed, and how it was modulated by temperature exposure, was not directly related to simple changes in locomotor activity.

Another factor preventing a detailed mechanistic interpretation of our results is an inability to relate the observed effects to the associated phases of memory consolidation, and the type of learning involved (see Marguiles et al. 2005; Hoedjes et al. 2011). Since the timing and duration of different memory phases is species-specific in insects, it is unknown whether the thermal stress in our first experiment was applied during the ASM phase or rather during a more stable memory phase (e.g., anaesthesia-resistant memory or long-term memory; see Marguiles et al. 2005). Furthermore, it is currently unclear whether the utilization of host-associated cues by *T. basalis* constitutes habituation (a decreased sensitivity to host-associated cues with increased exposure), associative learning (the association of walking traces with the absence of hosts; Takasu and Lewis 1996), or a combination of habituative and associative learning processes. Experiments are currently being performed to explore these possibilities. In any case, we suspect that the findings of our study could extend to both habituative and associative learning, since both learning types appear to have common underlying genetic and neurological mechanisms (Duerr and Quinn 1982; Engel and Wu 1996; Cho et al. 2004; Aztalos et al. 2007) and could thus have similar responses to thermal stress.

Predicting the responses of ecosystems to climate change necessitates a thorough understanding of how the foraging strategies of organisms will change when exposed to stressful temperatures. Any thermal effect that modulates time allocation strategies is likely to affect attack rates that would directly affect predator-prey or parasitoid-host population dynamics (Murdoch 1994; Hassell 2000). While more straightforward metabolic effects of temperature can certainly explain a certain proportion of the variation in time allocation and attack rates under different temperature regimes (e.g., Brown et al. 2004; LeLann et al. 2011; Sentis et al. 2013; LeLann et al. 2014), a consideration of realistic temperatures' effects on learning and memory is critical, since they may change foraging strategies in ways that cannot be predicted by changes in metabolic rates. Future studies in this area should attempt to (i) distinguish between the hypotheses for temperature-dependent memory windows that we have put forward in this paper, (ii) relate differences in memory retention at different temperatures to realized fitness gains, and (iii) examine whether ectotherm organisms can use behavioural thermoregulation to buffer any possible thermal constraints on memory retention.

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Chapter 8: The effects of temperature on ectotherm behaviour

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8.1 Abstract

In ectotherm animals, which cannot control their internal temperature, temperature imposes important constraints, and these organisms have evolved numerous adaptations to respond to these constraints. While the impacts of temperature on ectotherm physiology have been extensively studied, there are currently no reviews providing a framework to classify temperature's ultimate effects on ectotherm behaviour. Drawing from the literature on terrestrial arthropods (insects, mites, and spiders), we propose a framework that should apply to all ectotherm animals, generalizing temperature's behavioural effects into (i) direct or indirect kinetic effects, resulting from temperature's constraining influence on metabolism and neurophysiology, and (ii) integrated responses, where the direct integration of thermal information initiates or modifies a behavioural response (behavioural thermoregulation, thermal orientation, and thermosensory behavioural adjustments). For each type of temperature-induced behavioural change, the evidence for constraints vs. adaptive selection and the ecological relevance of the behaviour type are discussed. We also propose two complementary approaches to distinguish between adaptive behavioural modifications from constraints due to temperature: (i) behavioural "kinetic null modelling" and (ii) behavioural ecology experiments using temperature-insensitive mutants. Our framework should help to focus future research on the complex relationship between temperature and behaviour in ectotherms.

Key words: thermal biology, behavioural ecology, behavioural constraints, adaptive behaviour, thermosensation, metabolic theory of ecology

8.2 Introduction

Temperature influences all levels of biological organization. As temperature increases, the higher kinetic energy of biomolecules (e.g. enzymes) speeds up the rate of metabolic processes, scaling up to affect the physiology and behaviour of individual organisms (Angilletta 2009). This thermal rate dependency of physiology and behaviour is the most predictable for ectotherm organisms, which, in contrast to endotherms, do not typically maintain a constant body temperature through homeostatic processes.

While the effects of temperature on ectothermic organisms have long been recognized, there has recently been an explosion of research in this area (e.g., Deutsch et al. 2008; Dell et al. 2011; Dell et al. 2013; Huey et al. 2012; Gilbert et al. 2014; Sunday et al. 2014; Vasseur et al. 2014; Woods et al. 2014). In part, this renewed interest has been spurred by a need to understand the response of individuals and ecosystems to recent and incipient climate change, wherein average global temperatures are increasing, and extreme temperature events are becoming more commonplace (Stocker et al. 2013). Due to the undoubtedly massive effects these changes will have on ecosystems, it is critical to understand the basic details of how species and ecosystems cope with temperature.

In parallel, seminal studies by Gillooly et al. (2001) and Brown et al. (2004), among others, have demonstrated that the rates of many large-scale ecological phenomena (e.g. population growth rates, trophic interactions, biomass production) can be in large part predicted by metabolic rates of individual organisms that increase exponentially with increasing temperature, as predicted by first-principles enzyme kinetics (i.e., the Van't Hoff-Arrhenius equation). Furthermore, when correcting for organismal body mass, biological processes across many levels of organization show similar thermal dependencies (the "universal temperature dependence"; UTD). While this approach lends hope to the prospect of understanding large-scale processes from simple mathematical principles, it has a number of key deficiencies that have received substantial criticism (Cyr and Walker 2004; Clarke 2006; Irlich et al. 2009; O'Connor et al. 2007). One important critique is that this global view tends to ignore much of what is happening at intermediate levels of biological organization. For example, the many ectotherm organisms have the capacity to adapt their physiology and behaviour to modify or better tolerate their thermal environment. That is, temperature not only modifies how (e.g. how rapidly) behaviours are performed, but can determine whether or not a given behaviour is expressed or which one of a possible set of behaviours is performed in a given situation (i.e., behavioural “decisions”; *sensu* Dell et al. 2011).

Arguing that our empirical understanding of thermal biology has far outstripped our theoretical understanding, several comprehensive syntheses have been published that focus on physiological thermal adaptation (e.g., Chown and Nicolson 2004; Angilletta 2009). However, none of these works view the ensemble of empirical thermal biology research from an explicitly behavioural perspective. This is despite the fact that behaviour acts as the interface between an organism and its environment and is often seen as a ‘pacemaker’ of evolutionary change (reviewed in Duckworth 2009; Zuk et al. 2014). Critically, behaviour can modulate the thermal environments to which an organism is exposed and is at the heart of decision-making processes involved in foraging, reproduction, and dispersal. Behavioural responses to temperature have important consequences for the outcome of many ecosystem processes including species interactions (e.g., Dell et al. 2013; Harmon and Barton 2013) and food web structure (e.g., Sentis et al. 2014; Sentis et al. 2015).

In this review, we synthesize, for the first time, the empirical research on the effect of temperature on ectotherm behaviour, focusing on terrestrial arthropods. By distinguishing different categories of temperature-induced behavioural modifications, we develop a mechanism-based framework to categorize these behavioural responses. Although developed based on arthropod studies, this framework is applicable to all ectotherm animals. Our synthesis highlights that (i) the physiological mechanisms underlying temperature-induced behavioural changes are just beginning to be understood, (ii) because of a lack of any kind of organized understanding of thermal effects on behaviour, hypothesis-based research concerning temperature-induced behavioural modifications is rare, and (iii) there is currently relatively poor evidence to support the notion that individuals’ decisions take thermal information into direct account (apart from behavioural thermoregulation). We discuss how these impediments could potentially be overcome. This synthesis will hopefully serve as a guideline for future research into the effects of temperature on ectotherm behaviour, and will contribute to a more complete understanding of thermal biology across all levels of biological organization.

8.3 Methods and Scope

Definition of behaviour employed

In order to categorize the effects of temperature on ectotherm behaviour, what constitutes behaviour must first be defined. There are a variety of definitions used to describe the concept of behaviour and considerable disagreement among behavioural biologists about the details of these definitions and when they apply (Levitis et al. 2009), highlighting the ever-existing difficulty of distinguishing the concept of whole-animal behaviour from physiology and development. However, we will employ the general definition formulated by Levitis et al. (2009):

> "Behaviour is the internally coordinated responses (actions or inactions) of whole living organisms (individuals or groups) to internal and/or external stimuli, excluding responses more easily understood as developmental changes."

The key component of this definition is the concept of an "internally coordinated response by the whole organism", which distinguishes underlying physiological mechanisms behind behaviours – often at the level of a cell, tissue, or organ – from the whole-organism response (behaviour) that occurs when these physiological responses are coordinated (e.g., via the neural and endocrine systems).

For example, in the respiratory systems of some insects, the opening of spiracles occurs when the valve-closing muscle decreases in elasticity in direct response to rising carbon dioxide concentration (reviewed in Gillott 2005; Chown and Nicolson 2004). This process would not be considered a behaviour under the above definition, since it takes place at the level of a single tissue rather than the whole organism and does not involve higher-level integration. The whole-organism process of active ventilation during flight in some Lepidoptera, however (Miller 1981; Chown and Nicolson 2004), would be considered a behaviour, since it involves the coordinated actions of abdominal ventilatory movements and the opening and closing of several sets of spiracles; the control of which originates from a pacemaker center, whose impulse generation can be modulated by the central nervous system in response to sensory input. As per the latter portion of the definition regarding developmental changes, phenomena such as molting or metamorphosis would not be considered behaviour, but rather an ontogenetic physiological process, akin to the example of a rabbit growing hair in the winter discussed in Levitis et al. (2009). Movement to a new habitat to molt or undergo metamorphosis, however, would be considered behaviour.

Obviously, grey areas arise when defining development changes or what constitutes a “whole-organism” response. For example, the production and release of pheromones by insects is a cellular/physiological process often accomplished at the level of a single gland. However, the timing and quantity of pheromone release is a complex process modulated by the nervous integration of external (e.g. presence of mates, light cycles), and internal (e.g. ovariole maturation) information (e.g. Shorey et al. 1968; Delisle and McNeil 1987; Steiner and Ruther 2009). In this case, whether this process would be defined as a behaviour depends on whether it is framed in the context of integration of an internal or external stimulus (hence, a behaviour), or rather simply described as an isolated physiological event (not a behaviour). In addition, in many cases one’s ability to classify a process as a behaviour, and its relation to internal and external stimuli, can be limited by a lack of understanding of its underlying physiological processes, as will become apparent throughout this paper.

While keeping the above definitions in mind, it can be generally agreed that the effect of any external stimulus on behaviour is the manifestation of its effect on lower levels of biological organization. We attempted to construct our general framework of the effect of temperature on behaviour by categorizing the general mechanisms by which temperature may act on cell-, tissue-, and organ-level processes to produce the whole-organism responses generally considered as behaviours.

Scope of literature reviewed

Since the literature describing temperature’s effects on ectotherm behaviour and physiology is vast, we limited the scope of our literature search to insects, mites, and spiders; all of which we will hereafter refer to as arthropods. This group of ectotherms is well-studied in terms of their extremely diverse behaviours under different environmental conditions, the underlying physiological, cellular, and molecular mechanisms of these behaviours, and the understanding of behaviour’s connection to ecological processes.

We further limited our scope to thermal modifications of behaviour occurring within arthropods' non-aversive range of temperatures. In traditional thermal performance curves (see section 8.5 for a detailed discussion), this could be viewed as the exponential, increasing portion of the curve from the critical thermal minimum to the optimal temperature (Angilletta 2009). Therefore, we did not consider behavioural changes in response to heat or cold shock, hardening, or behaviours resulting from permanent or semi-permanent nervous or tissue damage caused by extreme low or high temperatures (e.g. Hance et al. 2007; Bowler and Terblanche 2008; Chown and Nicolson 2004 pp. 115-150). Furthermore, we did not include the processes of diapause or overwintering, which, although some times referred to as behaviours, we considered physiological/developmental states due to the relatively long time scale over which they occur. However, component behaviours involved in preparation or exit from diapause/overwintering could, in principle, fall within the scope of our discussion.

8.4 A framework to classify the effects of temperature on ectotherm behaviour

Kinetic versus Integrated effects

By identifying patterns in the literature, based on underlying mechanisms and behavioural outputs, we constructed a framework for the effects of temperature on behaviour intended to permit the categorization of all possible behavioural responses to temperature in ectotherms. We distinguished two general components of behavioural responses to temperature: "Kinetic" and "Integrated".

Kinetic components of behavioural changes with temperature are due solely to temperature's effect on biochemical reaction rates. While these effects often manifest themselves via metabolic rate as changes in the rate at which behaviours are performed (direct effects), their consequences for aspects of physiological state within or between life stages can influence behaviour indirectly (indirect effects).

Integrated components of behavioural changes with temperature, on the other hand, represent the organism's response to thermal information received by thermosensory organs/structures, which is then integrated by the central nervous system (particularly the brain) and used to modulate behavioural output, including "decision making". The behavioural output may in turn result in a change in the temperature experienced by the organism (behavioural thermoregulation), or may rather be the result of adjusted decision-making that compensates for, or takes advantage of, the temperature change.

To illustrate the difference between Kinetic and Integrated components of behavioural modifications due to temperature, it is helpful to consider how arthropod walking patterns may change across a hypothetical temperature gradient (Figure 8-1). Arthropod walking is controlled by clusters of neurons termed central pattern generators (CPGs) in the central or peripheral nervous system, which control the firing of motor neurons that activate the muscles responsible for limb movement (Delcomyn 2004; Büschges et al. 2008). The signals received by the motor neurons and the resulting muscle movements are further modulated by sensory feedback (e.g. from visual systems, proprioreceptors, mechanosensory organs, hygro- and thermo-sensory organs), resulting in changes of the characteristics of walking behaviour such as direction and speed.

When the arthropod walks across the temperature gradient, observed behavioural modifications due to temperature would have both Kinetic and Integrated components (Figure 8-1) (Dillon et al. 2012). In terms of Kinetic effects, changing temperature would result in changed neuronal conduction speed, a different firing rate of CPG neurons, a change in the rapidity of neurotransmission at neuromuscular junctions, and modified metabolic rates in muscle tissue due to differences in available kinetic energy (Robertson and Money 2012) (see section 8.5); all of which would result in the arthropod tending to walk faster at higher temperatures and slower at lower temperatures. Simultaneously, Integrated effects would also affect walking behaviour: the animal would receive feedback regarding temperature changes from thermosensory organs, the thermal information would be encoded by the brain and used to modulate muscular output, and this in turn would bias walking towards the individual's 'preferred' temperature (see section 8.7 for a detailed discussion). Thus, while Integrated and Kinetic effects are distinguished by whether direct thermal information is involved in a behavioural change, they occur simultaneously to produce the observed behavioural output.

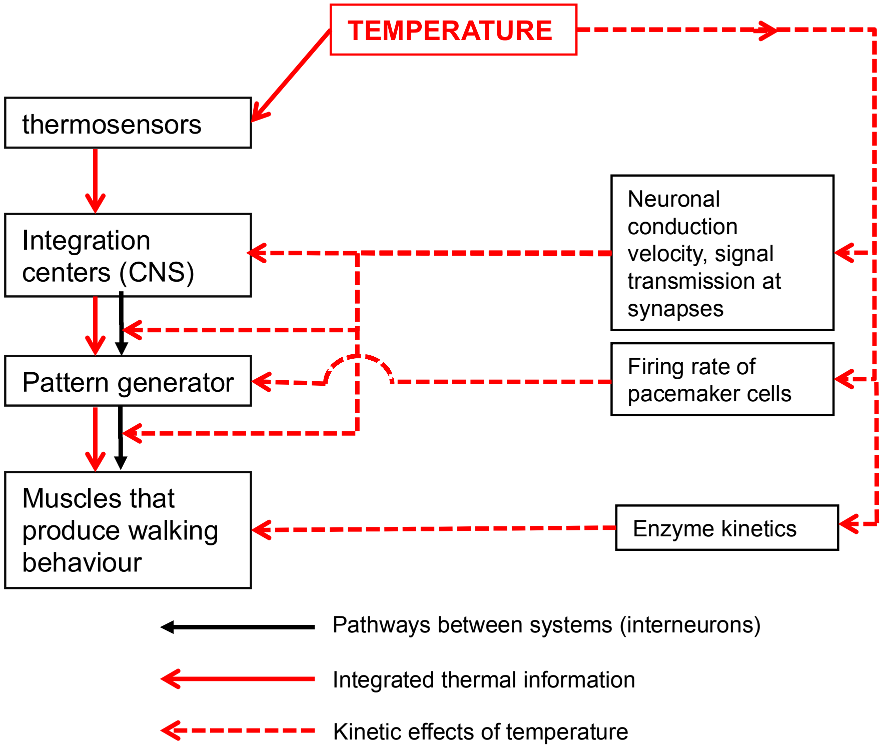

Figure 8-1. A simplified example demonstrating the mechanistic difference between Kinetic and Integrated effects of temperature on ectotherm behaviour. For a behaviour such as walking, temperature would firstly have Kinetic effects on neurons, synapses, pacemaker cells, and enzymes that would affect various parts of the nervous system pathways that coordinate walking movements. For an organism that is behaviourally thermoregulating (by walking towards its preferred temperature), in addition to the Kinetic effects on walking, thermal information is being directly integrated into the pathways involved in walking after it is received by thermosensors (Integrated effect) and processed in the central nervous system (CNS); this results in a bias in walking direction towards the preferred temperature.

The framework for temperature-induced behavioural modifications

Figure 8-2 presents the overall framework we have developed for the effect of temperature on ectotherm behaviour, showing the relationships between the different subtypes of Kinetic and Integrated effects, and how they can act simultaneously and/or sequentially to influence an animal's behaviour as temperature changes. In the following sections, we will explain each type of temperature-induced behavioural modification in turn, providing examples with a discussion of mechanisms and evolutionary patterns.

8.5 Direct Kinetic effects

Definition and examples of Direct Kinetic effects

The best-characterized and most obvious aspect of temperature-induced behavioural modification is the rate at which behaviours are performed. We classify these changes as Direct Kinetic effects (Figure 8-2) that can generally be considered constraints. For example, temperature affects the incidence and rate of locomotor (e.g. walking speed, flight performance), reproductive (mating, oviposition), and foraging (search, attack) behaviours (see Table 8-1 for examples). For the purposes of our framework, Direct Kinetic effects can invariably be described by "thermal performance curves" (Huey and Stevenson 1979), the application of which extends to any biological process whose rate is affected by temperature (e.g. development, digestion, egg production). Thermal performance curves are often applied to behavioural measures of performance, which are also strongly dependent on metabolic rate (Dell et al. 2011). Above a certain threshold (CT_{min}), behaviours tend to be performed more rapidly as temperature increases up to an optimum temperature (T_{opt}), their rate then decreasing up to a critical thermal maximum (CT_{max}) after which the behaviour can no longer be performed. Within an organism's non-aversive thermal range (the exponential, increasing section of the thermal performance curve), the relationship can be descriptively approximated by a linear curve; indeed, this is the basis of linear 'degree day models' of accumulating temperature units used, for example, in insect pest management (Pruess 1983). The change in the rate of metabolic or whole-organism processes over a 10°C change in temperature, or Q_{10}, has also been a commonly employed measurement of the thermal dependency of biological processes, however its precision is limited by the fact that it depends on the range of

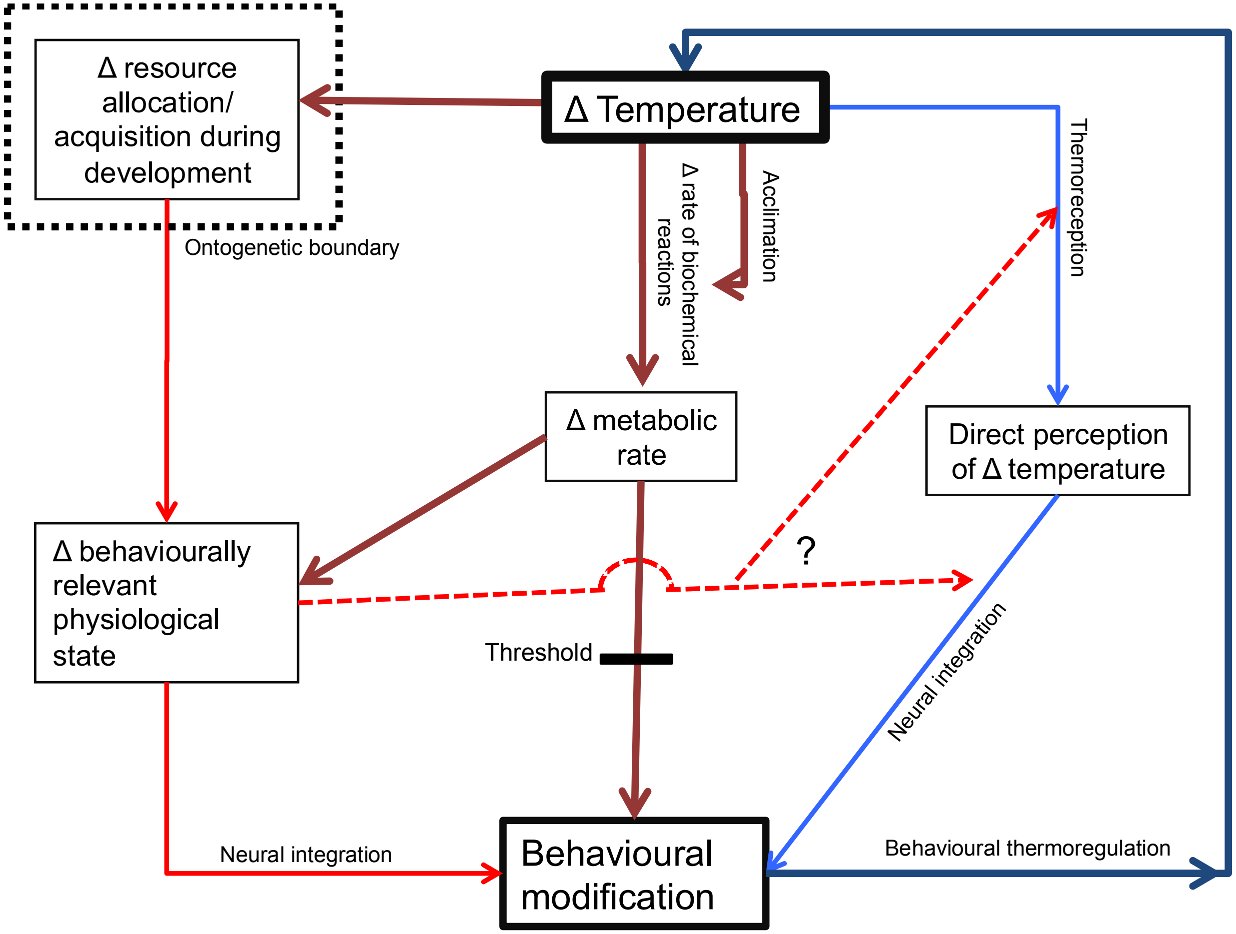

Figure 8-2. A general schematic for the effect of changes (Δ) in temperature on ectotherm behaviour. Arrows show the direction of cause among physiological factors affected by temperature, with solid-lined arrows representing pathways inferred from existing research, and dotted-lined arrows showing hypothetical pathways. **Kinetic effects** (red lines) can be either *Direct* (thick, dark-coloured) or *Indirect* (thin, light-coloured), and often operate above a certain thermal threshold (horizontal black bar). **Integrated effects** (blue lines) can induce *Behavioural Thermoregulation* (both blue colours), or a change in decision-making behaviour that does not change body temperature (exclusively thin, light-coloured), in which case the behavioural modification would be considered a *Thermoreceptory Behavioural Adjustment* or *Thermal Orientation*.

Table 8-1. Examples of Direct Kinetic effects, with their proposed mechanistic explanations.

Behavioural category	Species	Behaviour	Proposed explanation	Reference
Locomotion	*Aphidius ervi* (Hymenoptera: Braconidae)	Locomotor performance curve followed a typical performance curve and conformed to the "jack-of-all-trades, master of none" model. Performance breadth was heritable in males but not females.	There was a negative genetic correlation between performance breadth and maximal performance.	Gilchrist (1996)
Locomotion	*Drosophila melanogaster* (Diptera: Drosophilidae)	Fruit flies did not fly below 15°C.	Muscle mechanical power output and aerodynamic force increased with temperature, and matched the minimum power requirements at 15°C.	Lehman (1999)
Foraging	*Danaus chrysippus* (Lepidoptera: Nymphalidae)	Feeding rate increased with temperature.	Feeding rate followed the increase in metabolic rate.	Mathavan and Padian (1975)
Reproduction	*Dahlbominus fuliginosus* (Hymenoptera: Eulophidae)	Males rarely mated at high temperatures.	General activity of males, including their movements, were accelerated due to a higher metabolic rate to such a degree that they rarely mate.	Wilkes (1963)
Reproduction	*Callosobruchus chinensis* (Coleoptera: Chrysomelidae)	Negative relationships between ambient temperature and mating duration and sperm transfer duration. Female re-mating frequency at low temperature was lower than at other temperatures.	At low temperature, more difficult for the male to force seminal products through the aedeagus. Transfer of larger quantities of sperm and the longer mating duration at low temperature may have reduced the female's willingness to remate.	Katsuki and Miyatake (2009)
Reproduction	*Tibicina spp.* (Hemiptera: Cicadidae)	Positive correlation between the ambient temperature and the sound power of the calling song.	The timbal tensor muscle produced greater tension at higher temperatures.	Sueur and Sanborn (2003)
Reproduction	*Grapholita molesta* (Lepidoptera: Tortricidae) and *Pectinophora gossypiella* (Gelechiidae)	At high temperatures, males responded to a broader range of blend-dose combinations of female mating pheromones, initiating upwind flights more frequently than at low temperature when combinations differ from natural blends.	The effect of temperature acted directly on neural pathways involved in odor perception.	Linn et al. (1988)
Reproduction	*Achroia grisella* (Lepidoptera: Pyralidae)	Male song rate and female acceptance threshold exhibited parallel increases as temperature rises	Temperature coupling may have been an emergent property arising from the neuromuscular responses to temperature.	Greenfield and Medlock (2007)

temperatures over which it is measured (reviewed by Chown and Nicolson 2004, p. 76). More recently, the Van't Hoff-Arrhenius equation, originally formulated to statistically model the temperature dependency of enzyme kinetics and the corresponding rate of metabolic processes, has become the most common way to characterize the thermal dependency of whole-organism processes such as behaviours, whose rates depend on basal metabolism (Gillooly et al. 2001; Brown et al. 2004; Dell et al. 2011). This approach is considered by some to be 'mechanism-based' (but see Clarke 2004; O'Connor et al. 2007), in that the parameters represent the activation energy of a rate-determining enzyme and the Boltzmann constant, which specifies how temperature affects the kinetic energy of molecules involved in enzymatic reactions underlying behavioural rates. Working towards a "Metabolic Theory of Ecology" (MTE), Gillooly et al. (2001) and Brown et al. (2004) applied this relationship to cross-species analyses and larger-scale ecological processes, showing that activation energies of the fitted models tended to center around 0.60-0.70 eV (the so-called "universal temperature dependence", UTD). Later, Dell et al. (2011) performed analyses of intraspecific thermal dependencies of a variety of whole-organism and population-level processes, including behaviours involved in locomotion and foraging. This and other studies (e.g. Irlich et al. 2009) have demonstrated that there is substantial, systematic variation in temperature-rate relationships (including behaviours), and thus the true universality of the UTD is still a matter of significant debate. Whether the UTD applies differently to behaviours than other aspects of physiology and metabolism, or whether it applies to different kinds of behaviours differentially, is still an open question that should be investigated in the future.

The approaches to mathematically describe the thermal sensitivity of Direct Kinetic effects have the important limitation that they do not apply to the entire range of temperatures experienced by organisms in nature. If one wishes to model the non-linear, unimodal behavioural rate vs. temperature relationships present over the entire range of thermal sensitivity (i.e., the entire thermal performance curve), there are a variety of mathematical models to choose from, ranging from completely descriptive (e.g. second-order polynomials, Gaussian distributions, the Brière model) to those which incorporate principles of enzyme kinetics (e.g. the 'Sharp-Schoofield' model) (Angilletta 2006; Damos et al. 2012; Regnière et al. 2012). Although some of these models tend to be preferentially applied to developmental rates, there is no necessary reason why they could not also be fitted to temperature-dependent changes in behavioural rates. This would facilitate comparisons between the thermal dependencies (parameter values) of, for example, different behaviours performed by the same organisms.

Finally, the thermal sensitivity of behavioural rates can be changed by thermal acclimation, which modifies how the rate of biochemical reactions underlying behaviour responds to temperature (Figure 8-2). Reversible thermal acclimation (*sensu* Angilletta 2009, p. 126), which occurs within the same life stage, is observed when exposure to a given temperature regime induces a relatively rapid plastic physiological response by the organism. These physiological changes modify the subsequent thermal sensitivity of behaviour (i.e., the shape and location of the thermal performance curve). Since thermal acclimation does not have any special relevance to behaviour, we refer readers to other comprehensive reviews of this topic (Chown and Nicholson 2004; Angilletta 2009).

Proximate mechanisms behind Direct Kinetic effects

Within organisms' non-aversive thermal range, temperature's Direct Kinetic effects on ectotherm animal behaviour are ultimately a result of the amount of kinetic energy present in physiological systems. Higher kinetic energy (due to higher temperature) increases the rate of conformational change of proteins (including enzymes), and increases the proportion of reactants that reach the activation energy required for biochemical reactions to occur (reviewed in Fields 2001; Hochachka and Somero 2002). Higher temperature also increases the fluidity of cell membranes, affecting the transport of materials in and out of cells (e.g., Hazel and Williams 1990; Hazel 1995).

The above changes occur within the neural networks and effector muscles that produce behaviours, modulating the rate at which they are performed (Figure 8-1; Figure 8-2). For example, higher temperature increases the speed of neurotransmission through an increase in postsynaptic receptor affinity, a decrease in postsynaptic membrane resistance, a decreased refractory period, and an increased conduction velocity (reviewed in Montgomery and Macdonald 1990). However, as noted by Robertson and Money (2012), the effect of temperature on an animal's nervous system become less predictable as the complexity of the network increases, in part due to different thermal dependencies of the various components of each system, as well as built-in mechanisms to compensate for temperature differences and "shut behaviours off" below a certain threshold. For example, many behaviours have distinct lower thresholds (Figure 8-2; Table 8-1) that cannot be explained purely by the thermal dependency of biochemical processes (Robertson and Money 2012). Furthermore, some behaviours, such as the flight performance of locusts and dragonflies, show little to no thermal dependency, suggesting that some kind of compensation is taking place (May 1981; Xu and Robertson 1994; Robertson and Money 2012). In some instances, this kind of compensation may fit into the "Indirect Kinetic" or "Integrated" categories discussed in subsequent sections.

Evolution of Direct Kinetic effects

There is extensive evidence showing that the thermal sensitivity of behaviour (i.e., the position and shape of behavioural performance curves) responds to evolutionary selection from organisms' thermal environment (reviewed in Angilletta 2009). For example, patterns in the thermal sensitivity of behavioural rates have been found among different species (e.g. Carrière and Boivin 1997; Hurlburt et al. 2008; McGrannachan and Lester 2012) and within the same species from different habitats or geographic locations (e.g. Gilbert et al. 2001). There is relatively well-developed theory around predicting, characterizing, and comparing optimal thermal performance curves, which is discussed in detail elsewhere (Lynch and Gabriel 1987; Gilchrist 1995; Angiletta 2009). Briefly, characteristics such as the breadth of thermal performance and the relative thermal performance at a given temperature are thought to be shaped by environmental selective pressures, and balanced against the effect of constraints inherent in the mechanisms underlying thermal sensitivity. For example, cell membranes and enzyme structures that are most stable at low temperatures tend to have lower performance at higher temperatures, meaning that evolving increased behavioural performance at low temperatures should compromise performance at higher temperatures. This leads to the hypothesis that there could be a spectrum of thermal specialization, ranging from thermal specialists vs. thermal generalists; the latter of which would be a "jack of all temperatures" but also a "master of none". In general, this hypothesis has received mixed empirical support (e.g. Carrèire and Boivin 1997; Carrière and Boivin 2001; Izem and Kingsolver 2005), which has been synthesized in detail elsewhere (Angilletta 2009, Chapter 3).

8.6 Indirect Kinetic effects

Definition and examples of Indirect Kinetic effects

Some behavioural modifications due to Kinetic effects may be only indirectly due to temperature exposure, resulting in behavioural modifications that cannot be described by thermal performance curves (e.g., thermally-dependent discrete switches between alternative behaviours). These types of behavioural modifications can be a result of the thermal rate-dependency of a labile aspect of physiological state (within a life stage) or a life history trait that was fixed during development (across life stages) (Figure 8-2). In both cases, the behavioural change is a result of the change in some aspect of thermally dependent physiological state, not a direct response to temperature. Thus, this category can broadly be considered as thermal constraints acting on the underlying mechanisms that contribute to decision-making behaviour.

Within arthropod life stages, temperature exposure can change subsequent behavioural processes via its effects on various aspects of physiological state, which themselves have effects on the fitness payoffs of different behaviours or alternative variations on a single behaviour (Table 8-2). For example, many aspects of insect behaviour are sensitive to egg load (Minkenberg et al. 1992), and the rate at which eggs are synthesized is temperature-dependent (e.g., Berger et al. 2008). Therefore, behavioural variables such as searching intensity, clutch size, and host/prey handling time can be indirectly influenced by temperature, via its effect on egg load (Rosenheim and Rosen 1991; Berger et al. 2008; Table 8-2). Sometimes, the temperature-dependent aspect of physiological state responsible for modulating behaviour is less clear, and is inferred rather than directly measured. The death-feigning intensity of *Callosobruchus* beetles, for example, decreases with increasing temperature, with the authors arguing that this behavioural outcome was due to the temperature dependence of amine synthesis in the brain, which is correlated with death-feigning behaviour in other species (Miyatake et al. 2008). Traniello et al. (1984) found that temperature influenced prey size selection in the ant *Formica schaufussi*, with smaller, less profitable prey items being more selected at higher temperatures, at which foraging costs outside the nest are higher. These researchers suggested that the ants may have been tracking metabolic costs and resulting

Table 8-2. Examples of Indirect Kinetic effects within a life stage, with their proposed mechanistic explanations.

Behavioural category	**Species**	**Behaviour**	**Proposed explanation**	**Reference**
Foraging	*Formica schaufussi* (Hymenoptera: Formicidae)	At high temperature, workers were more likely to accept small and thus less profitable prey items.	Because of the increased metabolic cost of foraging at high temperatures, ants minimized the cost of a trip outside the nest rather than maximizing the income during any one trip.	Traniello et al. (1984)
Reproduction	*Aphytis lingnanensis* (Hymenoptera: Aphelinidae)	Parasitoids with higher egg loads (having previously been held at higher temperatures) took less time to handle hosts, laid larger clutches, and spent more time searching vs. resting/grooming.	Temperature affected behavioural decisions indirectly through its influence on egg maturation rates.	Rosenheim and Rosen (1991)
Anti-predator strategy	*Callosobruchus maculatus* and *C. chinensis* (Coleoptera: Chrysomelidae)	Death-feigning intensity decreased with increasing ambient temperature.	At low temperatures, beetles are not able to run away as well as at high temperatures so death-feigning may be more effective at low temperatures. Temperature-dependent brain amines, including dopamine involved in death-feigning behaviour, may be involved in this response.	Miyatake et al. (2008)
Reproduction	*Pararge aegeria* (Lepidoptera: Nymphalidae)	Warm temperatures increased early reproductive output.	Both egg maturation and oviposition rates increased with time spent at day and night warm temperatures.	Berger et al. (2008)
Foraging	*Coleomegilla maculata* (Coleoptera: Coccinellidae)	Handling times of aphid prey increased slower than expected with increasing temperature.	The thermal dependency of digestion rate limited the rate of prey handling, and thus indirectly affected the functional response of the predator	Sentis et al. (2013)
Nesting activity	*Lasioglossum malachurum* (Hymenoptera: Halictidae)	Duration of pauses in nesting activity decreased as temperature increased.	Short pauses were the consequence of a faster development of progeny in heated patches.	Weissel et al. (2006)
Learning/Foraging	*Trissolcus basalis* (Hymenoptera: Platygastridae)	Parasitoids learning reliability of host-associated chemical cues retained memories longer at high and low temperatures, with consistently lower residence times on patches of host kairomones compared to naïve individuals.	Wasps responded to thermal stress by lengthening their memory window, indirectly affecting patch time allocation behaviour.	Abram et al. (2015)
Reproduction	*Adalia bipunctata (L.)* (Coleoptera: Coccinellidae)	Ladybeetles were less likely to oviposit in the presence of conspecific infochemicals when (i) the infochemicals were emitted at higher temperatures, and (ii) the perception of infochemicals took place at lower temperatures	Temperature indirectly influences oviposition behaviour by affecting (i) the composition and quantity of released infochemicals, and (ii) the receiver's perception of the infochemicals and/or its motivation to oviposit	Sentis et al. (2015)

energy expenditure associated with high temperatures, and adaptively modifying their behaviour as a consequence (but see discussion in section 8.8).

Indirect Kinetic effects can also act across life stages to shape behavioural phenotypes. Developmental temperature changes the relative allocation of developmental resources to different functions, resulting in phenotypic trait/character variation that can be described by thermal reaction norms (Angilletta et al. 2003; Kingsolver et al. 2004). In contrast to thermal performance curves, the phenotypic traits described by thermal reaction norms are typically fixed and irreversible for an individual within an ontogenetic stage. This phenotypic trait variation due to developmental temperature can have a wide range of consequences for behaviour, affecting locomotion, foraging, and mating, among others (Table 8-3). For instance, individuals that develop at lower temperatures tend to be larger than those that develop at high temperatures (i.e., the size vs. developmental temperature reaction norm is positively sloped) (Atkinson 1994). Since size itself has large effects on behavioural strategies, the temperature-size rule is a common way that developmental temperature can indirectly mediate behavioural strategies. For example, insect parasitoids that are smaller as a result of developing at higher temperatures may have altered fecundity, basal metabolic rates, and life expectancy, changing the efficiency with which they interact with hosts and exploit host patches (Colinet et al. 2007; LeLann et al. 2011). Due to their smaller size, warm-developing parasitoids tend to be less capable of subduing larger hosts (LeLann et al. 2011; Wu et al. 2011; Moiroux et al. 2015). Moiroux et al. (2015) found that *Aphidius ervi* Haliday (Hymenoptera: Braconidae) parasitoids reared at higher temperatures tended to adopt more ‘risk-prone’ behaviours, by more frequently attacking lower-quality host instars compared to individuals reared at lower temperatures. Since smaller parasitoids may have had a lower projection for future lifetime reproductive success (following the ‘Relative Fitness Rule’; Giraldeau and Boivin 2008), it is possible that this behavioural shift could represent adaptive behavioural plasticity in response to developmental temperature-induced size variation.

Beyond the temperature-size rule, Le Lann *et al.* (2011) considered the Metabolic Compensation Hypothesis as a possible source of indirect effects of temperature on insect behaviour. This hypothesis, which has been rarely investigated – but was experimentally confirmed in *Drosophila* (Berrigan and Partridge 1997) and parasitoids (Le Lann et al. 2011) – posits that cold-developed individuals tend to have a higher metabolic rate than warm-

Table 8-3. Examples of Indirect Kinetic effects across life stages, with their proposed mechanistic explanations.

Behavioural category	**Species**	**Behaviour**	**Proposed explanation**	**Reference**
Foraging	*Aphidius rhopalosiphi* (Hymenoptera: Braconidae)	Oviposition rate was higher, and patch residence time and host handling time were shorter in females reared at low temperature than females reared at high temperature when foraging at a common temperature.	A higher metabolic rate and greater size when reared at low temperatures meant that females were more active, moved faster and parasitized more hosts	Le Lann et al. (2011)
Locomotion	*Drosophila melanogaster* (Diptera: Drosophilidae)	Cold-reared fruit flies failed to perform a take-off flight less frequently than warm-reared flies.	The improved ability to fly at low temperatures was associated with a dramatic increase in wing area and an increase in wing length	Frazier et al. (2008)
Reproduction	*Aphidius colemani* (Hymenoptera: Braconidae)	Females that developed at low and high temperatures laid more eggs that females developing at intermediate temperatures.	The decrease in oviposition rate at high temperature may be the result of a decrease in fat reserves when development time was shorter. At low temperatures, standard thermal rate-dependency causes lower oviposition rates.	Colinet et al. (2007)
Reproduction	*Drosophila melanogaster* (Diptera: Drosophilidae)	Males reared at 25°C had greater territorial success than males reared at 18°C and males whose parents developed at 25°C were more successful than were males whose parents developed at 18°C.	Fruit flies raised at 25°C, even though phenotypically small, are physiologically more vigorous than flies reared at 18°C.	Zamudio et al. (1995)
Learning ability	*Apis mellifera* (Hymenoptera: Apididae)	Bees reared at 35°C had a better short-term memory than bees reared at 32°C, while rearing temperature had no significant effect on the long-term learning and memory abilities of workers.	Temperature had no effect on fluctuating asymmetry. Consequences of rearing temperatures are thus subtle neural deficiencies affecting short-term memory rather than physical abnormalities.	Jones et al. (2005)
Foraging	*Apis mellifera* (Hymenoptera: Apididae)	Honeybees raised at high temperatures showed an increased probability to dance, foraged earlier in life, and were more often engaged in removal of dead colony members.	Low pupal developmental temperatures might have lead to reduced juvenile hormone (JH) biosynthesis rates in adult bees, causing a delayed behavioural development and hence a later onset of foraging. Further, developmental temperature may have interfered with JH metabolism and/or octopamine levels in the bee's brain, affecting foraging preferences.	Becher et al. (2009)

developed individuals when tested in a common temperature, probably because of a higher production of mitochondria and associated enzymes at low temperature (Hochachka and Somero 2002). Le Lann et al. (2011) observed that this higher metabolic rate induced by low developmental temperatures resulted in decreased adult longevity, but was counterbalanced by a higher egg load in an aphid parasitoid. These authors proposed that this change in resource allocation may explain why cold-developed females exploited patches more intensively than warm-developed females, and suggested that metabolic rate should be considered more frequently when investigating the influence of developmental temperature on behavioural decisions.

Proximate mechanisms behind Indirect Kinetic effects

Within a life stage, behavioural changes due to Indirect Kinetic effects are simply based on the state of a thermally rate-dependent process (e.g., oogenesis, energy expenditure), whose underlying mechanisms are the same as for Direct Kinetic effects (see section 8.5). How these temperature-induced changes in physiological state would translate into behavioural modifications, however, is not currently well understood at a mechanistic level, reflecting a general lack of understanding about how physiological and neurological processes translate into decision-making processes.

Because temperature can influence resource allocation during development in a variety of ways, there is unlikely to be a universal mechanism by which Indirect Kinetic effects act across life stages. For instance, a wide variety of mechanisms have been proposed for the temperature-size rule in ectotherms, including a tradeoff between growth rate and asymptotic size (Berrigan and Charnov 1994), a differing temperature dependence of growth rates versus development rates (Van der Have and de Jong 1996), a temperature dependence of growth rate but not growth duration (Perrin 1995), and ontogenetic differences between growth and development rates (Forster and Hirst 2012). Davidowitz and Nijhout (2004) developed a detailed mechanistic model for the temperature dependence of body size in *Manduca sexta* (L.) (Lepidoptera: Sphingidae), although it does not seem to generalize across all taxa (e.g., Ghosh et al. 2013). There is likely not a general mechanism underlying the temperature-size rule (Angilletta and Dunham 2003; Forster and Hirst 2012; Ghosh et al. 2013). This conclusion of mechanistic context-dependence can likely be extended to the behavioural consequences of developmental temperature. Behavioural phenotypes resulting from different developmental temperatures probably arise from complex and varied mechanisms, which represent a combination of adaptive plasticity and temperature-induced biophysical constraints.

Evolution of Indirect Kinetic effects

Indirect Kinetic effects of temperature are likely to be important selective pressures acting on insect behaviour, since both mean temperature and its variability can change drastically over the life of an individual. For example, a large number of insect species first develop in more protected, less thermally variable habitats such as water, rotting fruit, or leaf litter, and then move to the air as adults. This condition, uncommon in other organisms, may have favoured the evolution of specific adaptations to temperature changes during immature and reproductive life. However, to what extent behavioural responses to Indirect Kinetic effects are actually adaptive, and thus reflective of such selective pressures, is still somewhat controversial. Studies of the evolutionary patterns of the Indirect Kinetic effects of temperature on behaviour have mostly been examined in the context of how developmental temperature affects the behaviour of adult arthropods (i.e., behavioural trait vs. developmental temperature reaction norms). Much of this research has been undertaken in the context of the “Beneficial Acclimation Hypothesis” (Leroi et al. 1994), which states that developing at a given temperature would improve subsequent performance of adults at that temperature, compared to individuals that developed at other temperatures (reviewed by Angiletta 2009). However, this cannot really be considered acclimation in the same sense as the type of acclimation that occurs within life stages, since completely different mechanisms are at play. Tests of the effect of developmental acclimation are then, in reality, tests of how developmental plasticity responds to temperature (Wilson and Franklin 2002). Additionally, although the Beneficial Acclimation Hypothesis has received a lot of attention, several experiments have challenged its generality (e.g. Zamudio et al. 1995, Gibert et al. 2001). Competing hypotheses have emerged (for a review, see Huey et al. 1999), in particular the optimal developmental temperature hypothesis (Cohet and David 1978) which states that ectotherms that developed at intermediate temperatures perform well at a wider range of temperatures because they are more robust. This hypothesis has been supported by several experiments in insects, notably in *Drosophila* (e.g. Zamudio et al. 1995; Gibert et al. 2001). Zamudio et al. (1995), for example, observed that *D. melanogaster* males reared at an intermediate temperature had greater territorial success at every temperature than males that were tested at the same temperature at which they developed.

Although there do not appear to be universal patterns of how developmental temperature affects adult behaviour, it is clear that there is at least considerable underlying genetic variation. For example, various behavioural responses to developmental temperature have been demonstrated when comparing different species (Krstevska and Hoffman 1994), inbred and outbred lines of the same species (Kjaersgaard et al. 2014), different colour morphs of the same species (Forsman et al. 2002), and laboratory populations of the same species reared for several generations at different temperatures (Good 1993). Not enough studies are available to extract any general trends, however. More often measuring behavioural outcomes of individuals developing at different temperatures, rather than only physiological traits (e.g. fecundity, longevity, metabolic rate), could yield a clearer picture of how Indirect Kinetic effects are shaped by evolutionary pressures.

8.7 Integrated effects – Behavioural thermoregulation

Definition and examples of behavioural thermoregulation

Ectotherms are not completely constrained by ambient temperatures, whereby they must tolerate or adapt to the metabolic and physiological constraints imposed by their current thermal environment. While ectotherms cannot typically adjust their body temperatures through endogenous homeostatic control (as in endotherms), the fact that natural environments are thermally heterogeneous allows the modification of body temperature via behavioural thermoregulation (May 1979; Woods et al. 2014). In this case, the ambient temperature experienced by an animal causes a behavioural response, which in turn changes ambient temperature, subsequently affecting physiological functions via Kinetic effects (Figure 8-2).

Thermoregulatory behaviours in arthropods are diverse and the literature describing them extensive, but they can be grouped into the broad categories of (i) microhabitat selection,

(ii) basking, (iii) modification of temporal activity cycles and (iv) endothermy (see May 1979 for a review). Arthropods use a given strategy, or a combination of several, to avoid aversive temperatures and maintain a body temperature that optimizes such diverse functions as reproduction (Hedrick et al. 2002; Spence et al. 1980; Stahlschmidt and Adamo 2013), growth and development (Clissold et al. 2013; Coggan et al. 2011), locomotion (Dorsett 1962; Kingsolver 1985; Whitman 1988; Dreisig 1981), and defense against parasitic infection (ie. ‘behavioural fever’) (Boorstein and Ewald 1987; Elliot et al. 2002; Ouedraogo et al. 2004). Some representative examples of thermoregulatory behaviours and their proposed functions are shown in Table 8-4.

Behavioural thermoregulation strategies are dynamic, since the optimal temperature for a given individual can depend on the thermal optima of different aspects of physiological state (Huey 1991). For example, *Locusta migratoria* (L.) (Orthoptera: Acrididae) nymphs move to lower temperatures along a thermal gradient with increasing degrees of nutrient deprivation, which increases the efficiency of protein and carbohydrate assimilation at the cost of lower rates of growth and development (Coggan et al. 2011). These locusts can even use this dynamic thermoregulatory behaviour to optimize assimilation of specific nutrients, which varies with temperature (Clissold et al. 2013). Similarly, the preferred temperature of *Triatoma* bugs (Hemiptera: Reduviidae) along a gradient depends on nutritional status, with bugs preferring cooler temperatures when starved, and warmer temperatures following a blood meal (Guarneri et al. 2003; Minoli and Lazzari 2003). *Triatoma* thermopreference also has a strong circadian component, with bugs preferring warmer temperatures just before the onset of darkness; this cycle persists even in the absence of light/dark cues, suggesting the existence of an internal clock for thermal preferences (Minoli and Lazzari 2003). These examples, along with the others provided in Table 8-4, support the hypothesis that the behaviours involved in thermoregulation likely receive complex neural inputs integrating different types of physiological information. In the case of social insects, state-dependent thermoregulation of the colony (e.g. Porter and Tschinkel 1993; Riabinin et al. 2004) could also involve pheromonal communication between individuals, although this has not been directly tested. The neural and physiological underpinnings of thermoregulatory behaviour, which are likely to be incredibly complex, are just beginning to be elucidated.

Table 8-4. Examples of thermoregulatory behaviours, with their proposed mechanistic functions.

Behavioural category	**Species**	**Behaviour**	**Proposed function**	**Reference**
Microhabitat selection	*Gryllus integer* (Orthoptera: Gryllidae)	Male crickets chose cracks in the ground at a temperature where the frequency and intensity of their calling songs were maximized.	Optimize attractive characteristics (e.g., chirp rate) of calling songs to mates.	Hedrick et al. (2002)
Microhabitat selection	*Locusta migratoria* (Orthoptera: Acrididae)	Locust nymphs deprived of protein or carbohydrate and then fed a meal subsequently chose different temperatures.	Maximize the assimilation of the nutrient that addresses their nutritional imbalance.	Clissold et al. (2013)
Microhabitat selection	*Bombus terrestris* (Hymenoptera: Apidae)	Bees parasitized by conopid flies stayed outside the nest at night and actively sought out cooler temperatures.	Retard the development of the parasitoid (prolonging the life of the bee) and reduce the parasitoid's developmental success.	Müller and Schmid-Hempel (1993)
Microhabitat selection	*Solenopsis invicta* (Hymenoptera: Formicidae)	When ant colonies were well-fed, workers and brood were kept at high temperatures; when colony was food-limited, brood were still kept at high temperatures but workers were not associated with brood care seek lower temperatures.	Increase the longevity of workers not directly associated with brood care, while continuing to optimize brood development temperature, when resources are limited.	Porter and Tschinkel (1993)
Basking	*Pieris* spp. (Lepidoptera: Pieridae)	Butterflies changed orientation of their wings to reflect solar radiation onto their thorax.	Attain body temperatures necessary for flight.	Kingsolver (1985)
Basking	*Gerris* spp. (Hemiptera: Gerridae)	Adult water striders submerged themselves underwater when water temperature was higher than ambient air temperature.	Increase the rate of gonad maturation and egg production.	Spence et al. (1980)
Daily activity cycle	*Drosophila subobscura* (Diptera: Drosophilidae)	Flies at warm locations were mostly active in the evening.	Avoid exposure of eggs to high daytime temperatures soon after oviposition.	Huey and Pascual (2009)
Microhabitat selection, basking, daily activity cycle	*Taeniopoda eques* (Orthoptera: Romaleidae)	Grasshoppers (i) selected sites with large amounts of solar radiation and (ii) "flanked" (adjusted body posture) to maximize absorption of solar radiation on cool mornings; (iii) sought shade at midday to avoid adversively high temperatures.	(i) and (ii) raised body temperatures above ambient levels in the morning and maximized the amount of time that they could engage in feeding and reproduction before needing to perform (iii) to minimize heating later on.	Whitman (1988)
Endothermy	*Deilephila nerii* (Lepidoptera: Sphingidae)	Moths beat and vibrated their wings pre-flight.	Warm up flight muscles to temperatures necessary to initiate flight.	Dorsett (1962)
Endothermy	*Vespa orientalis* (Hymenoptera: Vespidae)	Hornet workers beat their wings at the nest entrance when temperature increased.	Ventilate the nest to prevent it from overheating.	Riabinin et al. (2004)

Proximate mechanisms behind behavioural thermoregulation

Effective behavioural thermoregulation depends on the ability to sense temperature in some way. While, in principle, temperature sensation used in behavioural thermoregulation could be direct or indirect, rapid responses associated with arthropod behavioural thermoregulation and recent research on *Drosophila* thermotaxis (i.e., temperature preference; see below) strongly suggest that, at least for microhabitat selection, direct temperature-sensing mechanisms are at play.

External thermosensory organs (e.g. sensilla) have been identified for many insects, often residing on antennae but also found on other areas of the body such as the thorax, head, legs, and ovipositor (Altner and Loftus 1985; Brown and Anderson 1998; Zars 2001). Internal thermosensory anterior cell neurons located in the brain have also been identified in *Drosophila* (Hamada et al. 2008). Recent studies of *Drosophila* thermotaxis demonstrate that both external thermosensing organs and internal thermosensory neurons play a key role in behavioural thermoregulation. Temperature sensation by these structures is mediated by an array of Transient Receptor Protein (TRP) cation channels that are activated by temperatures above or below the flies' preferred temperature (~25°C) (Sayeed and Benzer 1996; Zars 2001; Hamada et al. 2008; Gallio et al. 2011; Shen et al. 2011; Fowler and Montell 2012). In *Drosophila* adults, most peripheral thermosensory neurons are located in the antennae. A subset of these neurons are excited by cooling and inhibited by warming ('cold-sensitive'), in opposition to 'warm-sensitive' thermoreptors, which are exited by warming and inhibited by cooling (Hamada et al. 2008; Gallio et al. 2011). These sensory inputs produce a thermal 'map' in the *Drosophila* brain (Gallio et al. 2011) which is further processed by projection neurons in the proximal antennal protocerebrum and passed on to several other brain regions implicated in sensory reception and behavioural modulation (Frank et al. 2015; Liu et al. 2015). Together, these sophisticated systems of thermoreceptors and neural networks provide fine-scale thermal information that is required for flies to undertake normal thermoregulatory behaviour (Frank et al. 2015; Gallio et al. 2011).

Other investigations have revealed that fine-scale temperature preferences in *Drosophila* larvae within their 'comfortable' range (18-24°C) are, in part, controlled by an indirect signaling cascade that involves rhodopsin, a protein previously implicated in photoreception (Kwon et al. 2008; Shen et al. 2011). In the relatively simple case of *Drosophila* larval thermotaxis, these sensory inputs are integrated to bias stochastic patterns in locomotion towards favourable temperatures (Luo et al. 2010). These investigations set the stage for future tests on more complex strategies, including those dependent on dynamic aspects of physiological state (e.g. nutritional status, parasitic infection; see above). If the mechanisms of temperature sensation underlying behavioural thermoregulation have similar mechanisms in *Drosophila* and other species of arthropods, dynamic changes in preferred temperature would imply that information from thermosensory neurons is somehow integrated with information regarding physiological state (as in Figure 8-2). It is currently unknown whether this integration would take place downstream of thermoreception or instead whether physiological state would somehow modify the sensitivity of thermosensory mechanisms to adjust preferred temperature (via neural/hormonal signaling cascades, for example).

Evolution of behavioural thermoregulation

Behavioural thermoregulation has a critical role in shaping ectotherms' evolutionary adaptations to environmental thermal variation. While behaviour is often seen as a driver of evolutionary change (e.g., Duckworth 2009; Zuk et al. 2014), Huey et al. (2003), building on longstanding hypotheses (Bogert 1949), elegantly demonstrated that behavioural thermoregulation might actually act as an inertial force for the evolution of adaptations to temperature variation (the "Bogert effect"). Because behavioural thermoregulation essentially functions to mitigate thermal variations experienced by individuals that do not thermoregulate, it would reduce the strength of selection on other temperature-dependent physiological or behavioural traits (Huey et al. 2003). In addition, behavioural thermoregulation could buffer many of the negative effects of climate change on animals, although this will depend on factors such as the degree of thermal specialization and the availability of opportunities for thermoregulation (Kearney et al. 2009; Huey and Tewksbury 2009; Huey et al. 2012; Sunday et al. 2014).

Thermoregulatory behaviours by ectotherms are expected to undergo strong selection because they generally permit these animals to attain appropriate body temperatures much more rapidly than physiological or morphological adaptations (Stevenson 1985). The evolutionary consequence is that the physiology (i.e., performance curves of various functions) of some arthropods is adapted not necessarily to the typical ambient temperature of their environment, but rather to the temperature these organisms are routinely able to achieve via behavioural thermoregulation (Willot and Hassall 1998; Whitman 1988; Sanborn and Maté 2000; Sunday et al. 2014). For example, several species of Orthoptera are almost completely dependent on behavioural thermoregulation to obtain the body temperatures necessary to permit critical physiological functions and avoid adverse temperatures (Whitman 1988, Willott and Hassall 1998). But what are the proximate factors that influence the evolution of behavioural thermoregulation? Optimal thermoregulatory strategies should reflect the evolutionary pressures imposed by abiotic and biotic environmental factors, as well as the constraints or opportunities inherent in a species's life history characteristics. Intra- and inter-species differences in thermoregulatory behaviours have been described and are often tied to differing characteristics of habitat, life history, morphology, and thermal sensitivity.

Several investigations have presented convincing evidence for local adaptation in thermoregulatory behaviour. *Melanoplus sanguinipes* (F.) (Orthoptera: Acrididae) originating from higher elevations exhibit a higher tendency to raise body temperatures through mobility and basking behaviour than individuals from lower elevations (Samietz et al. 2005). At higher elevations, butterflies would need to compensate for less available thermal energy by absorbing more solar radiation (Samietz et al. 2005). Similarly, Nevo et al. (1998) found marked differences in the preferred temperature for oviposition in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) from differing elevations of opposite slopes of a canyon in Israel. Isofemale lines from south-facing slopes and higher elevations preferred higher temperatures for oviposition than lines from north-facing slopes and lower elevations (Nevo et al. 1998). Invasive populations of *Drosophila subobscura* Collin (Diptera: Drosophilidae) at higher latitudes in Western North America have longer activity periods and are more active overall than those at lower latitudes, despite having a lower body temperature, implying local adaptation of daily activity cycles (Huey and Pascual 2009). The short time period since the invasion began implies that these clinal differences in thermoregulatory behaviours must have evolved extremely rapidly (Huey and Pascual 2009).

Behavioural thermoregulation can be costly (Huey 1974, Huey and Slatkin 1976). Microhabitat selection, for example, may cause arthropods to forego exploitation of resources (e.g. food, mates) that reside in thermally undesirable locations (Forsman et al. 2002) and must be balanced with ecological factors such as predation risk (e.g. Stahlschmidt and Adamo 2013). Behaviours such as basking may render arthropods more conspicuous and vulnerable to predators (Whitman 1988; reviewed in Chown et al. 2004 pp. 168-169), while endothermic behaviours like flight muscle warm-up carry large energetic costs (May 1979).

Finally, thermoregulatory behaviour coadapts with morphology and physiology. For example, Whitman (1988) postulated that the evolution of chemical defenses in the desert grasshopper *Taeniopoda eques* Burmeister (Orthoptera: Romaleidae) permitted this insect to subsequently evolve large body size, sophisticated thermoregulatory behaviours, and thermally beneficial colouration, all of which would make the grasshoppers conspicuous and vulnerable to potential predators in the absence of chemical defenses. In a series of investigations, Forsman (2000, 2002) and colleagues demonstrated that genes encoding different colour morphs and associated thermoregulatory behaviours are likely to have co-evolved in *Tetrix* pygmy grasshoppers (Orthoptera: Tetrigidae). Thermoregulatory behaviour also co-adapts with other aspects of thermal biology, including both Direct and Indirect Kinetic effects (reviewed in Angiletta 2009). For example, differences in thermoregulatory behaviours (e.g. temperature preferences) are sometimes observed between individuals having developed at different temperatures (Forsman et al. 2002; Samietz et al. 2005; Krstevksa and Hoffman 1994; Good 1993).

8.8 Integrated effects – Thermosensory Behavioural Adjustments and Thermal Orientation

Apart from behavioural thermoregulation, do ectotherms use direct thermal information to adaptively modify other aspects of their behaviour (Figure 8-2)? It has not yet been conclusively demonstrated that such behaviours arise from direct temperature perception (but see ‘thermal orientation’ below) rather than an indirect effect of temperature on some other aspects of physiological state (i.e., Direct or Indirect Kinetic effects). There is some tantalizing evidence that such behaviours may exist, mostly from vertebrate systems (Hertz 1982; Herrel et al. 2007; Ohlund et al. 2015). But even in vertebrates, supposedly adaptive temperature-induced behavioural modification has not been directly linked to direct temperature perception. Below, we summarize possible examples of Non-thermoregulatory Integrated effects of temperature on behaviour from the arthropod literature. We divide non-thermoregulatory behaviours that are informed by thermoreception into (i) Thermosensory Behavioural Adjustments and (ii) Thermal Orientation (Table 8-5). In the case of Thermosensory Behavioural Adjustments, the results of several studies imply that perception of ambient temperature causes the arthropod to modify its “decision-making”; the implied reasoning is that the optimality of an individual’s decision depends on temperature and therefore the animal has evolved behavioural mechanisms to adjust for this dependence. Thermal orientation, on the other hand, is very similar to the already discussed microhabitat selection in thermoregulation in that it seems to involve the same proximate mechanism (thermotaxis; see Ruchty et al. 2010a, 2010b), but serves an ecologically different function (resource localization rather than modification of body temperature; Table 8-5). Because of this mechanistic similarity between thermal orientation and microhabitat selection for thermoregulation, we will rather focus our discussion in this section on Thermosensory Behavioural Adjustments, which are probably distinct in ecological function and evolutionary history.

Possible examples of Thermosensory Behavioural Adjustments

Common explanations for thermosensory behavioural adjustments imply high or low temperatures being used as an indication of increased mortality risk (Dill et al. 1990), lower life expectancy (Moiroux et al. 2015), increased metabolic costs of activity (Traniello et al. 1994) or deteriorating environmental conditions (Dumont and McNeil 1992; Roy et al. 2003; Amat et al. 2006). One should be careful with these interpretations because the effect of other variables (e.g. humidity, photoperiod) is difficult to tease apart from the effect of temperature (Dill et al. 1990; Dumont and McNeil 1992). Furthermore, in all of the above cases, the observed changes in behaviour could have been due to temperature-induced metabolic/physiological constraints (Kinetic effects) rather than Themosensory Behavioural Adjustments; an effort is not often made to distinguish between the two possibilities. However, Traniello et al. (1994) did acknowledge that increased acceptance of lower-quality small prey items by foraging *Formica schaufussi* (Hymenoptera: Formicidae) at high

Table 8-5. Possible examples of Integrated effects that do not involve thermoregulation, and their proposed adaptive functions: Thermosensory Behavioural Adjustments (TBAs), and thermal orientation.

Behavioural category	**Species**	**Behaviour**	**Proposed function**	**Reference**
TBA	*Trichogramma euprocditis* (Hymenoptera: Trichogrammitidae)	Female parasitoids intentionally allocated more male offspring to hosts when foraging at high temperature.	Minimize fitness reduction of offspring caused by developing or emerging at high temperature (fitness of male offspring less affected).	Moiroux et al. (2014)
TBA	*Aphidius ervi* (Hymenoptera: Braconidae)	When at a high temperature, parasitoids more frequently attacked lower-quality host instars.	Adopting more risk-prone behaviours at high temperature was an adaptive response to a lower expectation of lifetime reproductive success due to a shorter life expectancy.	Moiroux et al. (2015)
TBA	*Venturia canescens* (Hymenoptera: Ichneumonidae)	Parasitoids spent longer on host patches following a drop in temperature.	Temperature drop used as indication of deteriorating conditions (increased cost of inter-patch travel).	Amat et al. (2006)
TBA	*Acyrthosiphon pisum* (Hemiptera: Aphididae)	Aphids were less likely to drop off host plants when temperature was high and humidity was low.	Avoid leaving the host plant when chances of subsequent mortality are high.	Dill et al. (1990)
TBA	*Pseudaletia unipuncta* (Lepidoptera: Noctuidae)	Males were less responsive to female sex pheromone (longer mating delay) when experiencing low-temperature, short day-length conditions.	Delaying mating under deteriorating end-of-season conditions provides a window for long-distance migration.	Dumont and McNeil (1992)
TBA	*Tetranychus mcdanieli* (Trombidiformes: Tetranychidae)	At high and low temperatures, spider mites allocated proportionally more female progeny.	An adaptive response to deteriorating conditions, since females have better dispersal capacity and survival under harsher conditions.	Roy et al. (2003)
TBA	*Gryllus bimaculatus* (Orthoptera: Gryllidae)	The responsiveness of female crickets to male songs at a given temperature was 'coupled' to the effect temperature has on song characteristics.	Permit mate recognition across range of variability in mating signals caused by temperature.	Doherty (1985)
Thermal orientation	*Atta vollenweideri* (Hymenoptera: Formicidae)	Worker ants were able to use thermal radiation as a learned orientation cue for food rewards.	Location and return to valuable resources such as sun-exposed leaves.	Kleidenham et al. (2007)
Thermal orientation	*Triatoma infestans* (Hemiptera: Reduviidae)	Bugs oriented towards heat sources that approximated the temperature of a food source.	Food source location.	Lazzari and Nunez (1989)
Thermal orientation	*Melanophila acuminata* (Coleoptera: Buprestidae)	Beetles oriented towards thermal radiation emitted from forest fires.	Location of sites for mating and reproduction.	Evans (1964)
Thermal orientation	*Aedes aegypti* (Diptera: Culicidae)	Mosquitos oriented towards heat produced by their hosts, associating them with odor plumes using visual information.	Location of feeding sites.	van Breugel et al. (2015)

temperatures may be due to temperature-imposed constraints on foraging (hyperthermia, dessication, impairment of sensory ability) rather than an adaptive behavioural adjustment taking into account temperature-dependent metabolic costs. In a rare effort to distinguish between a behavioural adjustment and physiological constraints, Moiroux et al. (2014) were able to measure 'intended' sex-ratio allocation and the corresponding secondary (realized) sex ratio by the haplodiploid egg parasitoid *Trichogramma euproctidis* (Girault) (Hymenoptera: Trichogrammatidae) at different temperatures by observing oviposition behaviour (pattern of abdominal contractions to indicate intended sex ratio) and rearing the resulting offspring to adulthood. Similar to several other studies of parasitoid sex allocation (reviewed in King 1987), they observed a more male-biased secondary sex ratio at high and low temperatures compared to the intermediate temperature. However, the mechanisms behind the more male-biased sex ratios at the two extremes were different. At high temperatures, the intended proportion of male offspring (as well as the secondary sex ratio) increased, suggesting a temperature-dependent behavioural adjustment. At low temperature, the intended proportion of male offspring was the same as at intermediate temperature, but a reduction in the rate of egg fertilization (a Kinetic effect) caused a more male-biased secondary sex ratio than intended. It was hypothesized that intentionally increasing the proportion of males produced at higher temperatures is adaptive because developing and/or emerging at high temperatures may be less costly for male offspring than for female offspring. This work supports the notion that a single behavioural parameter can be subject to both compensatory adjustments and physiological constraints due to temperature, and illustrates the importance of distinguishing between the two possibilities when attempting to assign adaptive explanations to temperature-induced behavioural differences – in order for an adaptive explanation to be at all plausible, one first has to show that the behaviour is not simply due to a constraint.

Perhaps the most thoroughly researched phenomenon involving temperature-induced behavioural adjustments is "temperature coupling" (Gerhardt 1978) in animals that produce acoustic (e.g. crickets, grasshoppers) or visual (e.g. fireflies) signals for mate attraction. Several studies have observed that, across a range of sexually signaling species, levels of female responsiveness to male signals at a given temperature parallel temperature's effects on the male signal; that is, temperature's effect on male signal and the female's evaluation of the signal are coupled (reviewed in Greenfield and Medlock 2007). The adaptive explanation behind temperature coupling is that in order to properly assess male signals (ie. permit species recognition and assess male quality), the evaluation of signals by females needs to be adjusted for the effect temperature has on male signals (Gerhardt 1978). Early on, two hypotheses were put forward to explain this phenomenon: the common neural elements hypothesis and the coevolution hypothesis (reviewed in Pires and Hoy 1992a). The common neural elements hypothesis proposes that male signal pattern generation and female song recognition are processed by homologous neural networks which are affected by temperature in the same way; for example, in crickets the female could use the temperature-dependent output from the thoracic CPG, which produces the neural signal used to generate stridulatory patterns in males, as a template for song recognition in the female brain. The coevolution hypothesis rather states that the neural networks underlying male pattern generation and female response are different, but have coevolved to sustain coupling of male signaling and female responsiveness over a range of temperatures. Despite more than 40 years of research and debate concerning the mechanisms behind temperature coupling (e.g., Gerhardt 1978; Pires and Hoy 1992a,b; Doherty 1985; Bauer and von Helverson 1987; Shimizu and Barth 1996; Ritchie et al. 2001; Greenfield and Medlock 2007), its underlying mechanisms and adaptive significance remain unclear. In the context of our framework for temperature's effects on behaviour, several of the behaviour types we outline have been proposed as a mechanism under the umbrella of both of the above hypotheses. For example, a modified version of the common neural elements hypothesis proposed by Pires and Hoy (1992b) states that female crickets integrate temperature-dependent input from the thoracic CPG in the brain to produce responses to male signals (Indirect Kinetic effect), possibly with input from thermosensors in the head or the thorax (Thermosensory Behavioural Adjustment). In both of these cases, it is unclear if signal generation in males would only be due to temperature's effect on metabolic processes and speed of nerve signal transmission in the CPG (Indirect Kinetic effect) or less plausibly, if CPG signals would also be integrated with thermosensory input in males' brains to adjust stridulatory patterns (Thermosensory Behavioural Adjustment). Greenfield and Medlock (2007) presented evidence that temperature coupling in the acoustically-mating moth *Achroia grisella* (F.) (Lepidoptera: Pyralidae) does not even constitute a true adaptation, and the apparent coordination of signal generation and reception simply represents an emergent effect of the common influence of temperature on the underlying neuromuscular physiology of males and females (Kinetic effects). Clearly, further investigation and debate concerning the physiological mechanisms behind temperature coupling are still needed.

Proximate mechanisms behind Thermosensory Behavioural Adjustments

As is evident from the preceding section, the proximate mechanistic basis of apparently adaptive behavioural shifts due to temperature is somewhat unclear. To date, the few investigations that have attempted to explore these mechanisms and their adaptive significance have made use of techniques such as hybridization studies (temperature coupling; reviewed in Doherty 1985), selective warming of different body segments (temperature coupling; Pires and Hoy 1992b, Bauer and von Helverson 1987), examination of genetic correlations (temperature coupling; Greenfield and Medlock 2007), and detailed behavioural observations (temperature-dependent sex allocation; Moiroux et al. 2014). While these techniques could be usefully applied to studies of other aspects of temperature-dependent behaviour, they have not yet consistently allowed investigators to conclusively support or eliminate competing mechanistic or evolutionary hypotheses. In many cases, the mechanistic basis and adaptive significance of these behaviours could be better informed by knowledge of whether direct temperature perception is involved (section 8.7). This potential approach will be discussed at the end of this article (section 8.9).

Evolution of Thermosensory Behavioural Adjustments

Under what conditions would we expect compensatory behavioural adjustments to temperature to evolve? Given the inertial evolutionary effect of thermoregulatory behaviours (Huey et al. 2003), we might predict that compensatory behavioural adjustments could evolve when the costs of behavioural thermoregulation are too high or thermoregulatory behaviours are not feasible. This could occur, for example: (i) when opportunities for reproduction or feeding are in a fixed location and the location of alternative opportunities in better thermal environments is impossible or suboptimal, so that continuing to exploit the resource necessitates behaviourally compensating for the imposed thermal environment; (ii) when temperature’s effect is external to the individual in question and cannot be changed by thermoregulatory means (e.g. in female crickets intercepting a male courtship call determined by his body temperature); or, (iii) when options for thermoregulation by microhabitat selection are extremely limited by, for example, very poor dispersal ability (e.g. in immature stages of some arthropods). Evolution of compensatory thermal behaviours under such conditions would also imply that these behaviours are more easily shaped by selection or are more effective than shifting physiological performance curves and/or reaction norms via metabolic evolution or acclimation.

8.9 Use of the framework in future research on thermal behavioural ecology

In building our framework for the effect of temperature on ectotherm behaviour, we have encountered two major themes. First, apart from thermoregulatory behaviours, there have been very few tests of whether temperature influences adaptive decision-making processes in arthropods. Second, even when an apparently adaptive behavioural modification is observed with changing temperature, it is unknown whether it is a direct response to temperature perception or rather just a direct or indirect kinetic effect of temperature on some aspect of the animal's physiological state. To help guide future research in this area, we suggest two general, complementary approaches to distinguish integrated effects from direct and indirect kinetic effects: behavioural kinetic null modelling and behavioural ecology experiments using temperature-insensitive mutants. In both cases, the principle is the same: *if one can demonstrate that a behavioural modification does not occur because of direct temperature perception, it is mostly likely due to a Kinetic rather than an Integrated effect.*

Behavioural Kinetic null modelling

To determine whether a change in an animal's behaviour is a direct response to temperature perception, one can ask: how would the organism behave in the absence of Integrated effects? This kinetic null model would contain relationships describing only Kinetic Effects of temperature on behaviour (i.e., constraints), both direct and indirect. Thus, the null model would treat the animal as a metabolic machine whose behaviours are driven only by the effects of temperature on the rates of biochemical processes. Then, one or more Integrated models could be constructed assuming that the animal's behaviour is optimized to the impacts of temperature on fitness payoffs; that is, the individual is monitoring temperature and adaptively modifying its behaviour to suit thermal conditions (i.e., there are likely Integrated effects acting on behaviour).

Null modelling has been used previously in the context of behavioural thermoregulation. Huey et al. (2003) considered how the body temperature and sprint performance of thermoregulating lizards would vary over an elevational gradient, compared to “null lizards” that did not perform any thermoregulatory behaviours. Because of thermoregulation, the empirically measured body temperatures of real lizards declined less rapidly with increasing elevation than would be expected of null lizards. Subsequent studies, using *Caenorhabditis elegans* (Anderson et al. 2007) and *Drosophila* (Dillon et al. 2012) as model organisms, formulated null models in order to disentangle temperature’s effects on movement from temperature preference on the spatial distribution of small ectotherms across a thermal gradient. Here, the null models would assume random (Brownian) motion whose rate was dependent on temperature’s Direct Kinetic effects on movement. These studies showed that the expected null distributions were not necessarily intuitive and were extremely dependent on experimental conditions (i.e., the range of temperatures on the gradient relative to the organism’s thermal tolerance). Thus, these null models are critical to be able to establish what proportion of the observed distribution of small ectotherm species on a thermal gradient depends on thermal preference (Integrated effects) and not Kinetic effects. So, Kinetic null modelling is beginning to be fruitfully applied to teasing out the Integrated component of thermoregulatory behaviours, but it has not been used to investigate potential adaptive components of other behavioural responses to temperature.

As an example of how the kinetic null-modelling approach could be extended to other complex thermally dependent behaviours, consider a time-limited forager in an environment where resources are patchily distributed – a classic behavioural ecology paradigm. Optimal foraging theory postulates that such a forager should adjust the amount of time spent in each patch (its Patch Residence Time; PRT) based on factors (e.g. internal physiological state, relative host or prey quality in each patch, travel time between patches, stochastic mortality risk) that influence the forager’s perception of future fitness gains (reviewed in Wajnberg 2006). A forager is predicted to leave a resource patch when its rate of fitness gain on the patch falls below the average rate of fitness gain in the environment (Charnov 1976). When PRT varies with temperature, potential explanations may include both Kinetic and Integrated effects. Kinetic effects may include increased movement speed, more rapid host or prey handling, higher digestion rate (herbivores and predators), and more rapid oogenesis (parasitoids). The thermal dependency of such parameters could be measured and used to parameterize a null model for the effect of temperature on PRT. Other parameters would have fixed values whose thermal dependencies are unknown to the forager, because they do not depend on some aspect of its own metabolic rate. Then, Integrated models could be built assuming that the forager's behaviour is adapted to the thermal dependency of one or more of these other factors (e.g. travel time, host quality, mortality risk, energy expenditure) affecting the tradeoff between current and future fitness gains. One would then test the effect of temperature on forager PRT and determine if the data best fit the predictions of the Kinetic null model or one of the alternative Integrated models. If the data do not fit any of the models, then there are two possibilities: (i) there are relevant Direct or Indirect Kinetic effects that are not being included in the null model, or (ii) the hypotheses underlying the Integrated models are flawed. The models could then be updated with new hypotheses concerning how the behaviour is affected by temperature and tested with new experiments, gradually eliminating competing hypotheses and improving the fit between the model and experimental data. Used judiciously, this approach could be extended to a wide range of behaviours including foraging (e.g. resource choice, resource defense), reproduction (e.g., clutch size, mate guarding duration), communication (e.g. calling behaviour, vibrational signaling), dispersal (e.g., selection or time allocation amongst different habitats), and anti-predator strategies (e.g., choice to attack or flee).

The null modelling approach has some limitations. Firstly, parameterizing a useful Integrated model requires an accurate knowledge of how temperature affects the fitness payoffs of different possible behavioural decisions and assumes that there have not been evolutionary constraints on the animal's ability to adapt to these differential payoffs. Thus, as with all models, these Integrated models will only be as good as the hypotheses contained within them. Second, to include all relevant Kinetic effects in a null model, one needs a complete understanding of how thermal physiology underlies behaviour to make sure that deviations from the null model reflect Integrated effects rather than Indirect Kinetic effects that have not been accounted for. This should sometimes be apparent by a poor fit of the experimental data to the Integrated models, although it is conceivable that an Indirect Kinetic effect could produce the same behavioural pattern as if the animal were directly perceiving temperature (for example, if an individual is "tracking" its own metabolic rate). So, in some cases, it may also be necessary to employ the next approach, the use of temperature-insensitive mutants.

Use of temperature-insensitive mutants in behavioural ecology experiments

The most direct way to test if direct temperature perception is involved in a behaviour would be to examine if individuals with dysfunctional temperature sensation still change their behaviour over a range of temperatures. So far, this has only been performed in the context of *Drosophila* behavioural thermoregulation (see Section 8.7). Temperature-insensitive *Drosophila* have been produced either by developing mutant lines deficient in thermoreceptor function (Hamada et al. 2008; Gallio et al. 2011; Shen et al. 2011), functionally inactivating cellular structures involved in temperature perception with transgenic expression of toxins (Gallio et al. 2011), or the removal/ablation (e.g. with lasers) of putative thermoreceptory organs (Sayeed and Benzer 1996; Hamada et al. 2008). While these studies have been made possible by the wide array of genetic tools available for *Drosophila*, the recent sequencing of many other arthropod genomes could facilitate the extension of these tools to other biological systems. Sayeed and Benzer (1996) described a relatively simple screening method involving (i) a choice-chamber apparatus to screen for mutants that do not avoid aversive temperatures and (ii) verification on a thermal gradient followed by the establishment of isofemale lines deficient in temperature sensation. This methodology could be used to develop lines of temperature-insensitive mutants of other arthropod species that could then be genetically characterized and tested for temperature-dependent behavioural adjustments. Of course, this proposed approach assumes that the thermoreceptory mechanisms involved in thermoregulation, including the location and function of specific thermosensory organs, are the same as those involved in other Thermosensory Behavioural Adjustments. This matter could be subjected to preliminary investigation by, for example, conducting investigations of temperature coupling in temperature-insensitive mutant lines of *Drosophila* (Ritchie et al. 2001). Insect parasitoids are potentially ideal biological systems for testing this approach. Using parasitoids, one could test whether temperature-dependent sex ratio allocation (Moiroux et al. 2014) or patch residence time adjustments with temperature (Amat et al. 2007) depend directly on temperature perception by comparing responses of mutant versus wild-type lines. This methodology could be particularly effective if used in combination with null modelling – one would expect the behaviour of temperature-insensitive individuals to follow the predictions of the null model, while the behaviour of wild-type individuals should follow the predictions of the integrated model.

8.10 Conclusions

To be able to predict the global effects of temperature changes on ecosystems, a detailed understanding of how it acts on all levels of biological organization is needed. When investigating the influence of temperature and the potential effects of global warming on communities and ecosystems, authors mainly consider physiologically-based changes in life histories of organisms such as changes in phenology which may result in seasonal mismatches between predators and their prey (for a review, see Parmesan 2006). However, the influence of temperature on behaviours may sometimes explain ecological changes associated with global warming better than its influence on physiology. As just one example, consider a recent study by Barton (2010), who was studying a grassland food web comprised of spider predators, grasshopper herbivores, herbs, and grasses. In this system, spiders had an indirect positive indirect effect on grasses and an indirect negative effect on herbs by causing grasshoppers to change their diet composition towards herbs when spiders were present. Considering physiological differences between spiders and grasshoppers, Barton predicted that warming would decrease top-down control and weaken these indirect effects of predators on plants, but field results demonstrated the opposite. The author then simulated warming in the lab and performed behavioural observations to understand this contradiction. It appeared that the degree of spatial overlap between grasshoppers and spiders decreased with warming because of a higher sensitivity of spiders to high temperatures and resulting behavioural thermoregulation. Spiders moved lower in the canopy than grasshoppers, allowing herbivores to increase their daily feeding time and resulting in even more damage to herbs. The enhanced indirect effect of spiders on plants due to warming could thus be explained thanks to behavioural observations. This study and others (see Harmon and Barton 2013) demonstrate that the influence of temperature on behaviours should be more frequently considered when investigating the influence of global warming on communities and ecosystems including, for example, the design of management programs against invasive species (Fahrner et al. 2015).

Temperature influences many aspects of complicated physiological systems, and organisms have also evolved adaptive responses to cope with temperature variation. Because of this complexity, it is difficult to tease out what parts of behavioural responses to temperature are adaptive and which are due to constraints. Distinguishing between different mechanisms is important because different underlying mechanisms may have different capacities to adapt to changing environments. The framework we have presented here constitutes a first attempt to organize our current understanding and focus future research on the role temperature plays in behavioural ecology. Researchers planning to investigate how ectotherm behaviour changes with temperature should use our framework as a way to think about how organisms *should* change their behaviour with temperature and how constraints will simultaneously act to masquerade as, or obscure, adaptive components of the behavioural response. As understanding advances about how thermal physiology underlies behaviour, our framework needs to be modified and built upon. Hopefully in the meantime it will help guide more focused, hypothesis-based research, and lead to a fuller consideration of temperature's effects on the behavioural ecology of ectotherms.

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Chapter 9: An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid

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9.1 Abstract

Invasive alien species can act as ‘evolutionary traps’ for indigenous parasites and predators when the alien species is accepted as prey or a host but is unsuitable for consumption or development. We tested the relationship between acceptance and suitability of eggs of the invasive alien *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) in North America relative to eggs of the indigenous *Podisus maculiventris* Say (Pentatomidae) for the indigenous generalist egg parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae). *Telenomus podisi* accepted 0-24h old *H. halys* eggs at a rate similar to *P. maculiventris* eggs (87.5 ± 6.0% and 70.2 ± 9.1%, respectively). Successful development of *T. podisi* occurred in 98.3% of attacked *P. maculiventris* eggs, but was not observed in *H. halys* eggs. Oviposition by *T. podisi* did, however, reduce the developmental success of *H. halys* embryos relative to unattacked controls by 24.1% in 0-24h old eggs and 29.6% in 24-48h old eggs. We suggest that as *H. halys* spreads and increases in abundance in North America, it could operate as an evolutionary trap for indigenous egg parasitoids, thereby indirectly causing an increase in population levels of indigenous pentatomids. This predicted indirect effect would be a result of *H. halys* eggs acting as an egg sink for *T. podisi*. We also introduce the concept of a ‘time sink’, which may be particularly relevant for parasitoids such as *T. podisi* that spend considerable time protecting their reproductive investments.

Key words: biological invasion, egg sink, evolutionary trap, *Halyomorpha halys*; host-parasitoid relationship, host acceptance, time sink, *Telenomus podisi*

9.2 Introduction

A forager's utilization of resources is shaped by past selective pressures that link the behaviours necessary to exploit a resource, such as the utilization of cues related to resource acceptance, and the profitability of that resource (i.e., the resulting fitness payoff) (Schlaepfer et al. 2002). Introduction of an invasive alien species may add a resource to the environment that a forager has never encountered and break the link between resource acceptance and profitability; for example, when cues for acceptance of an invasive alien prey by an indigenous predator are present but the prey is unsuitable for consumption (Schlaepfer et al. 2005). The invasive alien prey is then referred to as an 'evolutionary trap' (Schlaepfer et al. 2005) and causes indigenous predators to invest energy or reproductive resources that result in a lower profitability than that normally associated with the investment.

Evolutionary traps may have consequences for the population dynamics of indigenous parasitoids, their indigenous hosts, and the invasive alien host species by mediating indirect effects. For example, the North American parasitoid *Dinocampus coccinellae* (Hymenoptera: Braconidae) shows similar levels of acceptance of the invasive *Harmonia axyridis* (Coleoptera: Coccinellidae) and the indigenous *Coleomegilla maculata* (Coccinellidae) (Firlej et al. 2010; Hoogendoorn and Heimpel 2002), but the former host species has high levels of resistance to parasitism via haemocytic encapsulation while the latter does not (Firlej et al. 2012). Rather than the more common situation where the presence of *H. axyridis* would cause an increase in parasitism of *C. maculata* via augmentation of populations of *D. coccinellae* (i.e., apparent competition), *H. axyridis* rather acts as an 'egg sink' for *D. coccinellae*, reducing levels of parasitism of *C. maculata* by causing foraging *D. coccinellae* to become egg-limited (Hoogendoorn and Heimpel 2002; Heimpel et al. 2003). Thus, according to the model of Heimpel et al. (2003), the presence of *H. axyridis* actually increases the equilibrium population levels of *C. maculata*, while the presence of *C. maculata* has a small negative effect on population levels of *H. axyridis* (Hoogendoorn and Heimpel 2002). This situation is referred to as an indirect (+, -) interaction, or 'apparent predation' (Holt 1977; Heimpel et al. 2003). Similar examples of parasitoids showing high levels of acceptance of unsuitable invasive alien species and predictions about the resulting indirect ecological consequences are lacking in the literature.

Halyomorpha halys Stål (Hemiptera: Pentatomidae), the brown marmorated stink bug, is a pest of Asian origin that has recently become established in North America (Hoebeke and

Carter 2003) and Central Europe (Wermelinger et al. 2008). This species has already become very common in invaded areas and has caused substantial economic losses in fruit orchards in the Northeastern United States (Nielsen and Hamilton 2009; Leskey et al. 2012). *Halyomorpha halys* is also a nuisance for homeowners when large numbers of the bugs move indoors for overwintering (Leskey et al. 2012). *Halyomorpha halys* continues to spread and based on climate modelling is expected to expand its range to large geographic areas of North America and Europe (Zhu et al. 2012). The first established Canadian population of *H. halys* was found in the summer of 2012 in Southern Ontario (T.D.G. and H. Fraser, unpublished data). While there are some reports of indigenous parasitoids being reared from *H. halys* eggs in the United States (Hoelmer and Tatman 2011; Leskey et al. 2012), no published studies have directly tested the acceptance and suitability of *H. halys* eggs to these parasitoid species.

Telenomus podisi Ashmead (Hymenoptera: Platygastridae) is a common indigenous egg parasitoid usually responsible for the majority of parasitism of several pentatomid species' eggs in North America (Yeargan 1979; McPherson 1982; Orr et al. 1986; Koppel et al. 2009). In the current study, the relationship between acceptance and suitability of *H. halys* eggs for *T. podisi* relative to eggs of the indigenous predatory species *Podisus maculiventris* Say (Pentatomidae) was tested under laboratory conditions. Following Keeler and Chew (2008), there are three general outcomes (with intermediate outcomes possible between each): (i) *T. podisi* will not recognize the invasive *H. halys* as a host, (ii) *T. podisi* will recognize *H. halys* eggs as suitable hosts and offspring will successfully develop, and (iii) *T. podisi* will recognize *H. halys* as a suitable host but offspring will be unable to develop. Whether or not *H. halys* eggs are accepted by and suitable for *T. podisi* development could have important ecological consequences for the parasitoid, its indigenous pentatomid hosts, and *H. halys*.

9.3 Materials and Methods

Insects

Halyomorpha halys were originally collected from a population in Hamilton, Ontario, Canada in the summer of 2012 (~200 individuals). They were then reared continuously at a containment facility at the Southern Crop Protection and Food Research Centre in London, Ontario at 24 ± 1°C, 50 ± 10% RH and under a 16:8h L:D photoperiod. First-instar nymphs were housed in 3L plastic tubs and provided with lettuce and raw peanuts. Adults and 2nd-5th instar nymphs were housed in ventilated cages (47.5 cm^3) with water, lettuce, sunflower seeds, peanuts, and apples. Eggs laid by females on paper towel and the sides of the cages were collected daily for experiments and to perpetuate the rearing.

Podisus maculiventris were originally collected as adults (~50 individuals) from several locations in the London region in 2011-2012 and reared continuously. Nymphs and adults were reared in plastic tubs, fed with mealworm (*Tenebrio molitor* L.) larvae and lettuce, and provided with water. Crumpled newspaper was used as oviposition substrate and eggs were collected daily.

Telenomus podisi were originally reared from ~100 field-exposed sentinel egg masses of *P. maculiventris* and *Euschistus* spp. (Hemiptera: Pentatomidae) in the London region in 2011. No *H. halys* populations were detected in Ontario until the summer of 2012, so it is most likely that these *T. podisi* populations had never encountered *H. halys*. Identifications of colony foundresses were confirmed by Dr. Lubomir Masner (National Collection of Insects, Arachnids, and Nematodes, Ottawa, Ontario, Canada). *Telenomus podisi* were reared continuously on eggs of *P. maculiventris* by offering egg masses (< 3 days old) to groups of *T. podisi* housed in large ventilated cages for 48h. To collect females used in experiments, parasitized egg masses from the colony were kept in small Petri dishes (diameter: 5.0 cm, height: 1.0 cm). Within 24h of emergence, females that had emerged in the presence of males were separated into 1.2 mL Eppendorf tubes with a small drop of honey water for 2 days before being used in experiments. This allowed time for ovariole maturation, since this strain of *T. podisi* is largely synovigenic (most of a female's lifetime production of oocytes mature during the adult stage) and females often emerge without any mature eggs (P.K.A.; unpublished data). Two to three-day-old, naïve females have between 9 and 15 mature eggs in their ovarioles and are consistently able to parasitize up to 16 host eggs in the subsequent 24-hour period (P.K.A.; unpublished data). This colony of *T. podisi* retains the ability to parasitize and develop in other species of stink bugs indigenous to North America despite being reared for several generations on *P. maculiventris* eggs (T.D.G.; personal observations).

Test arenas and host treatments

Pentatomid eggs were removed from the oviposition substrate with forceps and glued to squares of filter paper in masses of eight (two rows of four) with a small amount of white non-toxic glue (LePage White Glue). The squares of filter paper with eggs were then glued to the center of the bottom of a small Petri dish (diameter: 5.0 cm, height: 1.0 cm). Artificial egg masses were either (i) 24-48h old *P. maculiventris* eggs, (ii) 0-24h old *H. halys* eggs, or (iii) 24-48h old *H. halys* eggs. Two ages of *H. halys* eggs were tested because preliminary observations indicated that eggs were accepted at a much lower rate when >24h old. 0-24h old *P. maculiventris* eggs were not tested (i.e., a control for egg age) because we have previously determined that acceptance and suitability of *P. maculiventris* eggs to *T. podisi* does not differ with host egg age, even until the eggs are 5 days old (Zhou et al. 2014). For each replicate exposed to parasitoids, an unexposed egg mass of the same host treatment was set up in an identical arena. These served as controls for host developmental success (see below).

Behavioural observations

Experiments were performed at ambient laboratory temperature (23-25°C) and lighting between 3 and 8 hours after the start of the photophase. It was ensured that no movement occurred in the vicinity of the trials, since this affected parasitoid behaviour in preliminary experiments. Single female *T. podisi* were released into the Petri dish arenas containing one of the three host egg treatments. Their behaviour was filmed for 2h, more than twice the time required to completely exploit a host patch of eight eggs (Table 9-1). Each treatment was replicated 21 times. Treatments were spread out evenly with respect to day and time of day over the 5-day experimental period. Films were played back and, using Cowlog software (Hänninen and Pastell 2009), the occurrence and duration of the following behaviours were recorded (see Field 1998 for a detailed description of scelionid patch exploitation behaviour): (i) adoption of oviposition posture, (ii) drilling (insertion of ovipositor into a host egg), (iii) marking of a host egg, indicating egg deposition, (iv) patch guarding (repeated bouts of stationary and patrol behaviour; see Field 1998). The assumption that host marking indicates a successful oviposition has been confirmed several times in different species of scelionid parasitoids attacking different host species and is a well-established convention in the study of scelionid behaviour (Rabb and

Bradley 1970; Bin et al. 1993; Colazza et al. 1996; Weber et al. 1996; Field 1998; Agboka et al. 2002; Hirose et al. 2003; Wajnberg et al. 2004). We defined each oviposition attempt as a: (i) successful oviposition (adoption of oviposition posture + drilling + marking), (ii) rejection (adoption of oviposition posture + drilling, not followed by marking) (Colazza et al. 1996; Wajnberg et al. 2004), (iii) unsuccessful oviposition attempt (adoption of oviposition posture not followed by drilling). All rejections were of unparasitized host eggs; drilling was not observed in already-parasitized eggs. Only one incidence of superparasitism was observed and was discarded from the analysis. Eggs on which rejections and/or unsuccessful oviposition attempts occurred were often subsequently parasitized by the same female. Therefore, rejections and incomplete ovipositions did not necessarily indicate levels of final acceptance; rather, they are indications of how readily/efficiently the wasps parasitized host eggs.

Assessment of developmental outcomes

Following the filming period, females were removed from the arenas and both exposed and unexposed control eggs were incubated under standard rearing conditions (see above). Emergence of host nymphs and parasitoids was recorded as it occurred. After 30 days (7-8 days after the last parasitoid emergence), all eggs from which there was no emergence were dissected to record the contents of the egg: (i) undeveloped (liquid contents with no discernible stink bug nymph or parasitoid), (ii) fully developed pentatomid embryo that failed to emerge, (iii) pharate adult parasitoid that failed to emerge. Category (i) may have included dead early-instar parasitoid immatures that we were unable to distinguish, but dead late-stage parasitoid larvae or pupae were not observed in any of the treatments. Since gluing the eggs to filter paper was previously observed to affect emergence rate (but not development) of pentatomid nymphs, and all unemerged parasitoids (12.0% of all observed) were completely developed and still alive inside the host egg shell, completion of development rather than emergence was used as the final measure of developmental outcome. These parasitoids may not have emerged if the egg mass was left undisturbed, but since we never found parasitoids of any stage in *H. halys* eggs or unattacked controls (see below), using completion of development rather than emergence as the developmental outcome did not meaningfully affect any of the comparisons of host suitability for *T. podisi*.

Statistical analyses

Acceptance rate of host eggs in each replicate was calculated by dividing the number of accepted eggs by eight (the total number of eggs in the patch) and treatment rank-averages were compared with a Kruskal-Wallis test; data could not be normalized with transformations. Rejection rate and incomplete oviposition rate for each replicate were calculated by dividing the number of each behaviour by the total number of initiated ovipositions and rank-averages were compared with Kruskal-Wallis tests. Following significant ($p < 0.05$) Kruskal-Wallis tests, post-hoc differences between rank averages were compared by calculating the Tukey least significant differences (LSD) between mean ranks ($\alpha=0.05$). For replicates where females parasitized every egg (see Table 9-1 for the number in each host treatment), the mean time to exploit host patches (the time from the initiation of the first oviposition to the end of the last) was calculated and compared between treatments with a one-way analysis of variance (ANOVA). Also, in replicates where every egg was parasitized, the number of females that exhibited patch guarding behaviour was tallied. The proportion of guarding vs. non-guarding females was compared between the three host-treatments with a 2×3 Fisher exact test.

For each replicate, whether or not each egg was parasitized (from analysis of the videos) was related to its developmental outcome. For each host treatment, the developmental outcomes of parasitized eggs were pooled from all replicates and the proportions of each developmental outcome (undeveloped, pentatomid embryo development, parasitoid development) were compared with the outcomes of the unexposed control eggs (also with replicates pooled) with 2×3 (when parasitoid development occurred) or 2×2 (when parasitoid development did not occur) Fisher exact tests. Statistical tests were performed with R software (R Core Team 2013).

9.4 Results

Behavioural observations

The mean acceptance rate of host eggs (Kruskal-Wallis test, $H = 18.29$, $p < 0.001$, $df = 2$), mean rejection rate ($H = 25.09$, $p < 0.001$, $df = 2$), and mean incomplete oviposition rate ($H = 19.63$, $p < 0.001$, $df = 2$) of *T. podisi* varied significantly among host treatments (Table 9-1). *Podisus maculiventris* eggs and 0-24h old *H. halys* eggs were accepted at similar levels, while acceptance of 24-48h old *H. halys* eggs was significantly lower (Tukey LSD, $p < 0.05$). The greatest proportion of initiated ovipositions resulted in rejections on 24-48h *H. halys* eggs, while rejection rate of 0-24h *H. halys* eggs was significantly lower than that of *P. maculiventris* eggs (Tukey LSD, $p < 0.05$). A significantly larger proportion of initiated oviposition attempts were unsuccessful on *P. maculiventris* eggs than on either age of *H. halys* eggs (Tukey LSD, $p < 0.05$).

In replicates where every egg in the patch was parasitized, the time required to exploit the patch did not differ significantly between host treatments (ANOVA, $F_{2,29} = 1.76$, $p = 0.19$) (Table 9-1). In these replicates, the proportion of females exhibiting patch guarding behaviour, ranging from 0 on 24-48h old *H. halys* eggs to 0.625 on 0-24h old *H. halys* eggs, was also independent of host treatment (Fisher exact test, $p = 0.19$) (Table 9-1).

Assessment of developmental outcomes

The proportion of each developmental outcome was significantly different between eggs in which parasitism was observed and unexposed controls in *P. maculiventris* eggs (Fisher exact test, $p < 0.001$), 0-24h old *H. halys* eggs (Fisher exact test, $p < 0.001$), and 24-48h *H. halys* eggs (Fisher exact test, $p < 0.001$) (Figure 9-1). Parasitoid offspring successfully developed in 98.3% (117/119) of *P. maculiventris* eggs in which *T. podisi* oviposition was observed. While *T. podisi* did not ever successfully develop in either age of *H. halys* eggs, successful host nymph development was reduced by 24.1% and 29.6% in parasitized eggs relative to unexposed control eggs for host ages of 0-24h and 24-48h old, respectively (Figure 9-1). The reduced proportions of pentatomid embryo developmental success were attributable to higher proportions of 'undeveloped' eggs, where there was no discernible development of either a pentatomid nymph or a parasitoid.

Table 9-1. Behavioural parameters of *T. podisi* exploiting patches of *P. maculiventris* (24-48h old) and *H. halys* (0-24h old or 24-48h old) eggs. Within a column, means (±SE) followed by different letters are significantly different ($p < 0.05$; see text for details of statistical tests).

Treatment	**Mean % acceptance**[a]	**Mean rejection rate**[b]	**Mean incomplete oviposition rate**[c]	**# patches fully exploited (/21)**	**Mean time to exploit patch (min)**[d]	**Proportion of females defending patch**[d]
P. maculiventris (24-48h old)	70.2 ± 9.1 **a**	0.232 ± 0.037 **b**	0.298 ± 0.039 **a**	13	47.81 ± 4.35 **a**	0.615
H. halys (0-24h old)	87.5 ± 6.0 **a**	0.097 ± 0.024 **c**	0.100 ± 0.026 **b**	16	40.73 ± 1.94 **a**	0.625
H. halys (24-48h old)	23.8 ± 9.0 **b**	0.276 ± 0.035 **a**	0.069 ± 0.027 **b**	3	50.72 ± 4.91 **a**	0

[a] Number of eggs oviposited by the parasitoid/total number of eggs in the patch
[b] Number of rejections /total number of initiated ovipositions
[c] Number of incomplete ovipositions/total number of initiated ovipositions
[d] Of replicates where host patches were fully exploited

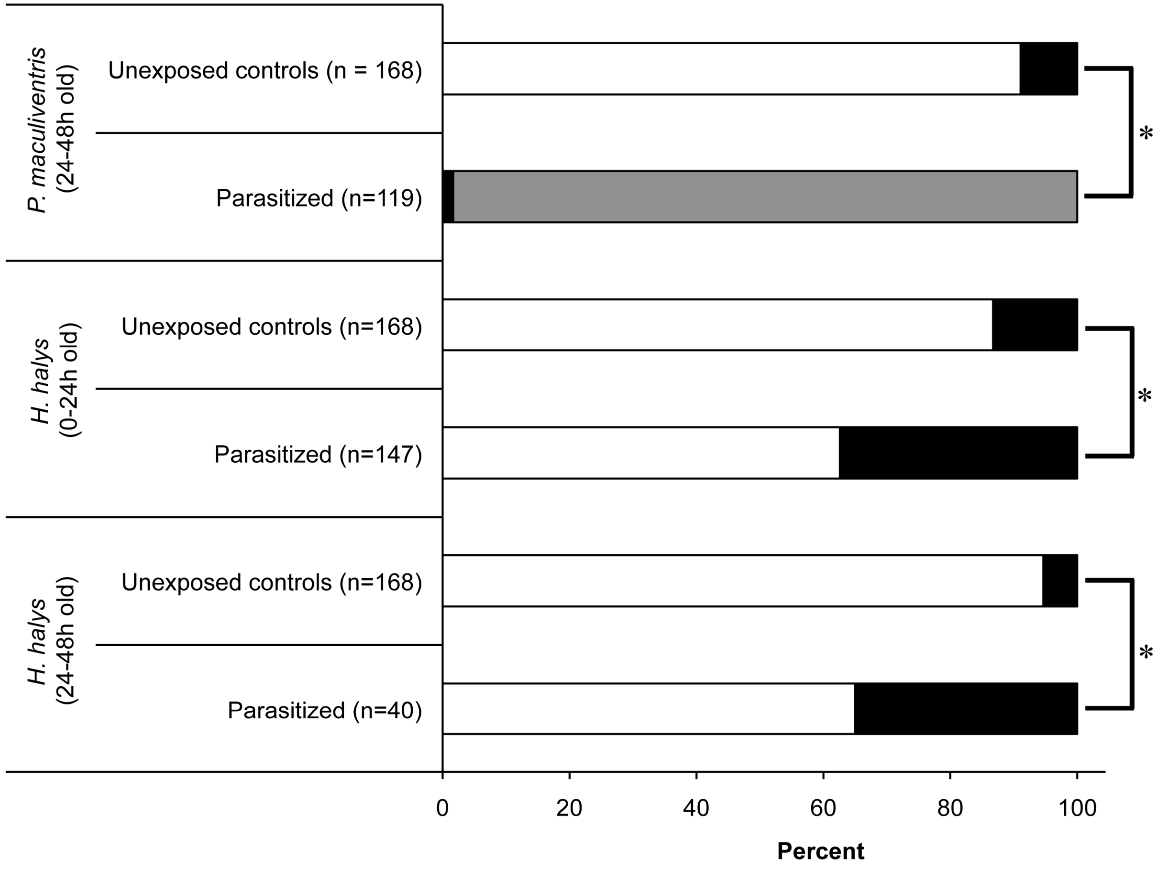

Figure 9-1. Percentage of each developmental outcome in parasitized eggs (in which parasitoid oviposition was observed) and unexposed control eggs of *P. maculiventris* (24-48h old) and *H. halys* (0-24h old or 24-48h old). White – pentatomid embryo; grey – parasitoid; black – undeveloped (no discernible development of pentatomid embryo or parasitoid). Asterisks (*) indicate significantly different proportions of each developmental outcome (Fisher exact test, $p < 0.001$).

9.5 Discussion

Telenomus podisi females accepted eggs (< 24h old) of the invasive alien *H. halys* as hosts at a high rate, but their offspring were unable to complete development. We propose that in invaded regions where *H. halys* becomes common, it could act as an evolutionary trap for *T. podisi*, with potential ecological consequences for the indigenous community of pentatomid hosts.

Behavioural acceptance of *H. halys* eggs by *T. podisi* indicates that the short-range cues necessary for parasitism are present. The fact that the presence of short range host cues alone seems to be sufficient for host location by *T. podisi* in nature (Okuda and Yeargan 1988; Bruni et al. 2000; Koppel et al. 2009) suggests that the attack of recently-laid *H. halys* eggs could commonly take place under natural conditions in environments where both species occur.

Acceptance of *H. halys* eggs was significantly lower when they were 24-48h old than when they were 0-24h old (Table 9-1). On 24-48h old *H. halys* egg patches, parasitoids often (14/21 replicates) rejected several eggs (2.71 ± 0.24; mean ± SE) and then left the patch, making no further parasitism attempts. This change in acceptance levels by *T. podisi* in different ages of *H. halys* eggs contrasts with our previous observations showing no difference in acceptance and suitability of different ages of *P. maculiventris* eggs. The mechanism behind this result is unknown, but the fact that the insertion of the ovipositor was used to mediate rejections indicates that the cue causing the rejection of older *H. halys* eggs is likely internal rather than, for example, being mediated by a change in the composition of external kairomones.

Parasitism by *T. podisi* prevented the development of some *H. halys* embryos, but never resulted in successful parasitoid development (Figure 9-1). Mortality of *H. halys* embryos could have been caused by damage to the host immature or the egg chorion by the developing parasitoid larva (Boivin 2010) or injection of compounds by the ovipositing parasitoid that liquefy the embryo or arrest its growth (Strand et al. 1983; Moreau and Guillot 2005). Although we did not determine the basis of the developmental unsuitability of *H. halys* eggs for *T. podisi*, the fact that we did not find dead late-stage parasitoid immatures in parasitized host eggs indicates that mortality occurred early in parasitoid development. This could be due to the absence of necessary developmental cues or nutritional resources (Lawrence 1990; Vinson 2010), a host egg chorion structure that prevented immature parasitoid respiration (Nénon et al. 1995), or an innate immune response by the host egg (Abdel-latief and Hilker 2008). To ensure that the complete developmental failure of parasitoids we observed was not due to the small number of females tested, we subsequently exposed over 1,200 0-24h old *H. halys* eggs (~45 egg masses) to a colony of >300 *T. podisi* over a period of three weeks, each egg mass being exposed for 48-72h. Although no parasitoid offspring emerged from these egg masses, corroborating our prior results, we found dead *T. podisi* pupae or dead pharate adults in 4 (<0.33%) of the exposed eggs upon subsequent dissection. Very low levels of developmental success may thus be possible. Indeed, there is a report of rearing *T. podisi* from field-collected *H. halys* eggs in the northeastern United States at very low rates (Hoelmer and Tatman 2011). Geographic populations/biotypes of *T. podisi* other than the ones in our colony may have the capacity to develop in *H. halys* eggs, a possibility requiring further testing.

Halyomorpha halys has the potential to become very common and widespread in its invaded ranges of Central Europe and North America (Zhu et al. 2012) and is already much more common than all indigenous pentatomid species combined at some sites in the Northeastern United States (Nielsen and Hamilton 2009). In such areas, it could act as an evolutionary trap for *T. podisi* by causing the parasitoid to invest eggs that do not result in offspring production. Since *T. podisi* is the most common parasitoid of several species of pentatomid bugs in North America (Yeargan 1979; McPherson 1982; Okuda and Yeargan 1988; Koppel et al. 2009), the invasive *H. halys* could have indirect effects on the populations of indigenous pentatomids. When a 'marginal' (relatively unsuitable for development) host species is introduced to a system comprising a parasitoid and a suitable host species, and when both species are accepted by the parasitoid at a similar rate, it is predicted that the equilibrium population levels of the suitable host species will increase (Heimpel et al. 2003). This effect is dependent on low parasitoid fecundity (egg-limitation), since the benefit to the suitable host species is due to the parasitoid allocating eggs to the unsuitable host and becoming egg-limited (Heimpel et al. 2003). The suitable host species is predicted to exert a slightly negative effect on the marginal host species, since it augments populations of the parasitoids that are able to cause some mortality of the marginal species (Hoogendoorn and Heimpel 2002; Heimpel et al. 2003). The overall interaction is of the (+, -) type, or 'apparent predation' (Holt 1977; Heimpel et al. 2003). The general conditions of the model predictions in Heimpel et al. (2003) are satisfied in the present system because: (i) *H. halys* (the marginal host) is attacked by *T. podisi* at a similar rate to *P. maculiventris* (the suitable host) but is less suitable for development; (ii) *T. podisi* has a low fecundity relative to the size of host egg masses and is therefore likely to experience egg-limitation in nature; (iii) the presence of suitable host species such as *P. maculiventris* is slightly detrimental to *H. halys*, since *T. podisi* is capable of causing some mortality (24-29%, Figure 9-1) of *H. halys* eggs. We therefore suggest that the invasive *H. halys*, via the indigenous parasitoid *T. podisi*, could cause an increase in population levels of pentatomid species such as *P. maculiventris* that are indigenous to North America. Whether this prediction is validated will depend on many ecological factors, including the importance of direct competitive interactions between *H. halys* and indigenous pentatomids. The fact that *H. halys* eggs are only accepted by *T. podisi* for a limited time during their development could also diminish the ecological impact of this interaction.

Post-exploitation patch guarding, including pre-emptive aggressive behaviours by *T. podisi*, occurred on patches of *H. halys* eggs with a frequency comparable to that on *P. maculiventris* eggs. The incidence and duration of patch guarding in scelionids increases with maternal investment (Field 1998) and the patch size used in this experiment was smaller than generally laid by both pentatomid species tested (Yeargan 1979; Orr et al. 1986; Nielsen et al. 2008). Our methodology did not allow a quantification of patch guarding duration (patch-guarding individuals were still on the patch at the end of the experiment), but we have previously observed up to 7 hours of patch guarding on patches of 12 *P. maculiventris* eggs (P.K.A.; unpublished data). *Halyomorpha halys* egg masses contain a median of 28 eggs (Nielsen et al. 2008). Therefore, in addition to acting as an ‘egg sink’ (following Hoogendoorn and Heimpel 2002), patches of *H. halys* eggs could be particularly potent evolutionary traps for *T. podisi* by also acting as a ‘time sink’, since they would cause parasitoids to invest time that does not result in any fitness payoff. The time sink effect is costly, and would potentially magnify the above-mentioned indirect ecological effects, if patch guarding increases predation risk or wastes time that could be used to find other host patches. This time sink concept should be integrated into models of indirect ecological effects mediated by species such as *T. podisi* that are likely to waste considerable time, in addition to reproductive resources, as a result of evolutionary traps.

There are two ways parasitoids could ‘escape’ an evolutionary trap. One possibility would be to evolve behavioural, physiological, or morphological barriers to acceptance of the invasive host species (Phillips and Shine 2004). Alternatively, parasitoids could evolve the capacity to develop in the invasive host species (Keeler and Chew 2008). The likelihood of either of these occurring depends, in part, on whether enough heritable genetic variability exists within and among populations of the parasitoid for the tendency to reject the invasive host species and the capacity to successfully develop in them (Henter 1995). In contrast, any (i) trade-off between a capacity to develop in the invasive host species and suitable indigenous host species, (ii) correlation between an increased tendency to reject the invasive host species and the tendency to reject suitable host species, or (iii) trade-off between increased virulence of the parasitoid in the invasive host species and other life history traits (Kraaijeveld and Godfray 1997), would prevent an escape from the evolutionary trap, since these trade-offs would inhibit the evolution of barriers to acceptance or increased developmental capacity.

Halyomorpha halys has also recently been accidentally introduced to central Europe (Wermelinger et al. 2008), and preliminary results indicate that the native European *Telenomus chloropus* Thomson accepts its eggs but is unable to develop in them (Tim Haye; personal communication). This emerging biological system thus presents the opportunity to study the ecological consequences, specifically the indirect effects, of evolutionary traps simultaneously in two invaded areas with different native host and parasitoid communities. In the coming years, indigenous pentatomid population levels before and after *H. halys* arrival in different regions should be measured, as should parasitism levels of *H. halys* and indigenous pentatomids. Behavioural acceptance levels and developmental success of indigenous egg parasitoids inside and outside of invaded areas over time could also be measured to test for between-population differences, and any evidence of escape from the evolutionary trap *H. halys* represents.

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Chapter 10: General Discussion

The responses of animals to their environment are incredibly complex, comprising both adaptive components and the products of constraints that act over a range of timescales and that combine to produce the phenotypes on which natural selection acts. In this thesis, I have conducted several investigations of how different types of phenotypic plasticity (behavioural, morphological, developmental) interact to shape the reproductive strategies of stink bugs and their parasitoids. My major findings are that: (1) Size-induced behavioural reaction norms of the parasitoid *Telenomus podisi* showed more evidence of compensatory plasticity than reaction norms of size and life history traits; (2) A predatory stink bug can selectively control the colouration of its eggs depending on where it is laying (the tops or undersides of leaves), with egg pigmentation protecting developing embryos against UV radiation; (3) Protective egg pigmentation in these predatory stink bugs may have evolved following a behavioural shift in oviposition site selection to the tops of leaves, where predation rates are lower than leaf undersides; (4) Extreme temperature regimes extend the memory duration of a parasitoid learning the reliability of host-associated cues; (5) The behavioural responses of ectotherms to temperature can be categorized within a relatively simple framework, according to mechanisms and outcomes; (6) An invasive stink bug could act as an evolutionary trap for an indigenous egg parasitoid in North America as a result of a mismatch between the level of behavioural acceptance by the parasitoid and the developmental suitability of the host. These results open up several promising avenues for future research.

Distinguishing adaptive components of plasticity from constraints

Not everything that animals do is adaptive – sometimes, they have to cope with the constraints imposed by environmental variation or make the best of a bad situation. In exploring the phenotypic plasticity of reproductive strategies of stink bugs and their parasitoids, I have found evidence of both adaptive responses to the environment as well as constraints. For example, plasticity in egg colouration and its association with oviposition site selection (Chapters 5 and 6) is clearly adaptive for *P. maculiventris*, while at the same time, the capacity to produce the UV-protective egg pigment appears to be compromised at high temperatures. Context is also important for determining whether a given strategy is adaptive – using egg kairomones as a host acceptance cue is normally adaptive for *T. podisi* when parasitizing co-evolved hosts, but becomes maladaptive in the context of the biological invasion by a new host, *H. halys* (Chapter 9). In other cases, the influence of adaptive plasticity versus constraints was less clear. For example, an extended memory window in response to thermal stress in *T. basalis* (Chapter 7) could represent an adaptive response to metabolic stress, or may simply reflect passive thermal dependence of the neurological mechanisms underlying forgetting. Here, the framework I developed in Chapter 8 could be used to test whether this phenomenon is due to Kinetic effects (constraints) or Integrated effects (i.e., the wasps are using direct thermal information to adaptively modify their memory retention). First, the method of Sayeed and Benzer (1996) could be used to develop lines of *T. basalis* that cannot sense temperature. Then, one could test whether the memory window of temperature-insensitive wasps is still extended in response to thermal stress. If the same response is observed in temperature-sensitive and temperature-insensitive wasps, one could conclude that thermally-dependent forgetting is a Kinetic, and not an Integrated response to temperature. While a “Kinetic null modelling” approach could be useful here as well, it would require a better understanding of the mechanisms underlying forgetting (and how they respond to temperature), an area of study still in its infancy (Berry et al. 2012; Hadziselimovic et al. 2014).

The interaction of different types of phenotypic plasticity: beyond verbal arguments

A given type of environmental variation often causes plastic responses in several different traits at once and all of these responses combine to produce the phenotype that will determine that organism’s reproductive success. In Chapter 4, for example, we discussed how life history and behavioural reaction norms of different-sized parasitoids would interact to determine their relative fitness. However, we relied on verbal arguments that are limited in their precision and predictive power, especially when addressing such a complex, multidimensional response. In the future, it would be interesting to use the reaction norms characterized here to parameterize a dynamic model that could be used to forecast the fitness of parasitoids of different sizes depending on environmental variables such as host availability, mortality risk, and competition levels. This approach, similar to that previously employed by Berger et al. (2012) for reproductive timing in a butterfly, could reveal that the relative importance of different traits (and thus the relative fitness of differently sized individuals) may change depending on the environment. For example, traits that increase the number of patches that can be visited during a lifetime (longevity, walking speed) should have greater importance in environments where host patches are rare or difficult to find, compared to traits that confer rapid host exploitation rates within patches (oogenesis rate, host handling rate). Such predictions could then be tested in mesocosm/field experiments. This general method could also be applied to other environmental variables that simultaneously influence both behavioural and life history traits, such as temperature.

The potential of comparative approaches to complement ecological and behavioural studies of plasticity

Conducting behavioural ecology studies on a single species, as we did in Chapter 4 (size-induced reaction norms) and Chapters 5-6 (evolution of selective egg pigmentation) can only go so far when attempting to characterize how plasticity in animal reproductive strategies has evolved. First of all, when working with a single species, we can only observe the final product of millions of years of evolution and have to make educated guesses regarding what the intermediate steps – whose traces could have been 'erased' along the way – might have been. Secondly, experimental reconstructions of evolutionary history are hampered by a lack of knowledge regarding the specific ecological context (e.g., predator species, host plants) in which the adaptation evolved, and thus an inability to reproduce it. An alternative method to complement the two studies mentioned above, and to identify more general patterns, would be to take a comparative approach. For the evolution of selective egg colouration, the best place to start would be to investigate the UV-sensitivity of developing embryos, egg pigmentation (never pigmented, always pigmented, selectively pigmented), and oviposition site selection (leaf tops versus leaf undersides) in several closely- and distantly-related stink bug species, then mapping their strategies onto a phylogenetic tree of the different stink bug species constructed (for examples in other taxa, see Scott et al. 2010; Penney et al. 2012). Differences in the lifestyles of the various species included in the phylogeny could then be used to infer what ecological factors are correlated with differences in egg pigmentation. For the study of size-induced reaction norms (Chapter 4), it could be informative to make comparisons of the slopes of reaction norms among several parasitoid species that are closely related, but differ in ecological or life history traits (e.g., koinobiont vs. idiobiont, host range generalist vs. specialist). These investigations could reveal a fuller diversity of reproductive strategies and give important insights into their evolutionary histories.

Behaviour-mediated evolutionary traps: implications for biological control

In Chapter 9, we found evidence that the indigenous egg parasitoid *T. podisi* could be "trapped" by its behavioural response to the eggs of the invasive stink bug *H. halys*. That is, the wasp's behavioural plasticity allowed adults to complete the behavioural host acceptance sequence, but their offspring's developmental plasticity was insufficient to allow them to successfully complete their development. This result adds to a growing body of literature showing that invasive species can act as evolutionary traps for indigenous natural enemies (Schlaepfer et al. 2005; Berthon 2015). The fact that *H. halys* is an economically important pest of agriculture brings up another important question that has not been given its due consideration in the past: what are the consequences of evolutionary traps for biological control? As discussed in Chapter 9, indirect ecological effects mediated by *T. podisi* could cause indigenous pentatomids (including both beneficial predatory species and occasional pests) to increase in abundance. But there is one more important finding here that could point to a new potential tool in biological control: *Telenomus podisi*, although incapable of completing development in the eggs of *H. halys*, 'aborted' (i.e., arrested the development and killed) about 25% of the eggs that it parasitized. Remarkably, since our study was published, Haye et al. (2015) obtained very similar results when studying the response of European scelionid parasitoids to *H. halys* eggs. Although this kind of "parasitoid-induced host egg abortion" has been previously described in other host-parasitoid associations (e.g., Chailleux et al. 2012; Pizzol et al. 2012), the idea of explicitly using parasitoids that cause abortion of host egg development for biological control purposes has not previously been investigated. It might be possible, for example, to improve the ability of indigenous scelionid parasitoids to abort *H. halys* eggs via artificial selection and release them against *H. halys* in an inundative or augmentative biological control context. At the very least, it will be critical to include parasitoid-induced host egg abortion when considering the ecosystem services provided by native natural enemies, developing an integrated management program for *H. halys*, and making predictions about host-parasitoid population dynamics. Indeed, classic host-parasitoid population dynamics models tend to assume that each parasitoid oviposition results in the production of an offspring (e.g., Hassell 1978). In addition to other factors such as intraguild predation (Brodeur and Rosenheim 2000), parasitoid-induced host egg abortion could be one way that the validity of this assumption would be considerably weakened. The current importance of this type of mortality for the suppression of *H. halys* populations in already-invaded areas could be quantified using the molecular markers developed by Gariepy et al. (2014) and used to parameterize modified host-parasitoid population dynamics models and predict the consequences for biological control.

Conclusions

Characterizing patterns of phenotypic plasticity in nature is an enormous challenge for biologists: the number of different species that exist, the number of environments they inhabit, the number of different factors that are constantly changing in these environments, and the incredible diversity of ways that organisms can adapt and respond to these factors is staggering.

With every organism and every phenotypic response we study (Chapters 4, 5, 6, 7, 9), we are gradually filling gaps in our understanding, while also creating new gaps that we did not know existed. Synthesizing what we know already (as was done in our synthesis of the effects of temperature on behaviour, Chapter 8) can also reveal important holes in our knowledge. Both approaches will continue to be necessary as we gradually advance our understanding of the ecological and evolutionary interactions between organisms and their environment.

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Appendix I: Other activities and accomplishments during Ph.D. research

Published articles

Abram PK, Doyon J, Brodeur J, and Boivin G (2015) Susceptibility of *Halyomorpha halys* (Hemiptera: Pentatomidae) eggs to different life stages of three generalist predators. The Canadian Entomologist 147:222-226

Zhou Y, **Abram PK**, Boivin G, and Brodeur J (2014) Increasing host age does not have the expected negative effects on the fitness parameters of an egg parasitoid. Entomologia Experimentalis et Applicata 151:106-111

Miall JH, **Abram PK,** Cappuccino N, and Mason PG (2014) Potential impact of the native hyperparasitoid *Conura albifrons* (Hymenoptera: Chalcididae) on the exotic biological control agent *Diadromus pulchellus* (Hymenoptera: Ichneumonidae). Biocontrol Science & Technology 24:611-624

Oral presentations at scientific conferences (=presenting author)*

Abram PK*, Burte V, Boivin G, and Brodeur J (2015) Parasitoid-induced host egg abortion: a potential tool in biological control? 4th International Entomophagous Insects Conference, Malaga, Spain, October 4-9, 2015

Abram PK*, Guerra E, Torres I, Boivin G, and Brodeur J (2015) A flexible egg pigmentation strategy by a predatory stink bug : a response to UV radiation? 100th Ecological Society of America Annual Meeting, Baltimore, Maryland, USA, August 9-14, 2015

Abram PK*, Cusumano A, Peri E, Brodeur J, Boivin G, and Colazza S (2015) L'effet du stress thermique sur la mémoire d'un parasitoïde des oeufs. XXVe Symposium du Département de sciences biologiques de l'Université de Montréal, Montréal, QC, March 11-12, 2015

Guerra-Grenier E*, **Abram PK**, Boivin G, and Brodeur J (2014) Plasticité intra-individuelle de la pigmentation des oeufs chez la punaise soldat: une adaptation face aux rayons ultraviolets? Réunion annuelle de la Société d'Entomologie du Québec, Hôtel-Musée Premières Nation Wendake, QC, November 6-7, 2014

- **Winner of best student oral presentation**

Abram PK and Brodeur J* (2014) Parasitoid-induced host egg abortion: a tool in biological control? Annual Meeting of the Western Regional Biocontrol Group, Poipu, Hawaii, October 2014

Abram PK*, Gariepy TD, Boivin G, and Brodeur J (2013) La punaise envahissante *Halyomorpha halys* : un piège évolutif pour le parasitoïde indigène *Telenomus podisi*. Réunion Annuelle de la Société d'Entomologie du Québec, Ste-Adèle, QC, November 21-22, 2013

- **Winner of best student oral presentation**

Abram PK*, Gariepy TD, Boivin G, and Brodeur J (2013) An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. Joint Annual Meeting of the Entomological Society of Canada and the Entomological Society of Ontario, Guelph, ON, October 20-23, 2013

- **Runner-up for best student oral presentation**

Abram PK*, Gariepy TD, Boivin G, and Brodeur J (2013) The invasive *Halyomorpha halys* as an evolutionary trap for the indigenous egg parasitoid *Telenomus podisi*. 3rd International Entomophagous Insects Conference, Orford, QC, June 2-6, 2013

- **Winner of best student oral presentation**

Poster presentations at scientific conferences (=presenting author)*

Doyon J*, **Abram PK**, Boivin G, and Brodeur J (2014) Susceptibility of *Halyomorpha halys* (Hemiptera: Pentatomidae) eggs to different life stages of three generalist predators. Réunion Annuelle de la Société d'Entomologie du Québec, Wendake, QC, November 6-7, 2014

Caron A-S*, **Abram PK**, Boivin G, and Brodeur J (2014) A role for parasitoid-induced host egg abortion in biological control? Réunion Annuelle de la Société d'Entomologie du Québec, QC, November 6-7, 2014

Abram PK*, Després-Einspenner M-L, Brodeur J, and Boivin G (2013) Conditional egg colouration by a predatory stink bug. Joint Annual Meeting of the Entomological Society of Canada and the Entomological Society of Ontario, Guelph, ON, October 20-23, 2013

- **Winner of President's Prize for best student poster presentation**

Zhou Y*, **Abram PK**, Boivin G, and Brodeur J (2013) Increasing host age does not have the expected negative effects on fitness parameters of an egg parasitoid. 3rd International Entomophagous Insects Conference, Orford, QC, June 2-6, 2013

Awards and scholarships

Bourse Marie Victorin 2015
Institut de Recherche en Biologie Végétale, Université de Montréal

Robert J. O'Neil Outstanding PhD in Biological Control 2015
International Organization for Biological Control (IOBC)

Centre SÈVE Conference Travel Bursary 2015
Centre SÈVE

Jacques-Rousseau Travel Bursary 2015
Institut de Recherche en Biologie Végétale, Université de Montréal

Entomological Society of Quebec Student Bursary 2014
Entomological Society of Quebec

FQRNT International Internship Award 2014
Fonds de Recherche du Québec/Quebec Centre for Biodiversity Science

Ed Becker Conference Travel Award 2013
Entomological Society of Canada

Jacques-Rousseau Travel Bursary 2013
Institut de Recherche en Biologie Végétale, Université de Montréal

QCBS Student Excellence Award 2012
Quebec Centre for Biodiversity Science

NSERC Post-Graduate Scholarship (PGS-D3) 2012-2015

Natural Sciences and Engineering Research Council of Canada