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The ecology of adaptive condition-dependent polyphenism

Flor T. Rhebergen

The ecology of adaptive condition-dependent polyphenism

by

Flor T. Rhebergen

Rhebergen, F. T. 2022. The ecology of adaptive condition-dependent polyphenism

PhD thesis, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, The Netherlands

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The ecology of adaptive condition-dependent polyphenism

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Chapter 1

General introduction

Flor T. Rhebergen

1.1 — Adaptive condition-dependent plasticity

Populations consist of individuals that are not all the same. Every individual organism has its own unique developmental history, because development responds to genetic and environmental stimuli, because organisms often differ genetically, and because each individual organism experiences its own environment (Lewontin, 1983; Walsh, 2014). The property that development responds to (environmental) inputs is called developmental plasticity, or phenotypic plasticity (West-Eberhard, 2003), and the general capacity of development to produce variation fuels adaptive evolution (West-Eberhard, 2003; Laland et al., 2015). Without variation, natural selection has nothing to select. Therefore, understanding how development maintains the capacity for variation, even though natural selection tends to reduce variation, is crucial to understand how adaptive evolution works (Box 1.1).

Plasticity is often just a consequence of the simple and obvious fact that organisms are part of the physical world, so their development inevitably responds to environmental forcing. For example, if you do not eat, you will not obtain nutrients to grow, and this must affect how you develop. However, many examples of developmental plasticity are not so easily explained. Organisms often act as if they proactively adjust their development to circumstances that have not happened yet, but will happen in their immediate future (Nijhout, 2003; West-Eberhard, 2003; Beldade *et al.*, 2011; Simpson *et al.*, 2011; Nettle & Bateson, 2015). For example, water fleas (*Daphnia* sp.) 'normally' do not defend themselves against predators, but if you rear them in an environment in which predators are present, they start developing spikey projections on their head (Krueger & Dodson, 1981). Tropical caterpillars (*Bicyclus* sp.) in the rainy season develop into butterflies with large and conspicuous eye-spots, but when the dry season is approaching, caterpillars from the same population develop into butterflies that mimic dead leaves (Roskam & Brakefield, 1999). Such phenomena can be understood as products of *natural selection for* developmental plasticity in response to (environmental) cues. They are examples of *adaptive* developmental plasticity, where organisms maximize their expected fitness (i.e. their individual contribution to the growth of the population) by developing flexibly, depending on environmental cues (Nettle & Bateson, 2015). This is part of the solution to the puzzle of persisting variation under natural selection: while in many cases selection tends reduce the developmental tendency to vary, occasionally selection promotes it. But how?

Adaptive developmental plasticity results in developmental variation in various ways. It can cause organisms to develop differently if they are members of different populations in different ecological circumstances (Stamp & Hadfield, 2020). It can

also cause developmental differentiation over time within a single population, if the ecological circumstances that the population experiences fluctuate predictably (Roskam & Brakefield, 1999). But perhaps most intriguingly, adaptive developmental plasticity can cause differently developing organisms to coexist alongside each other, within the same population, simultaneously (Gross, 1996). This is possible because organisms living simultaneously within the same population never really experience the exact same environment. For example, individuals that happen to be faster-than-average growers experience a world in which they are strong social competitors, where resources are relatively easily acquired, and trade-offs between various important developmental processes have less of an impact on development (van Noordwijk & de Jong, 1986). Individuals that happen to grow poorly possibly experience a very different world, in which social opportunities are rare, resources are hard to obtain, and trade-offs among developmental processes prevent the organism from optimizing several functions at once. Different individuals can therefore differ quite substantially in the ecological opportunities that the environment affords them, even if they live simultaneously within the same population (Lewontin, 1983; Walsh, 2014; Chiu, 2019). If such affordances vary predictably according to the organism's own individual history of growth and development (its 'condition'), a form of adaptive plasticity can evolve that could be summarized as 'Making the Best of a Bad Job': adaptive condition-dependent plasticity (Baldwin, 1896; Dawkins, 1980; Parker, 1982; Gross, 1996; West-Eberhard, 2003; Tomkins & Hazel, 2007).

But *how* do organisms develop plastically to Make the Best of a Bad Job'? How does the environment afford different opportunities to individuals with different histories of growth and development? What is it about the interaction between organism and environment that makes the optimal developmental trajectory context-dependent, even within populations? What maintains developmental variation in the face of selection? In this thesis, I aim to answer these questions. My focus is on laboratory-based populations of bulb mites *Rhizoglyphus robini* (Acari: Astigmata: Acaridae), but I aim to contribute to our conceptual understanding of the evolution of developmental plasticity in general.

1.2 — The fighter-scrambler polyphenism in male bulb mites

The bulb mite *Rhizoglyphus robini* is a gregarious mite species that naturally occurs in more or less dense populations on rotting underground plant structures, such as roots or bulbs (Díaz *et al.*, 2000). The adult males within such aggregations show conspicuous phenotypic variation: about 70-80 percent possess a strongly enlarged third leg

Box 1.1 – Plasticity, adaptive evolution, and the origin of variation

Developmental (phenotypic) plasticity is commonly defined as the capacity of development (the phenotype) to respond to environmental effects (e.g. Pigliucci, 2005; Beldade *et al.*, 2011). At first glance, it seems that this means that developmental plasticity is of no special consequence for evolution by natural selection. After all, natural selection acts on inherited (i.e. genetic) variation, not on variation induced by environmental differences. And since inherited variation is usually conceptualized as originating through random genetic mutation, there does not seem to be much room for developmental plasticity as an explanatory construct. Variation originates randomly, and recurs over generations through inheritance, before selection molds it into an adapted phenotype. To be sure, plasticity can *be* such an adapted phenotype, in which case it has evolved via initial random genetic mutation (genes for plasticity), and subsequent selection for developmental sensitivity to environmental cues. But in this common conceptualization of adaptive evolution, plasticity does not *explain* anything. It is an effect, not a cause, of evolution (de Jong, 2005).

But this is not a completely satisfying account of adaptive evolution. The reason why it is not satisfying is the emphasis on *randomness* underlying the origins of selectable variation. Evidently, randomness does play a role: variation accumulates in the genome through (nearly) random mutation. But genetic mutations cannot affect evolution unless they somehow affect development and become expressed in the phenotype. And genetic mutations affect development only if they have an effect on the existing cue-response mechanisms that regulate and canalize development (Waddington, 1957; West-Eberhard, 2003). Therefore, while genetic mutation is (nearly) random, developmental variation (i.e. variation in cue-response mechanisms) is functionally and biophysically constrained and very much not random (Waddington, 1957; West-Eberhard, 2003).

Understanding how selectable variation comes into existence implies understanding how developmental cue-response mechanisms are organized. For the origin of variation, it does not matter whether such cue-response mechanisms are cued environmentally (i.e. developmental plasticity as usually defined) or genetically, and on a molecular level this dichotomy may not even make sense (Griffiths & Gray, 1994). What first and foremost matters is that existing cue-response mechanisms canalize development, that variation is therefore generated in non-random patterns, and that such cue-response mechanisms are susceptible to effects of genetic mutation (West-Eberhard, 2003). When canalizing mechanisms become affected by genetic mutation, resulting phenotypic variation can be inherited, allowing an evolutionary response to selection. Thus, a closer look at how variation originates implicates developmental plasticity as an important determinant of selectable variation. We need to know how organisms plastically respond to environmental perturbations and cues, to predict how they produce selectable variation (Levis & Pfennig, 2019; Noble *et al.*, 2019). Plasticity is both a cause and an effect of adaptive evolution.

pair with sharp, dagger-like claws (the 'fighter' phenotype; Fig. 1.1), while 20-30 percent of the males have unmodified legs (the 'scrambler' phenotype; Fig. 1.1; Radwan, 1995; Smallegange, 2011a). Adult females always have unmodified legs, as do the

juvenile stages (Fig. 1.1). Fighters use their modified legs in contests with other males over resources, such as receptive females (Radwan *et al.*, 2000; Radwan & Klimas, 2001). In such contests, they attempt to forcibly pierce the cuticle of their rival in a 'lethal hug', using their potentially deadly legs as a pair of pincers. Scramblers cannot do this, as they lack the weaponry. The fighter leg pair is thought to evolve under sexual selection (i.e. selection due to variation in fertilization success), because it is a male-limited trait, of which the primary function appears to be to increase mating success by eliminating rivals (Radwan & Klimas, 2001; Skwierzyńska *et al.*, 2018).

The two male morphs in *R. robini* are the alternative outcomes of condition-dependent developmental plasticity during the metamorphic transition from the final juvenile stage (the tritonymph) to the adult stage, when the adult body develops (Fig.1.1). During this transition, relatively large males tend to become fighters, while relatively small males become scramblers (Smallegange, 2011a; Leigh & Smallegange, 2014). This polyphenic form of condition-dependence (i.e. condition-dependence with discrete alternative morphs) occurs in males of several acarid mite species, but is not unique to mites. Similar phenomena have been recorded in male traits across the animal kingdom, including enlarged horns or mandibles in beetles (Kawano, 2000; Emlen *et al.*, 2005), forceps in earwigs (Tomkins, 1999), claws in crustaceans (Karplus & Barki, 2019), facial morphology in orang-utans (Banes *et al.*, 2015), and head shape in salmonid fishes (Gross, 1985).

We assume that condition-dependent plasticity in male weaponry has evolved in response to selection for such plasticity (that is to say, it is an adaptation), because the alternative morphs occur so regularly. Indeed, experiments in bulb mites have all but confirmed that plasticity is shaped and maintained by selection. Using artificial selection for condition-*in*dependent male morph expression, it is possible to evolve populations that lack condition-dependence, containing males that more or less always develop as either fighters or scramblers (Radwan, 2003; Plesnar-Bielak *et al.*, 2014; Van den Beuken & Smallegange, 2018). It is particularly telling that such populations quickly evolve back towards condition-dependent plasticity producing the same proportions of fighters and scramblers as before, once the artificial selection protocol is abandoned (Skrzynecka & Radwan, 2016). This strongly suggests that there is something about the ecological interactions within undisturbed bulb mite populations that causes selection for condition-dependent expression of alternative male morphs. Nevertheless, it remains poorly understood how small and large male bulb mites differ in their affordances, so that large males maximize fitness by developing enlarged fighter legs, and small males by refraining from developing weaponry.

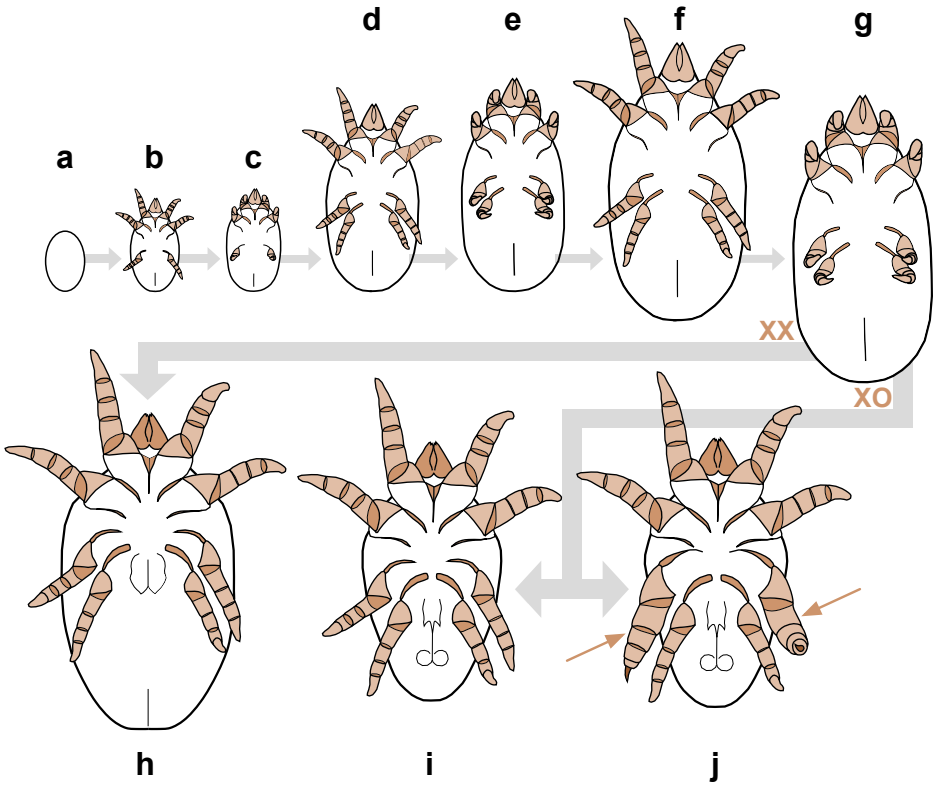

Figure 1.1 — Life cycle of the bulb mite (*Rhizoglyphus robini*). Bulb mites usually go through three juvenile stages (**b-c**, larva; **d-e**, protonymph; **f-g**, tritonymph) before they enter the adult stage as morphologically distinctive females (**h**), scrambler males (**i**) or fighter males (**j**). Females produce hundreds of eggs (**a**). Under severely challenging circumstances, a facultative dispersal stage (deutonymph, not illustrated) may be expressed between the protonymph and the tritonymph stage. Successive stages are interspersed with non-feeding quiescent phases (**c**, quiescent larva; **e**, quiescent protonymph; **g**, quiescent tritonymph), during which incomplete metamorphosis takes place. Sex determination is chromosomal (XX-XO), males being the heterogametic sex. Male morph determination is condition-dependent. Brown arrows denote modified legs in the fighter morph. Illustrations in ventral view, head above.

1.3 — Thesis outline

My thesis is inspired by the following central question: *How is condition-dependent development of alternative morphs maintained by selection?* Applied to the bulb mite system, a less formal way of putting this is asking: *Why do poorly-growing male* Rhizoglyphus robini *completely refrain from developing weaponry, while well-growing males develop lethal weapons?*

In Chapter 2, I discuss this question in more conceptual detail. I argue that there are two distinct research agendas involved in answering the question how condition-dependent plasticity is maintained under selection, corresponding to different levels of explanation. Firstly, there should be a focus on explaining the shape of the condi-

tion-dependent reaction norm (i.e. how the phenotype is expressed across the range of 'condition'). This is an ecological question, focusing on how individuals with different developmental histories (varying 'condition') experience different environmental affordances. It is also a question of optimality: a particular range of developmental histories should favour expression of one phenotype, and a different range of developmental histories should favour another phenotype, but where in the range of 'condition' is the optimal switch point? Secondly, there should be a focus on explaining how such developmental 'decision rules' can evolve towards optimality in the first place. Without genetic variation, evolution grinds to a halt. So, whenever we observe that reaction norms appear to be tailored to the prevailing pattern of environmental opportunities, genetic variation must have been present to enable evolution to track optimality. What maintains genetic variation associated with such decision rules, even though a single optimal rule is favoured by selection? This is a population-genetics question. Thus, ecology and population genetics both contribute to the study of adaptive developmental plasticity, on their own level of explanation. With regard to acarid mites with fighter-scrambler polyphenism, I argue that the ecology of adaptive condition-dependent plasticity has been underappreciated. We understand less about condition-dependent environmental affordances, than about mechanisms maintaining genetic variation.

In Chapter 3, I zoom further into the ecology of adaptive condition-dependent plasticity in the context of male secondary sexual traits, which, in many animal species, are characteristically condition-dependent in their development. I review how individuals within single populations accumulate variation over the course of their development, how such developmental variation has fitness consequences, and how organisms can adaptively adjust development to mitigate detrimental fitness effects of developmental variation ('Making the Best of a Bad Job'). I conceptualize two distinct adaptive components of 'Making the Best of a Bad Job' plasticity in secondary sexual traits. On the one hand, development anticipates variation in socio-sexual opportunities, and adapts morphologically to alternative mating tactics. On the other hand, under stress, development acts to maintain viability and optimal life history timing, and redistributes resources in a condition-dependent fashion to do so. Therefore, condition-dependent expression of secondary sexual traits can result from adaptation to alternative mating tactics, but also from the adaptive redistribution of resources under stress (somatic buffering). Finally, I present an inventory of published records of condition-dependent polyphenism in secondary sexual traits in arthropods, and review the

empirical evidence that such polyphenism is an adaptation to alternative mating tactics, and/or a consequence of adaptive somatic buffering.

To understand how condition-dependent polyphenism evolves, it is important to describe its reaction norm first. When during development does variation in growth and body size affect male morph expression; does this happen at the very last moment before metamorphosis, or is male morph expression determined earlier? And is this condition-cued switch fixed at a static critical body size, or does it shift with ecological circumstances (allometric plasticity)? In Chapter 4, I describe the shape of the condition-dependent fighter-scrambler reaction norm in *R. robini*. First, I test the hypothesis that male phenotype expression is determined at metamorphosis, and not earlier during juvenile development. Subsequently, I investigate whether size-dependent expression of alternative male morphs is subject to nutrition-dependent allometry plasticity. That is, not only does expression of enlarged fighter legs depend on body size at metamorphosis, this size-dependence itself may plastically shift with nutritional conditions, giving rise to plasticity at multiple levels (Moczek, 2002; Casasa & Moczek, 2019).

Repeatable and predictable (e.g. size-based) mating niche differentiation can drive the evolution of adaptive, anticipatory plasticity in morphological traits. In Chapter 5, I investigate whether social opportunities favour condition-dependent male phenotype expression in bulb mites. Specifically, I test the hypothesis that aggressive contests among fighter males over a mating opportunity are more often won by the larger and stronger male, so that smaller fighters are disadvantaged in aggressive interactions. If that is the case, small males may occupy a different mating niche than large males; small males have to do 'something else' than large males in order to mate (Gross, 1996). Such alternative mating tactics can select for functionally matching alternative morphologies and adaptive condition-dependent polyphenism.

Polyphenism can also be adaptive if it facultatively reduces developmental constraints to maturation, allowing life history flexibility. In Chapter 6, I investigate the link between condition-dependent development of alternative male morphs and the timing of maturity in bulb mites. Age and size at maturity are important life history traits, with large consequences for fitness (Stearns, 1992). This is particularly the case in bulb mites, which live in high densities in ephemeral habitats and experience high juvenile mortality. Mature too quickly, and you are too small to function appropriately in the bulb mite world. Remain juvenile for too long, however, and you might die before you reproduce. Metamorphosis is energetically expensive (Nijhout & Emlen, 1998), so mites need to reach a particular energy budget in order to mature (Day &

Rowe, 2002; Plaistow *et al.*, 2004). Thus, when the nutritional environment becomes poor, entering metamorphosis 'on time' could be facilitated by developmental plasticity in traits that are expensive to produce during metamorphosis: fighter legs. In this chapter, I first test the hypothesis that scrambler males are able to mature quicker than fighter males, given a particular growth trajectory. I then test the hypothesis that mites accelerate the onset of metamorphosis in response to the removal of food. Finally, I test whether increased competition for food among juveniles resulted in a larger proportion of males developing as scramblers, enabling them to mature relatively early and escape mortality. If a condition-dependent switch from an 'expensive' phenotype to a 'cheap' phenotype allows adaptively flexible maturation in a variable environment, polyphenism can evolve due to facultative 'polyphenic release' from sexually selected constraints.

In Chapter 7, I synthesize my findings and previously published results, to formulate a provisional answer to the question: *How is condition-dependent development of alternative morphs maintained by selection?*

Chapter 2

Cross-level considerations for explaining selection pressures and the maintenance of genetic variation in condition-dependent male morphs

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Abstract

Condition-dependent expression of alternative male morphs exists in many arthropods. Understanding their coexistence requires answering (at least) two questions: (i) what are the ecological selection pressures that maintain condition-dependent plasticity (polyphenism), and (ii) what maintains the associated genetic variation? Focusing on acarid mites, we show that the questions should not be conflated. We argue how, instead, answers should be sought by testing phenotype-level (question 1) or genotype-level (question 2) hypotheses. We illustrate that energy allocation restrictions and physiological trade-offs are likely to play a crucial role in condition-dependent polyphenism in acarid mites. We thus conclude that these aspects require specific attention in identifying selection pressures maintaining condition-dependent polyphenism, and evolutionary processes that maintain genetic variation in the underlying reaction norms.

2.1 – Introduction

Male secondary sexual traits are often differently expressed depending on nutrition during development (Brockmann & Taborsky, 2008; Lavine *et al.*, 2015). In many arthropods, such condition-dependent plasticity leads to male dimorphism (Gross, 1996; Brockmann, 2008; Radwan, 2009) or even trimorphism (Rowland & Emlen, 2009; Stewart *et al.*, 2018), with ensuing within-population male morph coexistence having evolved multiple times. Understanding the evolutionary causes of such condition-dependent male polyphenism represents an outstanding question in evolutionary biology, with direct relevance to the broader fundamental challenge of explaining the maintenance of variation in the face of Darwinian selection.

Arthropod male dimorphisms generally comprise 'minor' and 'major' male morphs (Moczek, 2003; Smallegange, 2011a; Buzatto & Machado, 2014; Buzatto et al., 2015). Majors are large and possess morphological structures that are used as weapons in male-male competition, while minors are smaller, lack large weapons and generally adopt a sneaker strategy to gain access to females (Oliveira et al., 2008). In such condition-dependent polyphenism, whether a male develops into an armed major male or an unarmed minor male depends on whether it reaches a critical resource or body size threshold during development. For example, in several horned dung beetles (Coleoptera: Scarabaeidae), large male larvae develop into horned majors (fighters) that guard tunnel entrances containing a breeding hornless female, whereas smaller male larvae develop into hornless minors (sneakers) that find females by tunnelling past the guarding fighters (Moczek & Emlen, 2000). In acarid mites, large male juveniles develop into majors (fighters) which have an enlarged and modified third leg pair with sharp tarsal claws that they use to kill competitors, whereas smaller male juveniles develop into minors (scramblers) with unmodified female-like third legs (Fig. 2.1a; Radwan, 2009; Smallegange, 2011a). Critical resource or body size thresholds underlying condition-dependent polyphenism can evolve rapidly in response to artificial selection (acarid mites: Radwan, 2003; Smallegange et al., 2014; Skrzynecka & Radwan, 2016) or after introduction to novel environments (dung beetles: Moczek et al., 2002; earwigs: Tomkins & Brown, 2004; acarid mites: Tomkins et al., 2011). This raises two questions. Firstly, what ecological selection pressures maintain condition-dependent plasticity and set the threshold for polyphenism (Tomkins & Hazel, 2007)? Secondly, since rapid evolutionary responses imply considerable threshold heritability, what maintains genetic variation associated with condition-dependent plasticity (Buzatto et al., 2012a)? Here, we illustrate that adaptive evolution of the mean threshold on the one hand, and genetic variation in thresholds on the other hand, have very different

Box 2.1 — Maintenance of genetic variation does not explain coexistence of alternative condition-dependent morphs

Within-population coexistence of different morphs, such as 'minor' and 'major' type males, elicits questions as to why and how selection maintains phenotypic diversity. The answer to these questions depends on whether the alternative morphs are directly linked to alternative large-effect alleles coexisting at intermediate frequencies (genetic determination independent of condition), or to a single condition-dependent reaction norm (condition-dependent plasticity). Coexistence of genetically determined condition-independent alternative morphs has been explained via balanced polymorphism in underlying genes (Sinervo & Lively, 1996; Lamichhaney *et al.*, 2015; Rios-Cardenas *et al.*, 2018). Condition-dependent alternative morphs are also likely to be associated with genetic variation, including balanced polymorphism, but in this case, genetic variation does not explain coexistence of alternative morphs (Tomkins & Hazel, 2007; Buzatto *et al.*, 2015). Instead, coexistence is explained by the shape of the underlying condition-dependent reaction norm, and the distribution of condition in the population, and genetic variation does not cause expression of alternative phenotypes except through its effect on condition and the reaction norm (Tomkins & Hazel, 2007; Buzatto *et al.*, 2015). Nevertheless, it has occasionally been proposed that we can understand why condition-dependent alternative phenotypes coexist by studying processes that maintain genetic variation (Skrzynecka & Radwan, 2016). This conflates levels of explanation at respectively the developmentally plastic phenotype and the genotype.

An illustrative case is that of the alternative male morphs in acarid mites of the genus *Rhizoglyphus*. In these species, males either develop as 'fighters' with enlarged third legs used as weapons in fights over mating opportunities, or as 'scramblers' without an enlarged third leg pair. This dimorphism is condition-dependent: male morph expression in *R. robini* and *R. echinopus* responds to food quality and somatic growth rate, with poorly growing males expressing the scrambler morph, and well-growing males becoming fighters (*robini*: (Radwan, 1995; Smallegange, 2011a); *echinopus*: (Tomkins *et al.*, 2011; Buzatto *et al.*, 2012)). Additionally, male morph expression has been found to be significantly heritable in *R. robini* (Radwan, 1995, 2003; Smallegange &

— *continued*

explanations on the level of the phenotype and genotype, respectively. These levels of explanation are sometimes conflated due to an apparent misunderstanding of condition-dependency and heritability, occasionally resulting in the misleading claim that balancing selection on the genotype-level drives coexistence of alternative morphs (Box 2.1). Focusing on acarid mites, where a number of species show condition-dependent polyphenism depending on juvenile nutrition and somatic growth (Table 2.1), we review hypotheses on each level of explanation: (1) which selection pressures shape the optimal condition threshold for condition-dependent polyphenism, and

Box 2.1 — continued from previous page

Coulson, 2011), but not in *R. echinopus* (Radwan, 2001). These contrasting heritability estimates helped fuel the narrative that male morph expression is genetically determined in *R. robini*, but environmentally cued in *R. echinopus* (e.g. Radwan, 1995, 2001, 2003), although the largely similar effect of body size actually indicates very similar condition-dependent plasticity in both species. The unhelpful dichotomy between 'genetically determined' male morph expression in *R. robini* and 'environmentally cued' male morph expression in *R. echinopus* is further complicated by the phenomenon that fighter expression in *R. echinopus* is additionally suppressed by chemical cues from dense mite populations (Radwan, 2001; Tomkins *et al.*, 2011). Density-dependent chemical cues do not seem to occur in *R. robini* (Radwan, 1995), which may have contributed to the idea that male morph expression is genetically determined in this species. As several authors have argued, it is inappropriate to use heritability estimates to infer genetic determination (Falconer & Mackay, 1996; Gross, 1996); a heritability estimate does not inform on whether a dimorphism is genetically determined or condition-dependent, and high heritability neither equals genetic determination nor refutes condition-dependence. In a condition-dependent dimorphism, high heritability instead indicates that the experimental set-up has effectively minimized phenotypic variation attributable to environmental effects.

Unfortunately, the assumption of genetic determination of alternative male morphs in *R. robini* is repeated in several articles (Radwan & Bogacz, 2000; Radwan & Klimas, 2001; Smallegange & Coulson, 2011; Plesnar-Bielak *et al.*, 2014, 2018; Skrzynecka & Radwan, 2016), and coexistence of alternative male morphs has consequently been interpreted to be due to balancing selection on genetic polymorphism (Skrzynecka & Radwan, 2016). This interpretation represents a conflation of phenotype-level and genotype-level explanations for variation, since male morph expression is in fact plastic and condition-dependent. Balancing selection may very well maintain genetic variation and heritability in the condition-dependent reaction norm (genotype-level), but cannot explain why the condition-dependent reaction norm has the shape that it has, nor can it explain why males differ in condition (phenotype-level).

thereby determine male morph frequencies given the distribution of condition (phenotype-level)? And (2) which processes maintain genetic variation underlying the condition-dependent developmental switch (genotype-level)?

2.2 — Selection pressures that favour condition-dependent plasticity in alternative male morph expression

In general, two non-mutually exclusive classes of hypotheses exist to explain adaptive condition-dependent developmental plasticity (Nettle & Bateson, 2015). Plasticity

is adaptive when it allows individuals to anticipate future 'selective' environments using information cues from the current 'predictive' environment during development (anticipatory, information-based plasticity; Fig. 2.1b). It is also adaptive when it minimizes detrimental consequences after some kind of harmful perturbation, mitigating current physiological problems (mitigating, somatic-state-based plasticity; Fig. 2.1b). In both cases, plasticity is adaptive if (i) individuals that experience a developmental input (i.e. a cue, or perturbation) have on average higher fitness if they develop an 'alternative' phenotype rather than the 'regular' phenotype, and (ii) individuals that do not experience a developmental input have on average higher fitness if they develop the 'regular' phenotype rather than the 'alternative' phenotype. In other words, fitness functions of different phenotypes should cross over the gradient of experienced developmental inputs (Nettle & Bateson, 2015). If we consider acarid mites as a model, plasticity is adaptive if males in poor condition maximize fitness by developing an unmodified third leg pair and expressing the scrambler morph, whereas good-condition males maximize fitness by developing an enlarged third leg pair and expressing the fighter morph (Radwan, 2009). The resulting scrambler and fighter fitness functions then cross over the condition gradient. Although conceptually similar, information-based and somatic-state-based hypotheses differ in what this condition gradient is, and why the fitness functions cross (Nettle & Bateson, 2015).

An influential information-based hypothesis is the idea that males in good and bad condition (e.g. body size) experience different mating niches, and prepare for distinct mating tactics (Gross, 1996; Oliveira *et al.*, 2008). According to the *alternative mating tactics hypothesis*, condition relates to expected future performance with regards to mating based on current body size, where male aggression and physical strength determine mating success. Good-condition males are relatively likely to win future contests over access to mates, and thus benefit most by expressing the fighter morph. Bad-condition males are relatively unlikely to win future fights, and benefit by expressing the scrambler morph, which is adapted for sneaking tactics to gain access to mates without engaging in fights (Fig. 2.1b). In acarid mites, the evolution of condition-dependent polyphenism is often assumed to be driven by the alternative mating tactics mechanism (Radwan, 2009), but without strong empirical support. Firstly, there is little evidence that fighters and scramblers occupy different condition-dependent mating niches, in which fighters monopolize females by fighting, and scramblers employ sneaking tactics to obtain matings (Radwan & Klimas, 2001; Smallegange *et al.*, 2012). Fighters and scramblers also perform similarly in sperm competition (Radwan, 1997), although under some conditions, food-deprived scramblers produce more offspring

Table 2.1 — Mite species in which males plastically develop as fighters or as scramblers. Excluded are species within the genera *Cheyletus* and *Schwiebia* where alternative male morphologies have been described, but no further life history information exists (e.g. Woodwring, 1969; Regev, 1974).

Species	**Fighter morphology**	**Condition-dependent male morph expression**	**Suppression of fighter expression in dense colonies**	**Crossing fitness functions**
Falculifer rostratus	Elongated first and second legs, and elongated lower cheliceral digits	Not detected (Proctor *et al.*, 2010)	Not detected (Proctor *et al.*, 2010)	Not known
Rhizoglyphus echinopus	Enlarged third legs with modified tarsal claws (Fig. 2.1a)	Yes (body size, temperature (Radwan, 2001; Buzatto *et al.*, 2012); Not detected (diet: Radwan, 2001)	Yes (Radwan, 2001)	Yes: in relation to colony size (Radwan *et al.*, 2014)
Rhizoglyphus robini	Enlarged third legs with modified tarsal claws (Fig. 2.1a)	Yes (body size, diet: Radwan, 1995; Leigh & Smallegange, 2014)	Not detected (Radwan, 1995)	Not known
Sancassania anomalus	Enlarged third legs with modified tarsal claws (Fig. 2.1a)	Yes (diet, temperature: Woodwring, 1969)	Yes (Woodwring, 1969)	Not known
Sancassania berlesei	Enlarged third legs with modified tarsal claws (Fig. 2.1a)	Yes (juvenile diet: Radwan, 1995; body weight: Radwan *et al.*, 2002)	Yes (Radwan, 1995; Radwan *et al.*, 2002)	Yes: in relation to population density (Michalczyk *et al.*, 2018); Not detected: in relation to body size (Michalczyk *et al.*, 2018)

than food-deprived fighters (Van den Beuken *et al.*, 2019a). Fighters can kill conspecifics, possibly to remove competitors (Smallegange *et al.*, 2018), but equally plausible is that they do so to feed on conspecifics (Łukasik, 2010). Secondly, it is unclear that an individual's juvenile body size predicts its performance as an adult. For example, there is currently no evidence that fighting or sneaking success scale with body size in acarid mites. An alternative, somatic-state-based, hypothesis posits that scrambler expression adaptively mitigates physiological problems due to poor condition (e.g. a small energy budget), and hence buffers development against variation in condition. According to the *somatic buffering hypothesis*, fitness functions of scramblers and fighters cross over the condition gradient because it is physiologically too expensive for bad-condition males to sustain prioritised physiological processes, such as somatic main-

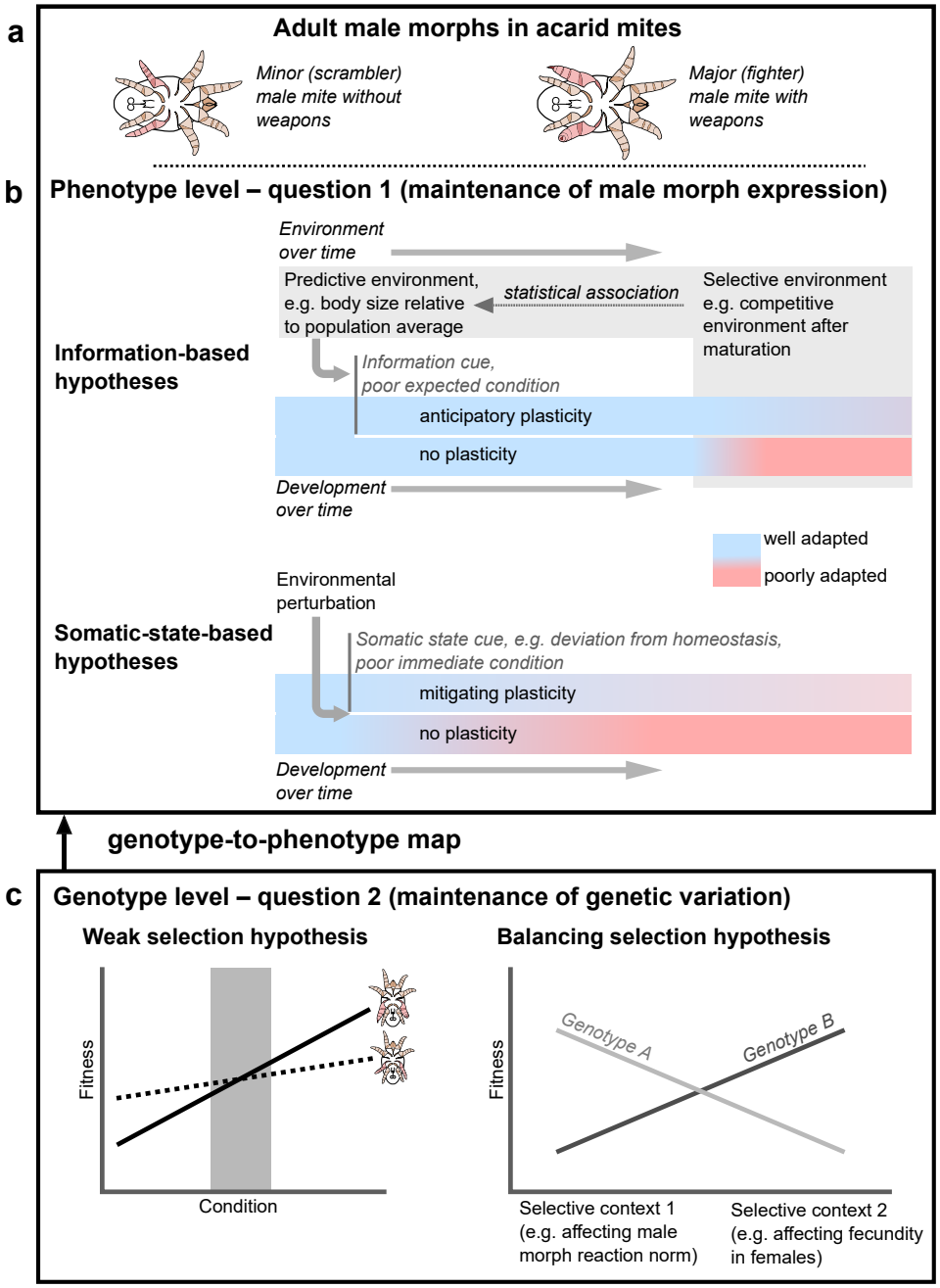

FIGURE 2.1 — Maintenance of genetic variation and maintenance of condition-dependent polyphenism require explanations at different levels of explanation. **a**, Alternative male morphs in the acarid mite *Rhizoglyphus robini*. **b**, Two (non-mutually exclusive) types of phenotype-level hypotheses are available to explain maintenance of condition-dependent polyphenism. Information-based hypotheses (e.g. the

— *continued*

tenance, growth and maturation, *and also* produce fighter morphology. Such bad-condition males can salvage somatic functioning by refraining from developing fighter morphology, redirecting resources to prioritized processes (Fig. 2.1b).

Empirically distinguishing between the two hypotheses is not straightforward. Whereas the alternative mating tactics hypothesis predicts that the condition threshold for polyphenism is evolutionarily regulated by how adult males perform in the mating arena, the somatic buffering hypothesis predicts that the threshold is regulated by the dynamics of population density, food competition and individual energy economies. Both mechanisms can act in concert, and it is not easy to predict which of the two will have a stronger effect on polyphenism threshold evolution. Through experimental evolution, Smallegange and Deere (2014) followed food-restricted *R. robini* populations while simultaneously harvesting either scramblers or fighters, allowing population density to respond to the harvesting treatments. Males evolved towards *unconditional scrambler expression* regardless of which morph was harvested. The evolutionary shift towards the scrambler phenotype was accompanied by an increase in average population density, likely from an overcompensatory population response to pulsed alleviations of average competition pressure (harvesting of large adults). Increased population density may have led to a decrease in per-capita resource uptake, and an increase in the relative costs of producing expensive fighter legs. When repeated under *ad lib* food conditions (reducing competition for food), Smallegange *et al.* (2018) still found a modest overcompensatory population reaction but with no evolution of the polyphenism threshold in response to the harvesting treatments.

Figure 2.1 — *continued from previous page*

alternative mating tactics hypothesis) assume that developing individuals adaptively anticipate an environment experienced later in life through a plastic response to information cues from the current environment (the 'predictive environment') correlating with the future environment (the 'selective environment'). Somatic-state-based hypotheses (e.g. the somatic buffering hypothesis) assume that individuals adaptively mitigate detrimental effects of environmental perturbation through a plastic response to somatic-state cues, which arise because of such environmental perturbation of development. The coloured bars show how plasticity improves adaptedness over the course of development (colour gradient), compared to a hypothetical 'no plasticity' situation. **c**, Two genotype-level hypotheses can explain maintenance of genetic varation associated with the critical condition threshold. Weak selection: selection for condition-dependent polyphenism is weakest near the critical condition threshold, because that is where the condition-dependent fitness functions of scramblers (dashed line) and fighters (solid line) cross, so fitness differences between the male morphs are small (dashed region). Genetic variation is then maintained through mutation. Balancing selection: genetic variation in polyphenism is the result of opposing selection pressures on genes in different selective contexts (e.g. expression in males and females).

Together, these experiments imply that variation in population density and internal energy economies during development may be more important evolutionary drivers of condition-dependent adaptive polyphenism than adult male mating performance.

2.3 — Processes that maintain genetic variation in condition-dependent polyphenism

Condition-dependent polyphenism evolves rapidly in response to artificial selection in acarid mites (Tomkins *et al.*, 2011; Plesnar-Bielak *et al.*, 2014; Smallegange & Deere, 2014), and assuming the current threshold is optimal, we should expect selection to remove associated genetic variation. Despite this, alternative male morphs are generally heritable (Radwan, 1995, 2003; Smallegange & Coulson, 2011). These heritabilities could reflect heritability of the polyphenism threshold (but also condition). Threshold heritability is difficult to measure (Tomkins & Hazel, 2007; Buzatto *et al.*, 2012), as it is generally unknown what the true proximate cue triggering alternative male morph expression is (Buoro *et al.*, 2012; Buzatto *et al.*, 2015); reported heritabilities are hard to interpret (Buoro *et al.*, 2012; Buzatto *et al.*, 2015). However, rapid evolutionary responses of the polyphenism threshold to novel selection pressures imply that genetic variation underlying the threshold must be substantial (Skrzynecka & Radwan, 2016). Such genetic variation can be explained if selection on the threshold is actually not particularly strong, or if balancing selection maintains genetic polymorphism. Both hypotheses have merit. Costly sexually selected male traits (e.g. fighter legs) are predicted to capture genetic variance (genic capture), as trait exaggeration inevitably increases the dependency on the resource budget and genes that contribute to resource acquisition, assimilation and allocation (Rowe & Houle, 1996). Genic capture enforces mechanisms that increase heritability of sexually selected male traits, by increasing the target for mutation or balancing selection (Radwan *et al.*, 2016).

Genetic variation in thresholds may simply be maintained under weak selection, through mutation-selection balance (Radwan, 2008). Condition-dependent threshold traits that evolve through crossing fitness functions have an interesting property: selection acts mainly on phenotype expression at the extremes of condition. Consequently, selection near the threshold is expected to be much weaker than far away from the threshold (Fig. 2.1c). It may be the case that selection on the threshold is too weak to purge mutations, because a relatively large proportion of the population is expected to express a value of somatic condition that is near the threshold.

The alternative hypothesis is that genetic variation in thresholds is under balancing selection (Fig. 2.1c; Radwan, 2008; Radwan *et al.*, 2016), including processes such as

heterozygote advantage (which is possible in male acarid mites as both sexes are diploid), fluctuating environments favouring different reaction norms, or frequency-dependent selection for reaction norms with different thresholds (e.g. coexistence of canalized and plastic male phenotypes; Buzatto *et al.*, 2012). Currently, none of these processes have empirical support in acarid mites. A more plausible process of balancing selection is intralocus sexual conflict. This arises when male adaptation has a negative effect on female fitness and vice versa, so that neither sex is optimally adapted and genetic variation is maintained (Bonduriansky & Chenoweth, 2009). Since male sexual traits and their condition-dependent reaction norms are likely associated with many genes through the process of genic capture (Rowe & Houle, 1996; Tomkins *et al.*, 2004b), since males and females share a genome, and since the sexes have different fitness optima for such traits, some degree of intralocus sexual conflict is likely inevitable (Connallon & Clark, 2013), potentially maintaining balanced polymorphisms in the fighter-scrambler reaction norm (Radwan *et al.*, 2016).

Showing that intralocus sexual conflict maintains genetic diversity underlying male morph expression requires the following demonstrations (Bonduriansky & Chenoweth, 2009). Firstly, male and female traits should be associated with the same genes, which may occur if development of both male and female traits is physiologically expensive and subject to allocation trade-offs (Radwan *et al.*, 2016). Male and female adaptation are then associated with resource economies that bias resources towards respectively male and female trait development. If the genetic underpinning of the resource budget is the same in males and females, male and female traits can be envisaged to be on a single resource allocation trade-off with a single set of associated genes. Secondly, given the existence of such a trade-off, males and females should have fitness optima on different positions on this trade-off. Lastly, neither sex should currently be at their fitness optimum, fuelling sexually antagonistic selection where male adaptation is detrimental to female fitness and vice versa.

In acarid mites, it is hypothesized that intralocus sexual conflict maintains genetic variation due to a trade-off between fighter legs and female fecundity (Plesnar-Bielak *et al.*, 2014, 2018; Joag *et al.*, 2016). Currently however, only the requirement that male fighter leg development and female fecundity are associated with the same genes has received some empirical support (Plesnar-Bielak *et al.*, 2014). Unfortunately, we do not know what the female fecundity optimum is. Increased egg production may not always yield higher female fitness, as life history theory predicts that some kind of female fecundity optimum must exist given juvenile mortality rates and the trade-off between offspring quality and quantity (Stearns, 1992; Roff, 1993). Furthermore, we

do not know what the condition-dependent male optimum is for fighter legs, and whether the male reaction norm deviates from the optimum due to sexually antagonistic pleiotropy. Candidate genes for such pleiotropy in fighter legs and female fecundity have not been conclusively identified (but see (Joag *et al.*, 2016)), and the presumed resource allocation trade-offs underpinning this genetic correlation have not yet been demonstrated. For these reasons, we cannot yet conclude that intralocus sexual conflict does indeed maintain genetic variation in fighter-scrambler reaction norms, and we should not discard alternative explanations.

Stewart *et al.* (2019) suggest balancing selection maintains genetic variation in *R. robini* through differential inbreeding depression between male morphs and sexes, with patterns (and intensities) fluctuating among environments. Notably, this research encompassed individual-level genetic diversity profiles that demonstrated that scramblers were more diverse than their highly inbred fighter counterparts, but that this differentiation decreased up to threefold in bad-nutrition environments. The severity of inbreeding depression may thus fluctuate with nutritional environment and with male phenotype, potentially resulting in balancing selection on alleles involved in various physiological processes. However, strong evidence for this hypothesis is lacking. Stewart et al.'s (2019) results may also be explained by differential inbreeding among male morphs and a collapse of male morph heritability in bad environments.

In either case, the next steps for understanding processes involved in the maintenance of genetic variation underlying condition-dependent plasticity would be increased diversity measures through genome-wide sequencing, including outlier analysis and gene discovery (e.g. genome-wide SNP analysis; Kocher *et al.*, 2018). By pinpointing the genetic basis and causal mechanisms of intraspecific condition-dependent variation within populations, such as polyphenism, researchers may identify the physiological processes and trade-offs involved in its development. This will not only contribute to understanding the potential roles of intralocus sexual conflict and differential inbreeding depression in maintaining genetic variation, but may also support somatic-state based selection pressures (e.g. somatic buffering) as drivers of condition-dependent polyphenism. In the acarid mite system, we envisage genetic evidence for somatic state mechanisms to comprise genes implicated in metabolic or other physiological processes involved in structural growth and somatic maintenance (Joag *et al.*, 2016). Finally, a developmental-causal understanding of polyphenism in acarid mites, combined with pedigreed populations, will allow us to more accurately measure how much genetic variation in polyphenism thresholds we actually have to explain.

2.4 – Implications and future directions

We find that only the alternative mating tactics hypothesis has been considered in acarid mites with regards to ecological selection pressures that maintain condition-dependent plasticity and set the threshold for polyphenism (Table 2.1), yet strong empirical evidence is lacking. The somatic buffering hypothesis provides a promising alternative explanation if condition-dependent polyphenism is a somatic-state-based mitigating response, rather than an information-based anticipatory response. This hypothesis shifts the emphasis away from adult-centrism, in which juvenile condition is just a predictor of future adult mating performance and polyphenism is an anticipatory adaptive response, to a life-cycle perspective in which trade-offs among physiological processes underlying maintenance and development take centre stage (Fig. 2.1b). An integrative view of adaptive plasticity that considers information-based hypotheses alongside somatic-state-based hypotheses moves the focus of evolutionary explanation (i.e. the idealized evolutionary individual) from the adult phenotype to the developing life cycle.

Balancing selection seems the most promising avenue to maintain genetic variation underlying polyphenism within mite populations. While adopting a population-genetic view to explain genetic variation is important to fully appreciate how alternative morphs can be evolutionarily stable, implicitly assuming a simple one-on-one genotype-to-phenotype linkage is fraught with misinterpretations (Box 2.1). In acarid mites, male morph expression depends on somatic condition. Condition, in turn, is largely environmentally determined, which renders male morph expression a case of developmental plasticity. This implies that the question of why genetic variation underlying condition-dependent thresholds is maintained, is at a different level of explanation than the question of why development is condition-dependent instead of canalised (Fig. 2.1). At one level of explanation, we seek to answer why heritability is maintained in the face of selection, while at the other, we seek to answer why alternative male morphs coexist.

Finally, condition-dependent polyphenism in some acarid mite species has been co-opted by an additional mechanism, where fighter expression is suppressed in large and dense colonies (Table 1). This may suggest that once a condition-dependent developmental switch has evolved, it is evolutionarily liable to be triggered by other cues that produce adaptive results. For example, in the soil mite *Sancassania berlesei* and the bulb mite *Rhizoglyphus echinopus,* polyphenism is cued by both somatic condition and chemical cues for population density (Radwan, 2001; Michalczyk *et al.*, 2018), the latter of

which appears to be adaptive (Michalczyk *et al.*, 2018). Thus, the causes and consequences of such evolutionary shifts in polyphenism cues would be an interesting research line to pursue, potentially revealing whether somatic state-based mechanisms of adaptive plasticity are an evolutionary precursor of information-based systems. Given that polyphenism in acarid mites can be cued by condition (either somatic state- or information-based) and/or colony size (information-based; Table 1), we foresee the acarid mite system to be at the forefront in answering such questions.

In conclusion, to understand drivers of condition-dependent polyphenism, we call for a research agenda that focuses on genotype-level explanations for genetic variation underlying reaction norms, and phenotype-level explanations for the reaction norm shape. While genetic variation could be maintained through various mechanisms of balancing selection, phenotypic variation (i.e. alternative male morphs) is maintained through selection for a single optimal condition-dependent reaction norm. What these selection pressures exactly are, remains to be investigated. Phenotype-level candidate hypotheses include information-based mechanisms (e.g., the alternative mating tactics hypothesis), and somatic-state-based mechanisms (e.g., the somatic buffering hypothesis). Ultimately, unravelling how selection maintains adaptive condition-dependent plasticity will improve our understanding of the evolution of sexual traits. Furthermore, understanding the relative role of information-based and somatic-stated based processes may improve prediction of population dynamics in the face of environmental change, as the dynamics of individual life-histories ultimately form the basis for the dynamics of populations as a whole.

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Chapter 3

'Making the Best of a Bad Job' revisited: the evolution of adaptive, condition-dependent expression of secondary sexual traits in arthropods

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Abstract

Male secondary sexual trait expression is often condition-dependent, sometimes resulting in discrete condition-dependent minor and major male morphs. Such polyphenism has been hypothesized to evolve as an adaptive conditional strategy, 'Making the Best of a Bad Job', allowing males in bad condition to salvage fitness by developing differently. Because secondary sexual traits evolve under sexual selection, it is regularly assumed that condition-dependent polyphenism in such traits also evolves under sexual selection for condition-dependent alternative mating tactics with corresponding phenotypes. Here, we conceptualize how developmental variation in condition results in fitness variation, and review different mechanisms through which condition-dependent plasticity in sexual traits can be adaptive. We identify a 'somatic buffering' component of adaptive plasticity that evolves under selection due to viability and life history timing differences, and an 'alternative mating tactics' component that evolves under sexual selection. Focusing on arthropods, we then present an inventory of genera in which condition-dependent sexual trait polyphenism has been recorded, and review the evidence that adaptive condition-dependent polyphenism in the secondary sexual traits of male arthropods is driven by alternative mating tactics, somatic buffering, or both. We find that condition-dependent polyphenism is widespread in arthropods. Yet, the available evidence is still insufficient to disentangle and quantify the relative contributions of alternative mating tactics and somatic buffering to the evolution of condition-dependent polyphenism.

3.1 – Introduction

The expression of morphologically exaggerated secondary sexual traits, such as ornaments or weapons, is conspicuously sensitive to the phenotypic state of the developing individual in many species, perhaps predominantly so in arthropods (Brockmann, 2008; Shuster, 2008; Lavine *et al.*, 2015). Common influences on sexual trait development include nutrition, oxidative stress, genetic effects, parasite load and pathogen infection, often grouped together under the umbrella of 'condition' or 'quality' (Box 3.1), resulting in the expression of condition-dependent alternative phenotypes (Table 3.1; Dominey, 1984; Gross, 1996; Hill, 2011). The evolution of such polyphenism is often explained by the hypothesis that individuals adopt a conditional evolutionarily stable strategy that is sometimes referred to as 'Making the Best of a Bad Job' (hereafter: MBOBJ; see Box 3.2 for historical context regarding the MBOBJ hypothesis). According to this idea, individuals in poor condition adopt an alternative developmental trajectory that maximizes fitness given their poor state. Hence, their fitness is still low compared to individuals in good condition, but not as low as it would have been had they not adopted an alternative developmental trajectory (West-Eberhard, 1979; Dawkins, 1980; Parker, 1982; Gross, 1996; Tomkins & Hazel, 2007). Therefore, the hallmarks of MBOBJ are condition-dependent expression of 'minor' and 'major' alternative phenotypes, and unequal average fitnesses of the alternative phenotypes (Dominey, 1984; Gross, 1996; Gross & Repka, 1998; Tomkins & Hazel, 2007). In this definition of the MBOBJ hypothesis, and throughout this review, 'condition' is defined as the phenotypic expectation of future reproductive success (i.e. residual reproductive value), which can be operationalized in a number of ways depending on context (Box 3.1).

Fitness differences arise due to variation in fertilization success in interactions with potential or actual mates (i.e. sexual selection), the capacity to survive to reproduce, and the timing of reproduction, so can be mitigated by adaptive plasticity underlying all of these fitness components. However, to date, the evolution of condition-dependent polyphenism in secondary sexual traits has often been assumed to be a consequence of sexual selection, and as an adaptation to alternative mating tactics (Gross, 1996; Shuster & Wade, 2003; Oliveira *et al.*, 2008). This raises an empirical question: do viability and the timing of life history events contribute to selection for alternative MBOBJ-phenotypes, or is sexual selection all that matters for MBOBJ plasticity in sexually selected traits?

In this review, we first summarize the biology behind a critical assumption of the MBOBJ hypothesis: the stable existence of variation in condition, so selection can

Box 3.1 — The definition and operationalization of condition

Different definitions for different explanatory purposes

The concept of individual 'condition' or 'quality', as a measure of how 'well' an organism performs, is commonplace in evolutionary literature. Yet, despite its central role in sexual selection theory, it is often unclear what 'condition' or 'quality' exactly means. Many authors have used these terms interchangeably without defining them precisely, often used to refer to the intuition that some individuals surely are 'better' than others within a population (Zahavi, 1977; Andersson, 1982, 1986; Cotton *et al.*, 2004b; Johnstone *et al.*, 2009; Zinna *et al.*, 2018b), with such heterogeneity being a prerequisite for evolution by natural or sexual selection. Some authors have attempted to provide a more restrictive definition for the concept of condition (Rowe & Houle, 1996; Wilson & Nussey, 2010; Hill, 2011; Lailvaux & Kasumovic, 2011; Clancey & Byers, 2014), over quality (commonly redefined as a genetic quality, or breeding value for total fitness (Hunt *et al.*, 2004)). Nevertheless, such attempts have not lead to consensus about what condition means. We identify two very different approaches to defining condition in the recent literature: the 'resource pool' and 'whole-organism performance' approach. We posit that the apparent controversy surrounding the concept of condition is mainly due to conflation of different explanatory purposes; that is, researchers are using the label 'condition' for different conceptual constructs that have very different explanatory functions.

Condition as the resource pool

Condition is often defined as the total pool of resources available for allocation, so that individual differences in condition relate to differences in the extent to which individuals acquire and assimilate energy (Rowe & Houle, 1996; Tomkins *et al.*, 2004b; Clancey & Byers, 2014). This particular conceptualization of condition is tied to the 'genic capture' hypothesis, which proposes that male traits evolving under female preferences retain genetic variance under strong sexual

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effectively shape condition-dependent norms of reaction. We explore the causes of individual differences in condition (i.e. how individuals diverge over ontogeny), their fitness consequences, and how those differences can persist over time in the face of selection. Then we conceptualize different mechanisms through which condition-dependent polyphenism in sexual traits can be adaptive, identifying 'somatic buffering' and 'alternative mating tactic' components in adaptive MBOBJ plasticity. Finally, we evaluate the evidence that condition-dependent polyphenism in the secondary sexual traits of male arthropods is solely driven by sexual selection and condition-dependent mating tactics, or also by viability selection and condition-dependent somatic buffering.

Box 3.1 — continued from previous page

selection because such traits depend on the resource budget with its ge-nome-wide mutational target (Rowe & Houle, 1996). Since 'genic capture' involves a mechanism that explicitly involves an individual's total pool of resources available for allocation, the definition of 'condition' as an individual's pool of resources is appropriate for this explanatory purpose. In the context of other hypotheses, however, condition may need a different definition.

Condition as whole-organism performance

Another common practice in evolutionary and behavioural ecology is to use the label 'condition' to indicate differences in individual performance, rather than differences in resource storage. 'Condition' or 'quality' are occasionally used to refer to the axis of multidimensional phenotypic variation that best explains variance in total fitness (Wilson & Nussey, 2010). This is particularly useful in trait-based evolutionary demography when researchers are interested in predicting an individual's reproductive output. However, when researchers are interested in dynamic phenomena that occur within an individual's life cycle, such as condition-dependent plasticity, condition cannot be defined as a post-hoc summary of fitness due to phenotype. The fact that organisms can mitigate expected poor performance through developmental plasticity (MBOBJ) implies that condition must instead be defined as a dynamic, state-dependent property of the individual; a plastic response to condition in early life becomes a determinant of condition in later life, potentially prompting further condition-dependent plasticity and condition change (Lailvaux & Kasumovic, 2011). A definition of condition that captured this dynamism was proposed by Hill (2011), in the context of explanations for the evolution of condition-dependent 'honest' sexual displays, a form of condition-dependent plasticity. Hill (2011) defined condition as an organism's capacity to withstand environmental perturbations (i.e. challenging environmental effects), at any

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3.2 — Individual differences in development, and their recurrence over time

Causes of individual differences: the accumulation of variation over ontogeny

Individual differences in condition are an inevitable consequence of development. Differences in environmental circumstances leave their mark on developmental trajectories, as developing organisms are part of the physical world. Additionally, phenotypes adaptively accommodate further development under stress, by responding plastically to perturbations of development. This leads to a diverging process, in which individual differences arise due to the feedback between episodes of developmental stress (here roughly defined as any developmental perturbation resulting in deviation from homeostasis; Crino & Breuner, 2015), developmental accommodation of stress, and genetic variation underlying both susceptibility to stress and accommodation of

Box 3.1 — continued from previous page

point in its life time. Environmental effects are challenges to the developmental system only to the extent that they have the effect of reducing fitness. Therefore, rephrased, condition *sensu* Hill is the current phenotypic expectation of future reproductive success, which is equal to residual reproductive value (Williams, 1966; Stearns, 1992).

Condition as residual reproductive value: different measurements in different selective contexts

In this review, we explore hypotheses explaining adaptive condition-dependent plasticity. Hence, we adopt the definition of condition as residual reproductive value. But what do we measure? Operationalization of the concept of condition requires careful consideration of *what it is about the organism's phenotype that forecasts future reproductive success at a particular point in their life cycle*. Key to this is an appropriate decomposition of condition into phenotypic causes of reproductive value (i.e. *why* individuals differ in condition, within a particular ecological context, at a particular time during their life cycle). These condition components include an individual's social state (i.e. its ability to obtain favourable outcomes from interactions with other individuals, determined by e.g. attractiveness or physical strength), and its physiological state (i.e. its ability to maintain homeostasis, determined by e.g. its resource budget). Variation in social state can be due to variation in body size; if so, body size is a useful proxy for condition. Variation in physiological state can be due to (adaptive) variation in how individuals time events in their life cycle (e.g. metamorphosis) under environmental fluctuations or variation in somatic growth rate. Finding a measurable proxy for physiological state is more difficult. If resource fluxes themselves cannot be measured, a reliable correlate might suffice. All of these condition components require careful consideration of what optimality is, whether and how individuals deviate from optimality, and given that individuals deviate from optimality in one or more condition components, how developmental plasticity might mitigate fitness costs. Although empirically challenging, combined measurements of individuals' relative social and physiological state, and their fitness consequences, will allow us to quantify *why* condition-dependent MBOBJ plasticity is adaptive.

development under stress. This can roughly be conceptualized as outlined below (Fig. 3.1a).

There is usually background genetic variation among individuals, arising by mutation in the germline, causing slight differences in how development responds to perturbations of it (Hunt *et al.*, 2004). The *genomic basis* of condition is expected to offer a very wide mutational target, so mutation pressure could be enough to maintain genetic variation associated with condition (Rowe & Houle, 1996; Tomkins *et al.*, 2004b).

Development is always vulnerable to *episodes of stress*: external or internal assaults on the physiology of development, causing phenotypic state changes. Such assaults include lack of developmental resources (i.e. chemical compounds) due to malnutrition or drought (Metcalfe & Monaghan, 2001; Reim *et al.*, 2006; Vickers, 2014); infection with pathogens, perturbation of the microbiome, or other alterations of the composition of the holobiont (Knell & Webberley, 2004; Rosenberg *et al.*, 2007; Gilbert *et al.*, 2015); oxidative stress and damage due to metabolic activity (Von Schantz *et al.*, 1999; Garratt & Brooks, 2012); expression of unfavourable alleles (Charlesworth & Charlesworth, 1987; Keller & Waller, 2002); toxification, i.e. co-option of detrimental compounds in development (Patra *et al.*, 2004); temperature shifts (Trudgill *et al.*, 2005); injury and mechanical damage (Duckworth, 2003); or functional interference by hormones and other signaling molecules secreted in response to perception of threat (Webster Marketon & Glaser, 2008).

Development accommodates further development under such perturbations, responding adaptively to minimize detrimental effects. Such responses, sometimes grouped together as phenotypic accommodation, are conserved adaptations. They canalize development under stress, as stress is inevitably part of the 'normal' environment in which populations evolve (West-Eberhard, 2003, 2005; Badyaev, 2005; Gerhart & Kirschner, 2007; Ellis & Del Giudice, 2019). Phenotypic accommodation includes several processes. For example, homeostasis in prioritized processes can be maintained by changing homeostatic set-points in others, redirecting resources (Glazier, 2002; McEwen & Wingfield, 2003; Romero *et al.*, 2009; Ng'oma *et al.*, 2017; Del Giudice *et al.*, 2018). Such stereotyped allostatic shifts lead to developmental robustness in prioritized processes, through plasticity in processes of lower priority. Developmental stability can also be maintained through somatic selection: developmental trial-and-error occurring when development overproduces variation in responses, evaluated by proxies for future performance (Hull *et al.*, 2001; West-Eberhard, 2003; Müller *et al.*, 2018; Snell-Rood *et al.*, 2018). This is sometimes referred to as 'exploratory behaviour' of development (Kirschner & Gerhart, 1998; Gerhart & Kirschner, 2007). Furthermore, development can be buffered through behavioural change. Adaptive behavioural change may come about through (i) essentially somatic selection on a behavioural level: overproduction and selective retention of behavioural variation (e.g. through klinokinesis or orthokinesis; Lagerspetz & Vainio, 2006). Such somatically selected behavioural patterns may be reinforced through cognition (learning by trial and error, conditioning; Dukas, 2004; Kawecki, 2010). Alternatively (ii), adaptive behavioural change may be a ritualized adapted process if a particular behavioural response

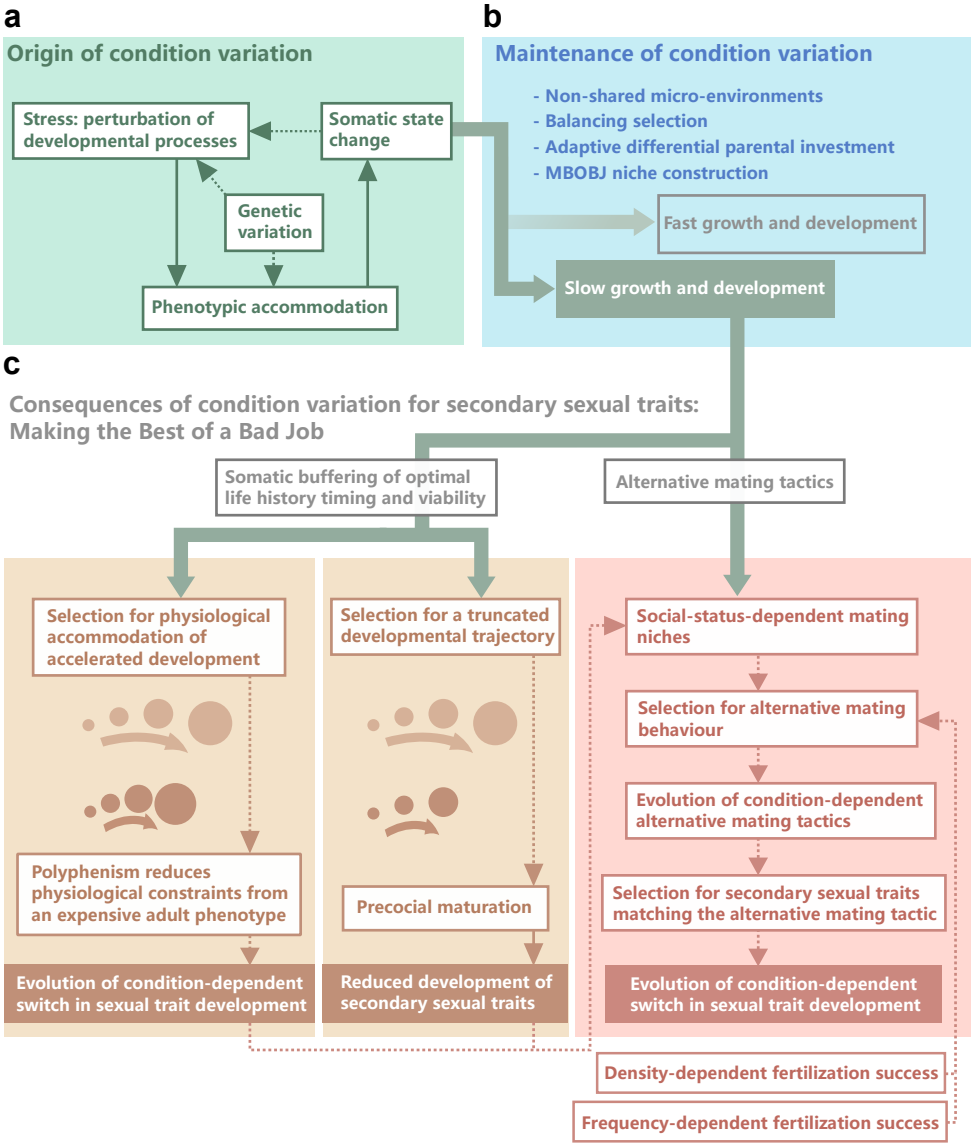

Figure 3.1 — Conceptual representation of the evolution of condition-dependent polyphenism in secondary sexual traits. Solid arrows denote direct causal relationships in development, dashed arrows denote indirect statistical effects. **a**, Condition variation originates in a feedback between recurring episodes of stress, phenotypic accommodation of further development under stress, and resulting somatic state changes. Since developmental stress responses are somatic-state-dependent, ontogenetic trajectories will diverge. Genetic variation affects condition through genetic effects on the susceptibility to environmental effects as well as the accommodating response. **b**, Several processes maintain condition variation under selection, affecting either the pattern of stressors that developing organisms experience (non-shared micro-environments, differential parental investment) or the selection pressures on genes associated with condition (balancing selection, MBOBJ niche construction). **c**, The MBOBJ hypothesis for

— *continued*

is part of a lineage's selective past (e.g. thigmotaxis, phototaxis, thermotaxis, hydrotaxis, chemotaxis, and schooling; Grünbaum, 2010).

Developmental responses to perturbations cause *phenotypic state changes*. Some of the state changes are immediately adaptive, accommodating development following perturbation. Inevitably, there will also be immediate and long-term side-effects of phenotypic accommodation due to trade-offs in allocation of resources and time, and accumulation of somatic damage due to adjustments of homeostatic set-points, habituation to signal hormone profiles, and mismatches between short-term adaptive responses and long-term environments, summarized under the concept of allostatic load (Metcalfe & Monaghan, 2001; McEwen & Wingfield, 2003).

In summary, (micro)environmental perturbations and developmental responses occur continuously along the ontogenetic trajectory, and alter the individual's developmental state. Furthermore, the developmental effects of perturbations are state-dependent. Development, as a state-dependent constructive process, thus tends to amplify small initial differences, causing divergence among even genetically identical (yet epigenetically distinct) individuals (Bierbach *et al.*, 2017; Bernadou *et al.*, 2018; Duclos *et al.*, 2019).

Consequences of individual differences for fitness

Differences among individuals, accrued over the course of ontogeny, lead to fitness differences along three pathways, thereby creating opportunity for the evolution of MBOBJ plasticity. The first fitness pathway is the *social* pathway: socially important phenotypic characteristics such as body size, physical strength and attractiveness cause variation in fertilization success in interactions with rivals or potential mates (West-

Figure 3.1 — *continued from previous page*

the evolution of condition-dependent polyphenism in secondary sexual traits, including 'Alternative mating tactics' and 'Somatic buffering' components of adaptive plasticity. In alternative mating tactics, (small) individuals in poor social status experience a differentiated mating niche, are initially under selection for alternative mating behaviour, and subsequently evolve a dedicated morphology matching the alternative mating tactic. Selection for alternative mating behaviour can additionally be shaped by density- or frequency-dependent fertilization success. In somatic buffering, individuals in bad condition salvage life history timing (e.g. maturation) and viability either by accelerating development relative to the slow growth trajectory, or by skipping developmental stages to mature precocially. Precocial maturation results in polyphenism because it reduces time for sexual trait development. Relative acceleration of development can result in evolution of polyphenism because polyphenism can adaptively reduce physiological constraints to life history flexibility. Somatic buffering and alternative mating tactics are not independent, as reduced sexual trait expression through somatic buffering could result in poor social status and subsequent selection for alternative mating tactics.

Eberhard, 1979). In the zero-sum game of sexual competition, such variation in fertilization success often causes strong sexual selection on mating behaviour and secondary sexual morphology (Shuster & Wade, 2003). The second fitness pathway is the *viability* pathway: variation in resource management can directly cause variation in how well vital physiological processes are maintained, and thus lead to susceptibility to disease and infection, reduced resistance to starvation, reduced cognitive performance or sensory ability, reduced movement, or reduced gamete production, all impacting the ability of an individual to survive to reproduce. However, viability is often protected against resource variation by various homeostatic mechanisms in physiological processes. This leads to the third fitness pathway, which is the *life history timing* pathway: variation in resource management results in differences in how growth rate, trait development rate, maturation rate and other life history processes emerge from the energy economy (Kooijman, 2010). The timing of life history events is thus constrained by the resource budget, and hence can show maladaptive variation under variation in resource budgets.

Variation in fitness components due to non-adaptive variation in development provides the substrate for adaptive MBOBJ plasticity, in which development acts to mitigate fitness costs of a suboptimal phenotype. However, mating success, viability and life history timing are generally not all optimized by a single developmental response; trade-offs occur. For example, natural selection will often favour large size, strong physiological or behavioural protection against environmental challenges (i.e. high viability), and fast maturation. Yet, all three fitness components cannot be optimized at the same time, since an acceleration of maturation will necessarily reduce time available for growth and investment in viability (Metcalfe & Monaghan, 2001). Under differential stress, such trade-offs may cause diverging optima for developmental trajectories, resulting in shifting developmental priorities depending on stress levels and on environmental features such as juvenile and adult mortality rates. To illustrate, nutrient-deprived slow-growing juveniles may optimize fitness by prioritizing accelerated maturation, as staying in the juvenile stage is inherently risky (no reproduction, but a non-negligible chance of dying) while extending the juvenile stage to acquire resources for additional growth before reproduction gives a poor return on time investment (Wilbur & Collins, 1973). In contrast, fast-growing juveniles may optimize fitness by prioritizing additional growth rather than accelerated maturation; a small time investment then results in relatively a large increase in body size, which improves future mating success (Day & Rowe, 2002; Plaistow *et al.*, 2004; Nilsson-Örtman & Rowe, 2021). Thus, developmental differences among individuals result in variation in

multiple fitness components, but it depends on context which fitness component is most important for total fitness and needs mitigation.

To summarize, individual differences in developmental trajectories are inevitable, and can have fitness consequences along the social, physiological and life-history-timing axes of fitness. The sensitivity of total fitness to particular fitness components will depend on environmental context. When individual differences have fitness consequences, we can meaningfully talk about condition differences (Box 3.1).

Generational recurrence of condition differences

Condition differences create opportunities for MBOBJ plasticity to evolve, provided that the distribution of condition is sufficiently wide for selection to effectively shape condition-dependent norms of reaction – but how? If natural selection is able to act on heritable variation underlying condition, variation is expected to diminish, and the distribution of condition could become too narrow to maintain selection for adaptive MBOBJ reaction norms. At least four non-mutually exclusive mechanisms can explain the recurrence of condition variation (Fig. 3.1b).

Firstly, individuals within a population may share an environment on a macroscopic scale, but experience different non-shared environments and accommodate different environmental challenges on a much smaller scale (e.g. initial parental investment (Benton *et al.*, 2005; Perez *et al.*, 2017), fine-grained variation in local environmental perturbations (Freund *et al.*, 2013), or stochasticity on a cellular level (Spudich & Koshland, 1976; Yvert *et al.*, 2013)). Due to the non-linear state-dependent constructive nature of development, small initial developmental differences become amplified over the course of ontogeny, 'trapping' genetic variation in a particular class of genotype-by-environment interactions that could be summarized as genotype-by-soma interactions. Therefore, a large portion of genetic variation in condition may not be available for cumulative natural selection: the soma with its unique developmental history is not inherited, so the fitness ranking of genotypes reshuffles each generation.

Secondly, genetic variation in condition, insofar as it is not trapped in genotype-by-soma interactions, may be maintained by balancing selection pressures due to a dynamic fitness landscape (Bell, 2010). Optimization of the phenotype is context-dependent, sometimes leading to opposing selection pressures on developmental processes depending on sex or environment. For example, divergent male and female optima (e.g. in body size) with incomplete sex-limited gene expression will maintain genetic variation (e.g. in growth (Connallon & Clark, 2013, 2014)). Alternatively, genetic variation can be maintained through local adaptation and subsequent migra-

tion or rapidly changing environments (e.g. the fast evolution of genes involved in pathogen or parasite resistance (Hamilton & Zuk, 1982; Balenger & Zuk, 2014)). Poor condition, then, could partially be a side-effect of accumulation of alleles that provide a context-dependent advantage. Additionally, gene-by-gene interactions underlying developmental processes may favour some alleles only in combination with a particular genetic background, leading to phenomena such as heterozygote advantage (Hedrick, 2012).

Thirdly, differences in condition may be due to adaptive differential parental provisioning of offspring, wherein selection acts primarily on parental strategies. Offspring number and per-offspring investment trade off against each other (Stearns, 1992), and parental provisioning of offspring depends on how parents navigate this trade-off (invest in many poorly provisioned offspring or a few well-provisioned offspring). Thus, environmental variation experienced by parents can lead to variation among offspring developmental trajectories (Rosenheim *et al.*, 1996; Hunt & Simmons, 1998; Benton *et al.*, 2005).

Finally, MBOBJ plasticity is a niche-constructing trait, since (by definition) its evolution changes the fitness consequences of condition variation. The evolution of MBOBJ plasticity may flatten the fitness landscape of condition so much that genetic variation in condition can simply be maintained by the mutation rate in the large mutational target of condition across the genome. Hence, the evolution of adaptive MBOBJ plasticity and the maintenance of a wide condition distribution may be involved in a positive feedback loop: MBOBJ plasticity flattens the fitness landscape of condition, so variation can accumulate, fuelling selection for MBOBJ plasticity (Odling-Smee *et al.*, 1996; Govaert *et al.*, 2019).

The MBOBJ hypothesis has been criticized for its assumption that a wide distribution of condition persists over generations, which, according to some authors, leaves MBOBJ 'outside the realm of conventional evolutionary principles' (Shuster and Wade, 2003, p. 397). We think that this criticism is based on the outdated and misleading metaphor of development as a 'program' that simply carries out the 'genetic code' to build a genetically determined phenotype. In contrast, current evolutionary biology increasingly emphasizes the decentralized, self-organizational, state-dependent and environment-responsive properties of development (Oyama *et al.*, 2001; West-Eberhard, 2003; Laland *et al.*, 2015; Schwab *et al.*, 2019). These developmental properties, together with the insight that the fitness landscape may generally be rather dynamic (Bell, 2010), make it predictable that developmental variation and fitness variation

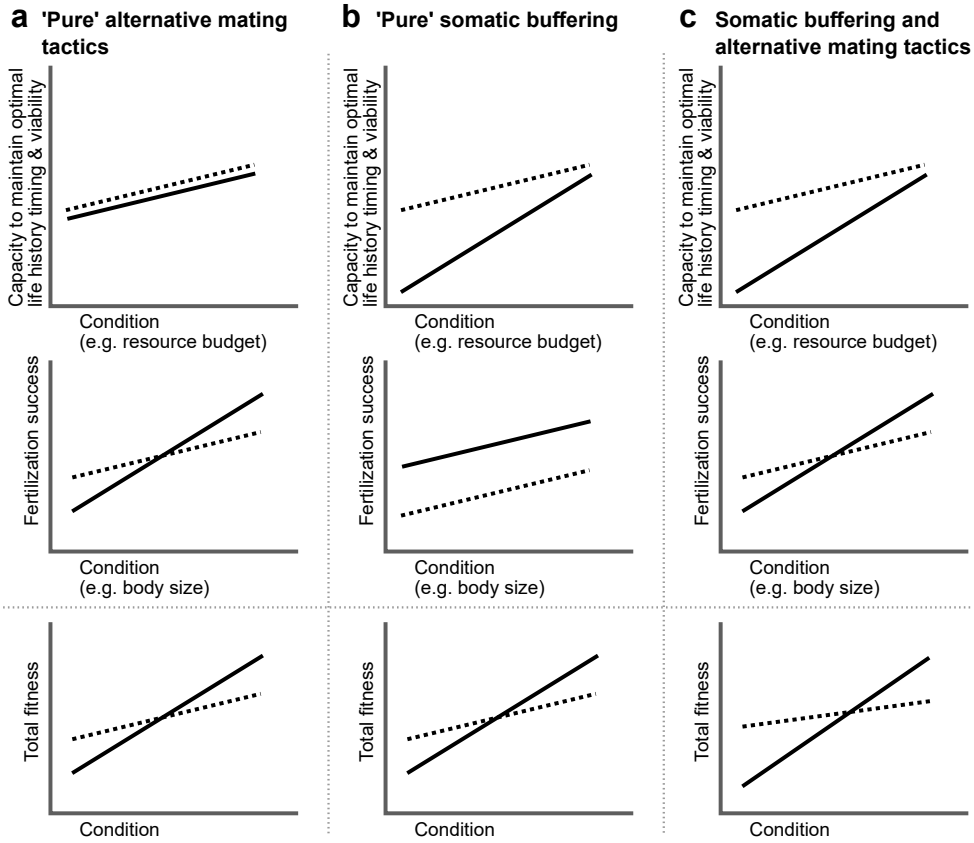

Figure 3.2 — The somatic buffering and alternative mating tactics components of MBOBJ plasticity are driven by different fitness components. Somatic buffering evolves through non-sexual selection (fitness differences due to viability and timing of life history events), while alternative mating tactics evolve through sexual selection (fitness differences due to fertilization success). **a**, In 'pure' alternative mating tactics, the fitness functions of alternative morphs (solid: major, dashed: minor) cross over the condition gradient because the fertilization success functions of both morphs do so, while major morph development is not associated with condition-dependent viability and life history costs. **b**, In 'pure' somatic buffering, the fitness functions of alternative morphs (solid: major, dashed: minor) cross over the condition gradient because of condition-dependent viability and life history costs of major morph development, while majors always have higher fertilization success than minors. **c**, In a more realistic scenario, both somatic buffering and alternative mating tactics contribute to crossing condition-dependent fitness functions.

recur over generations. Thus, the MBOBJ hypothesis resides firmly within the bounds of current Darwinian theory.

3.3 — Mechanisms of adaptive condition-dependent developmental plasticity in sexual traits

Selection for secondary sexual traits occurs whenever the fitness benefits of such traits in the mating arena outweigh the fitness costs of trait development and maintenance. Therefore, adaptive condition-dependent plasticity in sexual traits can evolve when fitness benefits or fitness costs are condition-dependent, so that the net fitness functions of males with and without sexual traits cross over the condition gradient (Fig. 3.2; Nettle & Bateson, 2015). Condition-dependent fitness benefits of sexual traits arise when males in different condition behave differently to acquire fertilizations, requiring behaviour-dependent morphological specialization (Gross, 1996; Oliveira *et al.*, 2008). For example, small males may not be able to compete with large males for mating opportunities using the same behaviours, and adaptively adopt alternative tactics with different levels of sexual trait expression. Condition-dependent fitness costs can arise when trait development has condition-dependent consequences for viability and/or maturation timing due to trade-offs. For example, a commitment to expensive trait development can preclude fast maturation, particularly in males in poor condition with relatively low somatic growth rates. Avoiding the fitness costs of suboptimal maturation timing by accelerating maturation relative to the growth trajectory necessarily results in a lower resource budget for development, potentially resulting in viability issues. Therefore, optimizing maturation timing may be possible without detrimental viability impact only if accommodated by a re-organization of developmental priorities (Metcalfe & Monaghan, 2001), for example a condition-dependent reduction of sexual trait development. These considerations lead to two hypothetical non-mutually exclusive casual pathways for how condition-dependent plasticity in sexual traits may optimize fitness (Fig. 3.1c): alternative mating tactics, and somatic buffering.

Alternative mating tactics

Often, fitness differences among males arise primarily due to variation in fertilization success (i.e. sexual selection). Condition-dependent variation in mating success can drive the evolution of alternative mating tactics, provided that the population's *mating system* constructs a secondary *mating niche* in addition to the dominant mating niche (Emlen & Oring, 1977; Shuster & Wade, 2003; Taborsky *et al.*, 2008; Shuster, 2009; Hendrickx *et al.*, 2015).

The mating system of the population is its distribution of mating opportunities as they relate to sex, condition, space and time (Emlen & Oring, 1977; Shuster & Wade, 2003), and is the ultimate ecological cause of sexual selection. The mating niche is the

manner in which an organism can exploit its environment to maximize mating success within a particular mating system (Shuster & Wade, 2003). Mating niches emerge from the structure of the mating system, which is in turn determined by life history traits, the distribution of phenotypic differences among reproductive individuals, and environment structure. Many mating systems contain only a single mating niche. Under high reproductive skew, a secondary mating niche may emerge, as disadvantaged individuals resort to different behaviours to mate and increase fertilization success, exploiting the efforts of dominant competitors (Shuster & Wade, 2003; Taborsky *et al.*, 2008) (e.g. by investing more in postcopulatory competition (Eberhard, 2009), or by sneaking behaviour (Dominey, 1984; Oliveira *et al.*, 2008; Neff & Svensson, 2013)). Note that secondary mating niches do not exist as 'empty' niches prior to the expression of different mating behaviours. Rather, they emerge from behavioural flexibility of disadvantaged individuals that are opportunistically Making the Best of a Bad Job, as they perceive their environment. When such disadvantage (and the need to Make the Best of a Bad Job) becomes predictable, initial opportunistic behavioural flexibility can evolve towards ritualized, condition-cued, adapted alternative mating tactics.

Mating niche differentiation has been observed in several arthropods with condition-dependent polyphenism. The alternative mating niches can generally be summarized as 'fighting' and 'sneaking' or fight-avoiding satellite behaviour (Shuster & Wade, 2003). In the dominant fighting niche, large males are successful at fighting, mate guarding and precopulatory courtship. Large males thus obtain a large number of copulations, or pair with a few preferred females which are subsequently assisted by the male in brood care (Cook, 1990; Rasmussen, 1994; Emlen, 1997a; Moczek & Emlen, 2000). In the alternative niche, small males circumvent fights and female mate preference by sneaking or satellite behaviour. Small males may get rejected relatively often by females, or be excluded from mating by male mate guarding (Cook, 1990; Rasmussen, 1994; Moczek & Emlen, 2000), but compensate by never rejecting females (Cook, 1990), by not investing time and energy in fighting (Rasmussen, 1994; Emlen, 1997a; Moczek & Emlen, 2000) or brood care (Cook, 1990; Hunt & Simmons, 1998), and by obtaining relatively high fertilization success from only a few copulations.

Generally, the emergence of fighting and sneaking niches is associated with resource- or female-defence polygynandry: females are strongly clustered, males guard clusters of females, both males and females mate with several partners, and reproductive skew among males is high. Female clustering, and subsequent emergence of male fighting and sneaking niches, can occur through patchy distribution of food (e.g. dung pads (Cook, 1990; Rasmussen, 1994; Emlen, 1997a; Moczek & Emlen, 2000; Cum-

mings *et al.*, 2018), sap streams on wounded trees (Hongo, 2007), fruits (Goldsmith & Alcock, 1993)), suitable substrate in sedentary species (Conlan, 1989), favourable nesting or oviposition sites (Danforth, 1991; Kukuk, 1996; Longair, 2004; Paxton, 2005; Buzatto & Machado, 2008; Buzatto *et al.*, 2011; Zatz *et al.*, 2011), or vectors in phoretic species (Zeh & Zeh, 2013), or through the benefits of sociality itself (Walker *et al.*, 1993).

Within the dominant (fighting) niche, mating success is generally not a lottery: it is condition-dependent, where condition relates to physical strength or attractiveness relative to other individuals in the population (sometimes referred to as 'status'; Dominey, 1984; Gross, 1996). Hence, males of poor condition maximize fitness through a niche shift and an alternative mating tactic, requiring a different set of morphological adaptations. In other words, fertilization success is a function of condition, and fertilization success functions of different male tactics and phenotypes cross each other over the condition gradient (Fig. 3.2a; Nettle & Bateson, 2015). Such crossing fitness functions lead to the evolution of condition-dependent plasticity in secondary sexual traits, preparing males for a fitness-optimizing mating tactic, provided that a perceivable cue preceding trait development (often in the juvenile stage) predicts condition during mating (in the adult stage). The body itself can be such a cue; continuity of the soma makes sure that there are always early signs for future performance. Therefore, in some mating systems, condition-dependent variation in mating success can drive the evolution of alternative mating tactics and corresponding alternative developmental trajectories, leading to condition-dependent adaptive plasticity in sexual traits.

Somatic buffering

Alternatively, fitness differences arise primarily due to variation in how individuals manage viability, while optimizing the timing of life history events under stress (i.e. non-sexual selection). Viability and life history timing should not be regarded separately, as it is the pressure from life history optimality that makes viability management a developmental challenge, particularly when environments are variable (Wilbur & Collins, 1973; Blanckenhorn, 1998, 2000; Morey & Reznick, 2000; Day & Rowe, 2002; Kooijman, 2010). In this scenario, the evolution of adaptive condition-dependent plasticity in secondary sexual trait development is not driven by future social performance and alternative mating tactics, but by the stress-dependent necessity to protect other physiological processes that occur simultaneously with sexual trait development: somatic buffering. Somatic buffering as an adaptive process is the consequence of

four properties of development: (i) the timing of developmental processes and life history events is often adaptively flexible within physiological constraints, responding to environmental cues; (ii) physiological processes interfere with each other; (iii) under stress, physiological processes differ in priority; and (iv) plasticity in physiology can protect viability under stress while accommodating flexible timing of life history events, potentially with morphological side-effects (details below).

Flexible timing of life history events

It has been well established that the timing of events such as metamorphosis or maturation is generally flexible, particularly in poikilothermic animals such as arthropods, which are not constrained by temperature-specific physiological rates (Stearns, 1992; Kooijman, 2010). Metamorphosis and maturation can be postponed relative to the growth trajectory when food is plentiful, temperature is favourable, and additional growth and resource acquisition before maturity is beneficial (Day & Rowe, 2002; Nilsson-Örtman & Rowe, 2021). Maturation can be accelerated when growth is slow, and it takes a long time to acquire the benefits of being large, or when growth circumstances perceivably deteriorate, projecting poor growth in the immediate future (Wilbur & Collins, 1973; Day & Rowe, 2002; Nilsson-Örtman & Rowe, 2021). After all, remaining a juvenile is risky, because reproduction is still impossible, and the probability of dying is not zero (Stearns, 1992). Such acceleration of maturation relative to the growth trajectory is generally physiologically constrained. Current models of maturation reaction norms assume that organisms need to acquire at least a minimally viable physiological state during ontogeny, in which they can enter metamorphosis and/or mature (Day & Rowe, 2002). Hence, in poorly growing organisms under stress, there can be selection to minimize physiological constraints to maturation imposed by the necessity to reach a minimally viable physiological state. Such constraints are minimized by an adaptive, condition-dependent re-organization of physiology at maturation, which reduces interference of vital physiological processes by shifting the relative priorities of different processes. Since those processes include development of expensive secondary sexual traits (Plaistow *et al.*, 2005; Hardersen *et al.*, 2011), adaptive accommodation of flexible maturation timing can be facilitated by condition-dependent polyphenism.

Adaptive flexibility in maturation timing is presumably common, yet under-recorded in species with condition-dependent male polyphenism. Maturation is often tied to a particular metamorphic moult during which the adult phenotype is developed (e.g. in nearly all holometabolous, and many hemimetabolous insects). In such cases, flex-

ibility in the timing of maturation is accomplished by postponing or accelerating the onset of metamorphosis relative to the growth trajectory, but leaving the time available to develop secondary sexual traits during metamorphosis unaffected. For example, dung beetle larvae (*Onthophagus taurus*) grow and develop inside brood balls of dung, and enter metamorphosis in response to exceeding a critical larval mass (determined by past food uptake; Emlen & Nijhout, 2001), but also in response to perceived and prognosed growth rate (determined by current food uptake; Hunt & Simmons, 1997; Shafiei *et al.*, 2001). Brood balls can differ quite dramatically in nutritional value due to variation in dung quality and parental effort (i.e. ball size), so larvae can enter metamorphosis at different sizes with different resource budgets depending on brood ball quality (Cotter *et al.*, 2008). In other species, such as rhinoceros (*Allomyrina dichotoma*) and stag beetle larvae (*Lucanus cervus*) that live freely in soil or rotting wood, the timing of metamorphosis is not only determined by food uptake, but also by seasonal time constraints (Plaistow *et al.*, 2005; Hardersen *et al.*, 2011). The larvae hatch throughout the breeding season, and differ in the time available to them before winter arrives and growth is no longer possible (Plaistow *et al.*, 2005), as well as in the growth period available to juveniles before conditions for mating become optimal (Hardersen *et al.*, 2011). Additionally, differently aged *A. dichotoma* larvae synchronize metamorphosis with nearby conspecifics, presumably reducing predation risk in the vulnerable pupal stage. As a result, some larvae postpone metamorphosis, while others accelerate it (Kojima, 2015).

In various species, maturation is not tied to a particular moult, but is free to vary heterochronically along the developmental trajectory. If that is the case, individuals can mature precocially in response to poor condition by becoming reproductively active at an earlier developmental stage than they would do under other circumstances. Precocial maturation burdens development with multiple challenges. Not only do precocial individuals have to accommodate maturation physiologically, such individuals also have greatly reduced time available to develop secondary sexual traits before maturation (if gradually) or even no time at all (if stage-specific), thereby generating polyphenism. Precocial maturation in response to poor condition has been shown to generate polyphenism in male sexual traits of several crustaceans (e.g. amphipods in the genus *Jassa* (Conlan, 1989; Kurdziel & Knowles, 2002), prawns in the genus *Macrobrachium* (Karplus *et al.*, 1992; Karplus & Barki, 2019), the shrimp *Rhinchocinetus typus* (Correa *et al.*, 2003)), and some hemimetabolous insects (e.g. weta *Hemideina crassidens* (Kelly & Adams, 2010)).

Physiological interference

The reason why polyphenism can facilitate flexible maturation timing, is that physiological processes underlying sexual trait trait development can interfere with other processes (e.g. maturation, metamorphosis). When different developmental processes draw resources from a shared resource budget, they are subject to allocation trade-offs (van Noordwijk & de Jong, 1986; Ng'oma *et al.*, 2017), which can have negative effects on process stability (i.e. homeostasis) when the resource budget is low. There are some theoretical reasons why we should expect development of sexually selected exaggerated morphological traits to be particularly energetically expensive, and subject to allocation trade-offs. Sexual signals and weapons often evolve in tandem with preference and assessment criteria under 'run-away' directional, social selection pressures. Hence, trait expression is driven towards its physiological limits, and equilibrium is maintained by developmental constraints deriving from costliness (West-Eberhard, 1979; Lande, 1981; Rowe & Houle, 1996; Chapman *et al.*, 2003). Direct empirical support for heightened energetic costs of sexual trait development remains relatively scant (Kotiaho, 2001; Cotton *et al.*, 2004a; Rico-Guevara & Hurme, 2019), although morphological enlargement and exaggeration must in some way be associated with increased allocation of resources (Rowe & Houle, 1996; Emlen, 2001; Simmons & Emlen, 2006; Lavine *et al.*, 2015). Indeed, enlarged and morphologically exaggerated sexually selected traits often show heightened sensitivity to nutrition quality during juvenile development, which has been interpreted as indicative of energetically expensive development (Rowe & Houle, 1996; Cotton *et al.*, 2004a-c; Johnstone *et al.*, 2009).

Allocation trade-offs are most likely to occur locally, between organs that directly compete for resources through proximity. Experimental suppression of horn development in the dung beetle *Ontophagus taurus* resulted in development of larger eyes compared to controls (Nijhout & Emlen, 1998). Corroborating a negative developmental correlation between eyes and horns (interpreted as resource allocation trade-off), artificial selection for larger horns in *O. acuminatus* resulted in evolution of relatively smaller eyes, and vice versa (Nijhout & Emlen, 1998). Using natural morphological variation in horns and neighbouring organs, it was subsequently shown that resource allocation trade-offs with horns affect different organs, depending on where exactly the horns develop in different dung beetle species (Emlen, 2001). However, these findings did not replicate clearly (Schwab & Moczek, 2014). Other studies find a multitude of positive, negative and statistically insignificant correlations between horn length and proximally and distally located morphological structures,

casting doubt on our ability to infer the physiological costs of horn development from uncontrolled morphological covariation (Pizzo *et al.*, 2012; Schwab & Moczek, 2016). In the acarid mites *Sancassania* and *Rhizopglyphus*, well-fed males develop a modified and enlarged third leg pair ('fighter' legs), while poorly-fed males develop normal unmodified legs. Leg development happens during an immobile metamorphic stage in which the male cannot feed, and fighter legs comprise a large part of the total body mass, so resource allocation trade-offs are expected. Indeed, in *S. berlesei* and in *R. robini,* the male body shrinks on average during metamorphosis if the male develops fighter legs, whereas males that do not develop such legs do not shrink (Radwan *et al.*, 2002; Smallegange *et al.*, 2012).

Allocation trade-offs may exist also between organs that are not physically close at all. Experimental suppression of horn development in *O. nigriventris* caused development of larger testes (Simmons & Emlen, 2006), and *Onthophagus* species with stronger plasticity in the horns appear to have more robust (i.e. less influenced by environmental stressors) testes sizes, suggesting that allocation to testes development may be buffered through plasticity in horns (Simmons & Emlen, 2006). However, it is difficult to exclude the possibility that testes development is *adaptively* plastic via cues for reduced horn development (Cirino *et al.*, 2021); after all, males without large horns often end up in a sneaking niche where larger testes confer higher fertilization success. Interestingly, experimental ablation of genital discs in *O. taurus* prepupae caused development of relatively longer horns, while genitalia did not develop at all (Moczek & Nijhout, 2004). This result supports the idea that a negative developmental correlation between horns and genitalia is non-adaptive and possibly caused by a resource allocation trade-off, as adaptive cued plasticity of horn development in response to the complete absence of genitalia is unlikely to evolve.

Trade-offs are not necessarily due to resource allocation from a shared budget; processes can also interfere functionally. Developmental processes that share physiological pathways may be mutually exclusive, if regulation of one process occurs through engagement of physiological pathways also involved in another process (Ricklefs & Wikelski, 2002; Hill, 2011). In rhinoceros beetles, for example, horn growth during the pre-pupal phase is primarily regulated by insulin-like growth factors (IGF) which are also heavily involved in somatic growth rate regulation in response to nutrient availability (Emlen *et al.*, 2012). IGF concentrations in the hemolymph decrease when nutrition is poor, preventing maladaptive allocation to growth when the resource budget is low, protecting prioritized resource fluxes (e.g. maintenance). Therefore, horn growth runs into large viability costs under poor nutrient availability

because it interferes with adaptive growth control, which relies on the same physiological machinery.

Functional interference of physiological pathways could be a general property of developmental systems, as novel traits are thought to evolve when pre-existing developmental pathways are co-opted into new functional contexts (Carroll, 2008; Shubin *et al.*, 2009). This makes development particularly vulnerable to functional interference of constituent processes, as old and new functions coexist within the same developmental system. Sexually selected traits in particular may be likely to involve physiological pathways that functionally interfere with high-priority somatic functions, such as maintenance under stress. Sexually selected traits often function as quality signals to potential rivals or mates, and are under selection for honest signaling (Andersson & Simmons, 2006). Functional interference with stress regulators provides an honesty guarantee at low energetic cost, and may therefore be a general feature of sexual signals (Hill, 2011; Emlen *et al.*, 2012; Warren *et al.*, 2013). Thus, sexual signals may capture functional interference over the course of their evolution, or emerge from pre-existing patterns of functional interference.

Adaptive prioritization

During development, not all physiological processes have equal priority under stress (Stevens *et al.*, 2000; Glazier, 2002; Kooijman, 2010; Hopwood *et al.*, 2013). Some yield fitness benefits in the more-or-less distant future, leaving scope for behavioural or developmental compensation if impacted by environmental perturbation (e.g. development of sexual traits), others are important for survival and reproductive success in the immediate future (e.g. maintenance, maturation). Some physiological processes take place in a short time frame during development, with irreversible fitness consequences if they are perturbed (e.g. adult morphology development in holometabolous insects), whereas other processes take place during extended developmental windows and can be compensated during development (e.g. growth; Metcalfe & Monaghan, 2001). Therefore, the potential for developmental or behavioural compensation mediates allocation priority under stress. This leads to a priority ranking of developmental processes, in which allocation to secondary sexual traits ranks relatively low compared to, for example, allocation to maintenance (which has immediate viability consequences) or morphological traits indirectly involved in maintenance, such as feeding traits.

Adaptive maintenance of viability through plasticity

When physiological processes interfere through allocation trade-offs or functional interference, and differ in priority, individuals in poor condition may maximize fitness by reducing lower priority processes, thereby maintaining prioritized processes. If interference is due to resource allocation trade-offs, adaptive plasticity may re-allocate resources (McEwen & Wingfield, 2003; Romero *et al.*, 2009; Ng'oma *et al.*, 2017). If interference is due to functional trade-offs, adaptive plasticity may reduce functional interference by e.g. signaling molecules such as hormones (Ricklefs & Wikelski, 2002; Hill, 2011; Emlen *et al.*, 2012; Warren *et al.*, 2013).

Morphologically exaggerated sexual traits are often energetically expensive to develop (i.e. require a large portion of the resource budget), may involve physiological processes that interfere with prioritized functions, and are of relatively low physiological priority (i.e. fitness return of resource allocation is in the distant future, and behavioural compensation for poor sexual trait expression is often possible), so are prime targets for complete or incomplete shut-down of allocation under stress. Indeed, this precise mechanism has been documented in various model systems in support of good-genes models of sexual selection by female preference, which assume differential marginal costs of sexual trait development and predict condition-dependent plasticity in sexual traits (Zahavi, 1977; Grafen, 1990; Penn & Számadó, 2020). Such condition-dependent plasticity not only provides a fitness benefit for female preference following good-genes models of sexual selection, but will also improve male fitness through the buffering effect of plasticity under stress. In such somatic buffering, different condition-dependent physiological decisions are adaptive because they immediately increase viability under stress, and/or because they enable life history flexibility while maintaining viability. Hence, in pure somatic buffering, expression of alternative male sexual trait phenotypes is a non-adaptive *side-effect* of adaptive developmental plasticity at a physiological level, and it is not maintained by adaptive alternative mating tactics (Fig. 3.2b).

Alternative mating tactics and somatic buffering as complementary components of adaptive condition-dependent plasticity

Distinguishing between somatic buffering and alternative mating tactics as evolutionary drivers of adaptive plasticity can be difficult, because adaptive plasticity is unlikely to be shaped by only one of the two processes (Fig. 3.1c). The prime criterion for classification is whether selection acts through viability and the timing of life history events, or through fertilization success (Fig. 3.2). These fitness components are often correlated at different episodes of selection during the life cycle (Arnold & Wade, 1984; Monaghan, 2008; Tigreros, 2013; Moore & Martin, 2019). Even so, their relative

importance likely differs in different populations, and they will result in different evolutionary patterns. For example, under pure somatic buffering, different male phenotypes are not necessarily expected to be associated with adaptive mating behaviours, but they should be if they evolved under selection for alternative mating tactics. Additionally, somatic buffering may pre-adapt populations to cope with novel stressors, whereas alternative mating tactics are not predicted to do so.

An additional difficulty is that somatic buffering, resulting in sexual trait variation as a side-effect, can very easily *result* in a differentiated mating niche for different phenotypic classes of males (Fig. 3.1c; Shuster & Wade, 2003). Males without well-developed sexual traits will behave differently than males with such traits, even if they (initially) do not adopt a ritualized adapted alternative mating tactic. Such males will not obtain mating success to the same degree within the dominant mating niche, resulting in increased male mating skew and opportunity for sexual selection. This will fuel sexual selection for an alternative mating tactic *following* the evolution of somatic buffering; developmental side-effects are rarely without evolutionary consequences (West-Eberhard, 2003). Conversely, it is also possible that 'alternative mating tactic' plasticity in sexual traits first evolves under sexual selection, later to be co-opted into an adaptive somatic buffering mechanism. An alternative mating tactic with dedicated morphology can release poorly growing males from energetic constraints as a side-effect, which may subsequently evolve into a generalized stress response. For example, if energetically expensive sexual traits develop during metamorphosis, co-optation of initially sexually selected plasticity could allow flexible metamorphosis timing without threatening viability under stress. Consequently, condition-dependent polyphenisms will show characteristics of both classes of adaptive plasticity (Fig. 3.2c).

We propose that somatic buffering and alternative mating tactics be regarded as two adaptive components of MBOBJ plasticity in secondary sexual traits. To understand and predict the eco-evolutionary dynamics of such traits (and co-evolving traits involved in trade-offs), it is important to distinguish between the two components of MBOBJ plasticity, and to disentangle their relative contributions.

3.4 — Empirical evidence for alternative mating tactics and somatic buffering as drivers of condition-dependent polyphenism in arthropods

Three empirical strategies can be used to infer the mechanisms of MBOBJ evolution in arthropod males. Perhaps most importantly (i), we can aim to provide a complete view of how fitness differences *causally* arise through viability, life history timing and mating success, leading to crossing fitness functions for alternative male phenotypes

over the condition gradient. Ideally, such approaches include experimental manipulations of both the ecological/social components and the developmental components of the system. A complementary and potentially illuminating approach (ii) is to compare related species with similar or different plasticity in sexual traits, and with different mating systems, nutritional ecologies and life histories. The extent to which plasticity covaries with the mating system, or with resource management and life history traits, can illuminate whether alternative mating tactics or somatic buffering drive the evolution and maintenance of plasticity. Clade-wide comparisons might even reveal what came first, somatic buffering plasticity as an adaptive stress response, or alternative mating tactics plasticity as an adaptation to diverging mating tactics. Such studies could shed light on the evolutionary importance of social selection pressures versus selection to overcome physiological constraints in a variable environment. Finally (iii), insight can be derived from observing contemporary evolution in which sexual trait reaction norms, mating systems, nutritional ecology and life histories are tracked, and ideally manipulated. Here, we evaluate the evidence coming from each of the three empirical approaches.

Measuring the causes of selection: do we understand why fitness functions cross?

A survey of the condition-dependent selection pressures on male sexual traits entails answering the following set of research questions within a study system.

1. What phenotypic characteristics determine an adult male's social status (i.e. its capacity to obtain favourable outcomes from interactions with conspecifics), and how is this adult social phenotype cued prior to sexual trait development?
2. Is fertilization success higher in the fighting niche than in the sneaking niche if males are in good social state, and is fertilization success higher in the sneaking niche than in the fighting niche if males are in poor social state (resulting in selection for condition-dependent mating tactics)?
3. Does mating success increase with sexual trait size or shape in the fighting niche, but not in the sneaking niche (resulting in tactic-dependent selection for sexual traits)?
4. Is development of the major phenotype associated with 'extra' viability and/or life history timing costs compared to the minor phenotype, and do such costs increase with decreasing physiological condition (i.e. condition-dependent costs for major phenotype development)?

Below, we illustrate to what extent these questions have been addressed in arthropods with MBOBJ plasticity with major and minor male forms (summarized in Table 3.1).

Q1 – What phenotypic characteristics determine an adult male's social status?

In virtually all male-diphenic species, major males adopt a fighting tactic in which rivals are removed by force in contests for mating opportunities. Therefore, in the context of mate acquisition, social status is generally best approximated by physical strength, which is assumed to relate to body size or mass. This general association between body mass, physical strength and social dominance has been demonstrated in a number of species (Lailvaux *et al.*, 2005; Kelly, 2008). Among arthropods with condition-dependent male polyphenism, the positive association between body mass and strength has explicitly been shown only in the dung beetle *Onthophagus taurus*, in which major males aggressively guard tunnels underneath dung pads containing females (McCullough & Simmons, 2016). In this species, adult body mass is predicted by juvenile mass, which is itself determined by nutrient uptake. Therefore, juveniles can forecast future access to the fighting niche using current body mass (or physiological correlates) as a cue.

Body size predicts social status not only if larger males are stronger than small males, but also if males that are much smaller than their opponent cannot use their weapons effectively. For example, in the rhinoceros beetle *Aegopsis curvicornis* (= *A. nigricollis)*, fighting major males grasp their opponent between a horn on the thorax and a horn on the head, which is impossible if the size difference between the fighting males is too large (Eberhard, 1987). Small minor males do not develop the thoracic horn, and do not fight.

Q2 – Is there sexual selection for condition-dependent mating tactics?

Major and minor males often exhibit very different behaviours, which raises the suspicion that major and minor forms are adaptations to different condition-dependent patterns of (mating) behaviour (Gross, 1996; Oliveira *et al.*, 2008). If this is the case, sexual selection favours alternative mating tactics, which in turn favour alternative morphologies. Alternatively, mating tactics do not drive the evolution of morphological plasticity, but morphological plasticity rather forces males to adopt and evolve compensatory behaviour. Thus, it needs to be shown that selection favours condition-dependent mating tactics, before we can conclude that alternative mating tactics drive the evolution of MBOBJ plasticity.

Assuming social status (condition) is best approximated by body size or mass, two practical hurdles make it difficult to measure mass-dependent selection for fighting or sneaking behaviour. Firstly, alternative mating tactics (e.g. sneaking and fighting) need to be frequent over the complete range of adult male mass, in order to measure mass-

dependent selection gradients for mating tactics. In natural circumstances, small fighting males and large sneaking males are often rare, and in the mid-mass range of overlap, mass-dependent sneaking and fighting success are predicted not to be that different (Gross, 1996). Therefore, it can be hard to obtain enough statistical power to distinguish mass-dependent selection gradients if males are sampled mainly from the mid-mass range (Michalczyk *et al.*, 2018). Secondly, mass is generally strongly correlated with sexual trait size. Therefore, studies that rely on natural variation have problems quantifying the effect of mass alone on fighting or sneaking success, as this is inevitably confounded with sexual trait size (Goldsmith & Alcock, 1993; Hunt & Simmons, 2001; Forslund, 2003; Hongo, 2007; Moore *et al.*, 2008; Cummings *et al.*, 2018). One solution is to sample males that are matched in sexual trait size, and investigate the residual contribution of mass to fighting or sneaking success (Emlen, 1997a; Forslund, 2003). This runs into, and exacerbates, the problem of solely sampling males in the mid-mass range. Another solution is to experimentally control sexual trait development through phenotypic engineering. Although this has been shown to be feasible in some model systems (Michalczyk *et al.*, 2018; Casasa & Moczek, 2019), it has only once been used to quantify mass-dependent selection for fighting or sneaking behaviour without sexual trait expression as a confounder (Michalczyk *et al.*, 2018).

Practical challenges aside, there is some evidence that fighting success is higher if a male is heavier than its rival, suggesting that mating success could increase with body size among males that fight. Fights among major males matched in horn size are more often won by the larger individual in the polyphenic beetle species *Onthophagus taurus* (McCullough & Simmons, 2016), *O. acuminatus* (Emlen, 1997a), *Allomyrina dichotoma* (Karino *et al.*, 2005; Hongo, 2007), and *Bolitotherus cornutus* (Mitchem *et al.*, 2019). Occasionally, body size determines fight outcomes among small minor males, but not among large major males. This has been recorded in the earwig *Forficula auricularia* (Forslund, 2000, 2003). Possibly, large male earwigs rely less on physical attacks and more on information gleaned from the rival's forceps to settle contests, whereas small males resort to fighting behaviour.

In some male-polyphenic species (particularly those that are not beetles), condition-dependent polyphenism may *not* be associated with size-dependent fighting or mating success. Researchers failed to find evidence for size-dependent fighting success in major males of freshwater prawns *Macrobrachium sp.* (Barki *et al.*, 1997), pteromalid fig wasps *Philotrypesis sp.*(Wong *et al.*, 2018), the harvestman *Serracutisoma proximum* (Buzatto & Machado, 2008), the pseudoscorpion *Semeiochernes armiger* (Zeh & Zeh, 2013), and the acarid mite *Sancassania berlesei* (Michalczyk *et al.*, 2018). In the related

mite *Rhizoglyphus robini*, fertilization success counterintuitively appears to depend more strongly on body size in the unarmed minor male form than in armed major males (Smallegange *et al.*, 2012). This pattern is independent of the presence of rivals, suggesting that fighting success does not mediate the association between body size and fertilization success (Smallegange *et al.*, 2012).

A small-size agility advantage could strengthen disruptive selection for mating tactics, facilitating evolution of alternative mating tactics, although competitively unsuccessful males in poor condition could consistently end up in a sneaking or satellite niche also if there is no relation between body size and sneaking efficacy. Among arthropods with condition-dependent polyphenism in sexual traits, the effect of body size or mass on agility and sneaking success is poorly understood. To our knowledge, it has been recorded only in the tunnel-digging dung beetle *Sulcophanaeus velutinus*, but here the larger males appear to be faster than the smaller males, except possibly in very narrow tunnels where large males get stuck (Cummings *et al.*, 2018). In other male-polyphenic beetles, small males have been recorded to avoid large males at aggregations of females (Siva-Jothy, 1987; Karino & Niiyama, 2006; Hongo, 2007). It is unknown whether size-dependent agility favours solitary or satellite behaviour in such males, or whether such males are simply forced to compensate behaviourally due to poor fighting ability.

Q3 – Is there selection for tactic-dependent sexual trait expression?

Assuming sexual selection favours distinct condition-dependent mating tactics, do adapted alternative mating tactics require different morphological adaptations in sexual traits? Answering this question entails showing that a large form of a sexual trait is favoured in the dominant mating niche, whereas a small form of the trait is favoured in the alternative (sneaking or satellite) niche. This runs into some of the same practical challenges as those pertaining to seeking evidence for condition-dependent mating tactics. With spontaneous phenotypic variation, sexual trait expression will be strongly correlated with body size, and body size is predicted to have an effect on fertilization success (at least with fighting tactics). Therefore, it is necessary to control for the effect of body size on fertilization success within mating tactics.

Within aggressive mating tactics, selection favours larger weapons if larger weapons (independently of body size) increase the probability of winning fights. In many male-polyphenic species, it seems obvious that large weaponry is an adaptation to fighting, as such traits are observed to play a role in fights, and because the morphology of such traits strongly hints at a function of deterring, displacing or even killing

rivals (Eberhard *et al.*, 2018; Rico-Guevara & Hurme, 2019). A concrete fighting advantage for larger traits has been recorded in major males of a number of male-polyphenic species (e.g. dung beetles *Onthophagus taurus* (Moczek & Emlen, 2000), *O. acuminatus* (Emlen, 1997a) and *Phanaeus difformis* (Rasmussen, 1994); rhinoceros beetles *Allomyrina dichotoma* (Karino *et al.*, 2005); pseudoscorpions *Semeiochernes armiger* (Zeh & Zeh, 2013); and earwigs *Forficula auricularia* (Radesäter & Halldórsdóttir, 1993; Forslund, 2003)). Occasionally, however, such large-weapon fighting advantage is not detected (e.g. earwigs *F. auricularia* (Styrsky & Van Rhein, 1999; Walker & Fell, 2001), indicating that supposed 'weaponry' may function in a more complex way than previously thought (e.g. sensory or courtship functions; Walker & Fell, 2001). Selection will also favour larger weapons in fighting tactics if larger weapons (independently of body size) increase the probability that rivals are deterred even before fighting, through behavioural displays of dominance (e.g. in rhinoceros beetles *A. dichotoma* (Hongo, 2003), pteromalid fig wasps *Philotrypesis sp.* (Moore *et al.*, 2009), freshwater prawns *Macrobrachium* sp. (Barki *et al.*, 1997; Karplus & Barki, 2019)).

Conversely, within sneaking tactics, selection favours a minor phenotype without sexual traits (independently of body size), if a minor phenotype facilitates speed, agility, or stealth. This has been demonstrated in a few male-polyphenic species (e.g. dung beetles *O. taurus* (Moczek & Emlen, 2000), *O. nigriventris* (Madewell & Moczek, 2006); acarid mites *R. echinopus* (Tomkins *et al.*, 2011), *S. berlesei* (Łukasik *et al.*, 2006); harvestmen *Serracutisoma proximum* (Buzatto *et al.*, 2011)). Indeed, experimental removal of horns in the horned dung beetle *O. nigriventris* improved agility, and subsequent re-attachment of horns reversed this effect (Madewell & Moczek, 2006), conclusively showing that agility, and thereby sneaking behaviour, is compromised by large weapons.

There is currently only one study that has attempted to measure tactic-dependent selection gradients on sexual traits directly via measurements of fertilization success. McCullough *et al.* (2018) manipulated *O. taurus* adult population density, and capitalized on a density-dependent shift in mating tactics to measure tactic-dependent selection for horn length. As predicted, they found that an increase in the density of rival males resulted in increased selection for shorter horns, as fighting and mate guarding became less effective and less common with an increasing number of competitors. However, they could not detect positive selection for horn length in low-density populations, in which fertilization success was more strongly determined by aggressive mate guarding.

Q4 – Do fitness costs of major phenotype development increase with diminishing physiological condition during trait development?

Selection for condition-dependent polyphenism arises not only when males of poor social status adopt an alternative mating tactic, but also when males in poor condition, with a low somatic growth rate, do not have enough time to acquire resources for sexual traits before maturation. Thus, when maturation timing is under selection, the fitness benefits of sexual traits may not balance the life-history and viability costs of expensive trait development. Such costs arise either because maturation timing becomes suboptimal, while the resource budget for sexual trait development is (partially) protected, or because maturation timing is (partially) protected (accelerated relative to the growth trajectory) while the resource budget for development is reduced.

Condition-dependent life-history or viability costs of developing the major rather than the minor phenotype have only rarely been demonstrated (in contrast to *maintenance* costs of such traits during adult life (e.g. McCullough & Emlen, 2013; Somjee *et al.*, 2018, 2021; O'Brien *et al.*, 2019), which do not contribute to selection for somatic buffering). Generally, costs are inferred based on phenotypic performance, not by measuring actual fitness. For example, metamorphosis into major phenotypes is sometimes associated with weight or size loss (stag beetles *Prosopocoilus inclinatus* (Ito *et al.*, 2017); acarid mites *Sancassania berlesei* (Radwan *et al.*, 2002)), a reduced immune response (earwigs *Forficula auricularia* (Körner *et al.*, 2017)) or with postponed metamorphosis (dung beetles *Onthophagus taurus* (Hunt & Simmons, 1997); acarid mites *Rhizoglyphus robini* (Smallegange, 2011a; Smallegange & Johansson, 2014)). Such effects likely have direct detrimental fitness consequences, and may additionally be symptoms of physiological problems during metamorphosis with negative effects on viability. If such fitness costs of sexual trait development increase with decreasing condition, development of a minor phenotype could be a way to mitigate the viability costs of poor condition ('polyphenic release from costs'). Evidence for condition-dependence of costs has been found in earwigs *F. auricularia* (Körner *et al.*, 2017) and stag beetles *P. inclinatus* (Ito *et al.*, 2017). Such condition-dependence was not detected in the rhinoceros beetle *Allomyrina dichotoma* and the dung beetle *O. taurus* (Cotter *et al.*, 2008; McCullough & Emlen, 2013), although these studies relied on natural variation in condition (measured in *O. taurus* as hemolymph protein level, in *A. dichotoma* as body size) and may have lacked the power to find condition-dependence.

Adaptive condition-dependence: evidence from related species with different ecologies

Comparing patterns of condition-dependent polyphenism in related species with different life histories and mating systems can be a complementary approach to assess how the MBOBJ strategy evolves. This is possible when condition-dependent polyphenism occurs in many species within a clade, and is most powerful when it has evolved multiple times independently. The two most daunting practical hurdles to this approach are a reliance on 1) clade-wide descriptions of condition-dependence, and 2) detailed observations of natural history across the clade (e.g. mating systems, but also species-specific life history challenges and adaptations). Unfortunately, these hurdles are probably prohibitively difficult to take, and no comparative studies currently exist that allow us to infer the ecological drivers of the evolution of condition-dependent polyphenism.

An important caveat in the comparative method is that differences among species in patterns of condition-dependence may be caused not only by different ecological selection pressures, but also by developmental biases towards dimorphism or monomorphism in particular lineages. For example, in scarabaeid dung beetles in the genus *Onthophagus* (Emlen *et al.*, 2005) and gonyleptid harvestmen (Buzatto *et al.*, 2014), condition-dependent polyphenism with minor and major male forms has evolved multiple times independently and occurs in various species, while being conspicuously absent in other species. Notably, the evolution of male polyphenism in *Onthophagus* and gonyleptids appears contingent on prior existence of sexual dimorphism (Emlen *et al.*, 2005; Buzatto *et al.*, 2014). This suggests that dung beetles and harvestmen evolve MBOBJ polyphenism by co-opting the developmental mechanism generating sexual dimorphism into male condition-dependence, as soon as the ecological circumstances favour male polyphenism (Emlen *et al.*, 2005; Kijimoto *et al.*, 2012; Buzatto *et al.*, 2014). This interpretation is corroborated by the observation that sexual dimorphism and male condition-dependent dimorphism are associated with similar sets of differentially expressed genes in the rhinoceros beetle *Allomyrina dichotoma* (Zinna *et al.*, 2018a). Thus, it could be that male condition-dependent polyphenism is generally favoured by selection, but often constrained by the lack of a pre-existing dimorphism-generating developmental mechanism.

Adaptive condition-dependence: evidence from contemporary evolution

A third approach to assess the ecological drivers of the MBOBJ strategy is to track contemporary evolution of condition-dependent reaction norms in response to eco-

logical change, and identify potential selective causes. This is sometimes possible in laboratory mesocosms, in which populations evolve in semi-natural but simplified environments, allowing the recording of evolutionary changes in condition-dependent plasticity as well as concurrent changes in ecological parameters (Tomkins *et al.*, 2011; Smallegange & Deere, 2014). Alternatively, one can capitalize on accidental introductions of species with condition-dependent polyphenism in novel environments, and track the evolution of plasticity alongside ecological change. From both approaches, population density has emerged as a mediator of selection for condition-dependent reaction norms. In acarid mites (contemporary evolution in mesocosms; Smallegange & Deere, 2014) and dung beetles (contemporary evolution in exotic populations; Moczek *et al.*, 2002; Moczek, 2003; Moczek & Nijhout, 2003), increases in population density are associated with the evolution of increased expression of the minor morph in intermediate-sized males (dung beetles) or overall (acarid mites). In contrast, in earwigs (contemporary evolution on isolated islands), increases in population density appear to be associated with increased expression of the *major* morph (Tomkins & Brown, 2004). Unfortunately, the reaction norm measurements in this study were not conducted in a common garden, which makes the pattern difficult to interpret.

In the known cases of contemporary density-dependent evolution of condition-dependent reaction norms, it is unknown how population density has shaped selection. It is possible that increased population density and competition for food makes it difficult for juveniles to acquire large resource budgets, so that juveniles prioritize fast maturation over additional resource acquisition in novel environments with increased population densities (i.e. a somatic buffering explanation). Alternatively, increased population density may shift the mating system and render fighting behaviour disadvantageous, so that major morph expression no longer improves mating success (i.e. an alternative mating tactics explanation). Indeed, experimental quantification of density-dependent sexual selection gradients in *O. taurus* showed that increased population density causes increased selection for testes size, and selection against secondary sexual traits (McCullough *et al.*, 2018). Thus, while population density appears to be an important driver of contemporary reaction norm evolution in male condition-dependent polyphenisms, little is known about *how* population density affects condition-, trait- and tactic-dependent fertilization success, or condition-dependent costs for trait development.

3.5 – Conclusions and outlook

Although condition-dependent male polyphenism in secondary sexual traits is widespread in several arthropod groups (and probably under-recorded), the selection pressures underlying its evolution have been studied only in a few arthropod model systems, most notably dung beetles *Onthophagus*, rhinoceros beetles *Allomyrina,* earwigs *Forficula,* mites *Rhizoglyphus* and *Sancassania*, fig wasps *Philotrypesis,* prawns *Macrobrachium* and harvestmen *Serracutisoma* (Table 3.1).

Only in *Onthophagus* do we have evidence for all three facets of the alternative mating tactics component of selection for MBOBJ plasticity: 1) a link between an individual's capacity to obtain favourable outcomes in social interactions (generalized as physical strength) and body size, so that body size can act as a developmental cue for trait development signaling (future) social status; 2) condition-(body size-)dependent fertilization success in the dominant mating niche, resulting in diversification of mating niches and tactics; and 3) tactic-dependent selection for secondary sexual traits. In all other model systems, at least two of the three facets of alternative mating tactics are missing evidence (Table 3.1).

The somatic buffering component of selection for MBOBJ plasticity is generally little investigated (Table 3.1). This may be because it is difficult to measure condition- and trait-dependent fitness differences that are not due to variation in mating success. It is more feasible to infer such fitness differences by measuring condition-dependent performance differences in males of different phenotypes. Such performance differences have been recorded in major male stag beetles *Prosopocoilus inclinatus* (condition-dependent trade-off between mandible and abdomen growth) and earwigs *Forficula auricularia* (condition-dependent trade-off between forceps growth and immune system capacity). In several other systems, such costs have been looked for but not detected, or detected but not found to be condition-dependent (Table 3.1).

In conclusion, there is a dearth of studies on the actual selection pressures shaping male condition-dependent polyphenism in arthropods. This is striking, because the pattern of male condition-dependent polyphenism is well known, and often explained as MBOBJ-type adaptations to alternative mating tactics (Gross, 1996; Oliveira *et al.*, 2008). Importantly, the claim that condition-dependent polyphenism evolves as adaptation to alternative mating tactics is not empirically validated by differences in mating behaviour between the male phenotypes. Such behavioural differences are expected also if polyphenism (initially) evolves for other reasons than mating niche differentiation, and divergent mating niches can be a consequence rather than a cause of developmental plasticity in secondary sexual traits.

The lack of empirical evidence for the precise selective mechanisms (except perhaps in *Onthophagus*) makes evolutionary trajectories of male reaction norms hard to predict. This is particularly pressing since rapid evolution of male polyphenism has been linked to changes in population density. The eco-evolutionary consequences of population density variation are notoriously complex and non-linear (Travis *et al.*, 2014; Reznick *et al.*, 2019; Than *et al.*, 2020), with important effects on both life history evolution (Wright *et al.*, 2018) and mating system dynamics (Shuster & Wade, 2003), potentially resulting in a major role for both alternative mating tactics and somatic buffering in shaping selection for male polyphenism.

We suggest the following routes to future understanding how selection shapes male MBOBJ plasticity. First and foremost, we need to disentangle the different pathways from phenotype to fitness components. This requires 1) linking individual capacity to obtain favourable outcomes from social interactions (social status) to aspects of the phenotype (e.g. body size); 2) investigating how such phenotypically caused variation in social status results in mating systems with distinct condition-dependent mating niches; 3) investigating how different mating niches select for different levels of secondary sexual trait expression; 4) looking for condition-dependent viability and life history costs caused by sexual trait development; and 5) investigating how polyphenism might release individuals in poor condition from such costs (e.g. reducing allocation trade-offs, allowing faster development, reducing functional interference of signaling molecules). Secondly, we need to incorporate novel methods that allow experimental phenotypic engineering of sexual trait development. The natural correlation between condition and sexual trait expression makes it difficult to rely on natural phenotypic variation in sexual traits and/or condition, without having one or the other as a confounder. Experimental manipulation of trait development improves our ability to uncover, for example, condition-dependent viability costs for development of exaggerated sexual traits. Such methods are now beginning to be available for a select few organisms, as the physiological mechanisms of condition-dependent trait plasticity are being uncovered (Zinna *et al.*, 2016; Casasa & Moczek, 2019; Casasa *et al.*, 2020). Finally, it is important that we do not solely rely on a few model organisms, but that we study a range of different species with different ecologies. Male condition-dependent polyphenism in sexual traits appears to be widespread in arthropods, among species that differ wildly in ecological characteristics. If there are salient common themes to be found, this should ideally appear from studies on ecologically very different animals.

TABLE 3.1 — Examples of arthropod (genera) condition-dependent polyphenism in male secondary sexual traits, including evidence for questions posed in 'Measuring the causes of selection: do we understand why fitness functions cross?'. Criteria for inclusion* are 1) polyphenism in secondary sexual traits (i.e. discontinuous relation between trait and body size), and 2) plausible condition-dependence (e.g. allometry). Excluded are genera that lack evidence for condition-dependence, and male size dimorphisms or behavioural phenotypes that are not associated with sexual trait polyphenism. Blank cells represent absence of relevant studies.

N.B. tests for allometric discontinuity in trait-body size scaling relationships are sensitive to measuring and statistical modelling decisions (Tomkins et al., 2005, 2006; Moczek, 2006; Packard, 2021). Here, claims of allometric discontinuity are taken at face value, but it should be noted that some of these claims may be controversial (e.g. in dynastid beetles; Packard, 2021).

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
Insecta: Coleoptera								
Copris (Coleoptera: Scarabaeidae)	Cephalic horn	Allometry[1,2,3,4]						1. Eberhard & Gutierrez, 1991 2. Sugiura *et al.*, 2007 3. Akamine, 2016 4. Kerman *et al.*, 2018
Onthophagus (Coleoptera: Scarabaeidae)	Cephalic and/or thoracic horns	Nutrition manipulation[1,3], allometry[1,3,5], protein concentrations in hemolymph[8]	Yes[2,5]	Yes: *O. taurus*: physical strength depends on mass; low larval body mass forecasts poor adult strength[9]	Yes: *O. taurus*, *O. acuminatus*: fighting success increases with body size, small males experience poor fighting success[2,9].	Yes: *O. taurus*, *O. acuminatus*: among fighting males, mating success increases with horn length[2,5]. *O. taurus, O. nigriventris*: among sneaking males, agility decreases with horn length[5,7]. *O. taurus*: increased sneaking favours short horns[10].	Unclear: *O. taurus*: major males take longer to mature than minor males; unclear if fitness cost is higher in poor condition[4]. *O. taurus*: no evidence that immune response strength trades off with horn length in poor condition[8].	1. Emlen, 1994 2. Emlen, 1997a 3. Moczek, 1998 4. Hunt & Simmons, 1997 5. Moczek & Emlen, 2000 6. Emlen *et al.*, 2005 7. Madewell & Moczek, 2006 8. Cotter *et al.*, 2008 9. McCullough & Simmons, 2016 10. McCullough *et al.*, 2018.

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
Oxysternon (Coleoptera: Scarabaeidae)	Cephalic horn	Allometry						Rowland & Emlen, 2009
Phanaeus (Coleoptera: Scarabaeidae)	Cephalic horn	Allometry[1,2]	Yes[1]			Yes: *P. difformis*: among fighting males, mating success increases with horn length[1].		1. Rasmussen, 1994 2. Rowland & Emlen, 2009
Sulcophaneus (Coleoptera: Scarabaeidae)	Cephalic and thoracic horns	Allometry	Yes					Cummings et al., 2018
Aegopsis (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry[2]		Yes: *A. curvicornis* (= *A. nigricollis*): in smaller males, horns are mechanically less effective weapons; small larval size forecasts poor adult fighting ability[1]				1. Eberhard, 1987 2. Eberhard & Gutierrez, 1991
Allomyrina (= *Trypoxylus*) (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Nutrition manipulation[6], allometry[1,7] (*but see* ref[8])	Unclear[1,4]		Unclear: *A. dichotoma*: fighting success and territory quality increase with body size, small males experience poor fighting success [1,4,9]. However, small	Yes: *A. dichotoma*: among fighting males, mating success increases with horn length[2,3,9], particularly in large males[9].	Unclear: *A. dichotoma*: no evidence that immune response strength trades off with horn length in poor condition[5]	1. Siva-Jothy, 1987 2. Hongo, 2003 3. Karino *et al.*, 2005 4. Hongo, 2007 5. McCullough & Emlen, 2013 6. Johns *et al.*, 2014 7. McCullough *et al.*, 2015

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
— *continued*					satellite males mate only rarely, and typically also fight before mating[4,9].			8. Packard, 2021 9. del Sol *et al.*, 2021
Augosoma (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry (*but see* ref[2])						1. McCullough *et al.*, 2015 2. Packard, 2021
Chalcosoma (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry[1,2] (*but see* ref[3])						1. Kawano, 2002 2. McCullough *et al.*, 2015 3. Packard, 2021
Coelosis (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry[1] (*but see* ref[2])						1. McCullough *et al.*, 2015 2. Packard, 2021
Diloboderus (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry[1] (*but see* ref[2])						1. McCullough *et al.*, 2015 2. Packard, 2021
Dynastes (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry[1,2] (*but see* ref[3])						1. Eberhard & Gutierrez, 1991 2. McCullough *et al.*, 2015 3. Packard, 2021
Enema (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry[1] (*but see* ref[2])						1. McCullough *et al.*, 2015 2. Packard, 2021

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
Golofa (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry[1] (*but see* ref[2])						1. McCullough *et al.*, 2015 2. Packard, 2021
Heterogomphus (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry[1] (*but see* ref[2])						1. McCullough *et al.*, 2015 2. Packard, 2021
Megasoma (Coleoptera: Dynastidae)	Cephalic horn	Allometry[1,2] (*but see* ref[3])						1. Eberhard & Gutierrez, 1991 2. McCullough *et al.*, 2015 3. Packard, 2021
Oryctes (Coleoptera: Dynastidae)	Cephalic horn	Allometry[1] (*but see* ref[2])						1. McCullough *et al.*, 2015 2. Packard, 2021
Podischnus (Coleoptera: Dynastidae)	Cephalic horn	Allometry[2,3] (*but see* ref[4])	Yes[1]					1. Eberhard, 1982 2. Eberhard & Gutierrez, 1991 3. McCullough *et al.*, 2015 4. Packard, 2021
Scapanes (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry						Rowland *et al.*, 2005
Spodistes (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry[1] (*but see* ref[2])						1. McCullough *et al.*, 2015 2. Packard, 2021

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
Xyloryctes (Coleoptera: Dynastidae)	Cephalic horn	Allometry[1] (*but see* ref[2])						1. McCullough *et al.*, 2015 2. Packard, 2021
Xylotrupes (Coleoptera: Dynastidae)	Cephalic and thoracic horns	Allometry[1,2,3] (*but see* ref[4])						1. Eberhard & Gutierrez, 1991 2. Rowland, 2003 3. McCullough *et al.*, 2015 4. Packard, 2021
Lethrus (Coleoptera: Geotrupidae)	Mandibular tusks	Allometry						Rosa *et al.*, 2019
Dendrobias (Coleoptera: Cerambycidae)	Enlarged mandibles	Allometry[1]	Yes[2]					1. Goldsmith, 1985 2. Goldsmith & Alcock, 1993
Aegus (Coleoptera: Lucanidae)	Enlarged mandibles	Allometry[1]	Unclear[2]		Unclear: *A. chelifer:* Fight outcomes correlate with body size, but confounded with mandible length[2].			1. Songvorawit *et al.*, 2017 2. Songvorawit *et al.*, 2018
Cyclommatus (Coleoptera: Lucanidae)	Enlarged mandibles	Allometry[1,2]	Yes[2]					1. Eberhard & Gutierrez, 1991 2. Chen *et al.*, 2020
Dorcus (Coleoptera: Lucanidae)	Enlarged mandibles	Allometry						Iguchi, 2013

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
Lucanus (Coleoptera: Lucanidae)	Enlarged mandibles	Allometry[1,2,3]						1. Eberhard & Gutierrez, 1991 2. Hardersen *et al.*, 2011 3. Romiti *et al.*, 2015
Odontolabis (Coleoptera: Lucanidae)	Enlarged mandibles	Allometry[1,2,3]						1. Kawano, 2000 2. Rowland & Emlen, 2009 3. Matsumoto & Knell, 2017
Prosopocoilus (Coleoptera: Lucanidae)	Enlarged mandibles	Allometry[1]	Unclear[2]				Yes: *P. inclinatus:* Mandible growth trades off with body size, but only in small males[3]	1. Shiokawa & Iwahashi, 2000 2. Inoue & Hasegawa, 2013 3. Ito *et al.*, 2017
Bolitotherus (Coleoptera: Tenebrionidae)	Cephalic and thoracic horns	Allometry	Unclear[2]		Yes: *B. cornutus*: fight outcomes correlate with body size, small males experience poor fighting success[2].			1. Eberhard & Gutierrez, 1991 2. Mitchem *et al.*, 2019
Parisoschoenus (Coleoptera: Curculionidae)	Cephalic horn, sternal spines and sheath	Allometry[1,2]						1. Eberhard *et al.*, 2000 2. Rowland & Emlen, 2009
Hoherius (Coleoptera: Anthribidae)	Enlarged mandibles	Allometry	Yes					Painting, 2021.

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
Oxyporus (Coleoptera: Staphylinidae)	Enlarged mandibles	Allometry						Hanley, 2001.
Insecta: Hymenoptera								
Lasioglossum (Hymenoptera: Halictidae)	Enlarged mandibles	Nutrition manipulation, allometry	Yes					Kukuk, 1996
Macrotera (=*Perdita*) (Hymenoptera: Andrenidae)	Enlarged mandibles	Allometry[1,2]	Yes[1]					1. Danforth, 1991 2. Danforth & Desjardins, 1999
Cardiocondyla (Hymenoptera: Formicidae)	Enlarged mandibles	Allometry	Yes					Schrempf et al., 2007.
Synagris (Hymenoptera: Vespidae)	Mandibular tusks	Allometry	Yes					Longair, 2004.
Philotrypesis (Hymenoptera: Pteromalidae)	Enlarged mandibles	Allometry[1,3,5]	Yes[1,3,4,5]		Unclear: *Philotrypesis* sp. B: larger males win more fights than smaller males, but body size confounded with mandible size[2,3]. *Philotrypesis taida:* no evidence that fighting and	Yes: *Philotrypesis* sp. A: among size-matched males with threat displays, males with longer mandibles fight less and have higher mating success. However, males with smaller mandibles incur		1. Cook & Bean, 2006 2. Moore *et al.*, 2008 3. Moore *et al.*, 2009 4. Wong *et al.*, 2018 5. Wong *et al.*, 2019

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
— *continued*					mating success depend on body size[4].	fewer injuries when fighting[3].		
Sycorycteridea (Hymenoptera: Pteromalidae)	Enlarged mandibles	Allometry	Yes					Wong et al., 2019
Sycoscapter (Hymenoptera: Pteromalidae)	Enlarged mandibles	Allometry[1]			Unclear: *Sycoscapter* sp. A: larger males win more fights than smaller males, but body size confounded with mandible size[2].			1. Bean & Cook, 2001 2. Moore *et al.*, 2008
Insecta: Hemiptera								
Mictis (Hemiptera: Coreidae)	Enlarged hind femur and abdominal tubercle	Allometry						Emberts *et al.*, 2017
Insecta: Orthoptera								
Hemideina (Orthoptera: Anostostomatidae)	Enlarged mandibles	Allometry						Kelly & Adams, 2010
Insecta: Dermaptera								
Forficula (Dermaptera: Forficulidae)	Enlarged cerci ('forceps').	Nutrition manipulation[3], allometry[3]	Unclear[5,6]		Unclear: *F. auricularia*: fighting success	Mixed evidence: *F. auricularia*: mating success increases	Yes: *F. auricularia*: Immune response strength is	1. Radesäter & Halldórsdóttir, 1993

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
— *continued*					increases with body size, but only in small males[1,2].	with forceps length in major males[1,6], not in minor males[6]; *F. auricularia*: forceps size does not play a role in mating success[2,4,5].	inversely associated with forceps length, but only in poor condition[7].	2. Styrsky & Van Rhein, 1999 3. Tomkins, 1999 4. Forslund, 2000 5. Walker & Fell, 2001 6. Forslund, 2003 7. Körner *et al.*, 2017
Malacostraca: Decapoda								
Cryphiops (Decapoda: Palaemonidae)	Enlarged claws on first leg pair	Allometry						Rojas *et al.*, 2012
Macrobrachium (Decapoda: Palaemonidae)	Enlarged claws on first leg pair	Allometry[1,3,4]	Yes[1,2]		Unclear: *M. rosenbergii*: no evidence that fighting success depends on body size[5].	Yes: *M. rosenbergii*: among fighting major males, mating success increases with claw size[5]. Sneaking minor males are socially prevented from developing claws[3,4].		1. Ra'anan & Sagi, 1985 2. Barki *et al.*, 1991a 3. Barki *et al.*, 1991b 4. Karplus *et al.*, 1992 5. Barki *et al.*, 1997
Rhinchocinetes (Decapoda: Rhynchocinetidae)	Enlarged claws on first leg pair	Allometry	Yes					Correa *et al.*, 2003

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
Malacostraca: Amphipoda								
Jassa (Amphipoda: Ischyroceridae)	Enlarged 'thumbs' on second gnathopod	Nutrition manipulation[2], allometry[1]	Yes[2]					1. Conlan, 1989 2. Kurdziel & Knowles, 2002
Arachnida: Opiliones								
Paecilaemula (Opiliones: Cosmetidae)	Enlarged chelicerae	Allometry	Yes					Solano-Brenes *et al.*, 2018
Acrogonyleptes (Opiliones: Gonyleptidae)	Apophysis on fourth coxa	Allometry						Buzatto *et al.*, 2014
Arthrodes (Opiliones: Gonyleptidae)	Apophysis on fourth coxa	Allometry						Buzatto *et al.*, 2014
Cobania (Opiliones: Gonyleptidae)	Apophysis on fourth coxa, elongated fourth leg pair	Allometry						Buzatto *et al.*, 2014
Discocyrtus (Opiliones: Gonyleptidae)	Apophysis on fourth coxa	Allometry						Hernández & Acosta, 2021
Gonyleptellus (Opiliones: Gonyleptidae)	Elongated fourth leg pair	Allometry						Buzatto *et al.*, 2014
Goniosoma (Opiliones: Gonyleptidae)	Apophysis on fourth coxa	Allometry						Buzatto *et al.*, 2014

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
Heteromitobates (Opiliones: Gonyleptidae)	Apophysis on fourth coxa	Allometry						Buzatto *et al.*, 2014
Ischnoterus (Opiliones: Idealeptidae)	Elongated fourth leg pair	Allometry						Buzatto *et al.*, 2014
Longiperna (Opiliones: Gonyleptidae)	Elongated fourth leg pair	Allometry[1,2]	Yes[1]					1. Zatz *et al.*, 2011 2. Buzatto *et al.*, 2014
Multumbo (Opiliones: Gonyleptidae)	Apophysis on fourth coxa	Allometry						Buzatto *et al.*, 2014
Neosadocus (Opiliones: Gonyleptidae)	Apophysis on fourth coxa	Allometry						Buzatto *et al.*, 2014
Neoancistrotus (Opiliones: Gonyleptidae)	Elongated fourth leg pair	Allometry						Buzatto *et al.*, 2014
Progonyleptoidellus (Opiliones: Gonyleptidae)	Apophysis on fourth coxa	Allometry						Buzatto *et al.*, 2014
Promitobates (Opiliones: Gonyleptidae)	Apophysis on fourth coxa, elongated fourth leg pair	Allometry						Buzatto *et al.*, 2014

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
Ruschia (Opiliones: Gonyleptidae)	Elongated fourth leg pair	Allometry						Buzatto *et al.*, 2014
Serracutisoma (Opiliones: Gonyleptidae)	Elongated second and fourth leg pairs	Allometry[1,3]	Yes[1,2]		Unclear: *S. proximum*: no evidence that fighting success depends on body size[1]			1. Buzatto & Machado, 2008 2. Buzatto *et al.*, 2011 3. Buzatto *et al.*, 2014
Pantopsalis (Opiliones: Monoscutidae)	Enlarged chelicerae	Allometry						Painting *et al.*, 2015
Forsteropsalis (Opiliones: Neopilionidae)	Enlarged chelicerae	Allometry						Powell *et al.*, 2020
Arachnida: Pseudoscorpiones								
Semeiochernes (Pseudoscorpiones: Chernetidae)	Enlarged claws on first leg pair	Allometry	Unclear		Unclear: *S. armiger*: no evidence that fighting success depends on body size	Yes: *S. armiger*: mating success increases with claw size.		Zeh & Zeh, 2013
Arachnida: Schizomida								
Rowlandia (Schizomida: Hubbardiidae)	Enlarged pedipalps	Allometry						Santos *et al.*, 2013

TABLE 3.1 — *continued*

Genus	Polyphenic secondary sexual trait	How is condition-dependent polyphenism inferred?	Do minor and major males engage in distinct mating behaviours?	Q1: Evidence for social status predicted by early phenotypic characteristics?	Q2: Evidence for sexual selection for condition-dependent mating tactics?	Q3: Evidence for selection for tactic-dependent phenotypes?	Q4: Evidence for condition-dependent costs for major phenotype development?	References
Arachnida: Astigmata								
Rhizoglyphus (Astigmata: Acaridae)	Enlarged third leg pair	Nutrition manipulation[1,4], allometry[4,5]	Yes[2,7]		Unclear: *R. robini*: fertilization success increases more strongly with body size in minor males than in major males. This happens independently of rival presence, so this effect is not mediated by size-dependent competition success[6].	Yes: *R. robini, R. echinopus*: in major males, the third leg pair is used and adapted for stabbing conspecifics to death[2]; in minor males, the third leg pair is used and adapted for walking[2,3].	Yes: *R. robini*: slow-growing major males take longer to mature than slow-growing minor males, but difference disappears in fast-growing males[4].	1. Radwan, 1995 2. Radwan *et al.*, 2000 3. Tomkins *et al.*, 2011 4. Smallegange, 2011a 5. Buzatto *et al.*, 2012 6. Smallegange *et al.*, 2012 7. Van den Beuken *et al.*, 2019a
Sancassania (=*Caloglyphus*) (Astigmata: Acaridae)	Enlarged third leg pair	Nutrition manipulation[2], juvenile weight[3,4], allometry[4]	Yes[1]		Unclear: *S. berlesei*: no evidence that fertilization success depends differently on body size in fighting majors and non-fighting minors[7]	Yes: *S. berlesei*: in major males, the third leg pair is used and adapted for stabbing conspecifics to death; in minor males, the third leg pair is used and adapted for walking[1,5,6].	Unclear: *S. berlesei*: major males lose weight during metamorphosis, minor males do not; unclear if fitness cost is higher in poor condition[3].	1. Radwan, 1993 2. Radwan, 1995 3. Radwan *et al.*, 2002 4. Tomkins *et al.*, 2004a 5. Łukasik *et al.*, 2006 6. Łukasik, 2010 7. Michalczyk *et al.*, 2018

Relevant articles were found using a Web of Science search with the following query: (((male AND (condition OR size) AND (weapon* OR ornament* OR horn* OR claw* OR forcep* OR mandible*) AND (dimorph* OR polyphen* OR polymorph* OR polyphen* OR ((alternative reproductive tactic*) OR (alternative reproductive phenotype*) OR (alternative mating tactic*)))))). The resulting database was augmented with relevant references from the articles found in the Web of Science search.

Box 3.2 — Making the Best of a Bad Job: historical context

Baldwin's organic selection

The idea that individual organisms 'Make the Best of a Bad Job' (MBOBJ), responding plastically to environmental challenges so fitness loss is minimized, has a somewhat turbulent history. An early incarnation of the MBOBJ idea was James Baldwin's conception of organismic agency (Baldwin, 1896, 1897; very similar ideas were simultaneously formulated by C. L. Morgan (1896) and H. F. Osborn (1897)). Where Darwin was unsure whether developmental flexibility ("use and disuse") could play a role in adaptive evolution (Darwin, 1859), Baldwin envisaged adaptive evolution to be the outcome of the interaction between Darwinian natural selection on inherited variation, and the goal-directedness of organismic development and cognition. Baldwin assumed that organisms self-preserve, being products of selection for self-preservation, and therefore expected them to respond actively and adaptively to challenges from the environment. In Baldwin's terminology, such "organic selection" of developmental and cognitive solutions to environmental challenges provides the first response to "the storm and stress" of environmental change, keeping populations intact so natural selection may cement favourable developmental responses into the inherited phenotype (Baldwin, 1896; Morgan, 1896; Osborn, 1897). Baldwin's organic selection is essentially organisms Making the Best of a Bad Job, and, in Baldwin's thought, this organismic agency is fundamental for evolution. It provides, tests and adjusts the variation on which natural selection acts, greatly increasing the efficacy of Darwinian selection.

Evolutionary theories built around organismic agency (e.g. Russell, 1930, 1945; Waddington, 1957) were eclipsed by the emergence of population genetics as the theoretical backbone of evolutionary biology in the 1930s and 1940s (Pigliucci, 2007). The Modern Synthesis proposed that evolution could be understood more or less completely in terms of the dynamics of gene frequencies in a population. Genes were assumed to be causal not just to inheritance, but also to phenotypic variation, and development was largely treated as a poorly understood 'black box' that somehow connected genes to phenotypes. Evolutionary biology's seeming abandonment of the developing organism, in favour of alleles competing for space on chromosomes, left little room for a concept of organismic agency (Bruce, 2014). The goal-directedness of organisms, so obvious and important to Baldwin, was relegated to the rank of 'proximate mechanism': explained by evolution, but inconsequential for the evolutionary process (Mayr, 1961). Hence, MBOBJ did not play a big role in evolutionary biology until game theory re-introduced a (rather stripped-down) form of whole-organism thinking during the 1970s.

The conditional strategy in game theory

In game-theoretical models of evolution, organisms play a fitness-optimization game using genetically determined 'strategies'. In such scenarios, strategies are considered evolutionarily stable if they cannot be invaded by competing (mutant) strategies when adopted by the majority of the population. Initially, alternative morphs were assumed to exist only if competing alternative strategies coexist through frequency-dependent selection (Gadgil, 1972). However, several authors

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Box 3.2 — continued from previous page

soon took to the notion that organisms could adaptively respond to information from their environment (including environmental effects on their own development), instead of executing a program with a single pre-set outcome. This so-called 'conditional strategy' was subsequently shown to be evolutionarily stable when individuals of unequal physical strength or sexual attractiveness compete for mating opportunities (Warner et al., 1975; Maynard Smith & Parker, 1976; Dawkins, 1980; Maynard Smith, 1982; Parker, 1982; Dominey, 1984): disadvantaged individuals can maximize fitness, given their state, not by engaging in 'dominant' behaviour but by adopting an alternative tactic. This alternative tactic does not necessarily yield the same average fitness as the dominant tactic, but in poor phenotypic condition it should improve fitness compared to what the dominant tactic would achieve. Thus, the assumed presence of individual performance differences was demonstrated to favour a particular class of conditional strategies that Dawkins (1980) famously called 'Making the Best of a Bad Job' (MBOBJ).

The game-theoretical reincarnation of MBOBJ, later rebranded as the 'status-dependent selection' model (Gross, 1996; Lee, 2005; Tomkins & Hazel, 2007), differed substantially from Baldwin's developmental account of organismic agency. For Baldwin, organisms were autonomous agents which must flexibly develop and behave so as to preserve themselves (Baldwin, 1896; Russell, 1945). Hence, Baldwin treated MBOBJ as a given, and as a necessary process in evolution. In contrast, in post-Modern-Synthesis population genetics and game theory, the conditional strategy was seen as a special case, against an expectation of genetically determined unconditional strategies. It had to be proven that organisms could in fact evolve to plastically Make the Best of a Bad Job (Maynard Smith, 1982; Shuster & Wade, 2003).

Shuster and Wade's critique of MBOBJ as a conditional strategy

While the conditional strategy was under theoretical scrutiny and became accepted as a potential evolutionarily stable strategy, the origin and maintenance of the condition (performance) differences necessary for the evolution of MBOBJ was much less thoroughly explored. This eventually became a target for criticism. In an influential book, Shuster and Wade (2003) argued that MBOBJ is unlikely to evolve. They noted that condition is inevitably associated with genetic variation, and argued that since selection simply does not favour 'a bad job', the unequal average fitnesses of the alternative morphs within the MBOBJ strategy should drive the better performing morph to fixation. According to Shuster and Wade, the apparent commonness of the MBOBJ strategy in sexual traits and alternative mating tactics was an illusion, created by improper measurements of supposedly unequal fitnesses of alternative morphs (Shuster & Wade, 2003; Shuster, 2010).

Shuster and Wade's critique was rebutted by Tomkins and Hazel (2007), who pointed out that unequal average fitnesses of alternative condition-dependent morphs are not evolutionarily paradoxical, provided that condition differences recur every generation, and that genetic variation in condition is uncorrelated with genetic variation associated with the condition-dependent

— *continued*

Box 3.2 — continued from previous page

developmental switchpoint. As a quantitative genetic framework underlying the conditional strategy in general and MBOBJ in particular, Tomkins and Hazel offered the environmental threshold (ET) model (Hazel et al., 1990, 2004), which demonstrated their case. Hence, the clash of opinions mainly revolved around whether it was thought that condition differences can recur in a population under selection (Gross & Repka, 1998; Tomkins & Hazel, 2007), or not (Shuster & Wade, 2003). In the main text of this review, we explain several empirically supported mechanisms that cause retention of condition variance. Thus, it was demonstrated that MBOBJ could definitely evolve as a particular ESS whenever selection favours condition-dependent development, according to the quantitative genetics described by the ET model.

The return of the developmentally flexible organism

Current movements within evolutionary biology are re-embracing the developmentally flexible organism, and appear to vindicate Baldwin's emphasis on explorative developmental properties (Kirschner & Gerhart, 1998; West-Eberhard, 1998, 2003, 2005; Oyama et al., 2001; Gerhart & Kirschner, 2007; Piersma & van Gils, 2011; Gilbert et al., 2015; Laland et al., 2015; Schwab et al., 2019). Organisms do, in fact, act as adaptive problem-solvers in development. It is becoming increasingly clear such organismic agency is neither an evolutionarily inconsequential 'proximate' mechanism that has nothing to do with 'ultimate' evolutionary explanations, nor a special case of a conditional strategy evolving by invading a resident unconditional strategy. Authors such as West-Eberhard (2003) argue a case closely resembling Baldwin's (although informed by a century of evolutionary genetics, behavioural and developmental biology): developmental plasticity is part of the evolutionary process, as selection acts on developing organisms *responding* to their internal (somatic) and external environment. Organisms are thus (once again) increasingly considered to be poised to Make the Best of a Bad Job in interaction with their individually experienced environment. The MBOBJ hypothesis for maintenance of alternative morphs in sexually selected traits is then one particular example of this general phenomenon, with particularly conspicuous phenotypic consequences.

Acknowledgements

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Chapter 4

Nutrient-dependent allometric plasticity in a male-diphenic mite

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Abstract

Male secondary sexual traits often scale allometrically with body size. These allometries can be variable within species, and may shift depending on environmental conditions such as food quality. Such allometric plasticity has been hypothesized to initiate local adaptation and evolutionary diversification of scaling relationships, but is under-recorded, and its eco-evolutionary effects are not well understood. Here, we test for allometric plasticity in the bulb mite (*Rhizoglyphus robini*) in which large males tend to develop as armed adult fighters with thickened third legs, while small males become adult scramblers without thickened legs. We first examined the ontogenetic timing for size- and growth-dependent male morph determination, using experimentally amplified fluctuations in growth rate throughout juvenile development. Having established that somatic growth and body size determine male morph expression immediately before metamorphosis, we examined whether the relationship between adult male morph and size at metamorphosis shifts with food quality. We found that the threshold body size for male morph expression shifts towards lower values with deteriorating food quality, confirming food-dependent allometric plasticity. Such allometric plasticity may allow populations to track prevailing nutritional conditions, potentially facilitating rapid evolution of allometric scaling relationships.

4.1 — Introduction

Male secondary sexual traits are often conspicuously sensitive to variation in juvenile food quality (Emlen & Nijhout, 2000; Kodric-Brown *et al.*, 2006; Lavine *et al.*, 2015). This nutrition-sensitive plasticity generally manifests itself in hyper-allometric scaling of sexual traits with body size (Simmons & Tomkins, 1996; Kawano, 2000; McCullough *et al.*, 2015) or even in discrete alternative reproductive phenotypes (Emlen *et al.*, 2005; Smallegange, 2011a). As a consequence, large individuals in a population can have enormous secondary sexual traits, while small individuals can be inconspicuously endowed. Scaling relationships can vary across time and space within species (Miller & Emlen, 2010). Such variation is sometimes due to genetic variation; different populations can differ in genetic background due to drift or local adaptation, resulting in variation in how body size relates to trait size (Buzatto *et al.*, 2012). It is also possible that the scaling relationship itself exhibits plasticity in response to local environmental effects, a phenomenon that has been dubbed 'allometric plasticity' (Emlen, 1997b; Moczek, 2002; Casasa & Moczek, 2019). Allometric plasticity can occur in response to food variation (Emlen, 1997b; Moczek, 2002). In such cases, variation in nutrition not only causes variation in body size and therefore in sexual traits, but also variation in *how* organisms weigh the body size cue to develop sexual traits.

Food-dependent allometric plasticity has a significant role in driving ecological and evolutionary patterns in sexual traits. Ecologically, allometric plasticity causes divergence in scaling relationships among populations in different nutritional environments. Such inter-population variation is often interpreted as local adaptation (Tomkins & Brown, 2004) or speciation in progress (Kawano, 2002), but may instead reflect undetected allometric plasticity. Allometric plasticity can also obscure scaling relationships of interest within populations when environments are nutritionally heterogeneous on small spatial or temporal scales. Such intra-population variation in scaling can conceal condition-dependence, which is often inferred through static allometric scaling (Bonduriansky, 2007; Eberhard *et al.*, 2018), and leave the impression that the association of sexual trait variation with body size is much weaker than it actually is. Evolutionarily, allometric plasticity is hypothesized to be an important first step in adaptive divergence of scaling relationships in novel (nutritional) environments (Casasa & Moczek, 2019). Genetic accommodation of ancestrally plastic scaling relationships can allow rapid adaptive evolution, if allometric plasticity in the novel environment coincides with release of selectable cryptic genetic variation, and/or if the novel environment persists for long enough to allow genetic mutations to accumu-

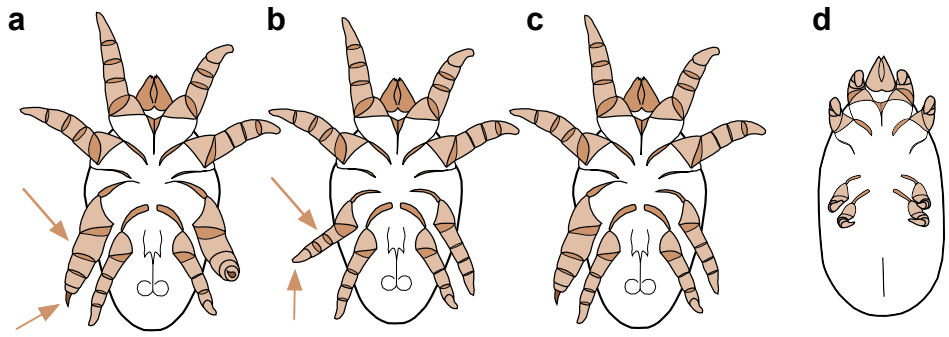


Figure 4.1 — *Rhizoglyphus robini* male morphology, ventral view, head above. **a**, Adult fighter male. **b**, Adult scrambler male. **c**, Adult intermorph male. **d**, Quiescent tritonymph stage, during which metamorphosis and fighter leg development takes place. Fighters and scramblers differ in the width of the third leg and in the shape of the third tarsal claw (arrows).

late and canalize the induced scaling relationship (West-Eberhard, 2003; Pfennig *et al.*, 2010; Moczek *et al.*, 2011; Casasa & Moczek, 2019).

Despite the hypothesized ecological and evolutionary importance of food-dependent allometric plasticity, it has been demonstrated only in a few species with continuous positive sexual trait allometries (Emlen, 1997b; Knell *et al.*, 1999; Moczek, 2002). In the majority of species with allometrically scaling sexual traits, such allometries have been measured in (non-)randomly sampled individuals from the natural environment, without information on environmental circumstances during development beyond body size (e.g. Kawano, 2000; Buzatto *et al.*, 2014; McCullough *et al.*, 2015). Therefore, to assess whether food-dependent allometric plasticity affects the eco-evolutionary dynamics of sexually selected condition-dependent traits, it is first and foremost necessary to assess whether such allometric plasticity occurs in other well-known model systems for the evolution of condition-dependent sexually selected traits.

Here, we tested whether food-dependent allometric plasticity is present in a species with discrete, condition-dependent, alternative male morphs: the bulb mite *Rhizoglyphus robini* (Radwan, 1995; Smallegange, 2011a). In *R. robini*, large males develop the adult 'fighter' morph, which has a proportionally thickened third leg pair with dagger-like claws that it uses in fights to kill rival males (Fig. 4.1a; Radwan *et al.*, 2000). Small males tend to develop the adult 'scrambler' morph, which has a normal, female-like, third leg pair and a modest motility advantage (Fig. 4.1b; Tomkins *et al.*, 2011). Although it is well established that the alternative morphs in *R. robini* are associated with variation in body size, and thus represent a discontinuous allometric scaling rela-

tionship (Smallegange, 2011a; Leigh & Smallegange, 2014; Smallegange & Deere, 2014), the correlation is noisy. A large range of overlap in body size exists between juveniles developing as fighters and juveniles developing as scramblers (Smallegange, 2011a; Stewart *et al.*, 2018). We hypothesize that these observations are, at least partly, explained by food-dependent plasticity in how body size scales with male morph expression. Such allometric plasticity, if present, could facilitate the repeatedly observed rapid evolution of how male morph expression relates to body size in *Rhizoglyphus* mites (Tomkins *et al.*, 2011; Smallegange & Deere, 2014; Casasa & Moczek, 2019).

To test the hypothesis that condition-dependent male morph expression is associated with food-dependent allometric plasticity, we first identified the sensitive ontogenetic stage at which male body size (or a physiological correlate of it) cues male morph expression. Assuming that growth rate and/or body size explain morph expression best during this sensitive stage, we exposed males to different fluctuating patterns of food availability during ontogeny, creating random variation in growth trajectories from larva to adulthood. We then assessed whether the resulting variation in male morph expression was fully explained by variation in growth rate and/or body size, at different time frames in ontogeny. Having identified the sensitive stage of morph determination, we then tested whether the allometry of adult third leg width and body size at the sensitive stage of morph determination shifts with food quality. We find that adult male morph expression is cued by growth rate and body size immediately before metamorphosis, and that this threshold body size at metamorphosis shifts towards smaller values with deteriorating food quality. Such allometric plasticity in *R. robini* can allow the body size threshold for male morph expression to adaptively track nutritional conditions and resulting shifts in the body size distribution.

4.2 – Materials and Methods

Study species and general procedures

Rhizoglyphus robini is a cosmopolitan agricultural pest mite on numerous crops and forms dense populations on subterraneous plant structures (Díaz *et al.*, 2000). The populations in this study were derived from *R. robini* samples that were collected from flower bulbs in storage rooms in Noord-Holland, The Netherlands, in December 2010. In favourable environments, *R. robini* has four morphological life history stages (larva, protonymph, tritonymph, then adult), interspersed with quiescent phases during which individuals do not move or eat, but undergo incomplete metamorphosis to enter the next stage (Fig. 4.1d).

We keep our *R. robini* populations in an unlit climate cabinet (25 °C; >90% humidity), in plastic containers (10 x 10 x 2.5 cm) with a plaster of Paris substrate. Twice a week, we clean one sixth of the substrate by removing all organic matter and add fresh water and food. We keep half of our populations (four containers) on a diet of baker's yeast and the other half on a diet of oats. Egg-to-egg time averages 11 days on yeast and 13 days on oats when food is *ad libitum*. Otherwise, mites from both diets are similar; no morphological or behavioural differences have been recorded between our yeast-fed and oats-fed populations (unpublished data). Populations on the same diet are mixed every six months to maintain genetic homogeneity.

In the experiments, individual mites were kept in individual plastic tubes (height 5 cm, diameter 1.5 cm) with a moistened plaster substrate. The tubes were sealed with fine mesh, kept in place by a plastic lid and placed in an unlit climate cabinet (25 °C; >90% humidity) throughout the experiment. We avoided bias due to genetic variation by randomly sampling the parental generation from our stocks, and allocating one offspring per parental pair to each experimental treatment – an isoline approach to test several genotypes explicitly would have been logistically infeasible. Mites were photographed ventrally and measured to the nearest 0.001 μm using a Zeiss Axiocam 105 color camera mounted on a Zeiss Stemi 200-C stereomicroscope, and ZEN 2 (Blue edition) software.

Experiment 1: When during ontogeny is male morph expression cued by body size and/or growth?

The mites in this experiment were reared on a diet of oats rather than yeast because development is slightly slower on oats than on yeast, increasing our power to find the critical developmental period in which morph expression is determined. The parental generation was formed by collecting 324 individual *R. robini* larvae from the oats-fed stock populations. The parental larvae were reared to adulthood on a diet of *ad libitum* oats in individual tubes. Adult females and males (90.1% fighters, 8.3% scramblers, 1.6% intermorphs, i.e. males that were fighter on one side and scrambler on the other (Fig. 4.1c)) were randomly paired to produce the focal generation. Pairs were kept in isolation in separate tubes for three days and were fed *ad libitum* oats. From each parental pair a single egg was collected and individually isolated. These individuals were reared to adulthood on a variable (n = 81) or a constant (n = 21) diet of powdered oats to obtain a wide range of growth trajectories. Focal individuals that were reared on a variable diet were transferred daily to a fresh tube that either did or did not contain food. The probability of changing to a different environment (i.e., moving from a food to a non-food tube, and vice versa) was set to 0.8 (n = 27), 0.5 (n = 27) or 0.2

(n = 27). Each individual was photographed and measured at 24-hour intervals to record growth trajectory. We measured idiosoma width and recorded morph expression upon maturation.

To test whether male morph expression was a response to food uptake and growth at the final moments of the tritonymph stage, versus earlier during juvenile growth, we constructed multiple logistic regression models. For each of six developmental stages, we constructed a generalized linear model (GLM) with a logit link function, with morph expression (fighter or scrambler) as response variable, and growth during that stage and size when entering that stage as explanatory variables. The six developmental stages we distinguished were the first and second half of the larval, protonymph and tritonymph stages (Table 4.1). We defined 'size when entering the first half of the larva stage' as the length of the egg, 'size when entering the second half of the larva, protonymph or tritonymph stage' as the idiosoma width of an individual when it had spent half of its total time in its respective stage, and 'size when entering the first half of the protonymph or tritonymph stage' as the idiosoma width of the quiescent larva or protonymph, respectively, 'growth during a particular stage' as the difference in idiosoma width when entering a particular stage (early/late larva, early/late protonymph or early/late tritonymph) and when entering the following stage. 'Growth during the early larval stage' was not available because we did not have idiosoma measurements at hatching. Therefore, we included idiosoma width at the end of the first half of the larval stage, rather than growth during the first half of the larval stage, as the first explanatory variable for the early-larva logistic regression model. We assessed the performance of the logistic regression models for the different stages by comparing AIC_c values (Akaike Information Criterion, corrected for small sample size) and calculating the percentage deviance explained. We tested whether the models performed better than chance by comparing the fitted models to null models using LRT. All analyses were done in R v. 3.4.4 (R Core Team, 2018), including packages *stats*, *dplyr* (Wickham *et al.*, 2018) and *ggplot2* (Wickham *et al.*, 2019).

Experiment 2: Is size-dependent male morph expression subject to allometric plasticity?

The parental generation was formed by individually isolating 200 larvae from the yeast-fed stock populations, in four experimental blocks of 50 larvae each. These larvae were reared to adulthood on a diet of *ad libitum* yeast in individual tubes. Per block, 15-24 adult males and females were randomly paired in a plastic tube with *ad libitum* yeast to produce the focal generation. Three days after pairing, eggs of each parental pair were transferred to a clean individual tube without food. Within 12 hours after

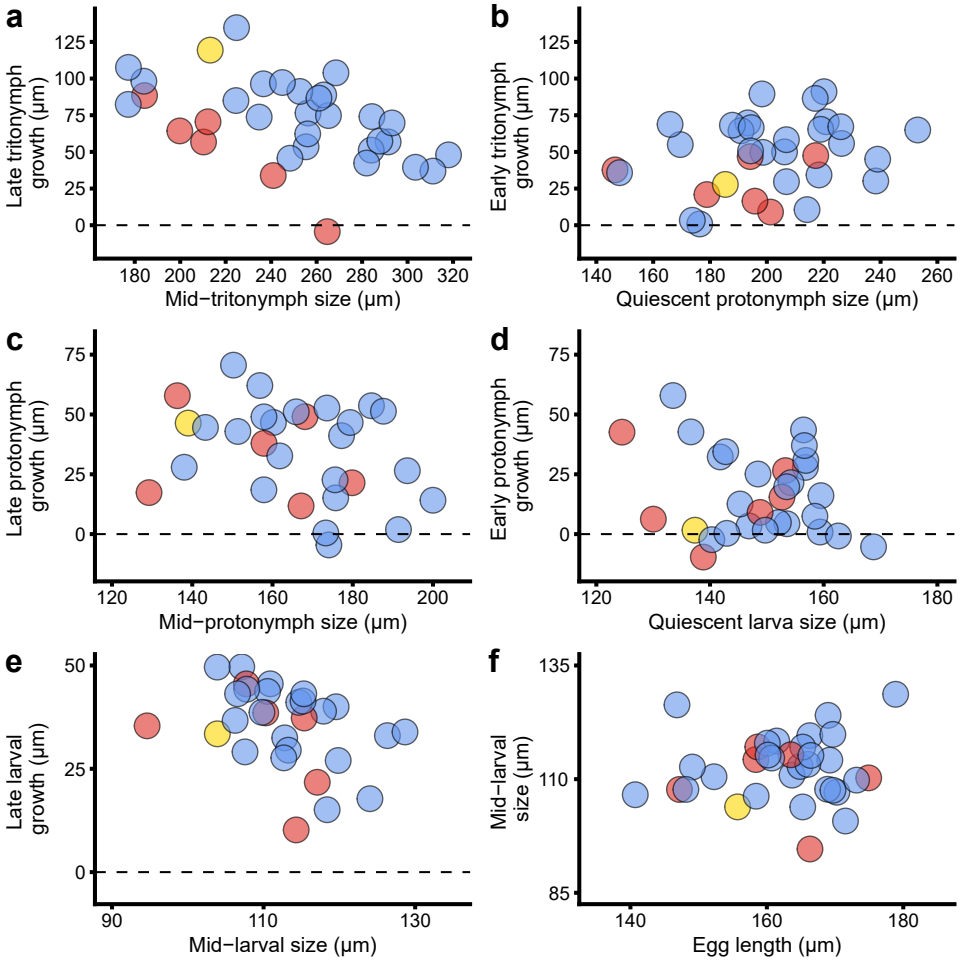


FIGURE 4.2 — Male morph expression (blue: fighter, red: scrambler, yellow: intermorph) as a response to experimentally induced variation in size *at the beginning* of a particular juvenile developmental stage, and growth *during* that stage. **a**, Second half of the tritonymph stage. **b**, First half of the tritonymph stage. **c**, Second half of the protonymph stage. **d**, First half of the protonymph stage. **e**, Second half of the larval stage. **f**, First half of the larval stage.

hatching, larvae were transferred to experimental tubes. Each parental pair contributed a single larva to each food treatment.

The focal individuals were individually isolated and reared to adulthood on quartered ~23 mm^2 circular disks of filter paper that were soaked for 20 seconds in 10 µL solution of yeast in water. Individuals were subjected to five food treatments, which represented a 2.5-fold dilution series (yeast solved in water): 40.0 mg/mL (n = 69), 16.0 mg/mL (n = 40), 6.40 mg/mL (n = 40), 2.56 mg/mL (n = 69) and 1.02

mg/mL (n = 40). Experimental blocks 1 and 4 contained all treatments, but due to logistical constraints, blocks 2 and 3 only contained the 40.0 mg/mL and 2.56 mg/mL treatments. Every two days, the substrate of each tube was cleaned with a moist brush and the yeast-soaked disk was replaced with a fresh disk. After individuals had entered the quiescent tritonymph stage, the disk was removed; adults emerged in an empty tube.

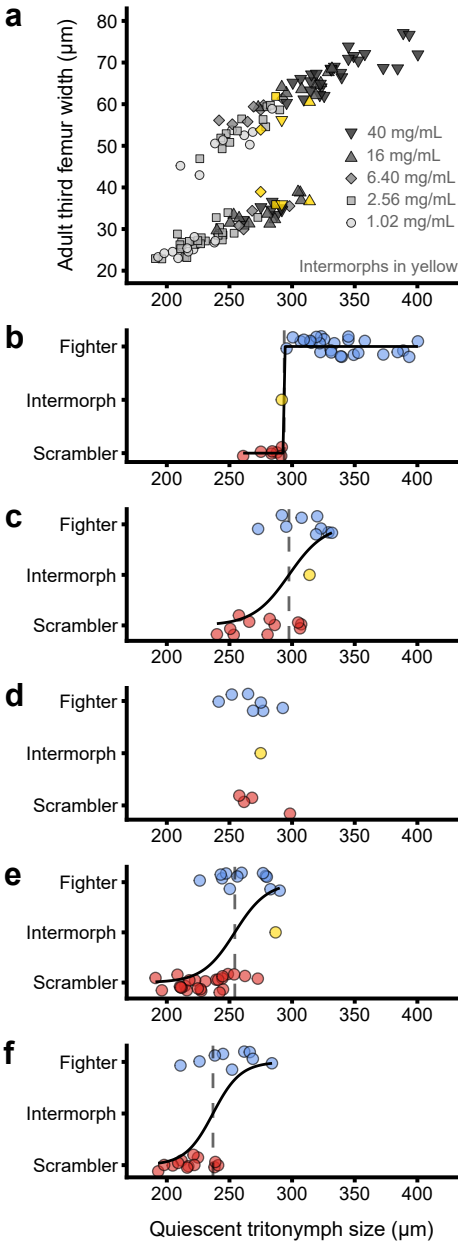
Each individual was photographed and measured in the quiescent tritonymph (Fig. 4.1d) and adult stage (Fig. 4.1a-c). We measured the width of the idiosoma (the white bulbous posterior part of the body) in the quiescent tritonymphs and adults (st. dev. = 3.3 μm; Appendix 4A) and the width of both third leg femurs in the adults (st. dev. = 1.1 μm, Appendix 4A), and recorded male morph expression.

To investigate the effect of food quality and QTIW on the probability of fighter expression, we constructed a logistic regression model with fighter expression as response variable, QTIW, food quality, the interaction between QTIW and food quality and block as fixed explanatory variables and parent ID as random intercept. Four intermorph males were excluded from this analysis. We centered QTIW around the overall mean QTIW value, so that the intercept represented the log-odds of fighter expression at the average QTIW. We tested significance of explanatory variables using LRT.

TABLE 4.1 — Logistic regression models with male morph expression (response variable), and size at the beginning of a juvenile stage and growth during that stage (explanatory variables), for each juvenile stage. The difference in deviance between the fitted models and null models without any explanatory variables is χ^2-distributed under the null hypothesis, with 2 degrees of freedom.

Juvenile stage	**AIC_c**	**Null deviance**	**Residual deviance**	**Difference in deviance (χ^2, df = 2)**	**P-value**	**% deviance explained**
Early larva	36.39	30.885 (31 df)	29.533	1.352	0.509	4.40%
Late larva	34.3	30.885 (31 df)	27.439	3.446	0.179	11.20%
Early protonymph	30.82	29.569 (28 df†)	23.86	5.709	0.058	19.30%
Late protonymph	32.97	29.569 (28 df†)	26.012	3.557	0.169	12.00%
Early tritonymph	32.31	30.885 (31 df)	25.455	5.43	0.066	17.60%
Late tritonymph	*16.37*	*30.885 (31 df)*	*9.516*	*21.369*	*<0.001*	*69.20%*

† Three fighter males passed the protonymph stage very quickly precluding multiple protonymph measurements. These males were removed from protonymph models.



4.3 – Results

Experiment 1: When during ontogeny is male morph expression cued by body size and/or growth?

We were able to follow the developmental trajectories of 69 focal individuals from egg to adulthood (67.6% of total isolated eggs). The individuals that we were not able to follow either died during experimentation or were accidentally lost during handling. Of these 69 individuals, 33 were males, 26 of which expressed the fighter morph. Adult morph expression was predicted by idiosoma growth and size in the last half of the tritonymph stage, immediately prior to metamorphosis (GLM: 69.2% deviance explained, χ^2 = 21.37, df = 2, P < 0.001; Table 4.1), but not in the first half of the tritonymph stage, the first and second half of the protonymph stage, or the first and second half of the larval stage (Table 4.1). Specifically, males that were large at the beginning of the last half of the tritonymph stage had a higher probability of expressing the fighter morph than small males, as did males that grew relatively faster during the last half of the tritonymph stage. The scrambler morph

FIGURE 4.3 — Condition-dependent enlarged fighter leg development shows food-dependent allometric plasticity. **a**, The allometric relationship of adult male third femur width (left and right leg averaged) and body size at metamorphosis. Colour and shape denote food quality (filter paper soaked in yeast solution; see legend). Intermorph males are shown in yellow; in this case, both third legs are shown. **b-f**, Male morph expression (blue: fighter, red: scrambler, yellow: intermorph) as a response to quiescent

— *continued*

was expressed only by males that were very small at the beginning of the last half of the tritonymph stage, or grew little during the last half of the tritonymph stage (Fig. 4.2a). Morph expression also did not depend on idiosoma growth and size in preceding juvenile stages prior to the second half of the tritonymph stage (Fig. 4.2b-f). For earlier stages, size-at-beginning-of-stage and growth-during-stage did not significantly explain variation in morph expression (Table 4.1).

Experiment 2: Is size-dependent male morph expression subject to allometric plasticity?

We reared a total of 128 males to adulthood on filter disks soaked in a yeast concentration of 40.0 mg/mL (n = 35), 16.0 mg/mL (n = 21), 6.40 mg/mL (n = 12), 2.56 mg/mL (n = 37) and 1.02 mg/mL (n = 23). The interaction between QTIW and food quality was statistically significant (LRT: $\chi^2 = 11.78$, df = 4, P = 0.019), indicating that the effect of QTIW on the probability of fighter expression varied across food treatments. Upon visual inspection of the data, the significant interaction between QTIW and food quality was likely caused by two artefacts of limited sampling: (i) the effect of QTIW on fighter expression had vanished in 6.40 mg/mL. However, in this treatment we could sample only 12 males, four of which became scramblers and one expressing the intermorph phenotype; (ii) QTIW completely separated fighters (n = 27) from scramblers (n = 7) in 40.0 mg/mL. Therefore, we assumed the true interaction effect to be zero (constraining the slopes of the logistic regression curves to the same value) and proceeded to test the main effects of QTIW and food quality.

The probability of fighter expression given the effect of food type increased significantly with QTIW (LRT: $\chi^2 = 75.83$, df = 1, P < 0.001; Fig. 4.3a-f). Food quality also had a highly significant effect on the probability of fighter expression given the effect of QTIW (LRT: $\chi^2 = 44.49$, df = 4, P < 0.001), showing a plastic body size threshold that scaled with food quality (Fig. 4.3a-f).

Figure 4.3 — *continued from previous page*

tritonymph body size, for males raised on a diet of 40.0 mg/mL yeast (**b**), 16.0 mg/mL yeast (**c**), 6.40 mg/mL yeast (**d**), 2.56 mg/mL yeast (**e**) and 1.02 mg/mL yeast (**f**). Solid black lines represent statistically significant (α = 0.05) logistic regression lines and denote the probability of fighter expression. Vertical dashed lines represent the quiescent tritonymph size threshold at which the probability of fighter expression is estimated to be 50%.

4.4 – Discussion

We hypothesized that food-dependent allometric plasticity underlies size-dependent male morph expression in *Rhizoglyphus robini*. If this is the case, the allometry of adult third leg width and body size at the developmental stage when male morph expression is cued should change depending on perceived food quality. We found that expression of the fighter phenotype is associated with body size and growth rate during the second half of the final juvenile stage before metamorphosis, but not earlier during ontogeny. This suggests that male morph expression is cued immediately before or during metamorphosis, in the quiescent tritonymph developmental stage. We then tested whether the allometry of quiescent tritonymph body size and the width of the adult third legs shifts with food quality. We found that the allometry did shift towards lower body sizes with a deterioration in food quality, confirming allometric plasticity in *R. robini* male weaponry.

The pattern of food-dependent allometric plasticity can emerge from different developmental mechanisms. Firstly, it is possible that male tritonymphs weigh and integrate multiple cues from the nutritional environment, to assess future mating chances and adaptively adjust development (Emlen, 1997b). Hypothetically, nutrition and growth inform not only on a male's own future physical strength, but also on the strength of potential rivals, provided that these rivals experience the same nutritional circumstances. Therefore, a male's developmental decision to prepare for an aggressive mating tactic should depend not just on its own body size, but on its body size relative to the expected average, favouring adaptive allometric plasticity (Emlen, 1997b).

A second explanation for the observed allometric plasticity is that males use only a single cue for fighter expression, which is not body size, but instead *correlates* with body size. If the association of the 'true cue' and body size depends on food quality, allometric plasticity will emerge. This could happen, for example, if male morph expression is cued by an individual's resource budget during metamorphosis (Chapter 2). Larger individuals will have larger resource budgets on average, but resource budgets are not expected to scale linearly with body size, because the energetic expenses associated with growth and metamorphosis also increase with size. Therefore, allometric plasticity could be a symptom of budget-dependent development of enlarged and expensive fighter legs.

Finally, apparent allometric plasticity could emerge if male morph expression and relative body size are both pleiotropically influenced by genes of large effect. If that is the case, allometries are effectively genetically determined, while nutrition simply

shifts the genetically determined allometries with average body size. Male morph expression in *R. robini* is certainly heritable (Radwan, 1995, 2003; Skrzynecka & Radwan, 2016), and fighter expression has been shown to be associated with genetic diversity and potentially coadaptive gene complexes (Stewart *et al.*, 2019) and the upregulation of genes involved in general metabolism (Joag *et al.*, 2016). However, to our knowledge, there is currently no evidence suggesting that the link between male morph expression and growth rate is caused by pleiotropy, rather than developmental plasticity of leg development in response to growth, size, the resource budget, or food quality. Pleiotropic genetic control of growth and male morph expression seems improbable given the results of our first experiment. Nonetheless, it is likely that the proportion of growth variation explained by genetic effects is larger when the nutritional environment is stable over time (as in our experiment 2) than when the nutritional environment is strongly fluctuating (as in our experiment 1). Likewise, male morph expression may be largely genetically influenced in unfluctuating nutritional environments, and more strongly affected by nutrition in fluctuating nutritional environments. More work is needed to exclude the possibility that apparent allometric plasticity in fact reflects a genetically controlled allometry, driven by genes of large effect, pleiotropically affecting both growth and male morph expression. However, to our knowledge, such a genetically controlled allometry would be a previously unrecorded phenomenon in arthropods.

Allometric plasticity is hypothesized to be an important first step in the evolutionary diversification of allometric scaling relationships (Casasa & Moczek, 2019), and has been recorded in a few species with conspicuous sexual trait allometries. In the dung beetles *Onthophagus acuminatus* and *O. taurus*, the allometry of male horns and adult body size plastically shifts with food quality and average body size (Emlen, 1997b; Moczek, 2002), so that the allometry tracks a shift in the body size distribution much like we describe for *R. robini*. These examples of allometric plasticity have been hypothesized to reflect adaptation to variation in the expected competitive environment, reflected by seasonally varying food (e.g. dung quality; Emlen, 1997b). Alternatively, they may reflect a developmental side-effect due to additional (but food-dependent) growth after secondary sexually selected traits have been cued by body size (Moczek, 2002). The opposite pattern of food-dependent allometric plasticity has also been recorded, where allometric plasticity does not track shifts in the body size distribution, but rather amplifies condition-dependence. For example, in the stalk-eyed fly *Diasemopsis aethiopica* and the armed beetle *Gnatocerus cornutus*, the allometric scaling of sexual trait size is lowered in individuals raised on poor-quality compared to individu-

als raised on good-quality food (Knell *et al.*, 1999; Okada & Miyatake, 2010). Allometries can also change in response to seasonal change: in the stag beetle *Lucanus cervus*, the allometry of male mandibles and adult body size changes over the course of summer, so that males pupating later in the year develop smaller mandibles for their body size than males pupating early in the year (Hardersen *et al.*, 2011). In this case, allometric plasticity is hypothesized to be adaptive due to time constraints on development in combination with allocation trade-offs: the mating season is short, so late-developing males should accelerate maturation at the expense of energetic investment in weaponry (Hardersen *et al.*, 2011). Yet another form of allometric plasticity occurs in the mite species *Rhizoglyphus echinopus* and *Sancassania berlesei,* which exhibit the same size-dependent fighter-scrambler polyphenism as the closely related *R. robini*. In these species, but curiously not in *R. robini* (Radwan, 1995), fighter expression in large males is suppressed by pheromones from dense colonies, so that the allometry collapses into complete scrambler expression in dense colonies (Radwan, 1993, 2001). This response has been demonstrated to be adaptive in *S. berlesei*: in large groups, the fighter phenotype no longer yields higher mating success than the scrambler phenotype even for large males (Michalczyk *et al.*, 2018), and instead becomes a liability (Radwan & Bogacz, 2000; Radwan *et al.*, 2000).

The different forms of allometric plasticity showcase the developmental flexibility associated with allometric scaling, but also highlight that these apparently similar patterns may have evolved under different selection pressures. Therefore, to understand how allometric plasticity evolves, it is necessary to study these selection pressures in detail – i.e., does the allometric scaling relationship of male sexual traits respond adaptively to different environmental cues for the expected future competitive environment? However, the evolution of allometric plasticity should be understood not just in terms of the selection pressures that might favour it, but also in terms of how allometric variation emerges from development. We may not even expect that allometries are food-independent, if we factor in the likely possibility that the energetic expenses of enlarged sexual trait production, relative to the total resource budget, depend on body size and growth rate.

Our observation of food-dependent allometric plasticity in *R. robini* adds to the limited number of recorded cases of allometric plasticity in male arthropod secondary sexual traits. Allometric plasticity is hypothesized to facilitate rapid local adaptation, adaptive divergence and speciation, if allometric plasticity occurs in the same direction as evolutionary change of trait allometries, and if plastic shifts in allometries coincide with release of cryptic genetic variation (Casasa & Moczek, 2019; Noble *et al.*, 2019).

To understand this potentially important evolutionary process, we should start looking for allometric plasticity in different species with conspicuous allometries in variable environments, and investigate whether the direction of allometric plasticity (Emlen, 1997b; Knell *et al.*, 1999; Moczek, 2002; Okada & Miyatake, 2010; Hardersen *et al.*, 2011) generally coincides with evolutionary shifts in the allometry (Moczek *et al.*, 2002; Moczek & Nijhout, 2003; Tomkins *et al.*, 2011; Smallegange & Deere, 2014). Only then can we start to understand how development produces allometric variation, how or whether selection favours particular plastic responses of the scaling relationship, and eventually whether allometric plasticity contributes to evolutionary diversification.

Acknowledgements

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Appendix 4A — Analysis of measurement error

Methods

We selected 7 adult male *R. robini* raised on 40 mg/mL (3 fighters, 2 scramblers) and on 2.56 mg/mL (2 scramblers) yeast solved in water. We photographed the mites ventrally and measured them to the nearest thousandth of a μm using a Zeiss Axiocam 105 color camera mounted on a Zeiss Stemi 200-C stereomicroscope, and ZEN 2 (Blue edition) software. We photographed each subject 10 times. Between photographs, we gently repositioned each subject on its back using a fine brush. We measured the maximum width of the idiosoma and the width of the left and right third femur. We measured the photographs not immediately after taking them, but after we were completely done with photographing our 7 subjects. We measured the photographs in blocks of 7, representing one photograph per subject, in pseudo-random order within blocks (the order in which subjects were measured was randomized within blocks) and among blocks (the order in which the photographs were collected into 7 blocks was randomized). Each block of measurements was recorded on a different spreadsheet, so we were blind to previous measurements.

To calculate the measurement error, we constructed a linear model for each of the three measurements, with the measurement as the dependent variable and individual ID as explanatory variable. We extracted the residuals and checked whether they were homogeneous across subjects using Bartlett's test of variance homogeneity. There was no evidence of variance heterogeneity across subjects in any of the measurements, so we proceeded by calculating the sample standard deviation of the residuals as our estimate of measurement error. If there would have been evidence of variance heterogeneity across subjects, we would have proceeded by investigating how the measurement error changed with body size.

Results and discussion

There was no evidence of variance heterogeneity in the model residuals for idiosoma width (Bartlett's test: $K^2 = 10.32$, df = 6, P = 0.112), right femur width (Bartlett's test: $K^2 = 8.57$, df = 6, P = 0.120) or left femur width (Bartlett's test: $K^2 = 4.84$, df = 6, P = 0.564), showing that the measurement error was the same for all subjects. The sample standard deviation of measurements (pooled for all subjects) was 3.3 μm for idiosoma width and 1.1 μm for both left and right femur width. Therefore, approximately 68% (normal distribution: $\mu \pm \sigma$) of the reported idiosoma width measurements are within 3.3 μm from the true value, and approximately 95% (normal distribution: $\mu \pm 2\sigma$) of the reported idiosoma width measurements are within 6.6 μm from the true

idiosoma width value. Approximately 68% of the reported femur width measurements are within 1.1 μm from the true femur width value, and approximately 95% of the reported femur width measurements are within 2.2 μm from the true femur width value.

Chapter 5

Size-dependent mating niche differentiation in the male-polyphenic mite *Rhizoglyphus robini*

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Abstract

Condition-dependent expression of alternative male morphs evolves when males of different sizes experience different mating niches, requiring different morphologies. Such mating niche differentiation can be due to competitive asymmetry between large and small males in contests over mating opportunities. Here, we tested the hypothesis that aggressive interactions among males cause size-dependent mating niches in an acarid mite with condition-dependent male polyphenism: the bulb mite *Rhizoglyphus robini*. In this species, large males mature as armed fighters with enlarged legs, and small males as scramblers without modified legs. We staged experimental dyadic contests over a mating opportunity between either a pair of fighter males, or a fighter and a scrambler male. We predicted that the larger male would have a higher likelihood of mating first in contests among fighters, that the fighter male would have a higher likelihood of mating first in fighter-scrambler contests, that fighters would have a higher likelihood of interrupting ongoing copulations if they are larger than their mating rival, and that copulations in the presence of a fighter rival therefore last shorter than copulations with a scrambler rival present. We found that in contests among fighters, the larger male had a higher probability of mating first. In contests among a fighter and scrambler, the fighter male was more likely to mate first regardless of the body size difference between the contestants. Ongoing copulations were only rarely interrupted by the rival male (always by a fighter), and the probability of interruption did not depend on the body size difference between the mating male and its rival. Copulations lasted shorter in the presence of a rival fighter, but this effect was not attributable to interruption of copulations. We conclude that the fighting niche is particularly accessible for large males, as larger males have a higher probability of winning pre-copulatory contests. Such mating niche differentiation likely contributes to evolutionary maintenance of condition-dependent male polyphenism, where small males are forced to adopt an alternative mating tactic and hence develop a dedicated morphology.

5.1 – Introduction

Male secondary sexual traits are often conspicuously variable within animal populations, with small males typically developing proportionally smaller or less exaggerated traits than larger males (Emlen & Nijhout, 2000; Kodric-Brown *et al.*, 2006; Lavine *et al.*, 2015). In several arthropods, such condition-dependent expression of sexual traits is represented by discrete alternative male phenotypes (Danforth & Desjardins, 1999; Tomkins, 1999; Emlen *et al.*, 2005; Cook & Bean, 2006; Radwan, 2009; Buzatto & Machado, 2014; McCullough *et al.*, 2015; Karplus & Barki, 2019). It is a long-standing hypothesis that such condition-dependent polyphenism in males is maintained by social-status-dependent sexual selection for alternative mating tactics in small and large individuals (Dawkins, 1980; Eberhard, 1982; Parker, 1982; Gross, 1996; Tomkins & Hazel, 2007). According to this idea, small males are physically weaker than large males, unlikely to compete successfully over mating opportunities in direct intrasexual competition with large males, and adaptively 'Make the Best of a Bad Job' by developing a morphology adapted for alternative, contest-avoiding mating behaviour such as sneaking (Gross, 1996; Brockmann, 2001; Taborsky *et al.*, 2008). Hence, condition-dependent expression of alternative male morphologies is hypothesized to be maintained by the existence of different mating niches (*sensu* Shuster and Wade 2003) for males of different sizes.

Evidence that condition-dependent polyphenism in male sexual traits evolves under disruptive selection due to size-dependent mating niches is sparse, despite the relatively widespread occurrence of such polyphenisms (Dominey, 1984; Gross, 1996; Oliveira *et al.*, 2008). Within arthropods, the evidence is largely confined to horned beetles in which small males develop a 'minor' phenotype without large horns. In these cases, fights among males over mating opportunities are generally won by the larger male, so that small males resort to fight-avoiding sneaking behaviour to gain access to a female (Emlen, 1997a; Moczek & Emlen, 2000; Hongo, 2007; McCullough & Simmons, 2016; Mitchem *et al.*, 2019). In contrast, tests for size-dependent mating niches in male-diphenic earwigs (Styrsky & Van Rhein, 1999; Forslund, 2003), prawns (Barki *et al.*, 1997), harvestmen (Buzatto & Machado, 2008) and acarid mites (Michalczyk *et al.*, 2018) have been inconclusive. Thus, while the evolution of male polyphenism can be explained by the existence of distinct and size-dependent mating niches in horned beetles, it remains poorly understood in other arthropods.

Here, we test the hypothesis that aggressive interactions among males cause size-dependent mating niches in an acarid mite with condition-dependent polyphenism in a male secondary sexual trait: the bulb mite *Rhizoglyphus robini*. Confirmation of this

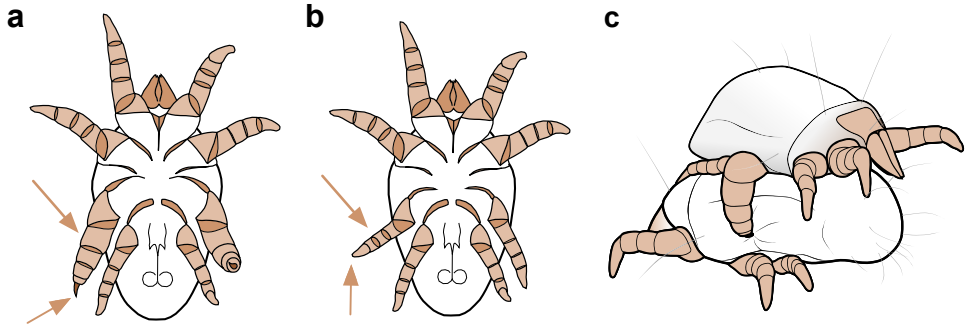

Figure 5.1 — *Rhizoglyphus robini* alternative adult male phenotypes. **a**, Large juveniles become fighters (ventral view, head above). **b**, Relatively small juveniles become scramblers (ventral view, head above). Note the difference in third leg morphology (arrows). **c**, Fighters use their enlarged legs with sharp tarsal claws as weapons in intrasexual fights.

hypothesis would strengthen the general idea that condition-dependent polyphenism in male sexual traits evolves under disruptive sexual selection due to diversifying mating niches. In bulb mites, large well-fed males develop a strongly enlarged third leg pair with sharp claws (the 'fighter' phenotype, Fig. 5.1a; Radwan, 1995; Smallegange, 2011a), which function as weapons in fights with rival males (Radwan & Klimas, 2001; Radwan, 2009). In contrast, small and poorly-fed males tend to develop an unmodified third leg pair, which is morphologically similar to the other legs (the 'scrambler' phenotype, Fig. 5.1b; Radwan, 1995; Smallegange, 2011a). Fighter males are more aggressive than scrambler males (Radwan *et al.*, 2000), and can monopolize females by killing rivals (Fig. 5.1c; Radwan & Klimas, 2001), while scramblers have a mobility advantage (Tomkins *et al.*, 2011) and can obtain higher fertilization success from a single copulation (Van den Beuken *et al.*, 2019a). Male morph expression depends on body size immediately prior to metamorphosis, with larger males becoming fighters and smaller males scramblers (Radwan, 1995; Smallegange, 2011a), although adult fighter males are on average not larger than adult scrambler males (Smallegange *et al.*, 2012); fighters shrink during metamorphosis while scramblers do not (Radwan *et al.*, 2002). In several closely related acarid mite species, fighter expression is additionally suppressed by pheromones signaling high colony density (Radwan, 1995, 2001; Michalczyk *et al.*, 2018); this does not happen in *R. robini* (Radwan, 1995). Copulating male *R. robini* have been demonstrated to be vulnerable to attacks from rivals, and the presence of fighter males prevents the evolution of prolonged mate guarding, where pairs remain in copula after sperm transfer (Radwan & Siva-Jothy, 1996; Skwierzyńska

et al., 2018). Thus, the male fighting niche can be size-dependent in two different ways: the probability of eliminating a rival before copulation may depend on the size difference between the rivals, or the probability of successfully disrupting rival copulations may be size-dependent.

From our hypothesis that the fighting niche is size-dependent, we derive the following predictions. Firstly, in fights among fighters over a mating opportunity, the larger fighter male should have a higher probability of mating first than the smaller male (i.e. only large males occupy the fighting niche successfully; *prediction 1*). Secondly, in fights between a fighter and a scrambler over a mating opportunity, the fighter male should be more likely to mate first regardless of its size (i.e. scramblers are not well adapted to the fighting niche; *prediction 2*). Thirdly, ongoing copulations are disrupted by rival fighters, not by rival scramblers, and fighters have a higher probability of dislodging their mating rival if they are larger than their rival (*prediction 3*). Therefore, copulations should last longer if rivals are scramblers than if rivals are fighters (*prediction 4*). To test these predictions, we staged experimental dyadic male contests for a mating opportunity (a virgin female) with fighter-fighter and fighter-scrambler dyads, noted body size of both contestants, and scored which male mated first, whether the copulation was subsequently disrupted by the other male, and how long mating lasted.

5.2 – Materials and Methods

Study species and experimental procedures

Rhizoglyphus robini occurs naturally on underground plant structures, where it can form dense populations that feed on fungi and rotting plant material (Díaz *et al.*, 2000). Our *R. robini* stocks come from flower bulbs in storage rooms in Noord-Holland, The Netherlands, and were collected in December 2010. The stocks are kept in an unlit climate cabinet (25 °C; >90% humidity), in plastic containers (10 x 10 x 2.5 cm) with a plaster of Paris substrate, and are kept on a diet of baker's yeast. The stock populations each contain tens of thousands of mites, and are mixed every six months to maintain genetic homogeneity. Waste is removed and water and food are added twice a week. In these circumstances, generation time (egg-to-egg) averages 11 days. *R. robini* has three juvenile stages (larva, protonymph, tritonymph), interspersed with quiescent phases during which individuals do not move or eat, but undergo incomplete metamorphosis to enter the next stage. Alternative male morphs (fighter or scrambler) are expressed only in the adult stage. In our stock populations, approximately 70-80% of the adult males are fighters.

We set up experimental contests between two males over a mating opportunity (a virgin female). The males in each contest were either both fighters, or a fighter and a scrambler. These males were randomly collected from the stocks, and then individually isolated in plastic tubes (height 5 cm, diameter 1.5 cm) with a moistened plaster substrate, containing *ad lib* food (yeast). The tubes were sealed with fine mesh, kept in place by a plastic lid and placed in an unlit climate cabinet (25 ˚C; >90% humidity). Because we wanted to distinguish the males individually in the experimental contests, we dyed the food of half of the males green, and the food of other half red, using food dye. As adult mites are slightly translucent, the colored food was visible inside of the digestive tract, which made them easily distinguishable. Since the males were collected as adults, and had likely mated before, we kept them isolated for 3-5 days before experimentation, which we assumed would build up motivation to mate. We obtained virgin females by randomly collecting quiescent tritonymphs from the stocks, and isolating them in plastic tubes (see above) containing *ad lib* food (yeast, undyed). The females emerged as virgin adults the next day, and were used in the experiments 3-5 days later.

The experimental contests took place in empty plastic tubes (height 5 cm, diameter 1.5 cm) with a moistened plaster substrate (hereafter: arena), positioned under a microscope. For each contest, we randomly picked a green-coloured and a red-coloured male from the males that had been isolated 3-5 days before. Before releasing the males in the arena, we photographed them ventrally to measure their body size (width of the idiosoma, at its broadest point) to the nearest μm, using a Zeiss Axiocam 105 color camera mounted on a Zeiss Stemi 200-C stereomicroscope, and ZEN 2 (Blue edition) software. After photographing them, we released the males in the arena, and left them to acclimate for ten minutes. We then added a virgin female to the arena, introduced at equal distance of both males. *Rhizoglyphus* males move rather slowly, and pairs have previously been shown to remain in copula from several minutes up to multiple hours, with a median of 20 minutes (Radwan & Siva-Jothy, 1996; Skwierzyńska *et al.*, 2018). Therefore, we monitored the arenas for approximately 10 seconds every minute. This allowed us to run multiple trials simultaneously, while being frequent enough to record which male mated first, the duration of copulation (to the nearest minute), and whether the non-copulating male was attempting to disrupt its rival's copulation. Experimental contests would last until one male had finished copulating with the female, and were aborted if no copulation had occurred within an hour. Our sample size was largely determined by logistical constraints; we managed to stage 57 fighter-fighter contests, and 14 fighter-scrambler contests. In fighter-scrambler con-

tests, males were easily distinguishable by phenotype, but were alternately dyed green or red anyway, to avoid confounding between contest type and the dying procedure.

Statistics

Our first prediction was that in contests between fighters of different sizes, the larger fighter has a higher probability of winning contests and mating first. To test this prediction, we constructed a logistic regression model (generalized linear models with logit link function), with the difference in size between the green (fighter 1) and the red fighter (fighter 2) as explanatory variable (i.e. size of the green fighter minus size of the red fighter); the response variable was whether the green male (fighter 1) mated first or not. If the size difference between the fighters does not explain which fighter mates first, then the probability of the green (or red) fighter winning should be 0.5 (since males were randomly assigned to green or red coloured food), and there should not be a significant effect of the size difference between the fighters on the probability that the green fighter mated first.

Our second prediction was that in contests between a fighter and a scrambler, the fighter has a higher probability of winning irrespective of the body size difference. To test this prediction, we constructed a logistic regression model with the difference in size between the fighter and the scrambler as explanatory variable (i.e. the size of the fighter minus the size of the scrambler); the response variable was whether the fighter mated first or not. If the outcome of the fighter-scrambler contest is explained by the body size difference rather than male morph, then there should be a significant effect of the size difference between the fighter and the scrambler on the probability that the fighter mated first, and the probability that the fighter mates first should be 0.5 at zero body size difference. On the other hand, if the outcome of the fighter-scrambler contest is explained by male morph, then there should not be a significant effect of the size difference between the fighter and the scrambler, and the probability that the fighter mates first should overall be higher than 0.5.

Our third prediction was that in contests between fighters of different sizes, a fighter has a higher probability of dislodging its mating rival if it is larger than its rival. To test this prediction, we constructed a logistic regression model with the size difference between the mated and the unmated fighter as the explanatory variable (i.e. the size of the mated male minus the size of the unmated male); the response variable was whether the unmated fighter disrupted the copulation (yes or no). In all logistic regression models, we tested significance of explanatory variables using likelihood ratio tests (LRT).

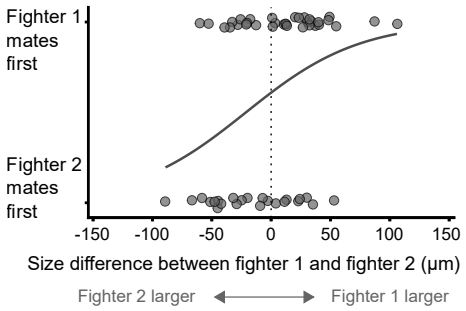

FIGURE 5.2 — In fighter-fighter contests, the probability that a male mates first depends on its body size relative to its rival's body size. Fighter 1 (the green male in experimental contests) mated first with a higher probability if it was larger than fighter 2 (the red male in experimental contests), and vice versa, so larger males were more likely to mate first. The black line represents the modelled probability of winning the contest and mating first. The dotted vertical line denotes equal body size, at which the probability that fighter 1 (the green male) mated first is estimated at 0.61 (95% CI: [0.47 – 0.73]).

Our fourth prediction was that copulations in the presence of a rival last longer if the rival is a scrambler rather than a fighter. We tested this prediction using a Kruskal-Wallis test, with as explanatory variable 'mating type' (i.e. either mated fighter with unmated fighter, or mated fighter with unmated scrambler; only three contests involved a mated scrambler with an unmated fighter, and were excluded from this analysis), and mating duration as response variable. For this analysis we could not use a parametric test, as the residuals were non-normally distributed even after log-transformation of the response variable.

All analyses were done in R v. 3.4.4 (R Core Team, 2018), including packages *stats*, *dplyr* (Wickham *et al.*, 2018) and *ggplot2* (Wickham *et al.*, 2019).

5.3 – Results

Our first prediction was that in contests between fighters of different sizes, the larger fighter has a higher probability of winning contests and mating first. In dyadic contests for a mating opportunity among fighter males, the probability of mating first depended significantly on the size difference between the males, so that the larger fighter male had a higher probability of mating than the smaller male (LRT: $\chi^2 = 7.54$, df = 1, P = 0.006; Fig. 5.2). The estimated probability that the green-dyed male (male 1) mated first at zero size difference was 0.61 (95% CI: [0.47 – 0.73]), not significantly different from 0.5 (z = 1.52; P = 0.128).

Our second prediction was that in contests between a fighter and a scrambler, the fighter has a higher probability of winning irrespective of the body size difference. In dyadic contests between a fighter and a scrambler, there was no evidence that the size difference between the fighter and the scrambler contributed to which male mated first (LRT: $\chi^2 = 0.00$, df = 1, P = 0.982; Fig. 5.3). Instead, the probability of mating first was overall significantly higher for fighters (0.79, 95% CI: [0.50 – 0.93]; LRT: χ^2

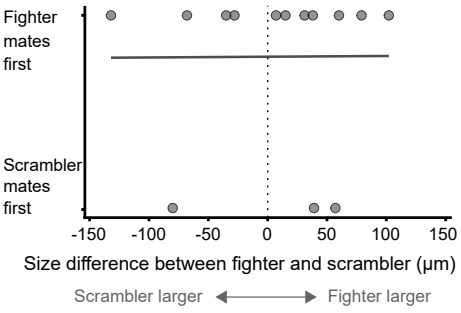

FIGURE 5.3 — In fighter-scrambler contests, there is no evidence that the probability of mating first depends on relative body size. Instead, fighters have an overall higher probability of winning the contest and mating first. The black line represents the modelled probability of winning the contest and mating first. The dotted vertical line de-notes equal body size, at which the probability that the fighter mates first is estimated at 0.79 (95% CI: [0.50 – 0.93]).

= 4.86, df = 1, P = 0.027; Fig. 5.3), regardless of the size difference.

Our third prediction was that in contests between fighters of different sizes, a fighter has a higher probability of dislodging its mating rival if it is larger than its rival. Copulations by fighters were only rarely interrupted by the rival that did not mate first (n=7; probability of interruption estimated at 0.12 (95% CI: [0.06 – 0.25]), and only in fighter-fighter contests. There was no evidence that the probability of such interruptions depended on the body size difference between the interrupting male and the mating male (LRT: χ^2 = 0.00, df = 1, P = 0.992; Fig. 5.4).

Our fourth prediction was that copulations in the presence of a rival last longer if the rival is a scrambler rather than a fighter. Copulations by fighters lasted on average shorter when the rival was a fighter (median 13 minutes) than when the rival was a scrambler (median 18 minutes; Kruskal-Wallis χ^2 = 6.07, df = 1, P = 0.014; Fig. 5.5). This was not due to interruptions of copulation in fighter-fighter contests, because also uninterrupted copulations by fighters lasted on average shorter when the rival male was a fighter, than when the rival male was a scrambler (Kruskal-Wallis χ^2 = 4.97, df = 1, P = 0.026). Only three scramblers mated, so copulations by scramblers (with a rival fighter) were not considered in this analysis.

5.4 – Discussion

We tested the hypothesis that condition-dependent male morph expression in *Rhizoglyphus robini* is associated with size-dependent alternative mating niches. Accordingly, only large males have access to the fighting niche, while smaller males resort to an alternative mating tactic. We found that males in fighter-fighter contests over a mating opportunity had a higher probability of mating if they were larger than their opponent, confirming that small fighters have a disadvantage in the fighting niche (*prediction 1*). In fighter-scrambler contests, on the other hand, fighters had a higher probability of mating regardless of the body size difference, supporting that scramblers are not

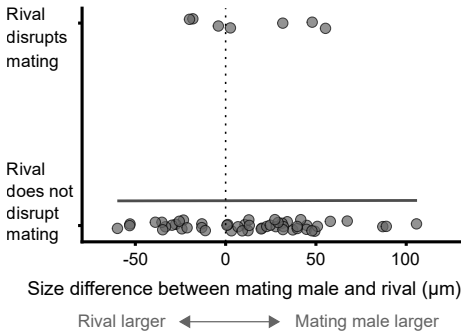

Figure 5.4 — In fighter-fighter contests, there is no evidence that the probability that an ongoing copulation is disrupted by the rival depends on relative body size; copulation disruptions are rare. The black line represents the modelled probability of a disrupted copulation. The dotted vertical line denotes equal body size, at which the probability of disruption is estimated at 0.12 (95% CI: [0.06 – 0.25]).

adapted to the fighting niche in a mating context (*prediction 2*). We also found that copulations by fighters were occasionally disrupted by rival fighters, but only rarely, and apparently independent of the body size difference between the mating male and its rival (*prediction 3*). Copulations by fighters lasted shorter on average when the rival male was a fighter than when the rival was a scrambler (*prediction 4*), which could be due to the disturbing effect of the presence of another fighter. However, both uninterrupted and disrupted copulations lasted shorter in the presence of fighters, so shorter copulation durations were generally not attributable to aggressive physical disruptions of copulation by rival fighters. All in all, we conclude that the fighting niche in *R. robini* is particularly accessible for relatively large males who are able to win contests for mating opportunities. Small males, on the other hand, likely have to resort to alternative behaviours to mate. We found size-structured mating niche differentiation to be mainly due to size-dependent fighting success prior to copulation, and not due to size-dependent interruption of ongoing copulations. Size-dependent access to the fighting niche could play a role in explaining the evolution of condition-dependent male morph expression in *R. robini*.

Our results align well with evidence from horned beetles that larger males are generally more successful fighters than smaller males (Emlen, 1997a; Hongo, 2007; McCullough & Simmons, 2016; Mitchem *et al.*, 2019), so that small males resort to sneaking behaviour to gain access to females, adaptively expressing a different, weaponless morphology (Moczek & Emlen, 2000). We currently do not have evidence that *R. robini* scramblers are sneakers, but they have been shown to obtain higher fertilization success from a single copulation than fighters (Van den Beuken *et al.*, 2019a). Furthermore, scramblers move quicker than fighters and are better able to navigate spatially complex environments (Tomkins *et al.*, 2011), which could benefit a hypothetical sneaking tactic. Hence, small males may be able to maximize fertilization success, given their poor fighting ability, by expressing the scrambler morph rather than the

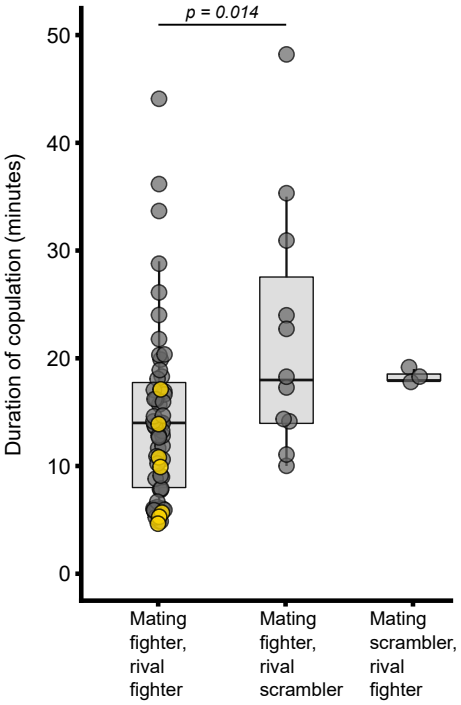

Figure 5.5 — Copulations by fighter males last longer if the rival male is a scrambler, than if the rival is another fighter. This effect is not driven by disruption of copulations in fighter-fighter contests, as the effect persists when disrupted copulations (shown in yellow) are removed from the analysis.

fighter morph, while large males benefit by expressing the fighter morph, given their high likelihood of winning fights. It should be noted that this scenario, in which fertilization success functions for scramblers and fighters cross each other over the body size gradient, has not been supported in a recent study. Specifically, Michalczyk et al. (2018) measured mass-dependent fertilization success of scramblers and fighters in the related mite *Sancassania berlesei,* which shows the same condition-dependent fighter-scrambler polyphenism as *R. robini*, but could not detect crossing fitness functions. On the one hand, this could indicate that reduced mating success in scramblers is not compensated by sneaking behaviour, and that condition-dependent scrambler expression might be adaptive for a different reason (e.g. to prevent immediate viability issues during metamorphosis; Chapter 2). On the other hand, Michalczyk et al (2018) may simply not have had enough statistical power to detect crossing fitness functions.

We found that fighter copulation time was shorter in the presence of a rival fighter than in the presence of a rival scrambler. This effect did not appear to be due to disruption of copulation by fighters. Social-context-dependent copulation duration in *R. robini* has been recorded previously (Skwierzyńska *et al.*, 2018), where copulation duration increased slightly when males had previously experienced rivals from a different morph than themselves. This is consistent with our results, although we did not test whether scramblers increased copulation time in the presence of fighters rather than scramblers. Whether such behavioural plasticity is adaptive remains to be seen. It seems intuitive that males of both morphs would increase copulation duration in the absence of fighters, released from risk of attack. It is less intuitive that scramblers

would adaptively increase copulation duration in the presence of fighters (Skwierzyńska *et al.*, 2018).

In conclusion, we demonstrate that an aggressive male morph in *R. robini* increases mating success mainly for large males. Small males, on the other hand, are disadvantaged in aggressive contests, and benefit less by developing adaptations for fighting. The pattern that larger individuals are better able to secure mating opportunities than smaller individuals in intrasexual contests seems to be a general one (Parker 1974; Maynard Smith and Parker 1976; Lailvaux and Irschick 2006). In species with condition-dependent male polyphenism, this competitive asymmetry, and ensuing mating niche differentiation, likely contributes to maintenance of condition-dependent expression of alternative male morphs and mating tactics (Gross, 1996; Oliveira *et al.*, 2008). However, size-dependent mating niches may not be the only factor involved in evolutionary maintenance of condition-dependent alternative morphs. Variation in growth (condition) impacts fitness in multiple ways beyond social status, and condition-dependent polyphenism may adaptively mitigate poor fitness prospects through e.g. reducing physiological constraints to rapid maturation, or maintaining viability by reducing allocation to expensive sexual traits (Chapter 2). It remains to be seen whether small males actually maximize fertilization success with an alternative mating tactic such as sneaking behaviour, and are under selection for scrambler expression because of that particular effect. Nevertheless, size-dependent mating niche differentiation, due to reduced fitness of a sexually selected phenotype in small males, undoubtedly facilitates the evolution of condition-dependent polyphenism.

Acknowledgements

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Chapter 6

Facultative release from developmental constraints through polyphenism promotes adaptively flexible maturation

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Abstract

The timing of maturation, a critical fitness determinant, is influenced by developmental and energetic constraints, particularly when growth is poor in adverse conditions. Such constraints can be altered through developmental plasticity. Thus, in theory, plasticity in energetically costly sexually selected morphologies can promote life history flexibility in variable environments. We experimentally tested this hypothesis in bulb mites *(Rhizoglyphus robini)* that polyphenically develop as armed fighters with enlarged legs or as scramblers without modified legs. We found that (i) mites enter metamorphosis earlier if they develop as scramblers, (ii) mites accelerate the onset of metamorphosis when they sense resource limitation, and (iii) scrambler expression increases under increased competition for food, enabling males to mature early and escape juvenile mortality. We propose that life history plasticity can evolve through polyphenic release from sexually selected constraints, making the evolutionary dynamics of secondary sexual traits and life history traits, typically studied separately, interdependent.

6.1 – Introduction

Age and size at maturity are life history traits with major fitness consequences, and are characteristically plastic (Stearns & Koella, 1986; Teder *et al.*, 2014). This plasticity evolves under environmental variation, as the balance between fitness costs (juvenile mortality risk, generation time length) and fitness benefits (larger body size at reproduction) of postponing maturation shifts with nutritional circumstances. If resources are abundant and growth is rapid, it generally pays to postpone maturation past the stage at which maturation is physiologically possible, to become large. However, if resources are scarce and growth is slow, fitness is optimized by maturing as early as physiologically possible (Wilbur & Collins, 1973; Morey & Reznick, 2000; Day & Rowe, 2002; Nilsson-Örtman & Rowe, 2021). Hence, in favourable circumstances, optimal age and size at maturity are generally determined by ecological opportunities and the benefits of being large (Blanckenhorn, 2000). In unfavourable circumstances, optimal age and size at maturity are often more strongly influenced by developmental demands and energetic constraints (Day & Rowe, 2002; Ricklefs & Wikelski, 2002; Berner & Blanckenhorn, 2007; Nilsson-Örtman & Rowe, 2021), which can be altered through developmental plasticity (West-Eberhard, 2003).

In the majority of animal species, maturation occurs through metamorphosis (Moran, 1994; Rolff *et al.*, 2019; Ten Brink *et al.*, 2019), during which several energetically expensive processes compete for resources (Nijhout & Emlen, 1998): organisms restructure their bodies, develop reproductive organs, and sometimes also develop secondary sexual traits to be used as weapons or ornaments in competition for mating opportunities (Emlen, 2008; Wiens & Tuschhoff, 2020). Secondary sexual traits are not only typically costly to produce and maintain (Zahavi, 1975; Emlen, 2001; Getty, 2006; O'Brien *et al.*, 2019), but often also developmentally plastic and nutrition-sensitive: poorly-fed individuals often develop proportionally smaller sexual traits, or no sexual traits at all (polyphenism; Moczek, 1998; Tomkins, 1999; Cotton *et al.*, 2004a; Smallegange, 2011a; Warren *et al.*, 2013). This suggests that individuals may not always be developmentally obliged to acquire a large resource budget and produce sexual traits. Instead, individuals may plastically tune trait expression to the state of the resource budget or the pace of resource acquisition, releasing maturation from developmental constraints and thereby accommodating optimal maturation timing. This hypothesis, which we coin 'plastic release hypothesis', or 'polyphenic release hypothesis' in cases of a sexual polyphenism, remains untested, despite its potential for understanding the poorly explored relationship between sexual traits and life history (Morehouse, 2014). A deeper understanding of how sexual trait development impacts

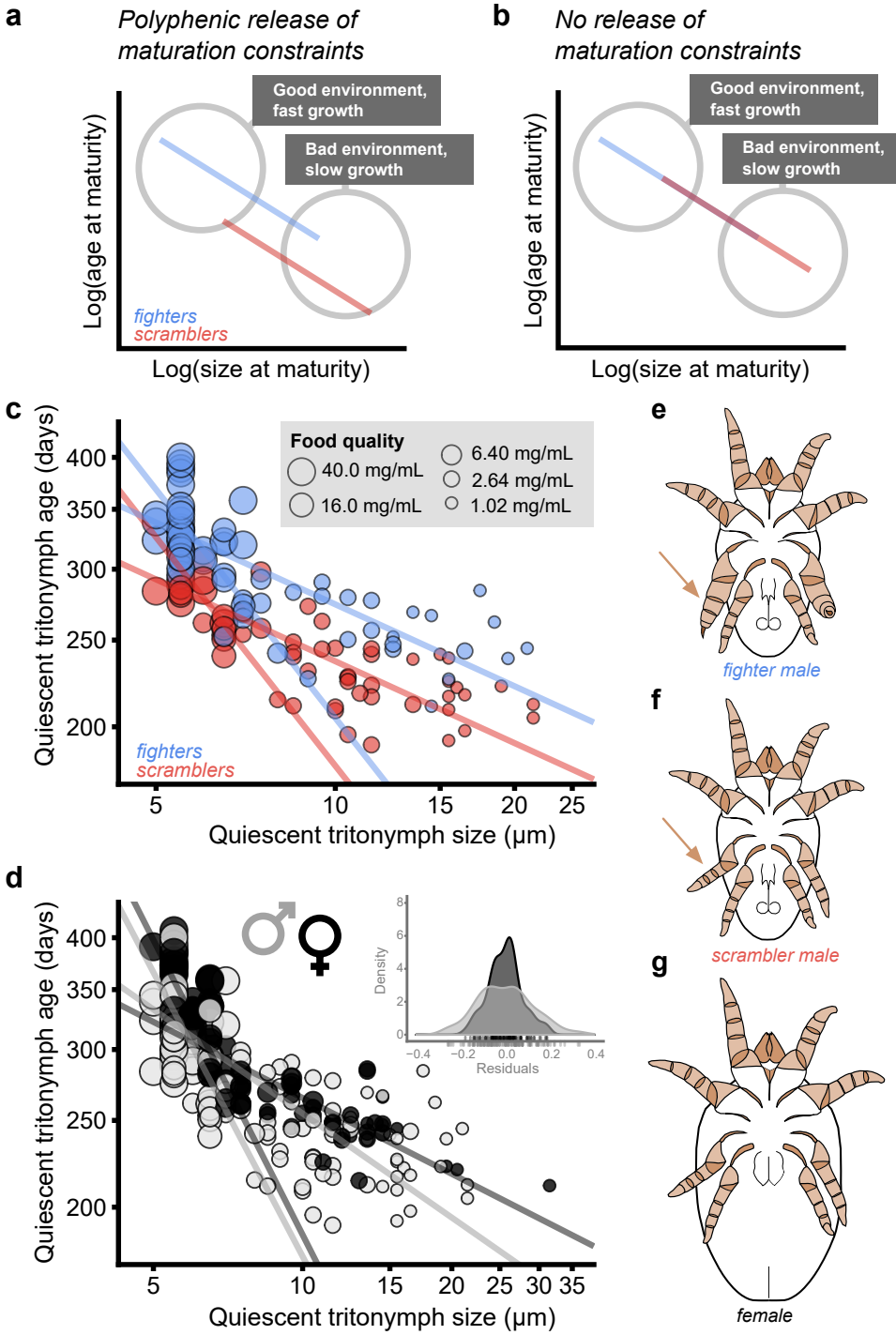

FIGURE 6.1 — *caption on next page*

the timing of life history events, and vice versa, could be an important step towards unravelling the contribution of developmental and physiological constraints to life history evolution (Zera & Harshman, 2001; Day & Rowe, 2002; Ricklefs & Wikelski, 2002; Nilsson-Örtman & Rowe, 2021).

Here, we test the polyphenic release hypothesis in bulb mites (*Rhizoglyphus robini*). These mites form large populations on ephemeral food sources such as rotting plant roots, and experience highly variable levels of competition for food as they go through boom-bust cycles (Díaz *et al.*, 2000). Hence, age at maturity is strongly plastic and nutrition-dependent, ranging from less than a week in well-fed large individuals to more than a month in poorly-fed small individuals (Smallegange, 2011b). Additionally, *Rhizoglyphus* mites show a conspicuous polyphenism in a male secondary sexual trait (Radwan, 2009). Large males typically mature as a 'fighter', armed with dagger-like tarsal claws at the end of a greatly enlarged third leg pair (Fig. 6.1) that they use to puncture the cuticle of conspecifics, thereby killing them. Smaller males mature as an unarmed 'scrambler' with unmodified legs adapted for walking (Fig. 6.1; Radwan & Klimas, 2001). In *R. robini*, scrambler expression is associated with poor growth immediately before metamorphosis (CHAPTER 4). In several related acarid mites that share the fighter-scrambler polyphenism, scrambler expression is additionally induced by pheromones from dense colonies (Radwan, 1995, 2001); this is not the case in *R. robini* (Radwan, 1995).

According to the polyphenic release hypothesis, if enlarged fighter legs are physiologically expensive to produce, the fighter-scrambler polyphenism lowers the physiological threshold for entering metamorphosis and maturation by facultatively removing a major resource allocation target (weaponry). Here, combining individual-

FIGURE 6.1 — Reaction norms of age and size at maturation for *Rhizoglyphus robini* male morphs. **a**, The polyphenic release hypothesis predicts that the reaction norm of age and size at maturity is elevated in fighters (blue), relative to scramblers (red). Axes are log-transformed to linearize the generally strongly L-shaped relationship between age and size at maturity (Day & Rowe, 2002; Smallegange, 2011b). **b**, Alternatively, if fighter expression does not constrain maturation timing, age and size at maturity will fall on the same curve for fighters and scramblers. **c**, Age and size at metamorphosis (log-transformed) of fighters (blue) and scramblers (red) under variation in food quality (circle width). The fighter reaction norm is elevated compared to the scrambler reaction norm (fast-growing males: Wald $\chi^2_1 = 29.5$, $P < 0.001$; slow-growing males: Wald $\chi^2_1 = 29.7$, $P < 0.001$). Lines represent SMA regressions for fast- and slow-growing fighters and scramblers separately. **d**, Age and size at metamorphosis (log-transformed) of males (grey) and females (black) under variation in food quality (circle width). Males follow the average norm of reaction less closely than females: the distribution of SMA residuals is wider for males than for females ($F_{126,109} = 2.67$, $P < 0.001$). Lines represent SMA regressions for fast- and slow-growing males and females separately. **e**, The male fighter morph. The third leg pair is strongly modified and enlarged (arrow). **f**, The male scrambler morph. The third leg pair is not modified (arrow). **g**, Female.

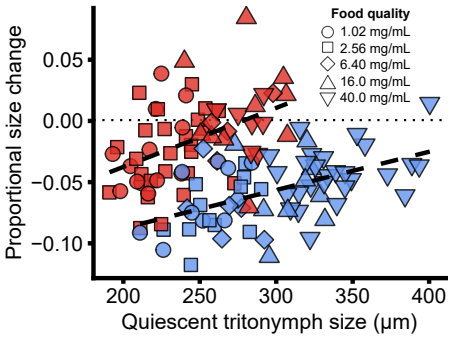

FIGURE 6.2 — Proportional shrinking or growing during male metamorphosis, as a function of body size at metamorphosis and male morph. Variation in quiescent tritonymph body size (i.e. size at metamorphosis) was experimentally induced by varying food quality (symbol shapes). Dashed lines represent the regression lines for fighters and scramblers. Four intermorph males are not shown in this figure. Proportional size change depended on size at metamorphosis (linear mixed model, LRT $\chi^2_1 = 27.05$, $P < 0.001$), and fighters shrank more than scramblers (LRT LRT $\chi^2_1 = 57.16$, $P < 0.001$).

level growth manipulations with population-level manipulations of the strength of competition for food, we investigate whether scrambler expression can prevent juvenile male *R. robini* from being trapped in the juvenile stage when resource acquisition is slow.

6.2 – Results

In our first experiment, we tested the prediction of the polyphenic release hypothesis that scrambler expression enables males to enter metamorphosis at an earlier age (and hence smaller size) than fighters on the same growth trajectory. Thus, the reaction norm of age and size at metamorphosis in response to variation in somatic growth rate should be elevated in fighters relative to scramblers; the difference in elevation being evidence for a sexually selected constraint imposed on the reaction norm (Fig. 6.1a,b). We also tested the assumption that fighter expression is physiologically more expensive than scrambler expression, particularly for poorly-grown males. We generated variation in growth rates by rearing males to adulthood on disks of filter paper soaked in a yeast concentration of 40.0 mg/mL (n = 35), 16.0 mg/mL (n = 21), 6.40 mg/mL (n = 12), 2.56 mg/mL (n = 37) and 1.02 mg/mL (n = 23). We recorded age and size at metamorphosis (i.e. the quiescent tritonymph stage), size change after metamorphosis (as a proxy for physiological cost of adult phenotype development that tests the assumption of fighter expression being costly), and male morph.

Fighters (n = 64) shrank on average significantly more than scramblers (n = 60) during metamorphosis (likelihood ratio test (LRT) $\chi^2 = 57.16$, df = 1, $P < 0.001$); the average fighter lost 6.1% of its body size (95% CI [5.1, 7.1]; Fig. 6.2), whereas the average scrambler lost only 2.0% of its body size (95% CI [1.0, 3.0]; Fig. 6.2). Shrinking was less for larger quiescent tritonymphs (LRT $\chi^2 = 27.05$, df = 1, $P < 0.001$); average shrinkage decreased 3.4 percentage points with each 100 μm body width (95% CI [2.2, 4.6]; Fig. 6.2). Consequentially, the largest fighters did not shrink, whereas the

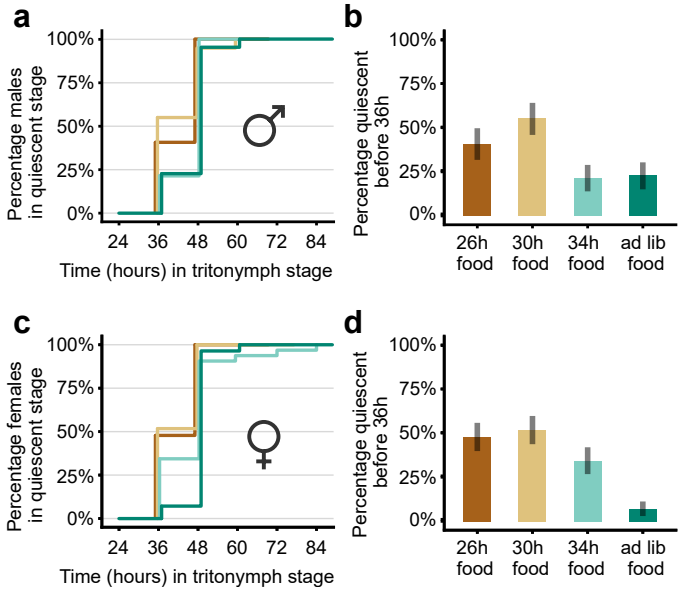

FIGURE 6.3 — Mites accelerate maturation in response to food removal; **a-b**, males; **c-d**, females. **a,c,** Percentage of male (**a**) and female (**c**) tritonymphs in metamorphosis over time, in four treatments: food removed after 26 hours in the tritonymph stage (dark brown), 30 hours (light brown), 34 hours (light green) or not at all (dark green). **b,d**, Percentage of male (**b**) and female (**d**) tritonymphs that had entered metamorphosis after less than 36 hours in the tritonymph stage, in response to food removal treatment. Error bars denote standard errors of the proportion. The percentage of tritonymphs in metamorphosis after less than 36 hours depended significantly on food removal regime ($\chi^2_3 = 20.17$, $P < 0.001$) but not on sex ($\chi^2_3 = 3.78$, $P = 0.286$).

smallest fighters lost up to 11.8% of their body size at the transition from juvenile to adult, supporting our assumption that the fighter morph is costly to develop for small males, and subject to resource allocation trade-offs. Such physiological costs may translate to substantial fitness costs, if only because body size could be an important determinant of mating success in fighters (CHAPTER 5; McCullough & Simmons, 2016). The interaction between quiescent tritonymph body size and male morph did not significantly affect shrinking (LRT $\chi^2 = 0.99$, df = 1, $P = 0.320$).

The reaction norm of age and size at maturity did not significantly differ in slope for fighter and scrambler males (fast-growing males: LRT $\chi^2 = 2.328$, df = 1, $P = 0.127$; slow-growing males: LRT $\chi^2 = 0.02$, df = 1, $P = 0.877$), but, in line with our prediction, was consistently shifted upwards for fighter males, compared to scramblers (fast-growing males: Wald $\chi^2 = 29.5$, df = 1, $P < 0.001$; slow-growing males: Wald $\chi^2 = 29.7$, df = 1, $P < 0.001$; Fig. 6.1c). Fast-growing males (40 μm/day, on the fast end of the spectrum) accelerated maturation by 0.4 days, and slow-growing males (8

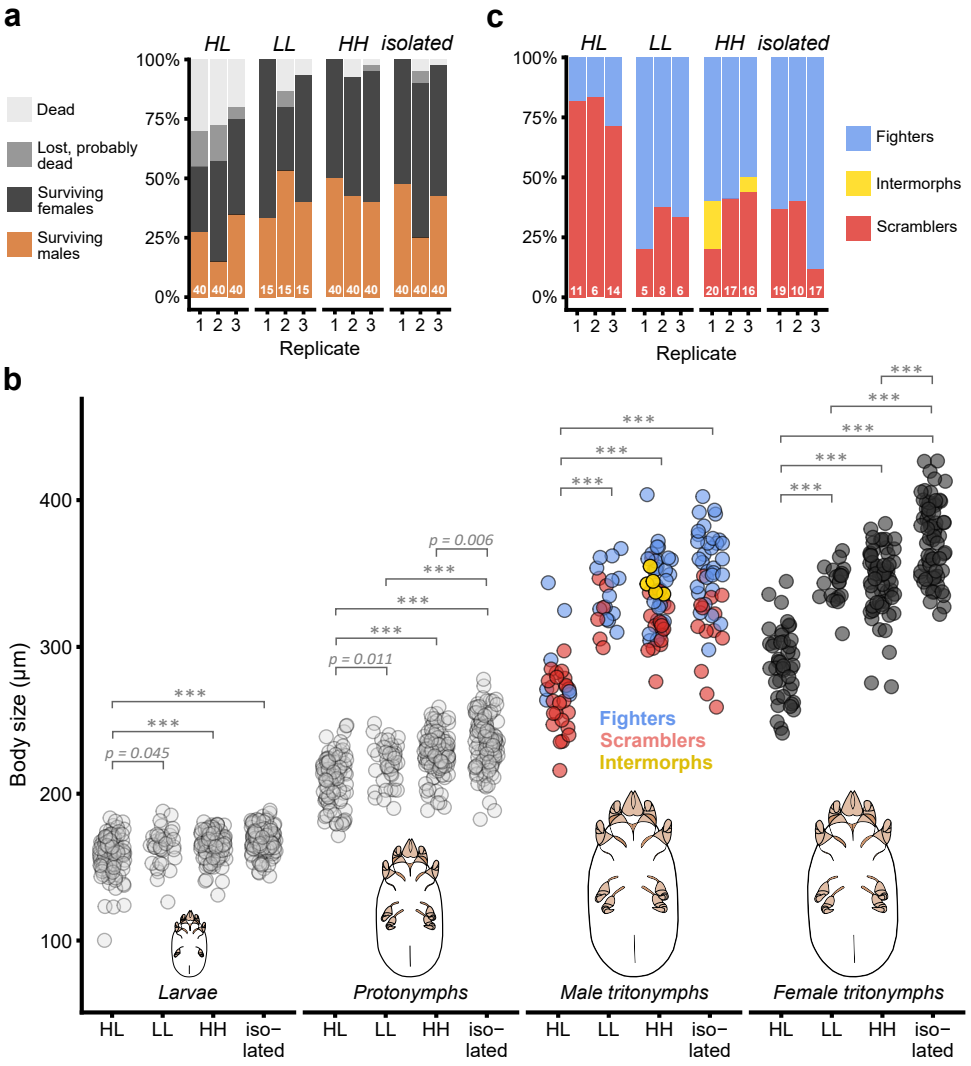

FIGURE 6.4 — Mortality, growth and male morph expression under competition for food. **a**, Mortality in four competition treatments: high population density, low food availability (HL); low population density, low food availability (LL); high population density, high food availability (HH); and individually isolated with ad libitum food (isolated). Numbers at the bottom of each bar denote initial population sizes. **b**, Body size (maximal idiosoma width) of quiescent larvae, protonymphs and tritonymphs in the four competition treatments, all replicates pooled. Size at quiescence differed among treatments (larvae: Kruskal-Wallis $\chi^2_3 = 36.0$, $P < 0.001$; protonymphs: Kruskal-Wallis $\chi^2_3 = 99.8$, $P < 0.001$; male tritonymphs: Kruskal-Wallis $\chi^2_3 = 64.6$, $P < 0.001$; female tritonymphs: Kruskal-Wallis $\chi^2_3 = 114.1$, $P < 0.001$). Brackets denote statistically significant post-hoc pairwise comparisons (Nemenyi's post-hoc test); all $p < 0.001$ except where indicated. **c**, Male morph expression depended on competition treatment (LRT $\chi^2_3 = 21.1$, $P < 0.001$). Numbers at the bottom of each bar denote the number of males that had survived until adulthood in each replicate.

μm/day, on the slow end of the spectrum) by 2.7 days, if they matured as scramblers rather than fighters. For slow-growing males in adverse circumstances when juvenile mortality is high, this represents a considerable timing advantage, presumably due to polyphenic release of developmental constraints. As a consequence, the residual distribution of body sizes at metamorphosis was wider for males than for females, which, unlike males, cannot switch to an energetically cheaper adult phenotype during metamorphosis and thus follow the average reaction norm more closely ($F_{126,109} = 2.67$, $P < 0.001$; Fig. 6.1d).

In interpreting the results from our first experiment, we assumed that bulb mites adaptively time the onset of metamorphosis cued by current, local circumstances, such as a perceived worsening of growth conditions (Wilbur & Collins, 1973; Morey & Reznick, 2000; Helm *et al.*, 2017). Under this assumption, polyphenic release from sexually selected constraints could be a powerful developmental strategy allowing early metamorphosis with even a very small energy budget. Thus, in our second experiment, we tested the hypothesis that the onset of metamorphosis is sensitive to diminishing food conditions. We predicted this effect to be stronger in males than in females, because males can plastically switch to a cheaper phenotype during metamorphosis, whereas females cannot. We reared freshly emerged tritonymphs (the final feeding juvenile stage) to adulthood on *ad lib* access to yeast, removed their food after 26 hours (n = 23 males, 27 females), 30 hours (n = 20 males, 29 females), 34 hours (n = 14 males, 32 females) or not at all (n = 22 males, 28 females) and recorded metamorphosis timing, sex, and adult male morph. We found that both males and females accelerated metamorphosis onset in response to food removal (Fig. 6.3a-d). After 36 hours, only 14% of the mites had entered metamorphosis in the non-removal treatment, whereas 30%, 53% and 44% had entered metamorphosis in the 34 hours, 30 hours and 26 hours treatment, respectively (LRT $\chi^2 = 20.17$, df = 3, $P < 0.001$; Fig. 6.3b,d). The sexes did not significantly differ in their response to food removal (LRT $\chi^2 = 3.78$, df = 3, $P = 0.286$; Fig. 6.3b,d), suggesting that females may employ developmental mechanisms of their own to facilitate accelerated metamorphosis. The sexes did not significantly differ in average metamorphosis timing (LRT $\chi^2 = 0.02$, df = 3, $P = 0.878$). We did not detect an effect of food removal on male morph expression (LRT $\chi^2 = 1.47$, df = 3, $P = 0.689$).

If polyphenic release is an adaptation to variable and occasionally unfavourable environments, it should result in an adaptive response to adverse population conditions. We tested this prediction in our third experiment. If competition for food is experimentally increased in laboratory populations, increasingly more males should

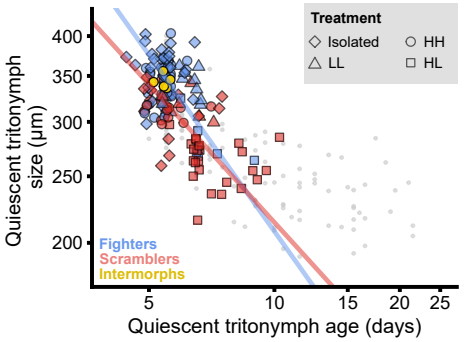

FIGURE 6.5 — Observed relationship between age and size at metamorphosis (log-transformed) in fighters and scramblers, under variation in competition for food. The SMA regression line for fighters is elevated compared to the regression line for scramblers (Wald $\chi^2_1 = 23.84$, $P < 0.001$). Different shapes denote competition treatments: *rectangle*, high population density, low food availability (HL); *triangle*, low population density, low food availability (LL); *circle*, high population density, high food availability (HH); and *diamond*, individually isolated with ad libitum food (isolated). Light grey points show the male reaction norm of age and size at metamorphosis as observed in experiment 1 (Fig. 6.1c).

express the scrambler morph rather than the fighter morph, because scramblers can mature earlier and at a smaller body size than fighters on the same growth trajectory. We prepared populations of newly hatched larvae in four treatments: low population density (15 larvae) and low food availability (two yeast rods, 0.25 mg; hereafter 'LL treatment'), high population density (40 larvae) and high food availability (10 yeast rods, 1.25 mg; hereafter 'HH treatment'), high population density and low food availability (hereafter 'HL treatment'), and individually isolated larvae (40 larvae) with *ad lib* yeast (hereafter 'isolated treatment'). Hence, competition for food was high in the HL treatment, and low in the other three treatments. We repeated this setup three times. We recorded size and age at the onset of metamorphosis (the quiescent phase) in each juvenile stage (larvae, protonymphs, tritonymphs), and we recorded sex and male morph in surviving adults. The effect of treatment on mortality changed with experimental replicate (LRT $\chi^2 = 14.6$, df = 6, $P = 0.024$). Over all replicates, however, mortality was considerably higher in the LH treatment (38%, Fig. 6.4a) than in the LL, HH and isolated treatments (9%, 4% and 4% respectively; Fig. 6.4a). Likewise, size at metamorphosis depended on treatment in all three juvenile stages (larvae: Kruskal-Wallis $\chi^2 = 36.0$, df = 3, $P < 0.001$; protonymphs: Kruskal-Wallis $\chi^2 = 99.8$, df = 3, $P < 0.001$; male tritonymphs: Kruskal-Wallis $\chi^2 = 64.6$, df = 3, $P < 0.001$; female tritonymphs: Kruskal-Wallis $\chi^2 = 114.1$, df = 3, $P < 0.001$; Fig. 6.4b), which was mainly due to poor growth in the HL treatment, but also the increased growth of females in the isolated treatment. Scrambler expression strongly responded to population treatment (LRT $\chi^2 = 21.1$, df = 3, $P < 0.001$), and was higher in the HL treatment (77%; Fig. 6.4c) than in the LL, HH and isolated treatments (32%, 41% and 28% respectively; Fig. 6.4c). This effect did not depend on experimental replicate (LRT χ^2

= 4.47, df = 6, P = 0.614). The difference between fighters and scramblers in the relationship between age and size at metamorphosis in the population experiment, pooled across all treatments, mirrored the difference in the reaction norms observed in our first experiment. The slope was not significantly different for fighters and scramblers (LRT $\chi^2 = 2.79$, df = 1, P = 0.095) but fighters consistently matured later and at a larger size than scramblers on the same growth trajectory (Wald $\chi^2 = 23.84$, df = 1, P < 0.001; Fig. 6.5). Bilaterally asymmetric 'intermorph' males, which were scrambler on one side and fighter on the other, occurred only in the HH treatment (n=5 over all replicates).

6.3 — Discussion

Our results provide the first evidence for polyphenic release of developmental maturation constraints. Slow-growing males were quicker to escape the risky juvenile stage, and avoided a considerable amount of shrinking (in line with earlier findings; Radwan *et al.*, 2002), if they did not develop a morphologically exaggerated trait. Based on this result, we hypothesize that scrambler expression is a consequence of adaptive re-allocation of resources to vital processes during metamorphosis (Lea *et al.*, 2017; Ng'oma *et al.*, 2017; Chapter 2), which is necessary when adverse environmental circumstances favour early maturation with a small resource budget (Wilbur & Collins, 1973; Rowe & Ludwig, 1991; Morey & Reznick, 2000). Thus, it may not be the scrambler morph *itself* that is adaptive, but rather the resource allocation switch underlying its condition-dependent expression. An alternative but equally intriguing explanation for our results is that poorly growing males anticipate poor future fighting success, refrain from developing into a fighter, and accelerate maturation for an adaptive sneaking tactic (Radwan, 2009). Accordingly, scrambler expression and accelerated maturation could both be part of an adaptive sneaker syndrome. Indeed, larger fighters have an increased probability of winning fights over mating opportunities (Chapter 5). However, there is currently no evidence that scramblers employ distinct sneaking behaviour to gain access to females, apart from their inability to kill rivals. Furthermore, in small males, the scrambler morph does not appear to improve fertilization success compared to the fighter morph (Michalczyk *et al.*, 2018). This renders the 'adaptive sneaker' hypothesis as yet unsupported, although it is not mutually exclusive with the polyphenic release hypothesis. All in all, scrambler males may truly have to 'make the best of a bad lot' (Eberhard, 1982). They cannot kill, and hence potentially miss out on mating opportunities. However, males that develop as scramblers are quicker to mature. In adverse environments, such accelerated maturation prevents juveniles from

dying before reproducing (Wilbur & Collins, 1973; Rowe & Ludwig, 1991; Morey & Reznick, 2000). This fitness benefit, accrued during early life, may partly, or possibly completely, compensate the fitness costs of not carrying weaponry later in the life cycle. The resulting life history flexibility may have contributed to the cosmopolitan spread of *Rhizoglyphus* mites, as well as their status as a major agricultural pest (Díaz *et al.*, 2000).

The selection pressures determining optimal maturation timing (Stearns & Koella, 1986; Rowe & Ludwig, 1991; Roff, 1993; Stearns, 2000; Day & Rowe, 2002) are often studied independently from the selection pressures that shape sexual trait expression (Shuster & Wade, 2003; Andersson & Simmons, 2006). Yet, our results imply that the evolutionary dynamics of secondary sexual traits and life history traits are likely intertwined (Cornwallis & Uller, 2010; Morehouse, 2014). Trait exaggeration due to sexual selection inevitably causes trait development to depend on an increasingly large portion of the energy budget (Rowe & Houle, 1996; Tomkins *et al.*, 2004b; Morehouse, 2014), and timing of life history events is typically energy-budget-dependent (Kooijman, 2010). Indeed, comparative evidence in birds (Ancona *et al.*, 2020) and fruit flies (Pitnick *et al.*, 1995a-b; Pitnick & Miller, 2000) suggests that the evolution of exaggerated secondary sexual trait results in prolonged maturation time, presumably because development of such exaggerated traits is energetically expensive. Hence, we need to consider the possibility that flexibility in the timing of life history events evolves by introducing plasticity in energetically expensive morphological traits, or that pre-existing plasticity in morphology facilitates the evolution of life history flexibility. To our knowledge, our results provide the first evidence that facultative expression of a male trait promotes flexible maturation timing through polyphenic release of developmental constraints.

6.4 – Methods

Study system and general procedures

The bulb mite *Rhizoglyphus robini* (Acari: Acaridae) is a common cosmopolitan mite species, which naturally forms dense colonies on rotting plant roots and flower bulbs (Díaz et al., 2000). The populations used in this study are derived from *R. robini* samples that were collected from flower bulbs in horticultural storage rooms in Noord-Holland, the Netherlands, in December 2010. *R. robini* normally has three juvenile stages (larva, protonymph, tritonymph), interspersed with quiescent phases during which individuals do not move or eat but undergo incomplete metamorphosis to enter the next stage (Díaz et al., 2000). Maturation occurs immediately after the

quiescent tritonymph stage (in this study referred to as: metamorphosis). In extremely adverse conditions, juveniles go through a rare fourth stage called the deutonymph, which is adapted for phoretic dispersal and is expressed between the protonymph and tritonymph stage (Díaz et al., 2000). None of the mites in our study expressed the deutonymph stage.

We keep our *R. robini* populations in an unlit climate cabinet (25 °C; >90% humidity), in plastic containers (10 x 10 x 2.5 cm) with a plaster of Paris substrate, on a diet of baker's yeast. Under these conditions, generation time averages 11 days. Twice a week, we clean one sixth of the substrate by removing all organic matter and add fresh water and food. We mix our populations every six months to maintain genetic homogeneity.

In the experiments, individual mites were kept in individual plastic tubes (height 5 cm, diameter 1.5 cm) with a moistened plaster substrate. The tubes were sealed with fine mesh, kept in place by a plastic lid and placed in an unlit climate cabinet (25 °C; >90% humidity) throughout the experiment. Experimental groups of mites (experiment 3) were kept in wider tubes (height 5 cm, diameter 2.5 cm). Mites were photographed ventrally and measured to the nearest 0.001 μm using a Zeiss Axiocam 105 color camera mounted on a Zeiss Stemi 200-C stereomicroscope, and ZEN 2 (Blue edition) software.

For statistical analysis and graphs, we used R v. 3.6.1 (R Core Team, 2018), including the packages stats, ggplot2 (Wickham et al., 2019), dplyr (Wickham et al., 2018), smatr (Warton et al., 2018), segmented (Muggeo, 2017) and PMCMR (Pohlert, 2015).

Experiment 1

The results of experiment 1 form part of an experiment of which the methods are reported elsewhere (Chapter 4). For clarity, we briefly report the methods here as well. The parental generation was obtained by individually isolating 200 larvae from the yeast-fed stock populations, in four experimental blocks of 50 larvae each. These larvae were reared to adulthood on a diet of ad libitum yeast in individual tubes, to eliminate bias due to parental effects. Per block, 15-24 virgin adult males and females of the parental generation were randomly paired in a plastic tube with ad libitum yeast to produce the focal generation. Three days after pairing, eggs of each parental pair were transferred to a clean individual tube without food. Within 12 hours after hatching, larvae were transferred to experimental tubes. Each parental pair contributed a single larva to each food treatment. Note that, to account for genetic variation associated with life history traits and male morph expression, we sampled the parental

generation randomly from our stocks. An isoline approach to test several genotypes explicitly would have been logistically infeasible.

The focal individuals were individually isolated and reared to adulthood on quartered ~23 mm^2 circular disks of filter paper that were soaked for 20 seconds in 10 μL solution of yeast in water. Individuals were subjected to five food treatments, which represented a 2.5-fold dilution series (yeast solved in water): 40.0 mg/mL (n = 69), 16.0 mg/mL (n = 40), 6.40 mg/mL (n = 40), 2.56 mg/mL (n = 69) and 1.02 mg/mL (n = 40). Experimental blocks 1 and 4 contained all treatments, but due to logistical constraints, blocks 2 and 3 only contained the 40.0 mg/mL and 2.56 mg/mL treatments. Every two days, the substrate of each tube was cleaned with a moist brush, and the yeast-soaked disk was replaced with a fresh disk to prevent mould from growing.

We checked whether individuals had reached the quiescent tritonymph stage every 12 hours. Once individuals had done so, we scored age at metamorphosis (in 12h intervals) and photographed each individual ventrally. To score size at metamorphosis, we measured the width of the idiosoma (the white bulbous posterior part of the body; measurement st. dev. = 3.3 μm; Appendix 4A). Roughly 24 hours later, when adults had emerged, we recorded sex and male morph, and photographed each individual again to measure size after metamorphosis. Of the 258 focal individuals, 17 died as a juvenile, yielding a total of 128 adult males and 113 adult females. We failed to obtain a quiescent tritonymph measurement of 1 scrambler male and 3 females.

To test whether (proportional) size change during metamorphosis depends on male morph and body size at metamorphosis, we constructed a linear mixed-effects model with proportional body size change during metamorphosis as response variable. Fixed explanatory variables were idiosoma width at metamorphosis, male morph (fighter or scrambler; 4 intermorph males were excluded from the analysis), the interaction between idiosoma width and male morph, and block. Parent identity was included as random intercept. We defined the response variable, proportional change in idiosoma width, as [adult idiosoma width – idiosoma width at metamorphosis] / idiosoma width at metamorphosis. We centred the explanatory variable 'idiosoma width at metamorphosis' around the mean value for fighters and scramblers separately, so that the intercept and morph parameters directly corresponded to the average amount of proportional shrinking in fighters and scramblers. We tested the assumptions of linearity, normality and homoscedascity by inspecting plots of the residuals against the fitted values and the explanatory variables, and by inspecting a normal

quantile-quantile plot of the residuals. We tested significance of model parameters using likelihood ratio tests (LRT).

To assess whether the reaction norm of age and size at metamorphosis differed in slope and elevation between fighters and scramblers, we performed standardized major axis (SMA) regression on the log-transformed age and size at metamorphosis of males (comprising fighters and scramblers; 4 intermorph males were excluded from this analysis), using the smatr package in R, with male morph as a grouping factor. As preliminary analyses indicated that the reaction norm was non-linear even when log-transformed, we first performed a breakpoint analysis using the segmented package to find the metamorphosis age at which the reaction norm changed. We then compared the breakpoint linear model with a regular linear model using the Akaike Information Criterion (AIC), and inspected the residuals of the breakpoint model visually to exclude remaining non-linearity. The breakpoint model performed considerably better than the linear model (ΔAIC = 20.2), so we proceeded by fitting SMA regressions for fast-growing and slow-growing males separately. Following Warton et al. (2018), we tested for a difference in the slope of the fighter and scrambler reaction norms using a likelihood ratio test (LRT), and for a difference in elevation using a Wald test. Finally, we compared the degree to which age and size at metamorphosis followed the average reaction norm in males (including the four intermorphs) and females, by fitting SMA regressions for slow- and fast-growing males and females separately, extracting the residuals, and comparing the variance of the male and female residuals using an F-test.

Experiment 2

We randomly collected 200 protonymphs from our stock populations over two days, and isolated them in individual tubes with ad libitum yeast. Once they had entered the quiescent protonymph stage, we removed their food. Every morning, we checked for freshly emerged tritonymphs, which emerged over the course of three days in tubes without food. The freshly emerged tritonymphs were randomly allocated to one of four treatments: 1) ad libitum access to food (yeast), but food removed after 26 hours (n=50); 2) ad libitum access to food, but food removed after 30 hours (n=50); 3) ad libitum access to food, but food removed after 34 hours (n=50); and 4) ad libitum access to food until metamorphosis (n=50). Every 12 hours, we scored whether tritonymphs had entered metamorphosis. Roughly 24 hours after the onset of metamorphosis, when adults had emerged, we scored sex and adult male morph.

The tritonymphs were observed to have entered metamorphosis on a limited number of time points, and the vast majority on only two time points (36h and 48h after

the start of the treatments). Therefore, we rescored the time of metamorphosis as 'early metamorphosis' (36h since the start of the treatment) or 'late metamorphosis' (>36h since the start of the treatment). To assess whether treatment influenced the proportion of tritonymphs entering metamorphosis early, we performed a logistic regression (generalized linear model) with metamorphosis timing (early or late) as binary response variable, and treatment, sex and day of tritonymph emergence as fixed explanatory variables. We used LRT to assess whether treatment, sex and their interaction had statistically significant effects.

Experiment 3

The population experiment was done in three replicates, each replicate containing each of the four treatments once. For each replicate, the parental generation was obtained following the same procedure as in experiment 1, except we collected 150 larvae from the stock populations per experimental replicate, forming 50 virgin male-female pairs to produce the focal generation. We isolated eggs following the procedure of experiment 1. Within 12h after hatching, a single larva per parental pair was transferred to each experimental treatment, to maintain genetic homogeneity across the treatments within each replicate.

Per experimental replicate, we prepared three population tubes containing three population treatments (HH: high population density, high food availability; HL: high population density, low food availability, and LL: low population density, low food availability). Additionally, we prepared 40 individual tubes for our fourth experimental treatment (individually isolated, no population effects). In the HH tube, we added 1.25 mg baker's yeast, which corresponded to 10 yeast rods. In the HL, LL and isolated tubes, we added 0.25 mg baker's yeast, which corresponded to 2 yeast rods. The HH, HL and isolated treatments received 40 newly hatched larvae each, which were placed on top of the food source using a fine moist brush. The LL treatment received 15 larvae. To prevent mould from growing, the population treatments (HH, HL and LL) were transferred to fresh tubes first after 3 days, then every 2 days, by carefully picking up each individual mite with a moist brush and putting them in a freshly prepared tube with fresh food in the quantities described above. Hence, the HH, HL and LL treatments received a pulse of fresh food first after 3 days, then every 2 days. The mites in the isolated treatment were not transferred to fresh tubes, but did receive fresh food at the same time as the population tubes.

We monitored all the tubes every 12 hours, and recorded the number of larvae, protonymphs and tritonymphs, the number of dead individuals and the number of

individuals that had entered the quiescent phase. We photographed all quiescent larvae, protonymphs and tritonymphs ventrally and measured the maximal width of the idiosoma (the large bulbous posterior part of the body) as a proxy for body size. We collected and isolated all quiescent tritonymphs in individual tubes, and recorded sex and male morph when the adult emerged. We then put the adult back in the population tube where it came from.

To assess survival until maturity, we performed a logistic regression (generalized linear model) with survival (yes/no) as binary response variable (we assumed lost individuals to be dead), and treatment and replicate as fixed explanatory variables. To assess the effect of treatment on male morph expression, we performed a logistic regression with male morph as binary response variable (note that 5 'intermorph' males were omitted from this analysis), and treatment and replicate as fixed explanatory variables. We used LRT to assess whether survival and male morph expression were significantly affected by treatment, and whether the effect changed with experimental replicate. To assess the effect of treatment on quiescent larval, protonymphal and tritonymphal body size, we used the non-parametric Kruskal-Wallis analysis of variance, as preliminary analyses showed evidence for non-normality and heteroscedascity. We used the Nemenyi post-hoc test for pairwise comparison of treatments.

Finally, to assess whether the reaction norm of age and size at metamorphosis differed in slope and elevation between fighters and scramblers, we performed standardized major axis (SMA) regression on the log-transformed age and size of male quiescent tritonymphs (pooled from all treatments and replicates), with male morph as a grouping factor. We tested for a difference in the slope of the fighter and scrambler reaction norms using a likelihood ratio test (LRT), and for a difference in elevation using a Wald test, following Warton et al. (2018).

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Chapter 7
Synthesis

Flor T. Rhebergen

7.1 — Introduction

Animal traits involved in the acquisition of mates are often rather variably developed, depending on an individual's condition: its phenotypic expectation of future reproductive success (Chapter 3). Such traits comprise weapons used in contests over mating opportunities, or ornaments used to attract potential mates. Occasionally, condition-dependent plasticity in sexually selected traits results in discrete alternative morphs, or polyphenism (Chapter 3). How is such condition-dependent polyphenism shaped and maintained by selection? Why do alternative developmental trajectories coexist?

In this thesis, I have investigated how selection favours condition-dependent development of male sexual traits, using laboratory-based populations of the acarid mite *Rhizoglyphus robini* as a case study. In *Rhizoglyphus* mites, selection maintains condition-dependent development of alternative male morphs during the transition from the final juvenile stage to the adult stage. Large males develop enlarged third legs (the fighter phenotype), while smaller males tend to develop unmodified third legs (the scrambler phenotype), following an underlying condition-dependent reaction norm (Radwan, 1995; Smallegange, 2011a; Buzatto *et al.*, 2012a). Experimental evolution has demonstrated that this condition-dependent reaction norm can evolve quite rapidly in response to ecological change (Unrug *et al.*, 2004; Tomkins *et al.*, 2011; Smallegange & Deere, 2014). Under artificial selection, it is even possible to create populations with (near) unconditional fighter or scrambler expression (Radwan, 2003; Plesnar-Bielak *et al.*, 2014; Van den Beuken & Smallegange, 2018). Yet, such populations readily evolve back towards the ancestral condition-dependent reaction norm, once artificial selection is abandoned (Skrzynecka & Radwan, 2016). What are the selection pressures maintaining a condition-dependent reaction norm in *Rhizoglyphus* populations?

Selection for condition-dependent polyphenism has a contribution from sexual selection, where fitness variation is due to condition- and trait-dependent variation in mating or fertilization success, and from natural selection, where fitness variation is due to condition- and trait-dependent variation in viability and fecundity, and/or the timing of life history events (Chapter 2, Chapter 3). The relative contribution of both sources of selection determines whether polyphenism evolves primarily as an adaptive response to alternative mating niches and mating tactics (Chapter 2, Chapter 3), or as an adaptive response to suboptimal viability, fecundity or life-history timing associated with poor condition (somatic buffering; Chapter 2, Chapter 3). In this Synthesis, I first summarize the evidence for the alternative mating tactics component and the somatic buffering component of selection for polyphenism in

Rhizoglyphus mites, from this thesis as well as previously published literature. I then discuss how we can estimate the relative contribution of sexual selection for alternative mating tactics, and natural selection for somatic buffering, to total selection for condition-dependent polyphenism. Ultimately, a better understanding of how selection maintains developmental plasticity will improve our ability to predict the course of evolution of plastic traits in changing environments. Moreover, understanding how niches diversify due to inevitable variation in condition will contribute to answering one of the big questions in evolutionary biology: how organisms maintain the capacity to produce variation under selection.

7.2 — Do adaptive alternative mating tactics contribute to selection for condition-dependent polyphenism in acarid mites?

The alternative mating tactics component of adaptive condition-dependent plasticity in sexual traits arises because of mating niche differentiation within populations (CHAPTER 3). Alternative mating tactics can *cause* the evolution of body-size-dependent polyphenism, if mating niches are differentiated along the body size gradient. Polyphenism then represents a form of information-based anticipatory plasticity, in which body size is used as a cue to increase the fit between phenotype and expected reproductive environment (CHAPTER 2). Alternative mating tactics can also evolve in *response* to prior evolution of condition-dependent sexual trait expression, if mating niches become differentiated among males with and without well-developed sexual traits. The latter process is a very likely consequence of polyphenism; males without sexual traits likely compensate for lack of mating success by adopting differentiated sexual behaviours, regardless of whether condition-dependent sexual trait expression is maintained by sexual selection. Because alternative mating tactics can be a cause or a consequence of polyphenism, it is not sufficient to show that alternative morphs are associated with alternative forms of sexual behaviour, to assess whether sexual selection for alternative mating tactics drives the evolution of condition-dependent polyphenism. It is also crucial to determine how the corresponding alternative mating niches originate: do they arise because of variation in body size, or are they a consequence of variation in sexual traits (CHAPTER 3)?

Are alternative morphs associated with alternative forms of sexual behaviour?

Previous studies have reported evidence for alternative mating behaviours in fighters and scramblers in acarid mites. In the presence of a female, fighters are more aggressive than scramblers, and regularly kill their rivals using their enlarged third leg pair as

a pair of pincers (Radwan *et al.*, 2000). Scramblers cannot kill. As a consequence, fighters can monopolize females in small populations, by simply killing all rival males (Radwan & Klimas, 2001). Whether fighter aggression has evolved in the context of mate acquisition remains an open question; fighters are aggressive also towards juveniles and cannibalize conspecifics (Łukasik, 2010; Van den Beuken *et al.*, 2019b), preferentially targeting quiescent (metamorphosing) tritonymphs as an easily accessible food source (Łukasik, 2010). Fighter aggression in a mating context could therefore be a side-effect (exaptation) of overall higher levels of aggression and adaptive cannibalism, or the other way around. Scramblers do not appear to show distinct fight-avoiding behaviour (Smallegange *et al.*, 2012), although they move somewhat quicker than fighters (Tomkins *et al.*, 2011). Scramblers do obtain more offspring from a single copulation than fighters (Van den Beuken *et al.*, 2019a), and may even contribute to female investment in offspring via nuptial gifts (van den Beuken & Smallegange, 2018). These traits could be adaptations to a low probability of gaining access to females, and a differentiated mating niche. Whether scramblers consequently fare better than fighters in sperm competition or cryptic female choice remains an open question; there is currently no evidence that this is the case (Radwan, 1991, 1997). All in all, fighters and scramblers clearly occupy distinct mating niches, as the two male phenotypes experience different types of opportunities to fertilize females. Whether these mating niches contribute to selection for the fighter-scrambler polyphenism, depends on whether mating niches are predicted by the cues that trigger male morph expression during development.

Are mating niches size-dependent?

In Chapter 5, I present evidence that the fitness return of fighting behaviour depends on the body size difference among rival fighter males, supporting the hypothesis that body size influences which mating niche is available to a male. This effect could be sufficient to drive the evolution of size-dependent fighter expression, adaptively anticipating an aggressive mating tactic. If small males win fewer fights than large males, they will not benefit by developing weaponry, and Make the Best of a Bad Job by anticipating a different reproductive tactic (Chapter 3). In this scenario, the mating niche depends on a male's body size relative to the population average. Accordingly, the mating niche is more accurately predicted not just by a male's own body size, but also by the expected body size distribution of its rivals. Because food quality affects body size at reproduction in acarid mites, it might act as an additional cue during juvenile growth, anticipating the body size distribution of future rivals (Emlen, 1997b;

TABLE 7.1 — Unassessed assumptions of the alternative mating tactics hypothesis, and of the somatic buffering hypothesis.

a) *Unassessed assumptions of the alternative mating tactics hypothesis*
– Fighter legs are adapted for winning fights with rivals over mating opportunities: males with larger legs win more fights
– Large-size mating advantage is due to increased fighting efficacy in larger males (e.g. due to increased physical strength, or functional size constraints associated with fighting)
– Body size at metamorphosis and food quality prior to metamorphosis are reliable cues for mating niches
– In net selection shaping the condition-dependent reaction norm, the sexual selection component is larger than the other selection components
b) *Unassessed assumptions of the somatic buffering hypothesis*
– Possessing stronger and thicker legs increases fitness, so selection would favour thicker legs in the absence of developmental costs
– Metamorphosis timing is under strong selection in natural populations
– There are developmental mechanisms causing trade-offs between fighter legs and prioritized developmental functions
– Scrambler expression is induced by stress signals in prioritized physiological processes, while fighter expression occurs in the absence of stress
– Observed covariation of male phenotype expression and life history traits are due to side-effects of alternative male morphs caused by phenotypically accommodated trade-offs. They do not reflect co-adapted life history patterns
– In net selection shaping the condition-dependent reaction norm, the sexual selection component is smaller than the other selection components

Moczek, 2002). Selection for mating-niche-matching adult male morphs could then favour a body-size-dependent reaction norm that adaptively shifts with experienced food quality (Tomkins et al. 2011). Indeed, such allometry plasticity is what we see in *R. robini*: in CHAPTER 4, I show that the 'switch point' body size at which males start developing the fighter phenotype rather than the scrambler phenotype is not fixed, but depends on food quality. Thus, the available evidence is consistent with size-dependent mating niches in *R. robini*.

Alternative mating tactics as evolutionary drivers of polyphenism in acarid mites: untested assumptions

The hypothesis that body-size-dependent mating niche differentiation regulates the adaptive evolution of anticipatory condition-dependent plasticity relies on several untested assumptions (Table 7.1a). A first assumption is that enlarged fighter legs are adaptations for winning contests with rivals over a mating opportunity (CHAPTER 3). This is a common assumption in research on acarid mite mating behaviour (Radwan,

2007, 2009) and follows studies on other arthropods with male-limited weaponry (Emlen, 2008; Lavine *et al.*, 2015; Rico-Guevara & Hurme, 2019). Yet, we do not have much support for it. For example, to my knowledge, there is currently no study that shows that more aggressive fighters with thicker legs kill more rivals, and mate more often, than less aggressive fighters with relatively thinner legs. We do know that fighters kill conspecifics not just in a mating context, but also in order to feed when food is scarce (Łukasik, 2010; Van den Beuken *et al.*, 2019b). Is cannibalism a side-effect of adaptive sexual aggression, or is it the other way around?

A second assumption is that larger fighters are more likely to mate than smaller fighters because they are physically stronger, and therefore better at fighting. If a large-size mating advantage is due to a different mechanism (e.g. female preference for larger males (Basolo, 2004), increased mobility in larger males, or a small-size disadvantage due to inability to achieve a functional mating position when males and females differ too much in size), small males will still experience a differentiated mating niche, but it may no longer be the case that smaller males 'Make the Best of a Bad Job' by preparing for a non-fighting tactic. The assumption that large males are physically stronger than smaller males is often made in arthropods that fight over mating opportunities (Chapter 3), but has been tested (and supported) in only a few species, and not in acarid mites (Lailvaux *et al.*, 2005; McCullough & Simmons, 2016).

A third assumption is that body size and experienced food quality can evolve as cues for adaptive, information-based anticipatory plasticity. This depends on how reliable the association between the putative cue (body size, experienced food quality) and the selective context (differentiated mating niches) is (Scheiner, 2013). The association between body size and the mating niche seems straightforward and is supported (Chapter 5). However, it remains to be seen whether the nutritional environment is homogeneous enough within generations (one to several weeks), yet variable enough over longer time scales, to allow juveniles to reliably predict growth trajectories of rivals from food uptake (Chapter 4). If the nutritional environment is spatially heterogeneous within a population, or if the population does not experience strong recurring variation in average food quality, selection for adaptive anticipatory plasticity will be too weak to produce adaptive evolution of the reaction norm (Scheiner, 2013).

All in all, the available data are consistent with the hypothesis that alternative mating niches contribute to selection for condition-dependent polyphenism in acarid mites. However, consistence with data on its own does not constitute a test of the hypothesis, and several underlying assumptions remain unassessed.

7.3 — Does adaptive somatic buffering contribute to selection for condition-dependent polyphenism in acarid mites?

The somatic buffering component of condition-dependent plasticity in sexual traits arises because trade-offs among allocation targets occur during trait development (Chapter 3). Since morphologically exaggerated secondary sexual traits can be energetically expensive to produce, their development implies that other allocation targets could be compromised if the resource budget is limited. Males in poor condition have small resource budgets, so are particularly vulnerable to the negative fitness effects of such trade-offs. The somatic buffering hypothesis posits that male sexual traits are evolutionarily maintained at condition-dependence because males with small resource budgets optimize viability, and thereby fitness, by not developing sexual traits. Therefore, unconditional sexual trait development does not evolve, unless either the distribution of condition (i.e. the resource budget) changes (e.g. through evolution of increased energy assimilation efficiency, or environmental change in nutrient availability), or the energetic cost of trait development diminishes (Chapter 2, Chapter 3). Condition-dependent polyphenism then represents a form of state-dependent mitigating plasticity that adaptively reduces detrimental effects of environmental perturbations, by altering and alleviating trade-offs associated with fighter expression (Chapter 2). Such trade-offs exist within the male developmental system, but, since males and female share a genome, also between the sexes.

Trade-offs within males

Supporting the somatic buffering scenario, male fighter leg development is associated with postponed metamorphosis, and results in a size reduction during metamorphosis that gets stronger with decreasing condition (Chapter 6). Since age and size at maturity are life history traits with large effects on fitness (Stearns, 1992; Day & Rowe, 2002), fighter leg development may not be favoured by selection in males in poor condition. Males are thus 'released' from the life history costs of fighter leg development through polyphenism. Such polyphenic release could allow males to optimize fitness given poor condition, and to adaptively accelerate metamorphosis when the nutritional environment deteriorates. Indeed, scramblers mature relatively earlier and at a smaller body size than fighters, given a particular somatic growth rate, and increased competition for food results in a higher proportion of males developing as scramblers (Chapter 6).

The somatic buffering hypothesis is fully consistent with, and potentially explains, the observed allometry plasticity in *R. robini* male morph expression (Chapter 4). Male

morph expression is correlated with body size at metamorphosis, but the 'switch point' body size at which males start developing the fighter phenotype rather than the scrambler phenotype is not fixed, but depends on food quality (Chapter 4). In a 'pure' somatic buffering scenario, the onset of alternative developmental trajectories is cued by stress signals (i.e. deviations from homeostasis) in prioritized resource fluxes during metamorphosis (Del Giudice *et al.*, 2018). A food-independent scaling of body size at metamorphosis and male morph expression, with a single absolute body size switch point, is then simply not expected. Males reared on a low food quality, which grow slowly and remain on average small, likely experience developmental resource stress at a smaller body size than males reared on high food quality, which grow fast and become large.

Trade-offs between the sexes

If trade-offs between weaponry and other developmental functions constrain male phenotype expression at condition-dependence (somatic buffering), such trade-offs should be apparent not only within the male developmental system, but also between the sexes. After all, males and females share a genome, so developmental adaptation facilitating condition-independent fighter expression in males could have detrimental effects not just in poor-condition males, but also in females (Bonduriansky & Chenoweth, 2009; Connallon & Clark, 2013; Radwan *et al.*, 2016). Likewise, developmental adaptation facilitating (for example) high female fecundity may increase male vulnerability to environmental perturbations, resulting in increased condition-dependent scrambler expression in males. Such intersexual trade-offs could lead to intralocus sexual conflict, and contribute to preventing evolution of condition-independent fighter expression in males (Bonduriansky & Chenoweth, 2009; Connallon & Clark, 2013; Plesnar-Bielak *et al.*, 2014; Radwan *et al.*, 2016). Indeed, evidence for intersexual trade-offs associated with experimental evolution of fighter expression has been found in earlier studies on *R. robini* (Plesnar-Bielak *et al.*, 2014; Joag *et al.*, 2016; Van den Beuken & Smallegange, 2018). Under artificial selection for unconditional fighter expression, males readily evolve a canalized unconditional fighter phenotype. Females from such selection lines typically evolve substantially reduced fecundity (Plesnar-Bielak *et al.*, 2014; Van den Beuken & Smallegange, 2018), and produce smaller eggs (Van den Beuken & Smallegange, 2018). Furthermore, fewer adult females are found in selection lines fixed for fighter expression, possibly because of increased female mortality (Van den Beuken & Smallegange, 2018). This suggests that experimentally evolved allocation processes unconditionally prioritizing male fighter leg development

have detrimental consequences when expressed in females. Perhaps this happens because such allocation patterns prioritize fighter legs by de-prioritizing other physiological processes, such as those involved in development of the female reproductive system, potentially giving rise to intralocus sexual conflict (Plesnar-Bielak *et al.*, 2014; Joag *et al.*, 2016; Radwan *et al.*, 2016).

Importantly, intersexual trade-offs uncovered by experimental evolution do not *necessarily* imply sexual conflict, and do not necessarily constrain male or female adaptation. Trade-offs between male and female traits lead to sexual conflict only if male and female traits are, at equilibrium, not at their fitness optima because of these trade-offs (Bonduriansky & Chenoweth, 2009). Trade-offs *within* the male developmental system may already prevent fighter expression from evolving condition-independence (somatic buffering, Chapter 2, Chapter 3, Chapter 6), and a condition-dependent reaction norm may therefore represent the male fitness optimum. Likewise, intermediate levels of fecundity and egg size may simply represent the female fitness optimum. Females do not necessarily benefit from ever increasing egg production, due to trade-offs between offspring size and number and current versus future reproduction (Stearns, 1992). Hence, intersexual trade-offs may not cause intralocus sexual conflict, and hence may not contribute to evolutionary maintenance of condition-dependence in *Rhizoglyphus*, despite recent suggestions that trade-offs imply conflict (Plesnar-Bielak *et al.*, 2014; Joag *et al.*, 2016; Radwan *et al.*, 2016). Whether intersexual trade-offs result in intralocus sexual conflict depends on how close to the fitness peak condition-dependence and intermediate fecundity are, at equilibrium, for respectively males and females.

Somatic buffering as an evolutionary driver of polyphenism in acarid mites: untested assumptions

Like the alternative mating tactics hypothesis, the somatic buffering hypothesis for the evolution of condition-dependency in male traits relies on several untested assumptions (Table 1b). Firstly, it implies that there is selection for enlarged fighter legs, with thicker legs yielding higher fitness at least in large males, and possibly in males in general (Chapter 3). While it has not explicitly been tested whether thicker legs yield higher fitness, the fighter phenotype has been shown to result in higher mating success than the scrambler phenotype in small populations of the related species *Sancassania berlesei* (Michalczyk *et al.*, 2018). Interestingly, this is not the case in large *S. berlesei* populations (Michalczyk *et al.*, 2018). In this species, unlike in *R. robini*, high population density adaptively suppresses fighter expression (Radwan, 1993, 1995), casting doubt

on whether patterns in *S. berlesei* generalize to *R. robini*, despite the morphologically similar fighter-scrambler polyphenism.

A second assumption is that selection strongly acts on life history timing (Chapter 3). Mites should prioritize maintaining optimal timing of metamorphosis, and cannot compensate for lack of resources by postponing reproduction and additional growth. This scenario is untested, but seems plausible in acarid mite populations. Juvenile mortality is high (Smallegange & Deere, 2014), while the reproductive environment (i.e. rotting underground plant structures) deteriorates over time as food is consumed and population density increases (Díaz *et al.*, 2000). Selection thus likely favours early maturation (Stearns, 1992).

A third assumption is the existence of developmental mechanisms causing trade-offs between sexual traits and other developmental functions (Chapter 3). These trade-offs can be 'Y-model' type resource allocation trade-offs, in which resources not invested in sexual traits might instead go towards maintaining growth or accelerating metamorphosis (de Jong & van Noordwijk, 1992). They can also be functional-interference tradeoffs, in which development of sexual traits involves processes also involved in other developmental functions, and therefore inhibits adaptive employment of such processes (Hill, 2011; Warren *et al.*, 2013). While Chapter 6 demonstrates that fighter leg development trades off with growth and development time, the mechanisms underlying these trade-offs are unknown.

A fourth untested assumption is that male scrambler expression is cued by stress signals (i.e. deviations from homeostasis) in prioritized functions (Chapter 3). Hence, fighter expression should occur in the absence of stress. Assessing this assumption requires mechanistic understanding of metamorphosis and male phenotype development, which is currently lacking. We do know that the fighter-scrambler polyphenism in *R. robini* is associated with differential gene expression in conserved metabolic processes (Joag *et al.*, 2016), but the precise developmental functions of the differentially expressed genes during metamorphosis are unclear (Joag *et al.*, 2016).

Finally, a fifth untested assumption is that males experience developmental effects of polyphenism (i.e. flexible metamorphosis timing, condition- and phenotype-dependent reduction in body size during metamorphosis, Chapter 6) directly *because* of development of alternative morphs that differ in energetic costs (Chapter 3). That is, the developmental effects of polyphenism on growth and life history timing represent unavoidable knock-on consequences of development, rather than evolved and co-adapted variation within an adaptive 'scrambler syndrome' or 'fighter syndrome' consisting of life history traits in concert with morphology. Assessing this assumption

entails an experimental approach, in which fighter expression is triggered in poor-condition males that would otherwise have become scramblers, and suppressed in high-condition males that would otherwise have been fighters. Unfortunately, such phenotypic engineering experiments are not yet possible in *R. robini*, because the mechanisms underlying metamorphosis and male phenotype development are unknown. An observational approach that does not control male phenotype expression (as taken in Chapter 6) cannot test causal assumptions.

All in all, the available data fit the somatic buffering hypothesis rather well. The somatic buffering hypothesis provides a single evolutionary mechanism for several striking phenomena in acarid mites: condition-dependent polyphenism (Chapter 4), trade-offs between male weaponry and male life history traits (Chapter 6), trade-offs between male weaponry and female life history traits (Plesnar-Bielak *et al.*, 2014; Van den Beuken & Smallegange, 2018), a connection of male polyphenism with differential expression of genes involved in conserved metabolic processes (Joag *et al.*, 2016), and allometry plasticity (Chapter 4). Without somatic buffering, these phenomena have no unifying mechanism; they would have to be explained by separate hypotheses if somatic buffering is not an important evolutionary mechanism underlying the maintenance of condition-dependence. However, again, mere consistence with data does not test a hypothesis, and the somatic buffering hypothesis entails a considerable list of untested assumptions.

7.4 — Can we estimate the contributions of somatic buffering and alternative mating tactics to selection for condition-dependent polyphenism?

Adaptation to alternative mating tactics and adaptive somatic buffering can both contribute to selection for adaptive condition-dependent polyphenism. In my thesis, I aimed to provide evidence for these evolutionary mechanisms. However, to understand how these processes contribute to shaping net selection on condition-dependence, it is ultimately necessary to measure selection itself, and to partition it into the contribution from somatic buffering (viability selection, selection due to life history timing) and alternative mating tactics (sexual selection). There are two main approaches for inferring selection that may be (or become) feasible in laboratory populations of acarid mites.

Firstly, selection can be inferred bottom-up, from phenotype to performance to fitness (Arnold, 1983). In bottom-up inference of condition-dependent selection gradients, the main empirical focus lies on how condition-dependent expression of alternative male morphs impacts different components of performance (e.g. timing of

metamorphosis, age and size at maturity, probability of mating, walking speed, susceptibility to infection, feeding efficiency) for both morphs, over the range of condition. Selection gradients are then inferred by statistically modelling the links between performance and fitness (Irschick *et al.*, 2008). Because the contributions of sexual selection for alternative mating tactics and viability selection for somatic buffering are modelled separately, inferring the relative importance of both processes to total selection is possible. Evidently, such bottom-up estimates of selection are sensitive to modelling choices, missing components of performance and overlooked correlations among performance components. Yet, the phenotype-performance-fitness paradigm has been quite popular and fruitful in evolutionary ecology (Lailvaux & Husak, 2014).

Secondly, selection for condition-dependent polyphenism can be inferred top-down (Arnold & Wade, 1984). In top-down inference of selection, the empirical focus is on measuring lifetime reproductive success for males of different morphs, over the complete condition gradient. Using two distinct population treatments, it is in principle possible to decompose total selection into a contribution from sexual selection (alternative mating tactics) and a contribution from viability selection and selection due to life history timing (somatic buffering). In a 'natural population structure' treatment, lifetime reproductive success of phenotyped males (condition at metamorphosis, fighter or scrambler) should be measured in naturally structured populations, and such males should be followed from the juvenile stage until death, allowing maturation timing to affect fitness. This would allow measuring total selection for condition-dependent polyphenism. In a 'relaxed mate competition' treatment, populations should be set up exactly the same way as in the 'natural population structure' treatment, but males should be isolated at maturation and provided with *ad libitum* mating opportunities, to reduce opportunity for sexual selection as much as possible. Residual variation in lifetime reproductive success then reflects viability and maturation timing differences, and the difference between the selection gradients in both treatments should reflect the contribution of sexual selection for alternative mating tactics.

The top-down approach faces at least two substantial challenges. Firstly, focal males should have all possible combinations of condition at metamorphosis and adult phenotype. This requires experimental induction or suppression of fighter or scrambler expression, to prevent natural collinearity of adult male phenotype and condition. Secondly, it is necessary to track a focal male's lifetime reproductive success, and acarid mites are too small to allow non-destructive genetic sampling. A potential solution is

that adult males can be made sterile through irradiation: eggs fertilized by irradiated males do not hatch (Radwan, 1991; Michalczyk *et al.*, 2018). It is therefore possible to construct populations in which all males are sterile except one focal male, allowing measuring a focal male's lifetime reproductive success as the number of viable eggs in the population (Michalczyk *et al.*, 2018).

7.5 — Conclusions and outlook

Condition-dependent alternative developmental trajectories are commonplace in sexually selected traits. An often-made default assumption is that because the high-condition phenotype is a particular adaptation to mate acquisition, both alternative morphs are adaptations to alternative mating tactics (Gross, 1996; Shuster & Wade, 2003). This assumption should be evaluated. Poor-condition developmental trajectories may well evolve under different selection pressures, because poor-condition individuals potentially face very different ecological challenges than high-condition individuals. Individuals in poor condition may experience an environment in which they should primarily salvage viability while maintaining optimal life history timing. On the other hand, individuals in good condition experience an environment in which they should primarily maximize social capital and/or mate acquisition. In general, such condition-dependent diversification of environmental affordances likely contributes to initial evolution of the capacity of developmental systems to generate variation, and hence to adapt under natural selection.

The results presented in this thesis represent the first steps towards unravelling the selection pressures shaping condition-dependent polyphenism in acarid mites. Both sexual selection for alternative mating tactics and viability selection for adaptive somatic buffering are likely to contribute, but their relative importance is not yet understood. In this Synthesis, I have sketched the outlines of a research program that could shed further light on how selection maintains condition-dependent development of sexual traits, using the alternative male morphs of *R. robini* as a case study. Both the alternative mating tactics component and the somatic buffering component of adaptive plasticity rely on assumptions still lacking empirical support (Table 1). Furthermore, it is ultimately necessary to measure selection on condition-dependent polyphenism itself, and dissect it into the contribution from sexual selection (selection attributable to variation in fertilization success), and the contribution from residual natural selection (selection attributable to variation in viability, fecundity and life history timing). Evidently, male secondary sexual traits evolve under sexual selection (Andersson & Simmons, 2006). But *adaptive condition-dependent variation* in such traits can

only fully be understood from a holistic perspective, with an emphasis on trade-offs among fitness components.

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Summary

The ecology of adaptive condition-dependent polyphenism

Individual organisms vary within populations. Much of this variation is directly due to the fact that individual organisms represent developing entities. In development, the organism (re)constructs itself in continuous interaction with its individually experienced environment. Since each individual organism experiences its own unique environment, some developmental variation is inevitable. This can have substantial fitness consequences; individuals varying in development are not likely to survive and reproduce at the same rate. Hence, they differ in *condition*: their phenotypic expectation of future reproductive success. Such variation in condition tends to correspond with variation in the kind of opportunities an organism gets during its life cycle. When that is the case, a form of adaptive condition-dependent plasticity can evolve that 'makes the best of the situation': condition-cued alternative developmental trajectories. But it remains poorly understood *how* environmental affordances vary with condition, *how* condition-dependent plasticity adaptively anticipates ecological opportunity and mitigates physiological stress, and hence how organisms maintain the adaptive capacity for condition-dependent flexibility under selection.

One of the more conspicuous forms of condition-dependent plasticity can be found in the morphology of secondary sexual traits. In many animals, and particularly in arthropods, male secondary sexual trait development is disproportionally sensitive to an individual's history of growth and development, and thereby its condition. Within populations, relatively large individuals generally possess well-developed sexual traits, while small individuals often develop proportionally smaller traits, sometimes even a discretely different 'minor' phenotype. *How does selection maintain such condition-dependent plasticity in morphological traits?* In my thesis I address this question, focusing on the acarid mite *Rhizoglyphus robini* as a case study. Male *Rhizoglyphus* mites show a condition-dependent polyphenism, with discretely different phenotypes. Large, well-fed males develop an enlarged third leg pair used as lethal weapons (the fighter morph), while small poorly-fed males develop unmodified legs (the scrambler morph). How does selection favour scrambler expression in smaller males, and fighter expression in larger males?

In Chapter 2, I argue that the evolution of condition-dependent plasticity should be understood at multiple levels of organization. Genotype-level explanations focus on maintenance of genetic variation underlying reaction norms, while phenotype-level

explanations focus on the shape of the condition-dependent reaction norm and thereby coexistence of alternative phenotypes. I briefly review genotype-level and phenotype-level hypotheses for condition-dependent polyphenism pertaining to the bulb mite system. Genetic variation in condition-dependent reaction norms can be maintained through various mechanisms of balancing selection, or through selection-mutation balance. The shape of the reaction norm evolves by selection for adaptive plasticity, which emerges through differences in how small and large individuals engage with their environments. Such selection pressures can result in *information-based mechanisms* of adaptive plasticity, in which organisms adaptively anticipate variable future environments as they perceive cues from the current environment, by developing different morphologies. However, development of morphological traits can interfere with allocation to other (prioritized) developmental functions, and the degree to which such trade-offs affect development is predicted to be condition-dependent. Therefore, adaptive condition-dependent plasticity can also evolve in the form of *somatic-state-based mechanisms,* in which organisms adaptively mitigate current physiological stress associated with poor condition by developing different morphologies, allowing maintenance of viability while preventing detrimental life history delays.

In CHAPTER 3, I zoom in on the ecology of condition-dependent sexual trait polyphenism, and the selection pressures favouring condition-dependent alternative developmental trajectories. I review how individuals within populations accumulate variation over the course of their development, how such developmental variation has fitness consequences (i.e. condition variation), and how organisms can adaptively adjust development to mitigate detrimental fitness effects of condition variation (Making the Best of a Bad Job) including non-mutually-exclusive information-based (i.e. *alternative mating tactics*) and somatic-state-based (i.e. *somatic buffering*) hypotheses. Finally, I make an inventory of published records of condition-dependent polyphenism in secondary sexual traits in arthropods, and review empirical evidence supporting the alternative mating tactics and somatic buffering hypotheses for the evolution and maintenance of condition-dependent polyphenism.

In CHAPTER 4, I describe the shape of the condition-dependent reaction norm in *R. robini*. Firstly, I test the hypothesis that male morph expression is determined at metamorphosis, and not earlier during juvenile development. Secondly, I test whether size-dependent expression of alternative male phenotypes is subject to nutrition-dependent allometry plasticity. That is, not only does expression of enlarged fighter legs depend on body size at metamorphosis, this size-dependence itself may plastically shift with nutritional conditions, giving rise to plasticity on multiple levels. I find that

male morph expression is best explained by variation in body size and growth immediately before metamorphosis, and not earlier in development, supporting that condition-dependent male morph expression is determined at metamorphosis. I also find that the body size threshold for male morph expression at metamorphosis shifts towards lower body sizes when mites are raised on nutrient-poor diets, confirming allometry plasticity. Nutrient-dependent allometry plasticity explains some of the considerable body size overlap between fighters and scramblers, and can be interpreted in the context of the alternative mating tactics hypothesis (food quality and body size may jointly cue future mating opportunities), or in the context of the somatic buffering hypothesis (physiological stress may occur at different body sizes depending on food quality and growth rate).

In Chapter 5, I investigate whether social opportunities favour condition-dependent male phenotype expression in *R. robini*. According to the alternative mating tactics hypothesis, repeatable and predictable (e.g. size-based) mating niche differentiation drives the evolution of adaptive, anticipatory plasticity in morphological traits. I test the prediction that aggressive contests among fighter males over a mating opportunity are more often won by the larger and stronger male, so that smaller fighters are disadvantaged in aggressive interactions. Additionally, I test the prediction that scramblers are not adapted to the fighting niche in a mating context, so contests among a fighter and a scrambler are won by the fighter regardless of the body size difference, and so copulations last shorter in the presence of a rival fighter than in the presence of a rival scrambler. I find that fighters in contests with other fighters are more likely to mate first, but not more likely to interrupt an ongoing copulation, if they are larger than their rival. I also find that that fighters in contests with scramblers are likely to mate first regardless of the body size difference between the two males. Finally, copulations do last shorter in the presence of a rival fighter rather than a scrambler, but this is not attributable to aggressive interruption of copulations by fighters. I conclude that that the fighting niche is particularly accessible for large males, as larger males have a higher probability of winning pre-copulatory contests. Such mating niche differentiation likely contributes to evolutionary maintenance of condition-dependent male polyphenism, consistent with the alternative mating tactics hypothesis.

In Chapter 6, I investigate the link between condition-dependent development of alternative male phenotypes and the timing of maturity in bulb mites. According to the somatic buffering hypothesis, since metamorphosis is energetically expensive, adaptively flexible timing of metamorphosis could be facilitated by developmental plasticity in sexual traits that are expensive to produce during metamorphosis. I first

test the hypothesis that scrambler males are able to mature quicker than fighter males, given a particular growth trajectory. I then test the hypothesis that mites accelerate the onset of metamorphosis in response to the removal of food, optimizing maturation timing with respect to growth conditions. Finally, I test whether increased competition for food among juveniles results in a larger proportion of males developing as scramblers, enabling them to mature relatively early and escape mortality. I find that males with similar growth rate are able to enter metamorphosis earlier and at a smaller body size if they develop as scramblers rather than fighters. Consequently, the reaction norm of age and size at maturity is more variable in males than in females, which lack polyphenism. I also find that males and females accelerate the onset of metamorphosis in response to deteriorating growth conditions, but I do not detect a direct effect of acceleration on male morph expression. Finally, I find that an experimental increase in competition for food is associated with increased juvenile mortality, decreased growth rate, and increased scrambler expression. I conclude that polyphenic release of sexually selected constraints likely facilitates adaptive condition-dependent life history flexibility, consistent with the somatic buffering hypothesis.

In my thesis, I have aimed to conceptualize a holistic perspective on the evolution of adaptive condition-dependent plasticity. Selection for adaptive condition-dependence involves alternative mating tactics as well as somatic buffering, and *Rhizoglyphus* mites show signatures of both selective mechanisms.

Samenvatting

De ecologie van adaptieve conditie-afhankelijke polyfenismen

Organismen, gedurende hun ontwikkeling, (re)construeren zichzelf in constante wisselwerking met hun individueel ervaren omgeving. En omdat ieder individueel organisme zijn eigen patroon van omgevingsstimuli ervaart, is het onvermijdelijk dat individuele ontwikkelingstrajecten onderling variëren. Dit simpele gegeven kan aanzienlijke consequenties hebben voor de fitness van een organisme: individuen die variëren in hoe ze ontwikkelen variëren waarschijnlijk ook in de snelheid waarmee ze reproduceren, en in de kans dat ze overleven. Variërende organismen verschillen daarom niet alleen in hoe ze eruit zien en wat ze doen (grofweg: hun fenotype), maar ook in wat we conditie noemen: hun fenotypische verwachting van toekomstig reproductief success. Zulke conditievariatie correspondeert vaak met variatie in het soort ecologische mogelijkheden en kansen dat een organisme vervolgens tijdens zijn levenscyclus krijgt. Als dat stelselmatig zo is, kan er een vorm van conditie-afhankelijke plasticiteit (grofweg: flexibiliteit in ontwikkeling en functie) evolueren, die een organisme in adaptief in staat stelt om 'er het beste van te maken': alternatieve ontwikkelingstrajecten, die in gang gezet worden door een schatting van conditie op een bepaald kritiek moment tijdens ontwikkelling. In mijn proefschrift onderzoek ik op welke manier omgevings-affordanties variëren met conditie, hoe conditie-afhankelijke plasticiteit vervolgens adaptief ecologische kansen anticipeert en fysiologische stress mitigeert, en daarmee hoe organismen onder natuurlijke selectie het adaptieve vermogen tot conditie-afhankelijke flexibiliteit behouden.

Een opvallende voorbeeld van conditie-afhankelijke plasticiteit is te vinden in de morfologie van dierlijke secundaire seksuele kenmerken. Bij veel diersoorten, en met name bij geleedpotigen, is het zo dat de ontwikkeling van mannelijke secundaire seksuele kenmerken buitenproportioneel gevoelig is voor variatie in groei en ontwikkeling, en daarmee conditie. Relatief grote individuen in een populatie laten dan vaak goed ontwikkelde seksuele kenmerken zien, terwijl relatief kleine individuen kleinere seksuele kenmerken hebben dan je op basis van proporties zou verwachten. Regelmatig hebben kleine individuen zelfs een kwalitatief verschillend 'minor' fenotype. *Op welke manier behoudt natuurlijke selectie zulke conditie-afhankelijke plasticiteit in morfologische kenmerken?* In mijn proefschrift richt ik mij op deze vraag. Mijn focus ligt met name op de acaride mijt *Rhizoglyphus robini*, de bloembollenmijt, als gevalsstudie. In het genus *Rhizoglyphus* laten de mannetjes een conditie-afhankelijk polyfenisme zien

(dat wil zeggen, plasticiteit resulterend in kwalitatief verschillende fenotypen). Grote, goed gevoede mannetjes ontwikkelen een verdikt derde pootpaar, dat ze gebruiken als dodelijk wapen (de vechter-vorm). Kleine, slecht gevoede mannetjes ontwikkelen een 'normaal' derde pootpaar, dat lijkt op de andere pootparen (de loper-vorm). Op welke manier geeft de loper-vorm een fitnessvoordeel aan kleinere mannetjes, en heeft de vechter-vorm een voordeel voor grote mannetjes?

In HOOFDSTUK 2 beargumenteer ik dat er verklaringen op meerdere organisatieniveaus nodig zijn om de evolutie van conditie-afhankelijke plasticiteit te begrijpen. Verklaringen op het niveau van het genotype richten zich op *genetische variatie* in reactienormen (grofweg: de curve die het verband beschrijft tussen het fenotype en een bepaalde omgevingsvariabele), en zijn daarmee belangrijk om te begrijpen waarom de erfelijke variatie niet erodeert onder selectie. Dat zou evolutie van de reactienorm tot stilstand doen komen. Verklaringen op het niveau van het fenotype richten zich op de *vorm* van de reactienorm, en zijn daarmee cruciaal om te begrijpen hoe verschillende fenotypen binnen een polyfenisme naast elkaar kunnen bestaan zonder dat de één de ander wegconcurreert. Ik geef een kort overzicht van van verschillende hypotheses op genotype- en fenotype-niveau met betrekking tot de evolutie van het vechter-loper-polyfenisme bij bloembollenmijten. Genetische variatie in conditie-reactienormen kan worden behouden door verschillende mechanismen van balancerende selectie, of door mutatie-selectie-evenwicht. De vorm van de reactienorm evolueert door selectie voor adaptieve plasticiteit, die ontstaat door verschillen in hoe grote en kleine individuen omgaan met hun omgeving. Zulke selectie kan resulteren in *informatie-gebaseerde* mechanismen van adaptieve plasticiteit, waarin organismen op basis van aanwijzingen uit hun huidige omgeving de toekomstige omgeving anticiperen door een passende morfologie te ontwikkelen. De ontwikkeling van dit soort morfologische kenmerken kan echter negatieve bijeffecten hebben (stress) op andere ontwikkelingsfuncties (met prioriteit), en de mate waarin dit soort trade-offs ontwikkeling beïnvloeden is conditie-afhankelijk. Daarom kan de evolutie van adaptieve conditie-afhankelijke plasticiteit ook gedreven worden door *somatische-staat-gebaseerde* mechanismen. Organismen mitigeren of voorkomen dan de fysiologische stress die geassocieerd is met slechte conditie, door op alternatieve wijze te ontwikkelen. Zulke mechanismen beschermen viabiliteit, terwijl ze tegelijkertijd voorkomen dat de afstemming van belangrijke levensgebeurtenissen (metamorfose, reproductie) nadelig vertraagt.

In HOOFDSTUK 3 richt ik mij verder op de ecologie van conditie-afhankelijk polyfenisme in seksuele kenmerken, en op de selectiedrukken die conditie-afhankelijke alternatieve ontwikkelingstrajecten vormgeven. Ik leg kort uit wat we weten over hoe

individuele organismen binnen populaties fenotypische variatie accumuleren gedurende ontwikkeling, hoe zulke variatie gevolgen heeft voor fitness (dat wil zeggen: conditievariatie), en hoe organismen adaptief hun ontwikkeling aan kunnen passen om nadelige fitnesseffecten van conditievariatie te mitigeren ('het beste maken van een slechte situatie') inclusief hypotheses over informatie-gebaseerde (alternatieve paartactieken) en somatische-staat gebaseerde (somatisch bufferen) mechanismen. Tot slot presenteer ik een overzicht van gepubliceerde gevallen van conditie-afhankelijk polyfenisme in secundaire seksuele kenmerken van geleedpotigen, en een overzicht van het bewijs voor de 'alternatieve paartactieken' en 'somatisch bufferen' hypotheses.

In HOOFDSTUK 4 beschrijf ik de vorm van de conditie-reactienorm in *R. robini*. Als eerste test ik de hypothese that de expressie van alternatieve mannelijke vormen bepaald wordt tijdens de (incomplete) metamorfose, en niet eerder tijdens juveniele ontwikkeling. Daarna test ik of grootte-afhankelijke expressie van alternatieve mannelijke vormen onderworpen is aan nutritie-afhankelijke allometrie-plasticiteit. Dat wil zeggen dat plasticiteit aanwezig is op meerdere niveaus: niet alleen hangt de expressie van verdikte vechterpoten af van lichaamsgrootte tijdens metamorfose, maar ook deze afhankelijkheid *zelf* kan veranderen in variërende voedingsomstandigheden. Mijn bevinding is dat de expressie van alternatieve mannelijke vormen het beste verklaard wordt door variatie in lichaamsgrootte en groei vlak voor metamorfose, en niet eerder gedurende ontwikkeling, wat de hypothese ondersteunt dat conditie-afhankelijke expressie van alternatieve vormen bepaald wordt tijdens metamorfose. Ik zie ook dat de lichaamsgrootte-drempel voor de expressie van de vechter-vorm tijdens metamorfose verschuift richting kleinere lichaamsgroottes wanneer de mijten opgroeien met een nutrienten-arm dieet, wat allometrie-plasticiteit bevestigt. Dit verklaart een deel van de aanzienlijke lichaamsgrootte-overlap tussen vechters en lopers, en kan worden geïnterpreteerd in de context van de alternatieve-paartactieken-hypothese (een combinatie van ervaren voedselkwaliteit en lichaamsgrootte zou toekomstige vecht- en paarkansen kunnen voorspellen), of in de context van de somatisch-bufferen-hypothese (kritische fysiologische stress zou op kunnen treden bij verschillende lichaamsgroottes, afhankelijk van voedselkwaliteit en groeisnelheid).

In HOOFDSTUK 5 onderzoek ik of conditie-afhankelijke expressie van alternatieve mannelijke vormen gedreven kan worden door sociale kansen. Volgens de alternatieve-paartactieken-hypothese wordt de evolutie van adaptieve anticiperende plasticiteit in mannelijke morfologische kenmerken gedreven door betrouwbare en voorspelbare (bijv. grootte-afhankelijke) differentiatie in de paarniche (grofweg: de manier waarop een individu zijn omgeving kan exploiteren om te paren). Ik test de

voorspelling dat conflicten tussen vechtermannetjes over een kans om te paren vaker gewonnen worden door het grootste mannetje, zodat kleinere vechters een nadeel hebben in agressieve interacties. Ook test ik de voorspelling dat lopers niet aangepast zijn aan de vechtniche, zodat gevechten tussen een vechter en een loper met hoge waarschijnlijkheid gewonnen worden door de vechter ongeacht het lichaamsgrootte-verschil, en zodat copulaties korter duren in de aanwezigheid van een concurrerende vechter dan in de aanwezigheid van een concurrerende loper. In gevechten tussen twee vechters om een vrouwtje heeft het grootste mannetje inderdaad een grotere kans om als eerste te paren. Er is echter geen bewijs dat de kans dat een vechter een bestaande copulatie interrumpeert afhangt van lichaamsgrootte; dit gebeurt zelden. In gevechten tussen een vechter en een loper om een vrouwtje is het inderdaad de vechter die een grotere kans heeft om als eerste te paren, onafhankelijk van het verschil in lichaamsgrootte. Tenslotte duren copulaties inderdaad minder lang in de aanwezigheid van een concurrerende vechter in plaats van een concurrerende loper, maar dit had niet te maken met agressieve interrupties van copulaties door vechters. De conclusie is dat de vechtniche vooral toegankelijk is voor grotere mannetjes, omdat grotere mannetjes een hogere kans hebben om gevechten vóór de paring te winnen dan kleinere mannetjes. Zulke differentiatie in de paarniche kan bijdragen aan het evolutionaire behoud van conditie-afhankelijk mannelijk polyfenisme, overeenkomend met de alternatieve-paartactieken-hypothese.

In Hoofdstuk 6 onderzoek ik het effect dat conditie-afhankelijke ontwikkeling van alternatieve mannelijke vormen heeft op wanneer bollenmijten volwassen worden. Volwassenheid wordt ingeluid door (incomplete) metamorfose, en metamorfose is een energetisch duur proces dat aangedreven wordt door opgeslagen reserves, geaccumuleerd tijdens de voorgaande juveniele groeifase. Tegelijkertijd hangt het van omstandigheden af wanneer een mijt het beste kan stoppen met groeien en beginnen met reproduceren, en de timing van volwassenheid is daarom flexibel. De somatisch-bufferen-hypothese stelt dat dit soort adaptieve flexibiliteit in de timing van volwassenheid gefaciliteerd kan worden door ontwikkelingsplasticiteit in secundaire seksuele kenmerken die energetisch duur zijn om tijdens de metamorfose te produceren. Ik test eerst de voorspelling dat, gegeven een bepaald groeitraject, loper-mannetjes eerder de metamorfose in gaan dan vechter mannetjes. Vervolgens onderzoek ik of mijten eerder de metamorfose in gaan wanneer hun voedsel wordt weggehaald, en zo de timing van volwassenheid aanpassen aan de hand van groeiomstandigheden. Ten slotte onderzoek ik of een toename in competitie voor voedsel onder juvenielen resulteert in een groter aandeel mannetjes dat zich als loper ontwikkelt, wat ze in staat zou stellen

om eerder volwassen te worden en juveniele mortaliteit te ontwijken. Mijn bevindingen zijn de volgende. Ten eerste: mannetjes met gelijkende groeisnelheid kunnen de metamorfose tot volwassenheid eerder beginnen als ze vervolgens loper worden in plaats van vechter. De relatie tussen leeftijd en grootte bij volwassenheid is daarom variabeler bij mannetjes (die polyfenisch zijn) dan bij vrouwtjes (die niet polyfenisch zijn). Ten tweede: zowel mannetjes als vrouwtjes beginnen eerder met metamorfose zodra de groeiomstandigheden verslechteren. Ik kon echter geen bewijs vinden dat deze voedsel-geïnduceerde versnelling een direct effect had op de expressie van alternative mannelijke vormen. Ten slotte, een experimentele toename in competitie voor voedsel onder juvenielen resulteert in hogere juveniele mortaliteit, lagere groeisnelheid, en een hoger aandeel mannetjes dat zich als loper ontwikkelt. Mijn conclusie is dat polyfenisme seksueel geselecteerde constraints kan verlichten, en zo bij kan dragen aan adaptieve flexibiliteit in de timing van volwassenheid. Dit komt overeen met de somatisch-bufferen-hypothese.

In mijn proefschrift heb ik mij gericht op het conceptualiseren van een holistisch perspectief op de evolutie van adaptieve conditie-afhankelijke plasticiteit. Selectie voor adaptieve conditie-afhankelijkheid in secundaire seksuele kenmerken omvat zowel selectie voor alternatieve paartactieken als selectie voor somatisch bufferen, en mijten in het genus *Rhizoglyphus* laten het signatuur van beide mechanismen zien.

Acknowledgements

This is a thesis about the evolution of developmental plasticity. It was supposed to be about something else. When I joined the DynaMite lab back in 2015, my first supervisor Isabel Smallegange was busy working on mechanistic eco-evolutionary population models, to study the role of eco-evolutionary feedbacks in population dynamics. It fell on my shoulders to put these theoretical ideas to the test, using a popular model organism for population dynamics research: the tiny bulb mite. At the time, our thinking was that these mites enabled us to track the evolutionary fate of alternative alleles without doing too much molecular work. The mites had a striking male polymorphism, in which alternative morphs were supposed to be associated with alternative alleles (which seemed to be backed up by the literature). So, we dreamt of inferring evolutionary change from change in male morph frequencies, and of inferring eco-evolutionary feedback from population consequences of shifts in male morph frequencies. But Isabel had noticed something funny about the polymorphism: while alternative male morphs were certainly heritable, their expression was also strikingly sensitive to food quality, and this had been downplayed in the literature. The evident plasticity of male morph expression in the bulb mite complicated things regarding the original plan. But I also found it fascinating! It became a major distraction, and eventually my thesis topic. Perhaps for the better, because it would have been difficult to study the eco-evolutionary dynamics of alternative male phenotypes without understanding how the underlying plasticity evolves. I am immensely grateful to Isabel, for letting me figure out what I felt I needed to do, for allowing me to change course, and for the many interesting and stimulating conversations we have had along the way. It must be difficult to see your PhD student go off-road. But I have learned so much!

As my project evolved, so did my supervisory team, and I want to thank them all. In the very early stages of my project, when I was still figuring out how to approach my study of bulb mite males, André de Roos was (briefly) involved as my promotor. After Isabel's DynaMite lab had moved to the Evolutionary and Population Biology group, Peter de Ruiter took over from André. Peter had a hands-on style of being promotor, which meant regular meetings and many conversations, and invariably he would say something that would made me think, *hmm, I haven't quite thought this through yet*. 'Read Lewontin,' said Peter. I did (and am continuing doing so) and I am very happy that I did it; Richard Lewontin has become a major influence on my perspective on evolutionary biology. Later still, Kathryn Stewart and Astrid Groot became

involved in my project. Kat became second supervisor, and Astrid took over Peter's role as promotor when he retired. The addition of Kat and Astrid to the team was great, because my project was sliding towards organismic biology, and away from Isabel's comfort zone of theory-rich population biology. Kat in particular made a major contribution to my work, by reading drafts, discussing ideas, and giving the occasional pep talk when I needed one. Thanks to André, Peter, Kat and Astrid.

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Now it is finished.

Author contributions

2. Cross-level considerations for explaining selection pressures and the maintenance of genetic variation in condition-dependent male morphs
   Isabel M. Smallegange, Flor T. Rhebergen* & Kathryn A. Stewart*
   IMS, FTR and KAS contributed ideas and wrote the manuscript.
   * Shared first authorship

3. 'Making the Best of a Bad Job' revisited: the evolution of adaptive, condition-dependent expression of secondary sexual traits in arthopods
   Flor T. Rhebergen & Isabel M. Smallegange
   FTR conceptualized the ideas and wrote the manuscript. IMS critically evaluated the argumentation and commented on manuscript text.

4. Nutrient-dependent allometric plasticity in a male-diphenic mite
   Flor T. Rhebergen, Kathryn A. Stewart & Isabel M. Smallegange
   FTR designed and conducted the experiments and analysed the data. FTR, KAS and IMS wrote the manuscript.

5. Size-dependent mating niche differentiation in the male-polyphenic mite *Rhizoglyphus robini*
   Flor T. Rhebergen, Maarten Wijstma & Isabel M. Smallegange
   FTR and MW designed the experiments and analysed the data. MW conducted the experiments. FTR and IMS wrote the manuscript.

6. Facultative release from developmental constraints through polyphenism promotes adaptively flexible maturation
   Flor T. Rhebergen & Isabel M. Smallegange
   FTR designed and conducted the experiments and analysed the data. FTR and IMS wrote the manuscript.