

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.

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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 (Jennions et 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. monandrous) 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

initially used 574 female crickets; however, 18 monandrous trials were terminated because the male died before the second mating opportunity. We checked female crickets daily for loss of the saltorial hindlimbs and mortality throughout the trial. At the end of each trial (Day 15 at 1200 h) we euthanized surviving females by freezing them at -20°C and immediately quantified the number of eggs within their ovaries. Total fecundity for each female was calculated as the sum of all eggs laid by Day 15 and those stored within the ovary. We chose this time frame as it corresponds with the upper limit that adult crickets survive in the wild (Cade, 1976) and therefore more accurately reflects the predicted lifetime fecundity of females in the field. Finally, we photographed and measured the length of the five most-posterior (i.e. most developed) eggs in the right ovary as an estimate of egg quality.

We later dissected spermathecae of frozen females to determine how many females retained sperm at the end of the 15 days oviposition period. Each spermatheca was transferred to a 1.5 ml microcentrifuge tube containing 75 μl of distilled water, crushed three times using fine forceps, and mixed by vortexing for three 1 s pulses. The entire sample was then spread evenly over a microscope slide, allowed to air-dry, and observed at $400\times$ magnification (Leica DM 2500; Leica Microsystems, Germany) for the presence of sperm.

2.3. Female mating frequency

In a separate experiment, we determined female mating frequency by observing 36 pairs of crickets throughout a 6-h mating period to quantify the number of successful copulations completed during that time. Copulation was counted as successful only if a spermatophore was transferred to the female and remained attached after the female dismounted the male. After 6 h, we placed females in oviposition containers to mirror the experimental design used in our previous experiment. One week after the initial matings, we compared mating rates during a second 6-h mating period to ensure that fecundity and oviposition differences between monandrous and polyandrous females are not confounded by an increased tendency of females to mate with familiar or novel mates. Therefore, females were paired with either the same or a different male and observed for 6 h to quantify the number of successful copulations.

2.4. Effect of ejaculate replenishment on lifetime fecundity and longevity

To determine the effect of ejaculate replenishment on female lifetime fecundity and longevity, we paired male and female crickets in a clear plastic container under a 25-W red light and counted the number of copulations observed during 6 h. Afterward, females were placed alone in individual oviposition containers. One week after the initial mating opportunity, females were paired with a novel male and again observed for 6 h. Females were then housed in oviposition containers until natural death. During this time we counted the number of eggs laid each week and checked females daily for mortality. Longevity was measured as the number of days a female survived after the first mating bout. To examine the effect of ejaculate replenishment on lifetime fecundity, oviposition rate, and female lifespan, females were separated into three post-hoc treatment groups: (1) mated during the first period but failed to mate during the second ($n = 12$); (2) failed to mate during the first period but mated during the second ($n = 17$); or (3) mated during both periods ($n = 15$). We observed a total of 53 pairs of crickets, but females that did not mate during at least one period were removed from the final analysis.

2.5. Statistical analyses

2.5.1. Effect of number and type of mating bout on female fecundity and survivorship

We quantified the effect of mating type (monandry vs. polyandry) on the quantity (total fecundity and number laid) and quality (i.e. size) of eggs of females in the 'twice-paired' and 'continuously-paired' treatments. When non-significant, monandrous and polyandrous females were pooled within their respective treatments and the factor was dropped from further analyses. We then investigated the effect of the mating treatment (virgin, 'once-paired', 'twice-paired', or 'continuously-paired') on female fecundity by using a generalized linear model (GLM), including female pronotum length as a covariate because cricket fecundity is positively influenced by female body size (Kelly et al., 2014). We compared full to reduced models using AIC, and simplified models by removing interaction terms when appropriate (i.e. homogeneity of slopes). Because our count data (total fecundity and number of eggs laid) suffered from over dispersion and an excess of zeroes, we used 'hurdle' from the 'pscl' package with either a quasi-Poisson or negative binomial family of errors. These analyses resulted in a two-stage analysis, in which we first determined if treatments differed significantly in the probability of laying eggs (i.e. compared the proportion of individuals that did not lay eggs vs. those that did), and then compared the effect of mating treatment on fecundity for only those individuals that laid eggs. When treatment significantly affected egg quantity, we grouped the data in order to examine treatment differences that were identified *a priori*: virgin versus mated (i.e. pooled 'once-', 'twice-', and 'continuously-paired' females), 'once-paired' versus 'twice-paired', 'once-paired' versus multiply-paired (i.e. pooled 'twice-' and 'continuously-paired' females), and 'twice-paired' versus 'continuously-paired'. We did this because there is currently no consensus on how to conduct planned contrasts on data analyzed using a two-stage GLM. We controlled for multiple comparisons using the Holm–Bonferroni method (Holm, 1979). To examine the effect of mating treatment and body size (i.e. pronotum length) on egg size, we analyzed our data using ANCOVA. We first examined the interaction between mating treatment and pronotum length in order to test the assumption of heterogeneity of slopes. If the interaction term was non-significant, it was removed from the model and an ANCOVA was performed. Sample sizes vary across analyses due to missing data (e.g. some females did not have any eggs in their ovaries). To examine how mating interactions affect female survival, we used a Pearson's Chi-squared test to compare the proportion of injured/dead females from each of the mating treatments. Finally, we compared the proportion of females in each treatment that had sperm absent from their spermatheca at the end of the 15-day trial using Pearson's Chi-squared test.

2.5.2. Female mating frequency

We examined whether type of mating significantly affects female mating rate by using Fisher's Exact test to compare the proportion of monandrous and polyandrous females that successfully copulated during the second 6-h mating period. Then, because our data were non-normally distributed, we used a Mann–Whitney *U* test to determine if mating type affects the total number of ejaculates that females accept across all mating opportunities.

2.5.3. Effect of ejaculate replenishment on lifetime fecundity and longevity

We examined the effect of multiple mating on female lifespan using a Cox regression (Cox, 1972; Fox, 2002) to compare the proportion of females surviving after mating for each treatment. We also used a Cox regression to examine the effect of mating treatment on the duration of time a female lays eggs after mating.

Table 1
Summary statistics (mean \pm SE) for the effect of number of mating bouts and number of mates (twice and continuously-paired treatments only) on all dependent variables.

Mating treatment	N	Total fecundity	% Laid eggs	# Eggs laid	Egg size (mm)	% Sperm limited	% Injured or dead
Virgin	83	246 \pm 12	66%	019 \pm 3	2.24 \pm 0.01	N/A	7%
Once-paired	85	250 \pm 14	91%	092 \pm 10	2.26 \pm 0.05	39%	3%
Twice-paired	162	311 \pm 11	93%	114 \pm 9	2.24 \pm 0.02	27%	10%
Monandrous	82	334 \pm 14	90%	107 \pm 13	2.22 \pm 0.01	37%	13%
Polyandrous	80	287 \pm 16	97%	122 \pm 13	2.26 \pm 0.04	16%	7%
Continuously-paired	155	279 \pm 19	97%	149 \pm 10	2.27 \pm 0.02	12%	22%
Monandrous	78	264 \pm 14	98%	159 \pm 14	2.26 \pm 0.03	10%	13%
Polyandrous	77	295 \pm 11	96%	140 \pm 13	2.25 \pm 0.01	13%	29%

Because even virgin females lay a baseline number of unfertilized eggs, duration of mating-induced oviposition was determined by the number of weeks a female continued to lay eggs at a rate higher than the average virgin (~ 10 eggs/week as determined from the first experiment). To compare changes in mating-induced oviposition through time, we used one-sample *t*-tests to compare the time it took for the mean oviposition rates of females in each treatment to equal that of virgins (i.e. ≤ 10 eggs/week). Next, we examined whether timing of mating and ejaculate replenishment increase the total number of eggs laid using a GLM with a quasi-Poisson distribution. Our post-hoc mating treatments were included as the main effect of the GLM and duration of mating-induced oviposition was included as a covariate. We compared full and reduced models using AIC with models being simplified by removing interaction terms when appropriate (i.e. homogeneity of slopes). We used treatment contrasts to examine the specific effects of mating early in the reproductive period and ejaculate replenishment on female lifetime fecundity. Finally, we used non-linear regression to model and plot the 2-parameter asymptotic exponential relationship of the cumulative number of eggs laid throughout female lifespan for each treatment. All statistical analyses were performed in R version 2.12 (R Development Core Team, 2009) with $\alpha = 0.05$, and all data are archived on Dryad.

3. Results

3.1. Effect of mating type on egg quantity and size

Mating type (i.e. monandry vs. polyandry) did not have a significant effect on the total fecundity (i.e. the sum of all eggs stored in the ovaries and those that were laid) of females in the 'twice-' or 'continuously-paired' treatments (GLM: $t = 0.235$; $p = 0.8141$, $df = 316$). Further, mating type had no significant effect on whether females laid eggs (binomial hurdle model: $z = 1.265$; $p = 0.2058$, $n = 317$) or on the total number of eggs laid when they did oviposit (negative binomial hurdle model: $z = 0.719$; $p = 0.4724$, $n = 302$). Mating type did not significantly affect egg size (ANCOVA: $F = 1.943$; $p = 0.1643$, $df = 3, 309$); however, egg size was positively related to the covariate pronotum length (ANOVA: $F = 7.589$, $p = 0.0062$, $df = 3, 302$). Therefore, females that mated monandrously or polyandrously were pooled within their respective mating treatments for the remaining analyses. See Table 1 for summary statistics of each treatment group.

3.2. Effect of mating bouts on egg quantity and size

Mating treatment and female pronotum length had a significant effect on the total fecundity of a female (Tables 1 and 2). Specifically, multiply-paired ('twice-' and 'continuously-paired') females had significantly higher total fecundity than did 'once-paired' females (GLM: $t = 3.562$, $p = 0.0004$, $n = 401$) and larger females had higher fecundity than smaller females (GLM: $t = 7.666$, $p < 0.0001$).

Mating treatment also had a significant effect on whether females were stimulated to oviposit (Tables 1 and 2, Fig. 1a),

Table 2

Effect of the number of mating bouts (explanatory factor) and female pronotum (covariate) on total fecundity (ANCOVA), the ability to stimulate oviposition and the number of eggs laid (GLM), and egg size (ANCOVA). *p*-Values remaining significant after a Holm–Bonferroni adjustment are bolded. *p*-Values are from contrasts testing for differences between specific treatment comparisons.

Model parameter	Estimate	SE	Test statistic	<i>p</i> -Value
Total fecundity				
Virgin vs. mated	0.0571	0.0560	$t = 1.020$	0.3080
Once- vs. twice-paired	0.0746	0.0445	$t = 1.679$	0.0942
Once- vs. multiply-paired	0.1877	0.0527	$t = 3.562$	0.0004
Twice- vs. continuously-paired	0.0747	0.0445	$t = 1.679$	0.0942
Pronotum (mm)	0.4904	0.0639	$t = 7.666$	0.0001
Laid vs. Did not lay eggs				
Virgin vs. mated	2.2922	0.3332	$z = 6.286$	0.0001
Once- vs. twice-paired	0.3512	0.4855	$z = 0.724$	0.4690
Once- vs. multiply-paired	0.7428	0.4582	$z = 1.621$	0.1050
Twice- vs. continuously-paired	1.0301	0.5986	$z = 1.721$	0.0853
Pronotum (mm)	0.2748	0.5081	$z = 0.541$	0.5886
Only those females that laid eggs				
Virgin vs. mated	1.7911	0.1796	$z = 9.970$	0.0001
Once- vs. twice-paired	0.2133	0.1793	$z = 1.190$	0.2341
Once- vs. multiply-paired	0.3863	0.1410	$z = 2.739$	0.0062
Twice- vs. continuously-paired	0.3168	0.0102	$z = 31.13$	0.0001
Pronotum (mm)	0.2580	0.0773	$z = 2.576$	0.0100
Egg size (mm)				
Virgin vs. mated	0.0013	0.0270	$t = 0.498$	0.6184
Once- vs. twice-paired	0.031	0.0102	$t = 1.393$	0.2442
Once- vs. multiply-paired	0.0016	0.0036	$t = 0.671$	0.5022
Twice- vs. continuously-paired	0.0085	0.0049	$t = 1.691$	0.0914
Pronotum (mm)	0.0422	0.0126	$t = 3.353$	0.0008

as mated females were more likely to oviposit relative to virgin females (binomial hurdle model: $z = 6.286$, $p < 0.0001$, $n = 485$). When females were stimulated to oviposit (i.e. lay ≥ 1 egg) the number of eggs that they laid increased significantly with both their number of mating opportunities (see Table 2, Fig. 1b) and their pronotum length (negative binomial hurdle: $z = 2.576$, $p = 0.0100$, $n = 485$). It is of note that although twice-paired females laid more eggs than once-paired females, this difference was not statistically significant.

3.3. Effect of mating bouts on female survival

The rates of female injury and death significantly differed between mating treatments (Pearson's Chi-squared: $\chi^2 = 26.507$, $p < 0.0001$, $df = 3$; Table 1), with risk of injury or death increasing as exposure to males increased. Further, females that mated polyandrously experienced increased rates of injury and death relative to females that mated monandrously in the 'continuously-paired' (Fisher exact test: $p = 0.0096$, $n = 155$) but not 'twice-paired' (Fisher exact test: $p = 0.2223$, $n = 162$) treatment.

3.4. Effect of mating bouts on sperm presence

The proportion of females with sperm present in their spermathecae after 15 days significantly differed between the mating

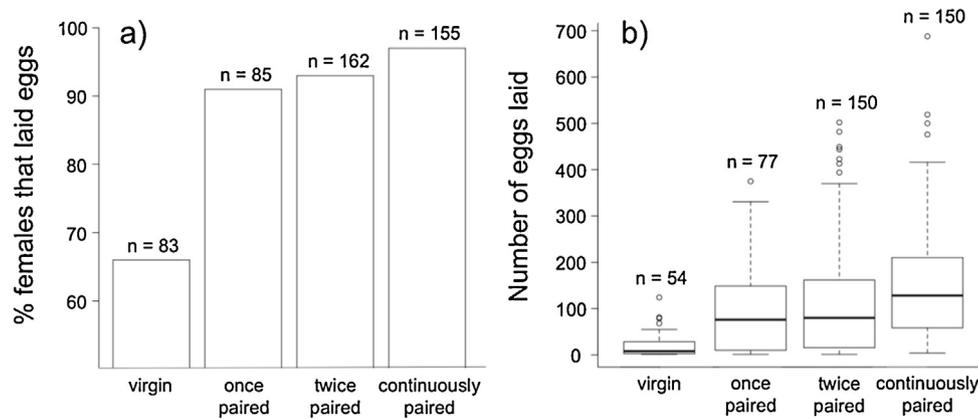


Fig. 1. Effect of the number of mating bouts on (a) the ability to stimulate oviposition and (b) the number of eggs laid in the two weeks after mating when oviposition was stimulated. For boxplots, the box represents the lower (25%) and upper (75%) quartiles, the solid dark horizontal line is the median, and the whiskers indicate 1.5 times the interquartile range. Data beyond the end of the whiskers are outliers and plotted as open dots.

treatments (Pearson's Chi-squared: $\chi^2 = 24.200$, $p < 0.0001$, $df = 2$; Table 1), where a greater proportion of 'twice-' or 'continuously-paired' females had sperm in their spermatheca compared to 'once-paired' females. Further, females that mated polyandrously were more likely to have sperm in their spermatheca relative to females that mated monandrously in the 'twice-paired' (Pearson's Chi-squared: $\chi^2 = 7.577$, $p = 0.0059$, $df = 1$) but not 'continuously-paired' treatment (Pearson's Chi-squared: $\chi^2 = 0.0783$, $p = 0.7796$, $df = 1$).

3.5. Female mating frequency

The average number of ejaculates a female received during 6 h was 1.89 ± 0.32 (mean \pm SE). Females given a novel male were not more likely to accept an ejaculate during their second mating opportunity compared to females provided with a familiar male (Fisher's Exact test, $p = 0.7949$, $n = 36$). Monandrous and polyandrous females did not significantly differ in the total number of ejaculates that they accepted from males (Mann-Whitney U test: $z = 1.0298$, $p = 0.3030$, $n = 36$).

3.6. Effect of ejaculate replenishment on lifespan, lifetime fecundity, and oviposition rate

We found that multiple mating did not significantly affect female lifespan (Cox regression: $z = 0.659$; $p = 0.5100$, $n = 44$), but did significantly affect the duration of mating-induced oviposition (Fig. 2), where females that mated during both opportunities laid eggs for significantly more weeks than females that mated only during the first (Cox regression: $z = 1.979$; $p = 0.0478$, $n = 44$) or second opportunity (Cox regression: $z = 3.318$; $p = 0.0009$, $n = 44$). The effect that our mating treatments had on the overall duration of mating-induced oviposition depended both on the frequency and timing (i.e. early or late in reproductive life) of mating. According to one-sample t -tests, females that mated during both opportunities laid eggs at a rate higher than virgins (10 eggs/week) for 4 weeks after the final mating ($t = 2.3248$, $df = 9$, $p = 0.0451$) compared to females that mated only during the first ($t = 2.9781$, $df = 11$, $p = 0.0126$) or second ($t = 2.3720$, $df = 14$, $p = 0.0326$) mating opportunity who exhibited only 3 weeks of mating-induced oviposition.

Both mating treatment and duration of mating-induced oviposition (measured in weeks) had significant effects on lifetime fecundity (Fig. 3). Specifically, we found that females that mated early in their reproductive life (i.e. only during the first opportunity) laid significantly more eggs than females that mated later in life (i.e. only during the second opportunity; GLM: $t = 2.532$

$p = 0.0154$, $df = 43$). Additionally, ejaculate replenishment significantly increased the total number of eggs laid compared to females that mated only during the first (GLM: $t = 3.516$, $p = 0.0009$, $df = 43$) or the second opportunity (GLM: $t = 3.627$, $p = 0.0008$, $df = 43$). Across all treatments, the number of weeks in which females laid eggs was positively related to female lifetime fecundity (GLM: $t = 3.101$, $p = 0.0035$, $df = 43$).

4. Discussion

As predicted, higher female mating frequencies significantly increased lifetime fecundity independent of whether females mated monandrously or polyandrously. Female *G. texensis* receive only a spermatophore and no other obvious fitness benefits from males (Loher and Dambach, 1989); therefore, our results clearly demonstrate that female field crickets gain direct benefits from the ejaculate. Further, because mating frequency did not increase female longevity, the greater number of eggs laid by multiply-mated females cannot simply be attributed to a longer lifespan, which concurs with a previous study in *G. vocalis* (Gershman 2007). Rather, we found that an increased rate of oviposition and a prolonged period of oviposition in the weeks after mating were responsible for the greater number of eggs laid by multiply-mated females.

Mating immediately increased the oviposition rate for all females, followed by a significant decline two weeks later, as demonstrated by our second experiment. However, the total number of eggs laid after mating was dependent on when a female mated. Females that mated on their first encounter with a male laid eggs at a higher rate than females that did not mate until their second encounter. Theoretical models show that the consequences of failing to acquire viable sperm in a reasonable amount of time after sexual maturation may considerably reduce an individual's fitness (Kokko and Mappes, 2005). Our results demonstrate this empirically, as females that failed to mate with the first available male suffered a significant decline in lifetime reproductive success. Further, these results suggest that optimally behaving females should readily mate any male early in the breeding season to ensure fertilization, and then continue mating at frequent intervals to maximize their subsequent offspring production.

We found that mating multiple times prolonged the high rate of oviposition that females experienced directly after mating. When females mated during only a single opportunity, their oviposition rates immediately and steadily declined each following week until they returned to rates equal to that of virgins during the third week. Females that mated during both opportunities, how-

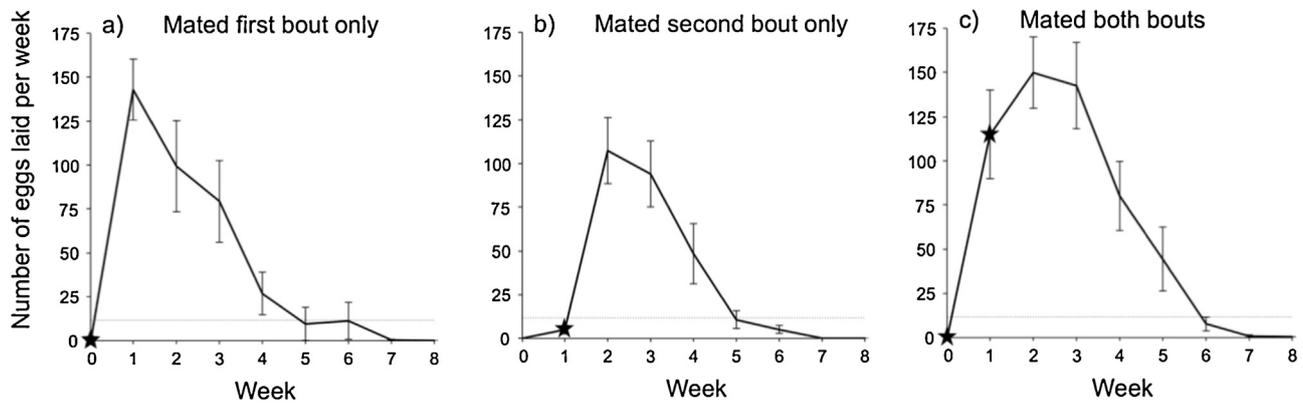


Fig. 2. The effect of the number of mating bouts on weekly oviposition rates (mean \pm SE) in females that mated during (a) the first opportunity only ($n = 12$), (b) the second opportunity only ($n = 17$), or (c) both opportunities ($n = 15$). Stars represent the week(s) that females in each treatment were mated and the horizontal dotted line identifies the average rate of oviposition exhibited by virgin females.

ever, experienced increased rates of oviposition not only after each mating bout, but also for an additional week for a total of four weeks of oviposition after their final mating. Consequently, females that mated during both opportunities laid a significantly greater number of eggs in the first three weeks of their reproductive life than females that mated only once during their entire lifespan. This result suggests that the immediate benefit of multiple mating could greatly outweigh the long-term costs imposed by mating on female condition and survival, and that mating multiply likely permits female *G. texensis* to replenish their sperm stores and other essential ejaculate-derived compounds (e.g. seminal fluid proteins, prostaglandins, etc.) in order to maintain high rates of oviposition.

We found that although mating at higher rates indeed increased female fecundity, mating had significant costs on female survival. Our first experiment demonstrated that females who spent more time in the company of males experienced significantly higher rates of injury and mortality, especially those females that mated with multiple males. Male aggression towards polyandrous females is a common, as males often increase mate guarding (Harts and Kokko, 2013; Wynn and Vahed, 2004) when the risk and intensity of sperm competition is high. In addition to male aggression, female crickets in nature also experience high rates of predation while searching for mates (Heller and Arlettaz, 1994; Sakaluk and Belwood, 1984) and high rates of infection from sexually transmitted diseases (Adamo et al., 2014; Zuk, 1987a,b). These past studies demonstrate that mating indeed has significant risks associated with it, which ultimately should select lower female mating rates.

If female crickets incur increased mortality from mating with numerous males, yet doing so does not increase their overall

fecundity, then what benefits do they gain by engaging polyandry (Bretman and Tregenza, 2005)? First, although our results demonstrate that mating once is enough to stimulate oviposition, an increased mating rate resulted in a greater likelihood of sperm presence in the spermatheca, especially in females that mated polyandrously. Therefore, mating with multiple males may reduce the likelihood of mating failures or receiving spermatophores devoid of sperm (Hunter et al., 1993). Evidence from orthopterans (Loher and Edson, 1973) and additional insect species (García-González, 2004) suggests that there is a high rate of mating failure. Males may experience sperm depletion after mating multiple times (Sturm, 2011) or strategically decrease the number of sperm that they invest in a given female (Simmons et al., 2007; Thomas and Simmons, 2007). Further, our study did not include an investigation of indirect benefits of mating, such as increased offspring hatching success, quality, or performance. Evidence for indirect benefits in field crickets are mixed however, with some studies showing support for the sexy sperm hypothesis (McNamara et al., 2014) or increased hatching success (García-González and Simmons, 2010; Simmons, 2001; Tregenza and Wedell, 1998), and others showing no evidence of increased hatchability (Jennions et al., 2007; Gershman, 2010), offspring performance (Jennions et al., 2007; Simmons, 2001; Tregenza and Wedell, 1998) or offspring quality (Jennions et al., 2007) related to polyandry. Regardless of the effect of indirect benefits, our study demonstrates that females readily engage in repeated mating with the same male and that the rate of mating does not differ between females that mate monandrously or polyandrously. These results suggest that females gain fitness

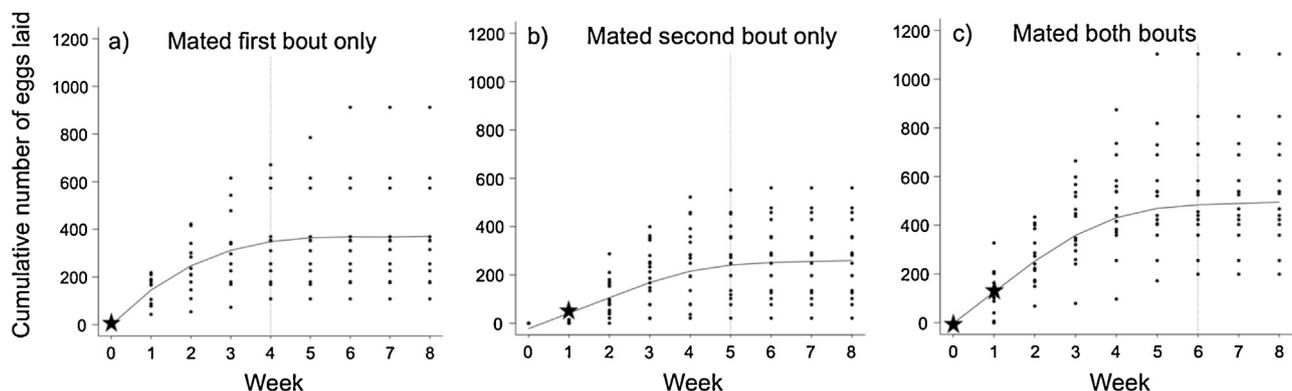


Fig. 3. The effect of the number of mating bouts on the cumulative number of eggs laid in females that mated during (a) the first opportunity only ($y = 384.17(1 - e^{-0.53x})$; $n = 12$), (b) the second opportunity only ($y = 334.83(1 - e^{-0.26x})$; $n = 17$), or (c) both opportunities ($y = 618.30(1 - e^{-0.33x})$; $n = 15$). Individual data points are represented by black dots. Stars represent the week(s) that females in each treatment were mated. The vertical dotted line identifies the point at which oviposition rate is equal to or less than that of virgin females (≤ 10 eggs/week).

benefits from ejaculate-derived substances, which might explain the high mating rate observed in female field crickets.

There are a number of ejaculate components that might be responsible for the direct benefits to fecundity and oviposition that we found. First and foremost, females may need to mate frequently to maintain supplies of viable sperm. To our knowledge, no one has examined the duration of time in which sperm remain viable in the spermatheca of crickets. If replenishment of viable sperm is indeed responsible for high mating rates, then we should find that the time at which females remate coincides with the time that the costs of sperm depletion (e.g. reduced egg fertilization) outweigh the costs of mating. However, the fact that females lay a large number of eggs for several weeks after mating, yet will readily remate immediately after removing a spermatophore from their genital tract, suggests that sperm replenishment is not solely responsible for the high rates of mating. Alternatively, seminal fluid proteins play a large role in reproduction in other species (Avila et al., 2011) and have significant effects in crickets as well. Recently, 21 seminal fluid proteins were identified in the cricket *Teleogryllus oceanicus*, many of which resemble those involved in post-mating changes to female reproduction in other insect species (Simmons et al., 2012). In *Gryllus*, however, isolated seminal fluid proteins induce only modest short-term oviposition. So although they may play a small role in increasing the fecundity of mated females, it is unlikely that seminal fluid proteins are driving increased oviposition or mating rates (Larson et al., 2012).

One other possibility to explain the reproductive benefits of multiple mating is that nutrients derived from the ejaculate might be directly responsible for the increased lifetime fecundity of females that mate frequently. Prostaglandin, a physiologically active lipid compound derived from C₂₀ polyunsaturated fatty acids, mediates oviposition behavior in crickets (Destephano and Brady, 1982; Loher et al., 1981; Stanley-Samuelson and Loher, 1983) and is required to stimulate egg laying after mating. Injecting prostaglandin into females initiates oviposition in a dose-dependent manner, where larger doses stimulate higher rates of oviposition and for a longer period of time than smaller doses (Destephano and Brady, 1982; Stanley Samuelson et al., 1986), similar to what is exhibited by females that receive multiple ejaculates. Because prostaglandin within the spermatheca is absent in virgins, only ephemerally available after mating, and maintained at large amounts by mating at high rates (Worthington et al., 2015), ejaculate-derived prostaglandin could provide the underlying reason as to why females remate frequently even when they have viable sperm stored in their spermatheca.

In a comparative context, orthopteran species in which ejaculates are larger also have lower mating rates (Jarčuška and Kaňuch, 2014; Vahed, 2006), perhaps because females do not need to replenish ejaculates as frequently. Interestingly, mating rates are not correlated with sperm number per se, but are predicted by overall ejaculate size (Vahed, 2006), suggesting that non-sperm components have significant effects on female mating rates across species. Because male investment in ejaculate size varies significantly across species (e.g. Sturm, 2014 demonstrates ejaculate size variation between four species of orthopterans), a comparative examination of female mating rates, sperm number, and ejaculate composition may provide additional insight into what role sperm, seminal fluid proteins, and accessory fluid components play in the evolution of female mating rates.

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