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Variation in male fertility in a polymorphic moth, *Parasemia plantaginis*

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The maintenance of multiple morphs in warning signals is enigmatic because directional selection through predator avoidance should lead to the rapid loss of such variation. Opposing natural and sexual selection is a good candidate driving the maintenance of multiple male morphs but it also includes another enigma: when warning signal efficiency differs between male morphs, why would females choose a phenotype with lower survival? We tested the hypothesis that indirect responses to selection on correlated characters through sexual selection may substantially shape the evolution of male coloration. If male phenotypes differ in their fertilization ability, female choice against the best surviving phenotype can evolve. The wood tiger moth, *Parasemia plantaginis*, has two coexisting male morphs in Europe. Previous studies have shown that yellow males are better defended against predators, but that white males have a higher mating success. We examined differences in fertility between white and yellow males in terms of sperm production, number of sperm transferred and rate of sperm replenishment, and association between these fertility traits, female mate choice and reproductive output. If white morphs have greater fertility than yellow males, then this could explain why females prefer to mate with white males. However, we did not find any difference between male colour morphs either in mating probability, fertility (i.e. sperm availability and sperm transferred) or reproductive success (i.e. number of eggs laid and hatching success). We discuss our results in relation to context-dependent mating success and maintenance of colour polymorphism within populations.

Colour polymorphism is widespread in both vertebrates and invertebrates (Roulin, 2004; Svensson & Abbott, 2005). The maintenance or coexistence of several colour morphs is of particular interest because the mechanisms involved are still a matter of debate (Bond, 2007; Roulin, 2004). Several, nonmutually exclusive, hypotheses have been proposed as drivers of colour polymorphism such as frequency-dependent selection, via for example predation, or sexual selection (Endler, 1983; Kingston, Rosenthal, & Ryan, 2003; Olendorf et al., 2006). In aposematic species, individuals display discriminable, generally conspicuous, warning signals to inform predators about their unprofitability (e.g. toxicity, unpalatability; Mappes, Marples, & Endler, 2005; Ruxton, Speed, & Sherratt, 2004). Because of the benefits for both prey (predation avoidance) and predator (avoidance of toxic prey), aposematic species are expected to show uniform conspicuous colour patterns driven by positive frequency-dependent selection (Beatty, Beirinckx, & Sherratt, 2004; Kapan, 2001; Rowland, Ihalainen, Lindström, Mappes, & Speed, 2007). Nevertheless, many aposematic species have polymorphic warning coloration. How warning colours are maintained in aposematic species may thus be an important step to understand how the general diversity of signals originates and is maintained in the wild.

Several hypotheses have been proposed to explain colour polymorphism in aposematic species such as effect of variable background on signal perception (Ruxton, Sherratt, & Speed, 2004), variation in predator tolerance or naivety towards toxicity (Endler & Mappes, 2004), and the cost of producing warning signals and toxic defences (Lindstedt et al., 2011; Speed & Ruxton, 2007). Surprisingly, very few studies have examined the role of sexual selection through female choice in the diversity of intraspecific colour patterns in aposematic species (but see Maan & Cummings, 2008; Nokelainen, Hegna, Reudler, Lindstedt, & Mappes, 2011). It has been suggested that aposematic signals, after first arising from

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natural selection (e.g. predation avoidance), could secondarily acquire a role in sexual selection. For instance, Maan and Cummings (2008) found in the polymorphic poison frog *Oophaga pumilio* that divergence in female mate choice has resulted in aposematic colour polymorphism in geographically distinct populations. Maintenance of colour polymorphism by female mate choice becomes puzzling when females prefer the less efficient aposematic morph in males (Nokelainen et al. 2011). Intuitively, females should prefer to mate with males with efficient warning coloration, as it would ensure better protection for their offspring if coloration is heritable and enhances survival against predators. However, female preference for weaker warning signals could be selected for if the male shows higher reproductive quality such as fertility (see for instance Kaitala, 1991; Simmons, 2011). In these conditions, females could prefer to mate with males displaying the weaker warning signal if it signals a higher lifetime reproductive success.

Here we investigated mechanisms that could explain female choice for males with a weaker warning signal. As a study species, we used the aposematic wood tiger moth, *Parasemia plantaginis* (family Erebidae, subfamily Arctiine). Males of this species exhibit discrete wing coloration on both a local and a broad geographical scale (Hegna, Galarza, & Mappes, 2015). European populations feature two distinct genetic male morphs, yellow and white (Galarza, Nokelainen, Ashrafi, Hegna, & Mappes, 2014). In previous experiments, females have been shown to have a preference for males with white hind wings compared to males with yellow hind wings when males were stressed before mating trials (Nokelainen et al. 2011), and when male colour frequency was white biased (Gordon, Kokko, Rojas, Nokelainen, & Mappes, 2015). Why females show such mating preference despite yellow males providing more efficient predation avoidance is not known. According to sexual selection models, females may gain benefits related to female fecundity ('direct' benefits) and/or offspring viability arising from the transmission of paternal genes ('indirect' genetic benefits; Johnstone, 1995; Kokko, Jennions, & Brooks, 2006). Among direct benefits, females may seek to maximize the fertilization success of her eggs, particularly in species producing hundreds of eggs, as in many insects. Because male sperm production can be costly, choosy females are expected to show preferences for males in good nutritional condition, free from parasites and nonsenescent, in order to target males with high fertility (Garratt & Brooks, 2012). In addition, females may also avoid mating with sperm-depleted males (Elzinga, Chevasco, Grapputo, & Mappes, 2011; Kaitala & Wiklund, 1994; Marcotte, Delisle, & McNeil, 2007; Velde, Damiens, & Van Dyck, 2011). As many insects are promiscuous, the ability of males to recover quickly from sperm depletion following mating might hence be important to ensure fertilization success in successive copulations (Wedell & Cook, 1999).

To study possible differences in fertilization benefits between white and yellow male morphs, we first conducted an experiment (the spermatophore experiment) in which we tested whether white males transfer larger spermatophores to females than yellow males. We then conducted an experiment (the fertility experiment) to investigate in detail the fertilization benefits that may arise from mating with white males. Specifically, we investigated differences between morphs in the amount of sperm produced and transferred to the female, the time needed by males to recover from sperm transfer after mating and the reproductive output. We assumed that if more efficient warning coloration is costly for yellow males to produce, they may have a lower reproductive quality (because of conflicting resource allocation) associated with lower reproductive success than white males. In this case, the reproductive costs of warning signal production could induce conflicting selection pressures from sexual and natural selection, potentially maintaining polymorphism in aposematic warning signals in this moth species. We thus predicted that white males would have higher fertility than yellow males and that females showing mating preference for white males would secure fertility benefits.

METHODS

Study Species

Colour polymorphism in *P. plantaginis* varies throughout the world. In Finland, males present two distinct morphs on their hind wings: yellow and white (Galarza et al. 2014). Both sexes produce defensive fluids to protect themselves against predators (birds, spiders, ants etc., Lindstedt et al. 2011) and yellow adult males have been shown to be more repellent to predators than white males (Nokelainen et al. 2011). Male mating attempts do not always lead to copulation suggesting a potential for female mate choice. Copulations usually take several hours between dusk and dawn. Males provide females with both fertile eupyrene and nonfertile apyrene spermatozoa contained within a spermatophore. Females mate multiply, providing an opportunity for sperm competition (Jennions & Petrie, 2000). Females lay on average 250 eggs within 3–4 days after copulation that hatch about 7 days later (at room temperature in the laboratory: minimum 17 °C, maximum 34 °C, mean 24 °C). About 50 days later (in laboratory conditions) larvae pupate and the pupal stage lasts ca. 10 days. As adults do not feed, all the resources needed for sperm production, wing colour pigment production and other traits are gathered during the larval stage and distributed to the different traits during metamorphosis.

Stock Conditions

Individuals used in the experiments originated from the laboratory stock population held in captivity at the University of Jyväskylä, Finland. Founders originated from 50 wild-caught already mated females sampled in Finland during summer 2010 (see Lindstedt et al. 2011). Genetic diversity was maintained by supplementing laboratory stock annually with wild individuals caught in Finland during the summers of 2011 and 2012. Three generations were produced per year during spring, mid-summer and late summer, with the latter one overwintering. Generations do not overlap. The breeding was conducted as follows (at room temperature, as described above, and natural photoperiod: length of day 10–19 h). Once adults emerged, one female and one male (either yellow or white) were placed together into a transparent plastic container (size 10 × 7 cm and 7 cm high) for mating. Adults died soon after egg laying and were removed from the container. Hatched larvae were reared in separate sibling groups (up to 30 individuals per box) and fed with dandelion and lettuce leaves. Food was available ad libitum and was changed daily. At the pupal stage, individuals were weighed and individually moved into plastic tubes (4 × 7 cm) until adult emergence. Subsequently, freshly emerged adults were placed in low-temperature-controlled cabinets (ca. 10 °C) with constant darkness until used in the experiments or in matings to maintain the captive stock. The species is a capital feeder (i.e. adults do not feed). During mating trials, all pairs were checked under dim red light. Conditions of experimental animals were kept as good as possible as we aimed to test their behaviour in optimal conditions. At the end of the experiments, individuals were killed by freezing and stored at −80 °C until dissection. The following experiments comply with current laws of Finland.

Experiment 1: The Spermatophore Experiment

During the summer of 2011, 171 pairs consisting of one virgin female and one virgin male were formed from captive-born adults.