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Direct costs and benefits of multiple mating: Are high female mating rates due to ejaculate replenishment?

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ABSTRACT

Females often mate more than is necessary to ensure reproductive success even when they incur significant costs from doing so. Direct benefits are hypothesized to be the driving force of high female mating rates, yet species in which females only receive an ejaculate from their mate still realize increased fitness from multiple mating. Using the Texas field cricket, *Gryllus texensis*, we experimentally test the hypothesis that multiple mating via monandry or polyandry increases female fitness by replenishing ejaculates, thereby allowing females to produce more offspring for a longer period of time. We found that higher rates of female mating significantly increased lifetime fecundity and oviposition independent of whether females mated with one or two males. Further, although interactions with males significantly increased rates of injury or death, females that replenished ejaculates experienced an increased rate and duration of oviposition, demonstrating that the immediate benefits of multiple mating may greatly outweigh the long-term costs that mating poses to female condition and survival. We suggest that ejaculate replenishment is a driving factor of high mating rates in females that do not receive external direct benefits from mating and that a comparative study across taxa will provide additional insight into the role that ejaculate size plays in the evolution of female mating rates.

1. Introduction

Sex consumes time and energy, can result in physical injuries, and drastically increases rates of parasitism, predation, and disease (reviewed in Arnqvist and Nilsson, 2000). Yet, females of many animal species mate more than is necessary to ensure reproductive success (Jennions and Petrie, 2000; Simmons, 1988) even though female fitness is generally limited by the number of eggs produced and not by access to males (Bateman, 1948; Trivers, 1971). Although male coercion might explain why females mate at surprisingly high rates (Arnqvist and Rowe, 2005), many qualitative (Rhainds, 2010; Simmons, 2005; Zeh and Zeh, 2001) and quantitative (Arnqvist and Nilsson, 2000; Slatyer et al., 2012a; South and Lewis, 2011) reviews suggest that females benefit from mating more than once. Multiple mating may increase overall fitness if females obtain indirect genetic benefits by mating with several males (i.e. polyandry) within a given a reproductive cycle (reviewed in: Jennions and Petrie, 2000; Zeh and Zeh, 2001). Such benefits include inbreeding avoidance (Hosken and Blanckenhorn, 1999), increased genetic compatibility (Tregenza and Wedell, 1998; Zeh and Zeh, 1996), and increased offspring fitness via "good" genes (Slatyer et al., 2012a) or heterozygosity at fitness enhancing loci (Brown, 1997). While many empirical studies support the indirect genetic benefits hypothesis, a recent meta-analysis of animal taxa (Slatyer et al., 2012a) found that the effect of indirect benefits obtained via polyandry on female fitness is relatively small and therefore unlikely to be the driving force behind the high rates of mating by females that are observed across taxa.

Alternatively, mating multiple times may increase female fitness if males provide direct benefits that boost female lifetime fecundity or survival (reviewed in Wagner, 2011). These benefits can take the form of protection from predators (Rodríguez-Muñoz et al., 2011), defense against harassing males (Cordero, 1999), assistance in defending resources (Slatyer et al., 2012b), male parental care (Knapp and Kovach, 1991), nutritional gifts (Gwynne, 1984; Fedorka and Mousseau, 2002), hydration (Ivy et al., 1999), replenishment of viable sperm (Ridley, 1988; Drnevich et al., 2001), increased immune function (Worthington and Kelly, in review at *Evolution*), or essential ejaculate-derived compounds (Boggs and Gilbert, 1979; Loher et al., 1981; Ursprung et al., 2009). Unlike indirect benefits, obtaining additional direct benefits through

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multiple mating does not necessarily require mating numerous males (Hunter et al., 1993). Females that repeatedly mate with the same male (i.e. monandry) have the potential to gain as much in fitness as females that mate polyandrously (Bybee et al., 2005; House et al., 2009; *but see* Noble et al., 2013).

Many studies examining the benefits of mating focus on species in which males provide females with a nutritious resource during courtship or copulation (i.e. nuptial gift), which females consume and use to increase the number or quality of their offspring (Gwynne, 1984; Fedorka and Mousseau, 2002; Simmons 1990; Tuni et al., 2013). However, there is evidence that ejaculates comprised only of sperm and accessory fluids can confer significant fitness benefits to females. For example, in some butterflies (*Heliconius helcale, Danaus plexippus,* and *Heliconius erato)*, nutrients derived from the ejaculate are incorporated into both the female's eggs and somatic maintenance (Boggs and Gilbert, 1979), increasing not only their fecundity but longevity as well. Alternatively, in *Gryllus* crickets, seminal fluid has been found to increase disease resistance in mated females (Worthington and Kelly, in review at *Evolution*).

Field crickets (*Gryllus* spp.) have been a useful model system for examining the fitness advantages of female multiple mating. Males provide an ejaculate comprised only of sperm and accessory fluid and offer no further contribution of resources, protection, or parental care. Empirical evidence for indirect benefits is contradictory in *Gryllus*, as studies show that polyandry increases (Simmons, 2001; Tregenza and Wedell, 1998), decreases (Jennionset al., 2007), or has no effect (Gershman, 2010) on offspring performance and hatching success. In contrast, there is strong evidence demonstrating that mating multiple times increases fecundity regardless of whether females mate monandrously or polyandrously (Gershman, 2010; Simmons, 1988; Wagner et al., 2001). This suggests that direct benefits obtained from the ejaculate are responsible for the increase in fitness. If ejaculatory substances (e.g. sperm or seminal fluid proteins) are required to stimulate oviposition, then females might remate frequently to replenish these substances. Failing to remate would thus result in decreasing offspring production because as ejaculatory substances decline in quantity through time, they limit female oviposition.

Here, we experimentally examine the fecundity benefit of multiple mating to female *Gryllus texensis* field crickets while also testing whether monandry or polyandry confer greater benefits. We partially quasi-replicate (Kelly, 2006) previous work (e.g. Simmons, 1988; Tregenza and Wedell, 1998; Wagner et al., 2001) by using an experimental design whereby females are paired with a male either once, twice, or continuously throughout a trial, and twice- and continuously-paired females are allocated either the same male (monandry) or two different males (polyandry) across matings. If females gain fecundity benefits from high mating rates, then we predict a positive relationship between female fecundity and number of mating opportunities. Additionally, if the fecundity benefits that females obtain from mating are derived from the ejaculate, then females within each treatment (twice or continuous) will exhibit equal fecundity regardless of whether they mated with one or two males. We also examine whether the act and type (i.e. monandry vs. polyandry) of mating negatively affects female survivorship. We predict that rates of injury and mortality will increase as females spend more time in the presence of males because pre and post-mating male aggression often results in loss of major limbs and even death (A. Worthington personal observation).

In a separate experiment, we test the hypothesis that multiple mating increases female fitness by increasing the rate of oviposition throughout the female's lifespan. If ejaculatory substances stimulate oviposition, then the number of eggs that a female lays after mating should decrease through time as the substances are depleted. However, we predict that re-mating will replenish these substances and allow females to not only lay eggs for a longer, but also at a higher rate. Additionally, we test whether multiple mating increases female lifespan, which could increase overall fitness if females that live longer also lay more eggs. Our study extends previous work on this topic by examining whether the immediate benefits of multiple mating outweigh the long-term costs that mating poses to female survival.

2. Materials and methods

2.1. Experimental animals

Crickets (long-winged *G. texensis*) were originally collected in Austin, TX in 2010 and have been maintained as a laboratory colony for 6 – 7 generations. Crickets were reared in large communal bins (73 × 41 × 46 cm) until their penultimate instar and were then kept individually in clear plastic 250 ml containers (10 cm diameter × 4.5 cm depth) to ensure virginity. Crickets were housed in an environmentally controlled room maintained at 28 °C on a 12:12 h light:dark cycle. All individuals were supplied cotton-plugged water vials and Special Kitty Premium Cat Food *ad libitum*. Experimental crickets were observed daily for eclosion. We standardized cricket age (7-day post-eclosion) and mated status (virgin) for all.

2.2. Effect of number and type of mating bouts on female fecundity and survivorship

We experimentally examined the fecundity benefit and survival cost of multiple mating in female *G. texensis*. We paired females with a male either zero times (i.e. virgin), once, twice, or continuously throughout a 15-day trial, and 'twice-paired' and 'continuously-paired' females were allocated either with the same male (monandry) or with two different males (polyandry) between each mating opportunity. At the beginning of the dark period (1200 h) on Day 0, we transferred each experimental female into a clear plastic mating arena (10 cm diameter × 4.5 cm depth) under a 25-W red light. Females in the virgin treatment remained solitary during this time, but those assigned to mating treatments ('once-paired', 'twice-paired', or 'continuously-paired') were randomly allocated an age-matched virgin male and given 6 h to mate. This method was useful in other studies examining the effect of mating on fitness (Sakaluk and Cade 1980; Wagner et al., 2001). Females may mate repeatedly while paired with a single male for 6 h. However, Simmons (1988) showed that mating multiple times on a single day does not result in increased oviposition in a closely related species, *G. bimaculatus*; therefore, repeated mating within the 6-h period should have no effect on how multiple mating affects female lifetime fecundity. At 1800 h, we measured female pronotum length to the nearest 0.001 mm (Leica Application Suite V3.8.0, Leica Microsystems. Switzerland), then transferred females to individual oviposition containers (16.5 × 10.5 × 7 cm) supplied with food, water, and moistened ReptiSand (ZooMed, San Luis Obispo, CA, USA) as oviposition substrate. Males were returned to their rearing containers, but those allocated to the 'continuously-paired' treatment were transferred into oviposition containers with their respective female. On Day 8, we again placed females in a mating arena at 1200 h. Virgin and 'once-paired' females remained solitary while 'twice-paired' and 'continuously-paired' females were provided with either the same male (i.e. monadrous) or with a different male (i.e. polyandrous) from their previous mating opportunity. At 1800 h, we transferred females to clean oviposition containers and males that were allocated to the 'continuously-paired' treatment were again housed with their respective females.

On Days 8 and 15 we collected, dried, and sieved oviposition sand, then counted the number of eggs that each female laid. We