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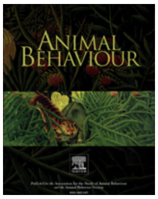

A polyandrous female moth discriminates against previous mates to gain genetic diversity

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Polyandry is common in many animal species. The hypotheses proposed to explain the evolution of polyandry generally fall into two categories: to gain material benefits and to obtain genetic benefits. Here we report our findings on a polyandrous moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae). The present study does not support the hypothesis that females gain material benefits from multiple copulations in terms of sperm replenishment, fecundity and longevity, because females that copulated once and females that copulated more than once with the same or different males had similar fertility, fecundity and longevity. Females used three strategies to gain possible genetic benefits: discriminating against previous mates probably via chemical cues, giving 60% chance of paternity to second males, and adjusting their oviposition patterns depending on whether they encountered new or previous mates after the first copulation. For the third strategy, females that encountered new mates after the first copulation saved eggs until the next day to facilitate fertilization of their eggs with the sperm of their new mates. Our findings do not support the hypothesis that polyandry increases offspring viability, because polyandry did not affect egg hatching success, offspring survival or weight. *Ephestia kuehniella* showed limited ability to disperse, and females laid all their eggs locally, mostly within 2 days, suggesting that offspring live together and that sib copulations are common in this species. Therefore, polyandry is probably a mechanism for reducing sib competition and inbreeding costs in offspring.

It is generally accepted that a male's reproductive success primarily depends on the number of females he can inseminate; however, whether females benefit from multiple copulations is still controversial (Simmons 2005). Copulations are costly, including the energy costs of sexual behaviour and risks of predation, disease transmission and injury inflicted by males (Daly 1978; Arnqvist & Nilsson 2000). Therefore, the benefits of multiple copulations in females must outweigh the costs to make the evolution of polyandry possible.

A number of studies show that multiple copulations may allow females to replenish sperm supply and/or obtain nutritional resources from males (e.g. Arnqvist & Nilsson 2000; Simmons 2001; Jimenez-Perez et al. 2003; Wang & Davis 2006). In many species, females may seek multiple mates for material benefits because of potential resource depletion from previous mates (e.g. Lemaitre et al. 2009).

The recent literature shows that females may gain genetic benefits from copulating with multiple mates, and a number of hypotheses have been proposed to explain the evolution of polyandry in this light (Simmons 2005). In the present study we focused on the genetic incompatibility (Zeh & Zeh 1996, 1997, 2001) and genetic diversity (Jennions & Petrie 2000) hypotheses, both of which explain a wide range of genetic benefits that females may gain from polyandry. The genetic incompatibility hypothesis suggests that polyandry enables females to bias paternity towards males with genes that confer higher fitness or that are more compatible with the females' genome; as a result, their offspring viability increases (e.g. Zeh & Zeh 1996, 1997; Tregenza & Wedell 1998; Konior et al. 2001; Pai & Yan 2002). The genetic diversity hypothesis proposes that polyandry allows females to increase the genetic diversity of their offspring, which may reduce sib competition (e.g. Ridley 1993) and inbreeding costs (Cornell & Tregenza 2007) and enhance disease resistance (e.g. Tooby 1982).

The genetic incompatibility model requires that sperm from multiple males are present at the site of fertilization (Simmons 2005), and the genetic diversity model necessitates that the offspring are fathered by multiple males (Jennions & Petrie 2000). Therefore, to gain genetic benefits from polyandry, females must have developed strategies to discriminate against previous mates in subsequent copulations (Zeh et al. 1998). However, so far only seven studies have explicitly tested whether females have any preference

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for new versus previous mates in their subsequent copulations. Discrimination against previous mates by females has been reported in four invertebrate (Bateman 1998; Zeh et al. 1998; Archer & Elgar 1999; Ivy et al. 2005) and two vertebrate (Eakley & Houde 2004; LaDage & Ferkin 2007) species. In a cannibalistic spider, however, females appear to have no discrimination against previous mates (Fromhage & Schneider 2005). Archer & Elgar (1999) suggested that hide beetle, *Dermestes maculates*, females choose new mates for subsequent copulations to gain the benefit of genetic diversity. In a follow-up study of Zeh et al.'s (1998) observations, Newcomer et al. (1999) obtained evidence that in the pseudoscorpion *Cordylochernes scorpioides*, females discriminate against previous mates for higher offspring viability.

The Mediterranean flour moth, *Ephestia kuehniella*, is a cosmopolitan pest of stored products. The moths do not feed on protein as adults (Norris 1934), and, as a consequence, all materials for spermatogenesis must be obtained during the larval stage. Both sexes of *E. kuehniella* become sexually mature within 24 h after emergence, copulation occurs in the first scotophase after emergence, and oviposition starts in the second scotophase (Xu et al. 2008). Females release a sex pheromone to attract males, and several males may arrive simultaneously to a receptive female (Kuwahara et al. 1971). Males always directly approach females, perform courtship display by jumping and fanning their wings over or around the females, and expose their genitalia, attempting to engage those of females (Xu et al. 2008). If receptive, females remain still when males approach and court, and then copulation occurs; if not receptive, females move away (Xu & Wang 2009).

Our previous studies showed that *E. kuehniella* females copulate more than once (Xu et al. 2008) even though they obtain more than enough sperm to fertilize their full egg load from a single copulation (Xu & Wang 2009). This raises two questions. Do *E. kuehniella* females choose between new and previous mates for subsequent copulations? Why do they copulate multiply? We hypothesized that *E. kuehniella* females (1) have developed strategies to discriminate against previous mates in subsequent copulations, and (2) gain material and/or genetic benefits from polyandry. We used two experimental designs to test whether females discriminated against previous mates: (1) simultaneous mate choice (e.g. Bateman 1998), in which once-copulated females were allowed to choose between a new and previous mate for a second mating, and (2) sequential mate choice (e.g. Zeh et al. 1998), in which once-copulated females were allowed to choose either a new or a previous mate for a second mating. We then carried out a series of experiments to examine the following possible benefits that females may gain from polyandry: (1) to replenish sperm supply for higher fertility, (2) to obtain nutritional resources for higher fecundity and/or greater longevity, (3) to increase offspring viability, and (4) to increase offspring genetic diversity. We describe and discuss strategies females used to discriminate against previous mates and to maximize reproductive benefits from polyandry in *E. kuehniella*.

METHODS

Insects

Insects were maintained in plastic cylinders (8 cm diameter × 10 cm height), each filled with 50 g of a standard diet (43.5% wholemeal wheat flour, 43.5% maize meal, 3.0% yeast and 10% glycerine), in the Entomology and IPM Laboratory of Massey University, Palmerston North, New Zealand. Cylinders were covered with two layers of nylon mesh. To start the colonies, 116 newly laid eggs (<24 h old) (Xu et al. 2007) were introduced into each cylinder with the standard diet. Two crumpled paper towels (25 × 25 cm) were placed in each cylinder for pupation. Adults were not given food or water because this was unnecessary (Norris 1934). The insect colony was maintained and all experiments were conducted at 25 ± 1 °C and 70 ± 10% RH, on a 14:10 h light:dark cycle.

We collected mature pupae (when they turned dark) from the crumpled paper towels in each cylinder and weighed them using an electronic dual range balance (Mettler AE100, Zurich, Switzerland) with a readability of 0.0001 g. The weighed pupae were kept individually in glass tubes (2 cm diameter × 7.5 cm height) until adult emergence to ensure virginity and age. To minimize weight changes over time, we used only those insects that emerged within 12 h after pupal weighing for experiments. The emerged moths were sexed and kept in the same glass vials before being used for experiments.

Pupal weight was considered adult body weight in this study. Mean ± SD pupal weight was 22.8 ± 1.9 mg for males and 25.1 ± 2.3 mg for females. We categorized body weight as average, light (<1 SD from the mean), or heavy (>1 SD from the mean). Unless stated otherwise, all adults used in this study were 1-day-old virgin moths with average body weight. Copulations were allowed under scotophase in the plastic cylinders. Illumination during observation was provided by a 30 W red light tube. We lined the plastic cylinders with porous plastic sheets (Wicket Bag, plain, perforated, 15 μm, Cryovac(tm); W.R. Grace Ltd, Auckland, New Zealand) to provide a substrate on which females could lay their eggs.

Mate Choice by Females for Their Second Copulation

We conducted two experiments to determine whether *E. kuehniella* females prefer new or previous mates for their second copulation: (1) simultaneous mate choice (e.g. Bateman 1998), in which once-copulated female were allowed to choose between both new and previous mates for a second mating, and (2) sequential mate choice (e.g. Zeh et al. 1998), in which once-copulated females were allowed to choose either a new or a previous mate for a second mating.

The once-copulated females and males were obtained by allowing virgin moths to copulate in the plastic cylinders (one pair per cylinder) in the first scotophase following emergence. Copulated pairs were separated and individually maintained in the cylinders for 14 h before their use in the following experiments at the beginning of the second scotophase: (1) in the simultaneous mate choice experiment, two once-copulated males (previous and new mates) were introduced into a female's cylinder, and (2) for the sequential mate choice experiment, only one once-copulated male (either previous or new mate) was introduced into the female's cylinder. In the simultaneous mate choice experiment, the previous and new males were marked randomly by different trace colour powders (Magruder Color Co., Elizabeth, NJ, U.S.A.) for discrimination. The mark did not influence mate choice (binominal test: $P > 0.05$).

We conducted observations hourly, with the red light tube as illumination, during the whole scotophase until copulation occurred (the mating duration of this species is about 2 h; Xu et al. 2008).

Influence of Recopulation on Female Lifetime Reproductive Output and Daily Oviposition Patterns

The once-copulated females and males were obtained by allowing virgin moths to copulate in the plastic cylinders (one pair per cylinder) in the first scotophase following emergence. The second copulation was allowed in the second scotophase by pairing