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Evolution of mate guarding under the risk of intrasexual aggression in a mite with alternative mating tactics

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Mate-guarding strategies are known to evolve in response to changes in the environment, but little is known about the genetic and plastic components of this source of variation. Here, we investigated how risk associated with aggression shapes postcopulatory association time between mates in the bulb mite, *Rhizoglyphus robini*, a species in which aggressive, armoured fighters often coexist with unarmoured scramblers. In some populations, scramblers have been reported to prevent females remating by remaining in copula for over 6 h. In this study, we investigated whether mate guarding by scramblers is affected by the presence of aggressive fighters in populations. We investigated whether guarding is riskier in the presence of fighters and found that guarding males were more likely to be attacked. Our data allowed us to determine whether the presence of fighters can affect mate-guarding duration, by comparing guarding duration between populations (both natural and artificially selected). We found that in both types of population, males guarded longer when fighters were absent. Comparisons between lines selected for the presence of fighters, lines selected for the presence of scramblers and their source populations indicated that scrambler morphs evolved prolonged guarding. We also investigated whether males show plasticity and shorten guarding in response to the presence of fighters in a social group. Surprisingly, we found that males in a mixed-morph context copulated significantly longer than males from single-morph groups. Our results demonstrate that mate guarding may evolve in response to the presence or absence of fighters in populations, but males are not able to adjust guarding behaviour to the risk of being attacked by fighters. The study provides insight into the role of genetics and plasticity in guarding strategies.

Male competition for mates drives sexual selection which leads to the evolution of many traits including aggressive behaviours, weaponry and mate attraction signals (Andersson, 1994). Mate guarding is one behavioural adaptation that may increase a male's paternity by preventing his female partner from mating with other males (Beecher & Beecher, 1979; Birkhead, 1979). Mate guarding is commonly observed in a wide range of taxa including both invertebrates (Burdfield-Steel & Shuker, 2014; Carazo, Font, & Alfthan, 2007; Chaudhary, Mishra, & Omkar, 2017; Siva-Jothy & Tsubaki, 1989; Sparkes, Keogh, & Pary, 1996) and vertebrates (Alberts, Altmann, & Wilson, 1996; Censky, 1995; Komdeur, 2001; Low, 2006; Matsubara, 2003; Willis & Dill, 2007).

Variation in mate-guarding strategies within and between species can be explained by several socioecological variables. For example, the incidence, nature and duration of mate guarding have been shown to be sensitive to variation in the operational sex ratio and male density and, thus, to the risk of sperm competition from potential rivals (Alcock, 1994; Clark, 1988; Low, 2006; McLain, 1980, 1989; Michiels, 1992; Møller & Birkhead, 1989; Radwan & Siva-Jothy, 1996; Rowe, 1992; Schöfl & Taborsky, 2002; Sillén-Tullberg, 1981; Simmons, 2001). Guarding intensity is also affected by the risk of intermale aggression as well as the risk of injury or death from predators while guarding females (Ancona, Drummond, & Zaldívar-Rae, 2010; Arnqvist, 1989; Fincke, 1982; Girard-Buttoz, Heistermann, Rahmi, Agil et al., 2014; Packer, 1979; Steele, Lehikoinen, Ost, & Kilpi, 2007; Waage, 1979b). Energetic costs due to reduced foraging may be another factor known to affect mate guarding (Censky, 1995; Girard-Buttoz, Heistermann, Rahmi, Marzec et al., 2014; Komdeur, 2001; Low, 2006; Schubert, Schradin, Roedel, Pillay, & Ribble, 2009; Weingrill, Lycett, Barrett, Hill, & Henzi, 2003; reviewed in Girard-Buttoz, Heistermann, Rahmi, Agil et al., 2014). However, although the costs of mate

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guarding are relatively well studied, how these costs shape the evolution of mate guarding has not been well investigated. In particular, the relative roles of phenotypic plasticity versus heritable differences in guarding behaviour are not well understood.

Postmating mate associations, where males remain physically associated with females much longer than the time necessary for insemination, may have several functions, the main one being to prevent females from remating, and hence to ensure increased paternity (Alcock, 1994; Mazzi, Kesäniemi, Hoikkala, & Klappert, 2009; McLain, 1980). For example, male bulb mites *Rhizoglyphus robini* (Astigmata: *Acaridae*) usually stay in copula for approximately 20 min (Radwan & Siva-Jothy, 1996), but mating associations lasting more than 6 h have also been reported (Radwan & Siva-Jothy, 1996). Prolonged copulations (lasting more than 90 min) do not result in the transfer of more sperm, but the proportion of eggs fertilized by a given male increases with longer copulation durations, that is, before the female remates with another partner.

Despite its benefits, prolonged copulation (as with other mate-guarding strategies) imposes substantial costs, as it consumes time that could be spent searching for other mates and may be associated with increased risks of injury and death (reviewed in Alcock, 1994). Thus, copulation duration is likely to depend on a cost-to-benefit ratio of guarding, which may vary in different social and demographic contexts. It has been shown that invertebrates are able to detect the presence of rivals, as well as potential mates, based on chemical cues and adjust the time they spend on mate guarding to the actual social situation (Burdfield-Steel & Shuker, 2014; Carazo et al., 2007; Chaudhary et al., 2017). Copulation duration in *R. robini* has been shown to increase in male-biased populations (Radwan & Siva-Jothy, 1996), in which the time needed for a male to remate with another female is longer on average (thus the probability of copulating with another female soon after mating is low, decreasing the cost of guarding) and the risk that an already inseminated female would copulate with another male is higher. These observations suggest that bulb mite males can assess social context and adjust their behaviour accordingly.

Copulation duration may also differ in populations with different proportions of the two male morphs observed in this species. In most populations, fighters, which possess a thickened third pair of legs used to stab rivals when fighting for access to a female, coexist with benign scramblers, which have unmodified, female-like legs and do not engage in fights (Gerson, Capua, & Thorens, 1983; Woodring, 1969). As bulb mites live on underground bulbs of plants such as onions and tulips, it is impossible to observe their behaviours in the wild, and all observations of their behaviour have been made in laboratory conditions. Fighters attack both scramblers and other fighters and, when present in small, isolated groups, may even kill all rivals to monopolize females, although no territorial behaviours have been observed (Radwan & Klimas, 2001; Radwan, Czyż, Konior, & Kolodziejczyk, 2000; Smallegange, Thorne, & Charalambous, 2012). Remaining in copula is likely to make males more vulnerable to attacks by fighters, the presence of which should thus select against prolonged postinsemination associations. Indeed, long mounting durations were observed by Radwan and Siva-Jothy (1996) in a population in which fighters had not been recorded, whereas prolonged mountings appeared rare in another population containing both morphs (Radwan, 1997). This association between the presence or absence of fighters in a population and the duration of postinsemination mate association has not yet been tested in a controlled, common-garden experiment. If the presence of fighters makes mate guarding less likely, the question arises whether the differences in guarding duration represent behavioural plasticity or fixed genetic differences that have evolved in response to the presence of fighters in a population. This is particularly interesting as little is known about the relative importance of innate (genetic) versus acquired behavioural adaptations to different social environments. To our knowledge, the current study is the first attempt to fill this gap with respect to mate-guarding behaviour.

In this study, we carried out a series of experiments on the bulb mite to investigate how increased costs of guarding shape the evolution of mate guarding. In the first experiment, we checked whether the presence of fighters made guarding riskier, thus increasing the cost of guarding. We determined whether fighters were more likely to attack copulating versus noncopulating scramblers. In the second experiment, we compared mate association times in populations in which fighters were either present or absent (using both natural populations and artificially selected lines). We predicted that mounting duration would be longer in populations without fighters. To disentangle plastic and genetic factors affecting mate-guarding time, we tested whether mating association duration depended on the presence of fighters in a social group. Differences in guarding time in the absence of behavioural plasticity would indicate genetic differences in guarding strategies. Finally, to determine the direction of evolution of mate-guarding duration, we compared mating association times in artificially selected lines versus those observed in their source populations, predicting that it would increase in lines selected for an increased proportion of scramblers.

METHODS

General Procedures and Stock Colonies

Our experiments used three types of population: (1) laboratory populations (more than 200 generations in laboratory conditions), (2) selection lines and (3) natural populations. Laboratory populations consisted of colonies of >1000 individuals maintained under laboratory conditions, which were established from a natural colony of individuals collected from onions in a garden near Kraków, Poland in 1998 and 2008 (Lab1998 and Lab2008, respectively). Replicate selection lines were derived from these laboratory-adapted colonies and were selected for either an increased or a decreased proportion of fighters (see Plesnar Bielak, Skrzynecka, Miler, & Radwan, 2014 for details). Natural populations contained individuals collected from two different locations 7 months prior to the experiment. Natural population K2011 came from onions in a garden near Kraków, Poland (the same source site as the Lab1998 and Lab2008 populations) and contained both male morphs. Natural population G2011, originated from tulip bulbs found in Gorlice, Poland (100 km southeast of Kraków) and at the time of collection consisted of scramblers only. As fighters did not appear within 7 months in the laboratory, which equated to 14 generations, this population was subsequently treated as being genetically fixed for this trait (i.e. scramblers only).

Large groups (>20 mites) were maintained in plastic containers (2 cm high and 2.5 cm in diameter), whereas individuals and pairs were kept in glass vials (2 cm high and 0.8 cm in diameter) with plaster-of-Paris bases soaked in water. Mites were fed dried yeast ad libitum and maintained at 24 ± 1 °C and >90% humidity.

Using invertebrates does not require any ethical review, permissions or licences according to Polish law. All the individuals we used came from laboratory populations. The experimental procedures were not invasive and after they were completed, the mites were killed by puncturing their body with a needle which causes instant death.

Aggressiveness of Fighters towards Scramblers

To determine whether guarding was associated with an increased risk of being attacked, we used naïve males (males that were isolated and housed in individual vials before reaching adulthood and, hence, lacked any contact with potential rivals before the