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Experimental removal of sexual selection leads to decreased investment in an immune component in female *Tribolium castaneum*

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ABSTRACT

Because of divergent selection acting on males and females arising from different life-history strategies, polyandry can be expected to promote sexual dimorphism of investment into immune function. In previous work we have established the existence of such divergence within populations where males and females are exposed to varying degrees of polyandry. We here test whether the removal of sexual selection via enforced monogamy generates males and females that have similar levels of investment in immune function. To test this prediction experimentally, we measured differences between the sexes in a key immune measurement (phenoloxidase (PO) activity) and resistance to the microsporidian *Paranosema whitei* in *Tribolium castaneum* lines that evolved under monogamous (sexual selection absent) *vs* polyandrous (sexual selection present) mating systems. At generation 49, all selected lines were simultaneously assessed for PO activity and resistance to their natural parasite *P. whitei* after two generations of relaxed selection. We found that the polyandrous regime was associated with a clear dimorphism in immune function: females had significantly higher PO activities than males in these lines. In contrast, there was no such difference between the sexes in the lines evolving under the monogamous regime. Survival in the infection experiment did not differ between mating systems or sexes. Removing sexual selection via enforced monogamy thus seems to erase intersexual differences in immunity investment. We suggest that higher PO activities in females that have evolved under sexual selection might be driven by the increased risk of infections and/or injuries associated with exposure to multiple males.

1. Introduction

Parasites can impose significant fitness costs on their hosts and may therefore exert strong selection pressure on hosts to defend themselves (Haldane, 1949; Price, 1980; Ewald, 1994; Schmid-Hempel, 2011). As the immune system is costly to maintain and use, trade-offs between immune function and other life-history traits can be expected (Sheldon and Verhulst, 1996; Rolff, 2002). Sexual selection is a powerful evolutionary force that can have major direct or indirect effects on a wide range of fitness traits, including immunity (e.g., Hamilton and Zuk, 1982; Andersson and Simmons, 2006). Sexual selection generally selects male traits that increase reproductive success in the face of pre- and postcopulatory male–male competition and that overcome female resistance (Andersson and Simmons, 2006). There is increasing evidence that males face a phenotypic trade-off between investment in reproduction and immune function (Blount et al., 2003; Faivre et al., 2003; Jacot et al., 2004) and such trade-offs can also have a genetic basis (Hosken, 2001; Simmons and Roberts, 2005). In many vertebrates, males are typically more susceptible to parasites (Zuk, 1990; Folstad and Karter, 1992; Poulin, 1996; Zuk and McKean, 1996; Schalk and Forbes, 1997; Moore and Wilson, 2002). The underlying mechanism of this pattern has usually been attributed to the immunosuppressive influence of testosterone (Alexander and Stimson, 1988; Zuk, 1990; Folstad and Karter, 1992). Nevertheless, in invertebrates, which lack testosterone and other steroid hormones, males are also often more susceptible to parasites than females (e.g., Gray, 1998; Wedekind and Jakobsen, 1998; Adamo et al., 2001; Schwarzenbach et al., 2005). In addition, recent data in sex-role reversed species in which females compete to mate with males suggest that sexually competitive females have a weaker immune response (Roth et al., 2011).

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An alternative and more general hypothesis suggests that sexual dimorphism in immunity can be expected because females and males of many species have sexually dimorphic life-history strategies and only a certain amount of resources are available for all life history traits (Sheldon and Verhulst, 1996; Rolff, 2002). The main assumptions of this hypothesis are that the immune system is costly, and females invest more in longevity than males, whereas mating rate and mating success are of much greater importance to males (Rolff, 2002). This hypothesis was recently referred to as the "susceptible male hypothesis" and predicts that females should invest relatively more in immune function than males and that the magnitude of sexual dimorphism in immunity increases with the strength of sexual selection (Rolff, 2002; Stoehr and Kokko, 2006). In the absence of sexual selection and conflict, as under scenarios with genetic monogamy, both sexes would therefore be expected to invest equally in reproduction and immunity (Zuk, 1990, 2009; Stoehr and Kokko, 2006).

In addition to different resource allocation strategies between the sexes, conflicting optimal fitness strategies between females and males can lead to evolution towards different fitness optima between the sexes (Parker, 1979). The importance of sexual conflict as an evolutionary force has become increasingly clear (Trivers, 1972; Parker, 1979, 2006; Arnqvist and Rowe, 2005), and sexual conflict is likely to be a powerful mechanism for the evolution of sexual dimorphism (Rice and Chippindale, 2001). Whereas recent studies have shown that sexual conflict can drive evolution in reproductive traits, and even speciation (Arnqvist and Rowe, 2002; Martin and Hosken, 2003; Ritchie, 2007; Hosken et al., 2009), the role of immune traits is less studied in the context of sexual conflict (Zuk, 1990, 2009) despite the fact that conflict over immunity can be expected as males and females are assumed to have different adaptive peaks in immunity, and, due to the costliness of immunity (Lessells, 1999; Zuk, 2009). In contrast, when sexual conflict is absent, such as under lifelong monogamy, the sexes would be expected to have similar adaptive peaks concerning immunity (Stoehr and Kokko, 2006; Zuk, 2009).

Insects have a complex innate immune system, consisting of humoral and cellular immunity, which can cope with a variety of parasites and pathogens (Schmid-Hempel, 2005). Melanisation is an important and well-studied immune response in many insects and phenoloxidase (PO) is a key enzyme of the melanisation cascade, which is often used to estimate immune function in insects (reviewed in Cerenius et al., 2008; González-Santoyo and Córdoba-Aguilar, 2012). PO activity has profound fitness consequences in several pathogen-host systems (Kraaijeveld and Godfray, 1997, 2001; Siva-Jothy et al., 2001; Rolff and Siva-Jothy, 2004; Cerenius et al., 2008). There is evidence for resource allocation trade-offs between PO and other fitness traits (Siva-Jothy and Thompson, 2002; Rantala et al., 2003; Cotter et al., 2004; Pomfret and Knell, 2006), and studies on narrow sense heritability have revealed that PO is a heritable trait (Cotter et al., 2004; Armitage and Siva-Jothy, 2005; Schwarzenbach and Ward, 2006; Gershman et al., 2010). It remains to be tested if sexual conflict can drive the evolution of sexual dimorphism in PO activity and if the evolution of PO activity is constrained by genetic trade-offs.

Experimental evolution experiments enable evolution to be captured in action, whilst assessing both direct and indirect responses to divergent selection. Experimental evolution applied to insect models and incorporating different intensities of sexual selection generally affects a host of morphological, physiological and behavioral reproductive traits in both sexes (e.g., Hosken, 2001; Morrow and Gage, 2001; Wigby and Chapman, 2004; Simmons and Garcia-Gonzalez, 2008; Michalczyk et al., 2011b; Demont et al., 2014). Due to the close links between reproduction and immunity, such experiments have considerable potential for advancing knowledge of immune traits (Kerstes and Martin, 2013). Our previous study using *Tribolium castaneum* lines that had been exposed to contrasting sexual selection intensities via different sex ratios revealed that females invest more in an immune measurement than males (Hangartner et al., 2013). Although the intensity of sexual selection varied among these lines, sexual selection was always present (Hangartner et al., 2013). Having established this dimorphism, in this study we test whether the complete removal of sexual selection via enforced monogamy generates males and females that have similar levels of investment into immune function.

We thus tested for evolutionary changes associated with immunity and parasite resistance in response to different mating systems using *T. castaneum*. These lines have been exposed experimentally to contrasting mating systems: monogamy versus polyandry (i.e. absence *vs* presence of sexual selection). These experimental evolution lines have been used previously to reveal consequences of evolving under polyandry and monogamy in both sexes (Demont et al., 2014; Grazer et al., 2014). In particular, the polyandry lines have evolved males who were quicker to achieve copulation when facing competition and females who are more resistant to multiple mating by delaying the first copulation when given a choice of males, revealing that adaptation to polyandry in both sexes provide benefits when choice and competition were present (Demont et al., 2014). We also found that females evolving under enforced monogamy incurred costs and gained benefits that were shaped by environmental quality (standard benign *vs* novel stressful environments, see Grazer et al., 2014). Polyandrous females generally had a higher fecundity than monogamous females, whereas monogamous females seemed to have reduced fecundity when mated with polyandrous males in standard benign environments (Grazer et al., 2014).

After 49 generations, males and females from all selection lines were tested for PO activity and resistance to their natural parasite *Paranosema whitei* (e.g., Milner, 1972; Berenos et al., 2009; Kerstes et al., 2013). *P. whitei* is a microsporidian pathogen of some species of *Tribolium* (previously *Nosema whitei* (Weiser, 1953), recently reclassified by Sokolova et al., 2005). *P. whitei* is an obligatory intracellular parasite that infects internal organs, and generally causes the host to die in the late larval or early pupal stage (Dunn and Smith, 2001; Blaser and Schmid-Hempel, 2005).

We expected evolutionary changes in immune function in response to adaptation to two contrasting mating systems (where sexual selection and conflict are either present or absent) and consider two scenarios: *Scenario 1:* sexual dimorphism in immunity is expected when sexual selection is present, but not under monogamy where sexual selection is absent. This scenario is in concordance with hypotheses based on different life history strategies between the sexes (Zuk, 1990, 2009; Rolff, 2002; Stoehr and Kokko, 2006), as well as with our previous work on the same species (Hangartner et al., 2013). It predicts that females have evolved to invest more in immunity than males in the polyandrous lines, with investment additionally fuelled by increased mating costs (e.g., stress, injury, sexually transmitted diseases). In contrast, the sexes are not expected to differ in immunity in the monogamous lines. *Scenario 2:* polyandrous lines with high sexual conflict show reduced immune investment, so individuals of both sexes in polyandrous lines would be expected to have evolved inferior immunity than monogamous lines. This would be indicative of a genetic trade-off between investment in reproductive traits and immunity, as found in other species (e.g., Hosken, 2001; McKean and Nunney, 2008; McNamara et al., 2013). Whereas scenario 1 predicts differences between the sexes depending on the selection regime, scenario 2 predicts differences between the selection regimes but not between the sexes.

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