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

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Condition-dependent expression of alternative male morphologies (AMMs) 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 of AMM expression, 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 AMM expression in acarid mites. We thus conclude that these aspects require specific attention in identifying selection pressures maintaining condition-dependent plasticity, and evolutionary processes that maintain genetic variation in condition-dependent phenotypic plasticity.

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Introduction

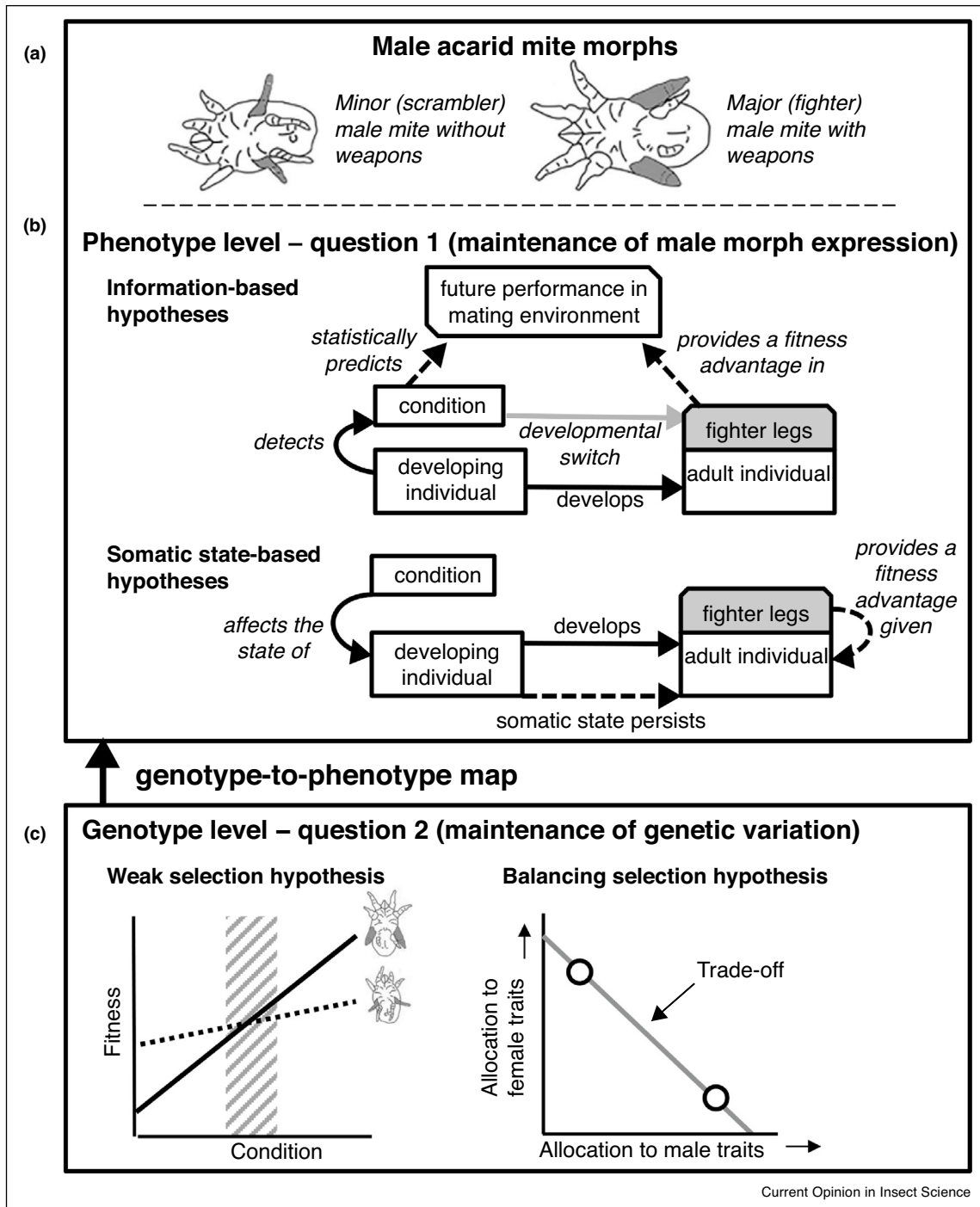
Male secondary sexual traits are often differently expressed depending on nutrition during development [1]. In many arthropods, such condition-dependent plasticity leads to male dimorphism [2–4], with ensuing within-population male morph coexistence having evolved multiple times. Understanding the evolutionary causes of male dimorphism 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 minors and majors [5–8, but see Ref. 9^{*}]. Often, majors are large and possess morphological structures that are used as weapons in male-male competition, while minors are smaller, lack (large) weapons and adopt a sneaker strategy to gain access to females. 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 many 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 tunneling past the guarding fighters [10]. 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 defenseless minors (scramblers) with unmodified legs (Figure 1a) [4].

Critical resource or body size thresholds underlying alternative male morph (AMM) expression can evolve rapidly in response to artificial selection (acarid mites [11–13]) or after introduction to novel environments (dung beetles [14]; earwigs [15]; acarid mites [11]). This raises two questions. Firstly, what ecological selection pressures maintain condition-dependent plasticity and set the threshold for AMM expression? Secondly, since rapid evolutionary responses suggest considerable threshold heritability, what maintains genetic variation associated with condition-dependent plasticity? Here, we illustrate how explanations for each question should be sought at either the phenotype or genotype level. Studies, however, often conflate the levels, or make unjustified inferences on how the two levels are linked. Focusing on the acarid mite model system, where a number of species show condition-dependent AMM expression depending on juvenile nutrition and somatic growth (Table 1), we review different hypotheses that can be employed to tackle each question. Our goal is to illustrate how a conceptual distinction between two levels of explanation (phenotype and genotype) will yield explanatory power to understand the evolution and

Figure 1



Conceptual diagram showing how, exemplified for the male dimorphism in acarid mites (a), answers to the two questions tackled in this review require explanations at different levels. (b) The first question — what are the ecological selection pressures that maintain condition-dependent plasticity and that set the threshold for AMM expression — can be tested using two, non-mutually exclusive hypotheses. *Information-based hypotheses* state that an individual develops fighter legs or not (grey block) cued by current condition (e.g. body size) as a predictor for future performance in the mating environment (grey arrow). The developmental switch is therefore an anticipatory mechanism allowing different behavioural mating tactics. *Somatic state-based hypotheses* state that individual condition may or may not prevent individuals from developing fighter legs, as investment in fighter legs would cause lasting harm if undertaken under bad condition. The developmental switch is therefore a stress-mitigating mechanism allowing maturation under adverse circumstances [27**]. Black solid arrows denote processes occurring in developmental time; dashed arrows denote relationships that hold over evolutionary time. (c) The second question — what maintains genetic variation associated with condition-dependent plasticity in AMMs — can be tested using two hypotheses. The *weak selection hypothesis* states

Table 1

Mite species in which males plastically develop as fighters or as scramblers. Excluded are species within the genera *Cheyletus* and *Schwiebia* where male morphologies have been described, but no further life history information exists (e.g. Refs. [16,17])

| Species | Fighter morphology | Condition-dependent male morph expression | Other cues for male morph expression | Crossing fitness functions |
|-------------------------------|--|---|--|-----------------------------------|
| <i>Falculifer rostratus</i> | Elongated first and second legs, and elongated lower cheliceral digits | Yes (exploitation competition) [18] | Not known | Not known |
| <i>Rhizoglyphus echinopus</i> | Thickened, muscular third legs (Figure 1a) | Yes (juvenile diet and temperature) [19]; No (juvenile diet) [19] | Population density [20]; colony size [19,20] | In relation to colony size [20] |
| <i>Rhizoglyphus robini</i> | Thickened, muscular third legs (Figure 1a) | Yes (juvenile diet) [21,23] | Not population density [21] | Not known [22] |
| <i>Sancassania anomalus</i> | Thickened, muscular third legs (Figure 1a) | Yes (juvenile diet and temperature) [24] | Population density [24] | Not known |
| <i>Sancassania berleseii</i> | Thickened, muscular third legs (Figure 1a) | Yes (juvenile diet [21]; body weight [25]) | Colony size [25] | In relation to male density [26*] |

maintenance of condition-dependent AMM expression. Definitions and concepts of important terms used throughout the review are presented in Box 1.

Selection pressures that favor condition-dependent plasticity in AMM expression

In general, two non-mutually exclusive hypotheses exist to explain adaptive condition-dependent developmental plasticity [27**]. Using acarid mites as a model, bad-condition males maximize fitness by developing unmodified legs and expressing the scrambler morph, whereas good-condition males maximize fitness by developing fighter legs and expressing the fighter morph (assuming expression is adaptive). This results in a crossing of scrambler and fighter fitness functions over the condition gradient. Although conceptually similar, the two hypotheses differ in what this condition gradient is, and why the fitness functions cross.

According to the *alternative mating tactics hypothesis*, condition relates to future performance with regards to mating, 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 [27**] (Figure 1b).

In acarid mites, the evolution of condition-dependent AMM expression is often assumed to be driven by the alternative mating tactics mechanism [4], 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 [22,37]. Fighters and scramblers also perform similarly in sperm competition [38], although under some conditions, food-deprived scramblers produce more offspring than food-deprived fighters [39]. Fighters can kill conspecifics, possibly to remove competitors [40], but equally plausible is that they do so to feed on conspecifics [41]. 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 [22].

According to the *somatic buffering hypothesis* (Figure 1b), fitness functions of scramblers and fighters cross over the condition gradient because it is physiologically too expensive for bad-condition males to sustain prioritized physiological processes, such as somatic maintenance and development, *and also* produce fighter morphology. Such bad-condition males can salvage somatic functioning by refraining from developing fighter morphology, redirecting resources to prioritized processes.

Consistent with the somatic buffering hypothesis in acarid mites, a resource allocation trade-off between fighter leg development and other somatic functions is implied via (i) fighter leg development happening at the same time as when adults molt into the adult stage in a closed developmental system, and (ii) observations that fighter males shrink during maturation, whereas scrambler males

(Figure 1 Legend Continued) that selection near the threshold where the fitness functions of scramblers (dashed line) and fighters (solid line) cross, is weak because in the associated region of condition, fitness differences between the male morphs are small (dashed region). The *balancing selection hypothesis* states that genetic variation in the AMM threshold is the result of opposing selection pressures, for example, due to intralocus sexual conflict (illustrated here). Under intralocus sexual conflict, such opposing selection pressures are the consequence of a shared allocation trade-off in males and females (black line) but different fitness optima for males and females (white circles).

Box 1 Glossary

Alternative mating tactic hypothesis: an *information-based* hypothesis for adaptive condition-dependent developmental plasticity. It assumes that AMM expression depends on an observable cue at the moment of morph determination (e.g. body size), which provides *information* on expected future mating performance given an individual's condition [27**]. According to the alternative mating tactic hypothesis, AMM expression is a predictive adaptive response to early cues for mating performance.

Balancing selection: the phenomenon that selection maintains multiple alleles at particular loci within a population. Balancing selection can be caused by many processes, such as heterozygote advantage, frequency dependence, intralocus sexual conflict or fluctuating environments [28,29].

Canalization: the propensity for development to produce the same phenotype regardless of environmental or genetic variation [30].

Frequency dependent selection: when the fitness of a phenotype or genotype varies depending on its relative abundance within a population [31].

Genic capture hypothesis: posits that, given condition-dependence of costly sexually selected traits, considerable genetic variance in them can be maintained because condition depends on many genes affecting resource acquisition and processing [32].

Genotype-to-phenotype map: this concept goes beyond a 1:1 genotype-to-phenotype association, whereby the genotype is only one of several causal factors that jointly affect the phenotype. Importantly, this concept takes into account developmental parameter space [33].

Heterozygote advantage: the phenomenon that individuals with heterozygote genotypes have higher fitness than homozygous individuals within a population [28].

Inbreeding depression: reduction in fitness of offspring derived from related individuals, due to largely recessive, deleterious mutations present in low frequencies within populations [33].

Information-based hypotheses for adaptive plasticity: a class of hypotheses that explain adaptive plasticity by assuming an information link between adult performance and environmental cues perceived by developing juveniles. Under information-based explanations for adaptive plasticity, trait expression in developing juveniles depends on whether they perceive cues that predict particular adult environments.

Intralocus sexual conflict: sexually antagonistic selection where male adaptation is detrimental to female fitness because the male trait is genetically correlated with the female trait and males and females have different fitness optima. It results in at least one of the sexes not reaching their fitness optimum, and in balancing selection on the genes underlying the male and female traits [34].

Phenotypic plasticity: the phenomenon that phenotype expression depends on environmental effects, given a particular genetic background [35].

Single Nucleotide Polymorphism (SNP): a single nucleotide variant that occurs at a specific position in the genome.

Somatic buffering hypothesis: a *somatic-state-based* hypothesis for adaptive condition-dependent developmental plasticity. It assumes that bad (poor) somatic condition directly threatens somatic functioning, which is then mitigated by an immediate adaptive developmental response with irreversible consequences for the adult phenotype as a by-product (e.g. stopping allocation to secondary sexual character traits). Under this hypothesis there is no informational link between expected future performance and AMM expression, and AMM expression is not an adaptive anticipatory response to future conditions [27**].

Somatic state-based hypotheses for adaptive plasticity: a class of hypotheses that explain adaptive plasticity by assuming a direct, causal link between an irreversible environmental perturbation of development and an immediate developmental response that mitigates the negative consequences of the perturbation. Under somatic-state-based mechanisms for adaptive plasticity, differential phenotype expression is not an anticipatory response to a future environment, but a consequence of adaptively coping with environmental perturbations.

Weak selection: an explanation for genetic variation, in which selection is not strong enough to purge weakly deleterious alleles that are introduced by mutation or migration [36].

do not [22,25]. However, it is unknown whether males with small resource budgets are particularly negatively affected by this trade-off and therefore refrain from developing fighter legs. To support the somatic buffering hypothesis, one also has to confirm that bad condition at maturation cannot be adaptively mitigated by postponing maturation and acquiring more resources [27**]. Particularly in growing populations, delayed maturation has a fitness cost [42,43]. However, it remains to be shown whether this delay will be more detrimental to male fitness than the possible compensatory benefits of weapons.

Empirically distinguishing between the two hypotheses is not straightforward. Whereas the alternative mating tactics hypothesis predicts that the AMM expression threshold is evolutionarily regulated by how adult males

perform, 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 AMM threshold evolution. Through experimental evolution, Smallegange and Deere [12] followed food-restricted *Rhizoglyphus robini* populations while simultaneously harvesting either scramblers or fighters, allowing population density to respond to the harvesting treatments. Males evolved towards *unconditional scambler expression* regardless of which morph was harvested. The evolutionary shift towards the scambler phenotype was accompanied by an increase in average population density, likely from an overcompensatory response to average competition pressure, 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 population density effects), Smallegange *et al.* [40] still found a modest overcompensatory population reaction but with no AMM expression evolutionary response to the harvesting treatments. Both experiments show that variation in population density and internal energy economies during development may be more important evolutionary drivers of condition-dependent adaptive AMM expression than adult male performance.

Processes that maintain genetic variation in condition-dependent AMM expression

Condition-dependent AMM expression evolves rapidly in response to artificial selection in acarid mites [11,12], and assuming the current threshold is optimal, we should expect selection to remove any associated genetic variation. Despite this, AMM expression is generally heritable, although heritability measures vary widely depending on population or study [13,44,45]. These heritabilities could reflect heritability of the AMM threshold (but also condition). However, threshold heritability is difficult to measure [46,47], as it is unknown what the true proximate cue triggering AMM expression is [8,48]. Therefore, reported heritabilities are hard to interpret [8,48]. However, rapid evolutionary responses of the AMM threshold to novel selection pressures imply that genetic variation underlying the AMM threshold must be substantial [13]. Such genetic variation can be explained if selection on the threshold is actually not particularly strong, or if balancing selection maintains genetic polymorphisms. 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 [32]. Genic capture enforces mechanisms that increase heritability of sexually selected male traits, by increasing the target for mutation or balancing selection.

Genetic variation in thresholds may simply be maintained under weak selection. 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 [49]. Consequently, selection near the threshold is expected to be much weaker than far away from the threshold (Figure 1c). It may be the case that selection on the threshold is too weak to purge mutations, particularly 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 is under balancing selection (Figure 1c), including processes such as heterozygote advantage (which is possible in these acarid mites as their sex determining

mechanism is an X0 type) or fluctuating environments, neither of which have currently been empirically tested. Frequency dependence is another possibility, but to date, there is no evidentiary support in acarid mites [37,45]. Two more plausible processes of balancing selection include differential inbreeding depression or intralocus sexual conflict.

To show that intralocus sexual conflict is maintaining genetic diversity in genes underlying male morph expression requires the following demonstrations [34]. Firstly, male and female traits should be associated with the same genes [50], which may naturally occur if development of both male and female traits is physiologically expensive and subject to allocation trade-offs. 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 (Figure 1c). Secondly, given the existence of such a trade-off, males and females should have fitness optima on different positions on this trade-off (Figure 1c). Thirdly, neither sex should currently be at their fitness optimum, fueling sexually antagonistic selection where male adaptation is detrimental to female fitness and vice versa. In acarid mites, currently, only the requirement that male fighter leg development and female fecundity are associated with the same genes has received some empirical support [13,51]. However, candidate genes for this pleiotropic effect have not been conclusively identified (but see Ref. [52]), and the presumed resource allocation trade-offs underpinning this genetic correlation have not yet been demonstrated. Therefore, we should not discard the alternative explanation that correlated phenotypic responses between sexes are driven by differential inbreeding depression.

Stewart *et al.* [53*] suggest balancing selection maintains genetic variation in *R. robini* through differential inbreeding depression between AMMs 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 [53*] results may also be explained by differential inbreeding among male morphs and a collapse of AMM heritability in bad environments (i.e. gene-by-environment interactions).

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) [54*]. By pinpointing the genetic basis and causal mechanisms of intraspecific condition-dependent variation within populations, such as AMM expression, 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 AMM expression. 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 [52]. Finally, a developmental-causal understanding of AMM expression in acarid mites, combined with pedigreed populations, will allow us to more accurately measure how much genetic variation in AMM thresholds we actually have to explain.

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 AMM thresholds, yet strong empirical evidence is lacking. The somatic buffering hypothesis provides a promising alternative explanation if condition-dependent AMM expression 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 mating performance and AMM expression is an anticipatory adaptive response with genetic variation as a boundary condition, to a life-cycle perspective, in which the physiological processes underlying maintenance and development take center stage (Figure 1b) [55**,56]. It thus moves the focus of evolutionary explanation from the external and contingent, to the internal and inherent [55**].

Balancing selection seems the most promising avenue to maintain genetic variation underlying AMM expression within mite populations. While adopting a population-genetic view to explain genetic variation is important to fully appreciate how AMMs can be evolutionarily stable, implicitly assuming a simple one-on-one genotype-to-phenotype linkage is fraught with misinterpretations [13]. In acarid mites, AMM expression depends on somatic condition. Condition, in turn, is largely environmentally determined, which renders AMM 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 canalized (Figure 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 AMMs coexist.

Finally, condition-dependent AMM expression in some acarid mite species has been co-opted by an additional mechanism, and cued by colony size (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 berlessei*, AMM expression is cued by both somatic condition and chemical cues for population density [27**], the latter of which appears to be adaptive [26*]. Investigating the causes and consequences of evolutionary shifts in AMM cues could reveal whether somatic state-based mechanisms of AMM expression that mitigate immediate threats to physiological functioning are an evolutionary precursor of information-based AMM expression where future conditions are anticipated via information cues. Given that AMM expression in acarid mites can be cued by condition (either somatic state-based or information-based) and/or colony size (information-based) (Table 1), we foresee the acarid mite model system to be at the forefront in tackling such investigations.

In conclusion, to understand drivers of condition-dependent AMM expression, we call for a research agenda that derives testable somatic buffering hypotheses from mechanistic approaches based on underlying physiological principles [e.g. Refs. 57–59]. This, in turn, would not only underpin approaches asking what maintains the underlying genetic variation, but also allow for accurate prediction of population dynamics in the face of environmental change, as the dynamics of individual life histories [e.g. Refs. 60,61] ultimately form the basis for the dynamics of populations as a whole.

Conflict of interest statement

Nothing declared.

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