Evidence for a third male type in a male-dimorphic model species

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Numerous macro- and micro-evolutionary mechanisms have been proposed to help explain the enormous intra- and interspecific diversity we see globally. These processes have similarly been presented to give rise to discontinuous phenotypic variation within populations or even sexes, raising important questions as to how these discrete variants originate and are maintained. For example, to improve reproductive success, males of some species either do or do not invest in conspicuous, risky, or energetically costly reproductive strategies that result in intrapopulation differences between groups of males in terms of physiology, behavior or morphology (Oliveira et al. 2008). Evolutionary theory posits that male-male competition (and associated trade-offs between increased reproductive potential and costs of those traits: e.g., increased predation risk or energy depletion) can promote the development of an inconspicuous, risk adverse, less energetically demanding "alternative reproductive tactic" (ART) such as sneakers (versus guards) or satellites (versus callers). Indeed, ARTs are pervasive across the animal kingdom and have extensively been studied in various taxa (Oliveira et al. 2008). Although awareness of ARTs is widespread throughout the scientific community, we still find ourselves discovering new mating tactics even for extensively studied organisms (e.g., Lank et al. 2013, Persons 2017), with laboratory populations proving indispensable for exposing their evolutionary foundations (e.g., Radwan 2009).

In our laboratory, we study the bulb mite (*Rhizoglyphus robini*), a common pest of numerous crops, as a model system to investigate the links between environmental influences and expression of ARTs. Importantly, this genus has been used as an archetypal organism for studying the cause and effect of ARTs in populations for nearly a century (Foa 1919). Because *Rhizoglyphus* species have a short generation time and are easily reared in laboratories, this taxon is an ideal system for studying the ecological-evolutionary underpinnings of ARTs. Male bulb mites display one of two discrete, reproductive tactics: (1) "fighters" with a thickened and sharply terminated third leg pair that can be used to fight and kill conspecifics, and (2) "scramblers" which lack such armaments (Fig. 1A). Research suggests that a body size threshold mediates morph expression (Smallegange et al. 2012), such that when the quality or quantity of food intake is high, males mature at a larger size, and are more likely to develop into a fighter (Smallegange et al. 2012). Importantly, we can easily identify these two ARTs on the basis of the presence or absence of the enlarged third leg pair (Fig. 1A).

We currently house stock populations consisting of tens-of-thousands of bulb mites obtained on two occasions from two locations in the Netherlands: 1992 from flower fields, and 2010 from flower bulbs in storage rooms, both near Anna Paulowna in North Holland. From these stock populations we have initiated numerous experiments that require the diligent inspection of population demographics, including individuals' morphology and reproductive tactic. Surprisingly, we recurrently encounter males that are morphologically similar to females (large body size, bulbous abdomen, relatively slim third leg pair) – a phenomenon we describe as "mega-scramblers" (Fig. 1A). What is more, some of these mega-scramblers are so large that their non-armamented legs are almost as wide as the armamented legs of fighters (Fig. 1A, B). While not as common as either fighter or scrambler males, we have observed these mega-scramblers in both independent stock populations, and under various environmentally-manipulated regimes during experimental rearing, suggesting that these mega-scramblers do not arise as a result of some artificial laboratory biotic or abiotic factor.

To distinguish ARTs in male dimorphic species where the male sexually selected trait is expressed on a continuous scale, like horn size in beetles (Rowland and Emlen 2009), allometric scaling analyses is typically used to characterize the discontinuity in the trait (Knell 2009). When we apply an allometric scaling analysis to all scramblers (of both the "normal" and "mega" types), we find a breakpoint that signifies an abrupt change in the scaling relationship between body length and leg width, separating scramblers from mega-scramblers (Fig. 1B). This suggests that these mega-scramblers comprise a third male morphology, or "trimorphism" (see Appendix S1 for methodology). Although trimorphisms in-and-of-themselves are not unheard of (e.g., Shuster and Wade 1991, Lank et al. 2013), allometric analyses of species previously classified as male-dimorphic has assisted in discovering cryptic diversity, as was done in the recent reanalysis of numerous beetle species that were thought to comprise two male morphs (Rowland and Emlen 2009). Considering that the bulb mite is widely used as a model organism for studying ARTs, we are surprised this phenomenon has not been noted previously, as it could call into question the tacit dichotomous nature of allocating males as either scramblers or fighters, with repercussions for our understanding of the evolution and maintenance of the bulb mite male morphs.

It remains an open question as to the origin of this plastic trait, and whether expression of the mega-scrambler phenotype is adaptive. Evidence to date suggests environmentally mediated threshold mechanisms moderate morph expression (Smallegange et al. 2012) and, while previous research indicates frequency dependent selection is unlikely in this system (Smallegange and Deere 2014), we cannot discount this explanation as of yet without experimentally manipulating frequencies of all three morphs (Friedman and Sinervo 2016).
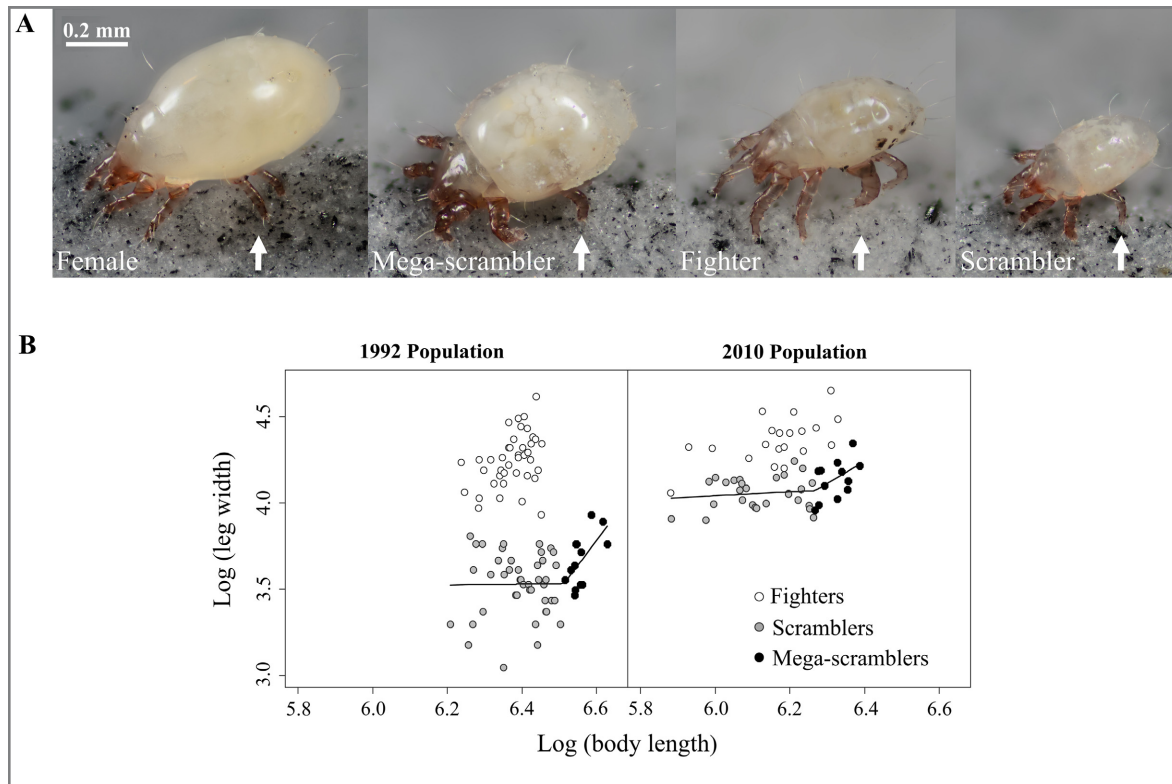
FIG. 1. (A) Dorsolateral images of adult bulb mites (*Rhizoglyphus robini*) including the female, and male trimorphism (mega-scrambler, fighter, and scrambler). Individuals are presented at the same scale (scale bar: top left) and aligned from largest to smallest (left to right). Arrows indicate structural differences in the third-leg pair among sexes and morphs. (B) Scatterplots of bulb mite male body length to leg width (log-transformed) collected from our 1992 (left) and 2010 (right) stock populations. Switch-point regressions between scramblers and mega-scramblers are represented by black trend lines. All measurements are in μm.

One potential adaptive explanation for the existence of female-like mega-scramblers is that they represent a female-mimicking ART. Males mimicking females as a strategy to achieve copulations and avoid aggressive encounters with other armamented males have frequently been observed in nature for various taxa, and are suggested to attain intermediate reproductive fitness compared to their higher-status or sneaker male counterparts (Oliveira et al. 2008). We have sporadically witnessed mega-scramblers being mounted by fighters indicating that other males may perceive them to be similar to females as well. Through general handling of our stock populations, we have not witnessed fighters or scramblers attempting to mount one another, although we have not empirically tested for such behavior. Given that the subterranean mites we study do not have eyes, but do have well-developed setal chemical sensory receptors, successful mimicry in these mega-scramblers may rely more on chemical than morphological cues, although we cannot exclude that tactile cues play a role. Chemical communication research could involve pheromonal profiling of adult mites; possibly mega-scramblers are more similar to females than other male morphs in the production of the sex-pheromone, α-Acaridial (females produce twice as much as males; Mizoguchi et al. 2003). Extensive ethological studies may further reveal that the levels of aggressive or sexual behavior shown towards mega-scramblers are more similar to those shown to females than to those shown to other males. With future research applying such approaches, it may be possible to show that mega-scramblers differ not only morphometrically but also chemically or behaviorally from other males; such findings would support our notion that the mega-scrambler should indeed be considered an ART.

Alternatively, mega-scrambler expression may be a consequence of adaptive somatic buffering against environmental fluctuations (Nettle and Bateson 2015). For example, juveniles that experience poor conditions may benefit by raising the threshold for fighter phenotype expression, thereby preserving resources that could be allocated for other prioritized functions. In spatially or temporally fluctuating environments, this may result in the expression of mega-scrambler phenotypes, particularly if the environment that sets the ART threshold early in juvenile life mismatches with the environment experienced later in juvenile life.

Finally, mega-scrambler expression could be a maladaptive side-effect of intralocus conflict between male tactics or sexes (Rice and Chippindale 2001). This could happen if optima for investment in particular somatic functions (e.g., reproduction, growth, body size) differ between male tactics

or between the sexes, maintaining genetic variation in energy allocation rules and thereby growth curves (Radwan et al. 2016).

Over the last century, a predominant number of biological investigations utilized either model systems or laboratory populations for experimentation. While model organisms are extensively studied from diverse perspectives (genetics, behavior, life-history, etc.) it would be imprudent to assume new organism-oriented discoveries are behind us. By means of a careful reassessment of ARTs in (model) species, not only may we expand our knowledge on the extent of natural diversity within species, we may also be able to connect certain morphological, behavioral or physiological states to each other on the basis of an underlying ART.

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