

POLYANDRY PROMOTES ENHANCED OFFSPRING SURVIVAL IN DECORATED CRICKETS

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Abstract.—Although female multiple mating is ubiquitous in insects, its adaptive significance remains poorly understood. Benefits to multiple mating can accrue via direct material benefits, indirect genetic benefits, or both. We investigated the effects of multiple mating in the decorated cricket, *Grylodes sigillatus*, by simultaneously varying the number of times that females mated and the number of different males with which they mated, measuring aspects of female fecundity and elements of offspring performance and viability. Multiple matings resulted in enhanced female fitness relative to single matings when females mated with different partners, but not when females mated repeatedly with the same male. Specifically, polyandrous females produced significantly more offspring surviving to reproductive maturity than did monogamous females mating once or mating repeatedly with the same male. These results suggest that the benefit females gain from multiple mating is influenced primarily by genetic and not material benefits.

Key words.—Crickets, genetic benefits, *Grylodes sigillatus*, multiple mating, offspring survival, polyandry, sexual selection.

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Although females often incur costs at mating through the increased risk of injury, disease, or predation, they frequently mate more often than is necessary to ensure the complete fertilization of their eggs (Daly 1978; Ridley 1988; Chapman et al. 1995; Blanckenhorn et al. 2002). Hypotheses that seek to account for this seemingly paradoxical behavior typically invoke two kinds of benefits to females, those that directly enhance a female's fitness (material benefits), and those that indirectly enhance a female's fitness through their effects on her offspring (genetic benefits). Material benefits to multiple mating include resources provided by males, reduced sexual harassment, and replenishment of sperm supplies (reviewed in Arnqvist and Nilsson 2000). Genetic benefits to multiple mating can accrue through the production of genetically diverse offspring, offspring of higher viability or quality, or offspring of higher sexual attractiveness, but only when females mate with different males (reviewed in Jennions and Petrie 2000). An accurate assessment of the relative contribution of material and genetic benefits to female fitness is essential, as there is still considerable debate as to their importance in explaining the evolution of polyandry (see Arnqvist and Nilsson 2000; Møller and Jennions 2001).

Although a number of studies have demonstrated a benefit to female multiple mating, in many cases it has been difficult to ascertain whether material benefits, genetic effects, or both, are responsible for the elevated fitness of multiply mating females. Some studies have not controlled for the number of matings experienced by females (e.g., Simmons 1988; Sakaluk et al. 2002), or have not included treatments involving females mating repeatedly with the same male or treatments involving females mating with different males (Keil and Sachser 1998; Watson 1998; Vahed 2003). While other studies strongly suggest a genetic rather than material benefit to polyandry, other explanations cannot be completely ruled out, as the inferences rely on correlation (i.e., number of partners or amount of extrapair paternity correlated to some aspect of

offspring viability or quality) (see Madsen et al. 1992; Olsson et al. 1994; and see Griffith et al. 2002 for discussion of this problem in studies involving birds).

Several investigators have sought to differentiate between the material and genetic benefits derived through multiple mating by holding constant the number of times that a female mates while varying the number of partners with which she mates (e.g., Tregenza and Wedell 1998; Newcomer et al. 1999; Evans and Magurran 2000; Baker et al. 2001; Simmons 2001; Wagner et al. 2001; Worden and Parker 2001; Fedorka and Mousseau 2002; Kamimura 2003). The rationale underlying these studies is that in species with no obvious paternal care, females need not mate with different males to secure material benefits through multiple matings (but see below), whereas genetic benefits can be obtained only by mating with different partners.

In an extensive review, Ridley (1988) noted that "impotent matings," matings in which the male fails to transfer any viable sperm to the female, frequently occur in a number of insect species. The acquisition of viable sperm with which to fertilize eggs is clearly a material rather than a genetic benefit, because it promotes the increased fecundity of females regardless of the genetic makeup of the male. In cases where the male produces normal sperm but simply fails to properly transfer sperm in the first mating, repeated matings with the same male should result in an increase in fecundity relative to females mating only once. However, if the male produces no sperm or abnormal sperm, repeated matings with the same male will not greatly enhance a female's reproductive success. In these cases, females would have to mate with multiple partners to secure viable sperm if male infertility was relatively common in a given population. Females should similarly seek out additional partners in those species in which males reduce their ejaculate size over successive matings, because the material benefit of sperm replenishment will be smaller for those females mating repeatedly with the

same male than for those mating with different males. For these reasons, the importance of genetic benefits in hatching success and fecundity may be inflated in studies that assess potential benefits to multiple mating by comparing females mating repeatedly with the same male and those mating polyandrously. Disentangling the material benefits of receiving viable sperm from indirect genetic benefits necessitates: (1) ensuring that, for each mating, females receive equal amounts of sperm and associated substances within the ejaculate, which can influence female reproduction (Chen 1984), and (2) including a treatment in which females mate only once in addition to repeated- and polyandrous-mating treatments because the mean hatching success of once-mated females provides a baseline measure of the average incidence of sterility or ejaculates of low sperm viability, without confounding material or genetic benefits. There have only been a few studies, all involving insects, that have separated the effects of number of copulations and number of mates while incorporating these design elements (Baker et al. 2001; Wagner et al. 2001; Worden and Parker 2001; Fedorka and Mousseau 2002).

We assessed the material and genetic benefits to female multiple mating in decorated crickets, *Grylodes sigillatus*, by simultaneously varying both the number of times that females mated and the number of different males with which they mated. We measured a number of fitness components representing both short-term (e.g., the number of offspring produced, egg hatchability) and long-term (length of offspring development, proportion of offspring surviving to adulthood, the mass of offspring at reproductive maturity) reproductive success. We report a significant increase in offspring survival of polyandrous females relative to monandrous females, suggesting that polyandry results in long-term genetic benefits to females.

MATERIALS AND METHODS

Study System

The decorated cricket, *Grylodes sigillatus*, occurs throughout the world in tropical and subtropical regions and is normally associated with human habitation (Smith and Thomas 1988). As is the case in other cricket species (Sakaluk and Cade 1980; Solymar and Cade 1990; Burpee and Sakaluk 1993), females mate repeatedly throughout their lives and with many different males, copulating as frequently as one or more times nightly (Sakaluk et al. 2002). Previous studies have shown that females may suffer increased predation while orienting to male calls (Sakaluk and Belwood 1984), and that nonvirgin females experience reduced longevity relative to virgin females (Burpee and Sakaluk 1993). Given the potential costs to female multiple mating, it is unclear why female *G. sigillatus* mate so frequently. Recent work in our laboratory suggests that polyandrous females may derive indirect genetic benefits via the production of male offspring of increased adult body mass (Sakaluk et al. 2002), but this study did not control for the number of matings and therefore could not eliminate the possibility of material benefits.

During mating, male *G. sigillatus* transfer a spermatophore consisting of a small sperm-containing ampulla surrounded by a large gelatinous spermatophylax. The spermatophylax

constitutes a nuptial food gift that the female detaches from the ampulla and consumes while the ampulla remains attached outside the female's genital opening after mating. Once the female has consumed or discarded the spermatophylax, she removes and eats the sperm ampulla, which terminates sperm transfer (Sakaluk 1984, 1987). Females can greatly influence the paternity of offspring through their ampulla-removal behavior, affording them an obvious mechanism by which they can selectively fertilize their eggs with the sperm of specific males (Sakaluk 1986; Sakaluk and Eggert 1996). Although the spermatophylax does not appear to provide *G. sigillatus* females with any detectable nutritional benefits (Will and Sakaluk 1994), previous work suggests that females benefit from ingesting the water contained in spermatophylaxes when they are water stressed (Ivy et al. 1999). Thus, multiple mating in *G. sigillatus* may enable females to secure both material and genetic benefits, making it an ideal study system with which to study the effects of polyandry on female fitness.

Experimental Protocol

Experimental *G. sigillatus* were the descendants of approximately 500 adults collected in Las Cruces, New Mexico, in May 2001. To prevent inbreeding and the loss of genetic variation, crickets were maintained in a large, panmictic population of approximately 5000 individuals. The experiment was carried out between March and October 2003. Adult crickets were housed in large plastic storage containers (59 cm × 43 cm × 30.5 cm) and provisioned with Flukers (Fluker Farms, Baton Rouge, LA) cricket chow, water supplied in 40 ml plastic tissue culture flasks plugged with cotton dental rolls, and egg cartons to provide shelter and to increase surface area. Moistened peat moss was made available both as an oviposition substrate and as a source of additional water. Late instar nymphs were separated by sex and reared to adulthood in an incubator at 32°C on a 16h:8h light:dark cycle.

Upon their adult molt, females were housed individually in 0.47 L containers. At three days of adult age, we weighed females to the nearest milligram and randomly assigned them to one of five treatments in which: (1) females were mated once to each of one, three, or five different males; or (2) females were mated three or five times to the same male. All experimental females completed their initial mating by the sixth day of adult age. Males used in experimental pairings were housed after they molted with nonexperimental, sexually mature females in plastic shoeboxes (10.5 cm × 7.5 cm × 3 cm) in a 2:1 female:male ratio for a period of six days to minimize variation in sexual experience among males. Although sperm numbers in *G. sigillatus* are repeatable across matings (Schaus and Sakaluk 2002), this sex ratio ensured that all males gained ample mating experience and concomitant resource depletion, based on mating frequencies observed in an earlier study of crickets held under similar circumstances (Burpee and Sakaluk 1993). On the seventh day of adult age, males were weighed to the nearest milligram and transferred to individual 0.47 L containers, where they were held until their use in mating trials. All experimental individuals were provisioned with food, water, and a dish containing moistened peat moss.

We observed matings in clear plastic viewing chambers (10.5 cm × 7.5 cm × 3 cm) lined with paper towels and viewed under red-light illumination. After each mating, females were allowed to consume the spermatophylax. When females discarded spermatophylaxes before they had been fully consumed, the partially eaten spermatophylax was recovered from the mating chamber with forceps and placed in the container housing the female. Females readily find and consume spermatophylaxes in this manner (Will and Sakaluk 1994; Ivy et al. 1999). We ensured that females of different treatments were fully and equally inseminated by allowing males to guard females for a period of 50 min, approximately the time taken for the transfer of a complete ejaculate (Sakaluk 1984). In cases where females eluded the guarding male before the 50 min period had elapsed, the female was removed from the mating arena and placed in a small centrifuge tube, thereby restricting her movement and preventing her from prematurely removing the sperm ampulla for the remainder of the 50 min insemination period. After the insemination period, females were returned to their respective containers where they were free to remove and consume the ampulla portion of the spermatophore. Thus, each mating resulted in the transfer of a complete ejaculate with equivalent amounts of sperm, seminal fluid, and accessory gland products, as well as the opportunity for females to derive any direct benefit from consumption of the spermatophore. Females assigned to treatments requiring more than one mating were given the opportunity to remate once or twice per day on each consecutive day in the manner described above until they had completed their schedule of matings. Females that did not complete their schedule of matings within six days of their initial mating were not included in the study ($n = 3$; no more than one in any treatment).

Mated females were housed individually in an incubator at 32°C on a 16h:8h light:dark cycle, and their position within the incubator was randomized daily. Experimental females were provided with egg carton, water, food, and oviposition substrate in the form of moistened peat moss. Oviposition substrate was removed after 12 days and placed in a separate container to facilitate the enumeration of newly hatched nymphs. Females were permitted to lay eggs until their death.

Upon their hatching, we reared a random sample of 20 of the first 300 nymphs produced by each female, housing them together in plastic shoeboxes under the same conditions as described above. Position within the incubator was randomized daily. Because the detection of genetic benefits to females may be manifest only when offspring are environmentally challenged, we imposed a nutritional stress on offspring by making food available to them only every third day. Food deprivation of this magnitude results in reduced offspring survival, increased developmental times, and lower mass at reproductive maturity (Sakaluk et al. 2002).

For each female, we recorded both the total lifetime number of offspring that hatched and the proportion of eggs failing to hatch within the first 12 days of oviposition. We restricted our analyses of hatching success to eggs laid in the first 12 days to avoid including infertile eggs that were a result of female senescence or a declining quality of stored sperm. Although replenishment of sperm is a potential direct benefit to multiple mating, our experiment was not designed

to examine this effect, as all females received their prescribed matings in a narrow time window, early in their adulthood.

We measured three aspects of offspring quality: (1) survival, calculated as the proportion of offspring surviving to sexual maturity; (2) developmental time, determined as the number of days from oviposition to hatch plus the average number of days from hatch until the first offspring had undergone adult eclosion; and (3) average adult mass upon the final molt. Because decorated crickets exhibit a sexual size dimorphism, with females being larger than males (Sakaluk et al. 2002), we considered adult mass of offspring separately for the sexes.

Data Analysis

Treatment effects were assessed with respect to a number of parameters related to female fitness: proportion of eggs that hatched over the first 12 days of oviposition, total number of nymphs, offspring developmental time, proportion of offspring surviving to sexual maturity, and mass of offspring at sexual maturity. We assumed that all of these parameters would be positively correlated with female fitness, except offspring developmental time, for which we assumed that more rapid development would be favored over a longer time to maturity. Because a preliminary ANCOVA revealed that both the number of nymphs ($F = 5.84$, $P = 0.0189$) and the proportion of offspring surviving to adulthood ($F = 12.2$, $P < 0.001$) were affected by the age at which experimental females completed their last mating, we used the residual values from these analyses for both variables in all subsequent analyses.

To assess differences in female reproductive success across treatments, we first analyzed our response variables with principal components analysis (PCA; Hatcher and Stepanski 1994) using PROC FACTOR in SAS (SAS Institute 1990). We then compared PC scores across treatments using multivariate analysis of variance (MANOVA; PROC GLM; SAS Institute 1990) to avoid the inflation in Type I error that results from the use of multiple nonindependent univariate tests (Scheiner 2001). Our reasons for employing PCA were threefold. First, PCA removes correlations from dependent variables by compressing original measures into a smaller number of independent expressions (Kleinbaum et al. 1988). This reduction in the number of dependent variables substantially increases the power of MANOVA, for which power declines as the number of response variables increases (Stevens 1992). Second, the uncorrelated predictors of reproductive output and offspring performance obtained through PCA ensure a more accurate analysis, as robustness of MANOVA is negatively influenced by the number of highly correlated responses (see Langsrud 2002). Third, uniting correlated responses under a single principal component permits an examination of possible trade-offs between different fitness components (Scheiner 2001).

Standardized canonical coefficients, which indicate by their magnitude the variables that contribute to significant MANOVA effects, were used to interpret the results of the MANOVA (Scheiner 2001). We employed multivariate contrasts to make a priori comparisons of the effects on female fitness of single mating versus multiple mating, polyandry

TABLE 1. Mean (\pm SE) survival and reproductive success of female decorated crickets mated one, three, or five times with the same or different males ($n = 12$ for each treatment).

Treatment	Longevity (days)	Number of nymphs	Proportion of eggs that hatched	Development time (days)	Proportion of offspring surviving	Male offspring mass (mg)	Female offspring mass (mg)
Once mated	48.67 \pm 3.13	818.3 \pm 71.9	0.963 \pm 0.010	67.79 \pm 1.28	0.45 \pm 0.04	177.9 \pm 7.0	215.5 \pm 6.7
3 \times same male	41.50 \pm 3.01	695.1 \pm 77.5	0.971 \pm 0.010	67.17 \pm 1.28	0.53 \pm 0.05	172.2 \pm 7.2	212.5 \pm 6.2
5 \times same male	49.42 \pm 4.38	752.2 \pm 115.9	0.977 \pm 0.006	68.17 \pm 1.39	0.57 \pm 0.04	181.5 \pm 7.2	216.3 \pm 8.8
3 \times different males	45.58 \pm 5.03	555.2 \pm 111.9	0.941 \pm 0.024	66.25 \pm 1.26	0.65 \pm 0.06	193.4 \pm 7.0	234.6 \pm 10.5
5 \times different males	51.00 \pm 5.53	810.3 \pm 109.2	0.970 \pm 0.010	69.13 \pm 1.62	0.68 \pm 0.03	171.3 \pm 5.4	228.7 \pm 8.0

versus monogamy, and mating three times versus mating five times. Least-square means were used to make pairwise comparisons among treatments within particular PCs.

In one family, all 20 offspring died before reaching sexual maturity, so we were unable to obtain direct measurements of offspring developmental time or offspring mass for this female. This posed a potential difficulty because MANOVA does not accommodate missing values for any of the original variables. To circumvent this problem, we replaced the missing values for these response variables with the mean value of the other females in the same treatment. Omission of this replicate from the analysis did not alter the conclusions.

RESULTS

The mean survival and reproductive success of females in the five treatments is shown in Table 1. There was no difference between treatments in the mean mass (\pm SE) of females ($F_{4,55} = 0.18$, $P = 0.946$), nor was there any difference in the average mass of males assigned as mating partners to females in the five treatments ($F_{4,55} = 1.05$, $P = 0.389$). There were no differences in female longevity across treatments ($F_{4,55} = 0.75$, $P = 0.560$).

Principal Components Analysis

Although the principal components analysis yielded only three factors with eigenvalues greater than one, we elected to use the first four factors because the fourth factor appeared to represent offspring development, an important component of offspring fitness. The removal of this factor did not change the results of subsequent analyses. These four principal components summarized 81.3% of the variation in female reproductive success (Table 2).

Rotated factor scores show that PC1 quantifies a trade-off between hatching success and offspring mass at sexual maturity, with high scores on PC1 resulting from higher offspring mass at adulthood and lower hatching success. Low scores of PC1 result from greater hatching success but lower offspring mass at adulthood (Table 3). PC2 quantifies female reproductive output, as demonstrated by the large positive

loadings of hatching success and lifetime number of nymphs produced. Thus, hatching success and number of nymphs are positively correlated, and large scores of PC2 indicate greater female fecundity. PC3 and PC4 quantify different aspects of offspring quality, with high scores on PC3 resulting from higher offspring survival to sexual maturity. The large positive loading of development time on PC4 indicates that high scores of PC4 represent longer development times.

Multivariate Analysis of Variance

The MANOVA revealed a significant effect of mating treatment on the four PC scores representing components of female fitness (Wilks' lambda = 0.609, $F_{16,159.5} = 1.76$, $P = 0.0413$). Multivariate contrasts showed no significant difference in the effect of multiple matings versus single matings (Wilks' lambda = 0.934, $F_{4,52} = 0.91$, $P = 0.46$), and no significant difference in the effect of mating three times versus mating five times (Wilks' lambda = 0.885, $F_{4,52} = 1.69$, $P = 0.17$). However, there was a significant difference in the effect of mating polyandrously versus mating monogamously (Wilks' lambda = 0.793, $F_{4,52} = 3.38$, $P = 0.0156$). Analysis of the standardized canonical coefficients for both the overall MANOVA and the multivariate contrast revealed that PC3 (offspring survival), and to a lesser extent, PC1 (trade-off between proportion of eggs hatching and offspring mass at maturity), contributed most to the overall treatment effect (Table 4).

Pairwise comparisons among relevant least-square means revealed no significant differences in PC1 scores across treatments (offspring masses vs. proportion of eggs hatched, all $P > 0.1$). However, analysis of PC3 (offspring survival to sexual maturity) revealed significantly higher scores for the polyandrous treatments than for the monogamous treatments (Fig. 1). Specifically, females mated to five different males produced a higher proportion of offspring surviving to sexual maturity than did once-mated females ($P = 0.0078$), females mated three times to the same male ($P = 0.0216$), and females mated five times to the same male ($P = 0.0228$). Similarly, females mated to three different males produced a higher

TABLE 2. Principal components analysis of female reproductive success in decorated crickets. Principal components listed in bold are those that were retained in the MANOVA of treatment effects on female reproductive output and offspring performance.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	1.67	1.34	1.03	0.83	0.62	0.50
Proportion of variance	0.28	0.22	0.17	0.14	0.10	0.08
Cumulative proportion of variance	0.28	0.50	0.67	0.81	0.92	1.00

TABLE 3. Principal components analysis of female reproductive success: factor loadings. Factor loadings in bold indicate those original variables that load strongly on each principal component (loadings > 0.4).

Original variable	Varimax rotated factor pattern			
	PC1	PC2	PC3	PC4
Hatching success	-0.52	+0.66	-0.00	-0.16
Number of nymphs	+0.13	+0.91	-0.08	+0.00
Development time	-0.11	-0.06	-0.02	+0.98
Offspring survival	+0.02	-0.09	+0.95	-0.02
Male offspring mass	+0.75	-0.10	-0.28	-0.20
Female offspring mass	+0.80	+0.09	+0.27	-0.03
Interpretation	Offspring mass vs. hatching success	Female reproductive output	Offspring survival	Offspring developmental time

proportion of offspring surviving to sexual maturity than did once-mated females ($P = 0.0347$), but did not differ significantly from females mated three times to the same male ($P = 0.0826$) and females mated five times to the same male ($P = 0.0865$). There were no significant differences in PC3 scores between any of the monogamous treatments (all $P > 0.5$), nor was there any difference in PC3 scores between the two polyandrous treatments ($P = 0.55$).

DISCUSSION

Multiple mating in *G. sigillatus* appears to provide significant fitness benefits to females (Sakaluk et al. 2002; present study), but these benefits become evident only when females mate polyandrously. Females mating polyandrously produced significantly more offspring surviving to adulthood than did either once-mated females or females mating monogamously. For that reason, we conclude that the benefits to multiple mating in this species are primarily genetic and not material. This conclusion is further supported by two other findings: (1) there was no difference in the fitness of females mating repeatedly with the same male and those mating only once, and (2) when compared to females mating three times, females mating five times (regardless of whether females mated with the same or different males) did not exhibit an increase in either offspring numbers or quality.

Although the genetic benefits resulting from polyandry in female *G. sigillatus* are consistent with evidence obtained for other crickets (Tregenza and Wedell 1998; Fedorka and Mousseau 2002), the lack of material benefits in this species is at odds with studies of other orthopteran species, particularly those in which males provide females with a nuptial food gift at mating. These gifts often have a positive effect on female fecundity (Gwynne 1984; Simmons 1990; Fedorka and Mousseau 2002), and in a recent review, Arnqvist and Nilsson (2000) reported stronger positive effects of multiple mating on egg production in insect species with nuptial feeding than in those lacking nuptial gifts. Unlike the gifts of

many species, however, the nuptial gifts of *G. sigillatus* appear to be devoid of significant nutritional content (Will and Sakaluk 1994; Warwick 2000). In addition to nuptial gifts, the ejaculates of male crickets often provide important material benefits to females, both in terms of replenishing sperm supplies and providing substances that stimulate egg production. These substances have been shown to increase both female fecundity (Loher 1979; Simmons 1988; but see Tregenza and Wedell 1998) and longevity (Bentur and Mathad 1975; Wagner et al. 2001). However, our results suggest that female *G. sigillatus* secure a sufficient amount of ejaculatory substances from a single mating to meet their reproductive needs.

In a recent meta-analysis of the incidence of multiple mating in insects, Arnqvist and Nilsson (2000) argued that polyandry, especially in gift-giving insects, can be explained primarily by direct benefits and that indirect benefits are unlikely to play an important role in the maintenance of multiple mating. Although this may be the case for some species, our results are inconsistent with this hypothesis because mating repeatedly with the same male did not lead to an improvement in any fitness measure relative to mating only once. Given that females apparently do not need to remate to maximize offspring production and that females in the field are subject to an increased risk of predation each time they seek out a novel mating partner (Sakaluk and Belwood 1984), it seems likely that genetic benefits play a predominant role in the maintenance of polyandry in this system.

Although our study was not designed to test the specific hypothesis that females remate throughout their lifetimes to replenish sperm supplies, we found no indication that female *G. sigillatus* are sperm limited or that they mate with multiple partners to ensure the acquisition of viable sperm. Females mating only once produced as many offspring as those mating multiply, and there were no instances in any treatment of females failing to leave offspring. The period over which nymphs hatched in this study was 22.2 days, and assuming

TABLE 4. Standardized canonical coefficients derived from MANOVA of four principal components representing different aspects of female fitness.

Source	Standardized canonical coefficients			
	PC1	PC2	PC3	PC4
Treatment (overall MANOVA)	0.66	-0.43	0.77	-0.16
Multivariate contrast polyandry vs. monogamy	0.61	-0.27	0.89	0.03

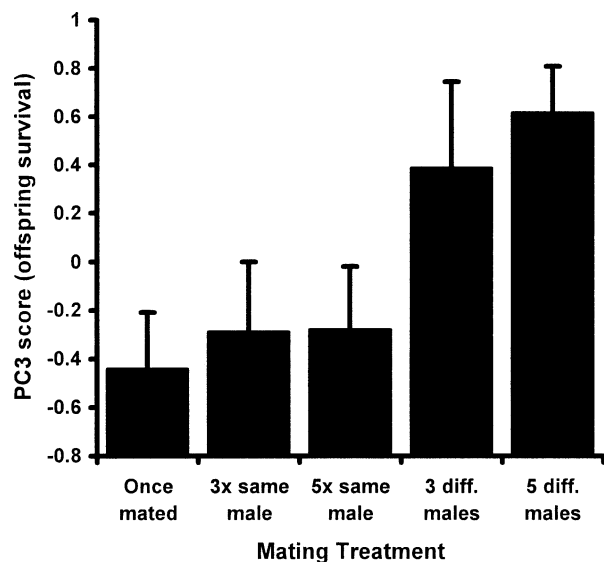


FIG. 1. Mean (\pm SE) PC3 scores (representing survival to reproductive maturity) of female decorated crickets mated one, three, or five times with the same or different males ($n = 12$ for each treatment).

that this value reflects the number of days over which females laid viable eggs, this period likely exceeds the time over which sperm must remain viable to ensure maximum fertility in nature. Free-living females live about three weeks under field conditions (Sakaluk et al. 2002), whereas females in the laboratory live more than twice as long (an average of about 47 days in the present study). Thus, it seems reasonable to assume that a free-living female could obtain a sufficient number of sperm from one mating to fertilize all of the eggs she is likely to lay in her lifetime.

Although the difference in fitness between females mating with three and five different males was not statistically significant, we suggest that females would enhance their fitness by maximizing the number of mating partners. Females mating with five males show a significantly smaller variance in proportion of offspring surviving to adulthood than females mating with three males ($F_{11,11} = 3.00$, $P < 0.05$). This decrease in variance may represent genetic bet hedging, whereby females mating with many males decrease their chances of suffering reproductive failure (Philippi and Seger 1989; Fox and Rauter 2003). The increased genetic diversity of offspring resulting from mating with multiple partners may result in higher offspring survival in stochastic environments, because females increase their chances of producing offspring possessing either genes that enhance survival in particular environments or genes that are expressed favorably in multiple environments (see Hunt et al. 2004).

Evidence that polyandry provides genetic benefits continues to mount (for reviews see Jennions and Petrie 2000; Griffith et al. 2002; and Hosken and Stockley 2003), but the precise nature of these benefits remains obscure. In distinguishing between two of the major classes of indirect genetic benefits, the avoidance of genetic incompatibility and the acquisition of “good genes” (review in Zeh and Zeh 2003), it has become customary to attribute any increase in hatching success resulting from polyandry to a reduction in genetic

incompatibility, whereas any increases in offspring viability and performance are typically attributed to genes obtained from superior sires (e.g., Tregenza and Wedell 1998; Kempenaers et al. 1999; Simmons 2001; Sakaluk et al. 2002). However, this distinction is probably overly simplistic because the effects of genetic incompatibility and “good genes” can be manifest at any stage of reproduction. For example, dominance effects are an unambiguous facet of genetic compatibility because they involve the nonadditive interaction of alleles at a locus, one from each parent. However, there is increasing evidence that traits related to fitness show high levels of dominance variance at all life stages, not merely those influencing hatching success (Crnokrak and Roff 1995; DeRose and Roff 1999; Merilä and Sheldon 1999). Further, we see no reason why characteristics inherited from superior sires could not play a role in embryonic development and survival, the primary determinants of hatching success. On a final note, failure to detect an effect of sire should not be taken as *prima facie* evidence that genetic compatibility is responsible for elevated fitness in polyandrous females, as good genes effects are generally small (Møller and Alatalo 1999), and thus large samples sizes may be needed to achieve adequate power to detect them (Griffith et al. 2002). Hence, regardless of which fitness parameters are influenced, additional experiments will be required to differentiate between the processes underlying the genetic benefits of polyandry, particularly those arising from the interaction of maternal and paternal genomes and those stemming from effects intrinsic to particular paternal genomes. Such experiments are critical to understanding mating system evolution because in mating systems that are characterized by genetic benefits, the strength and direction of selection on males is expected to differ greatly depending on which process leads to genetic benefits (Colegrave et al. 2002). For example, with respect to good-genes mechanisms, all females are expected to favor the same males, whereas the genetic compatibility hypothesis predicts that no single genotype will be optimal for all females (Zeh and Zeh 2003).

Although the results of our study add to a growing body of evidence that genetic benefits play a paramount role in the evolution of polyandry in crickets (Tregenza and Wedell 1998, 2002; Simmons 2001; Sakaluk et al. 2002), the fitness benefits that females obtain vary both across species and between studies of the same species. For example, some studies have demonstrated an increase in hatching success with an increase in polyandry, but no increase in offspring viability or performance (Tregenza and Wedell 1998; Simmons 2001), whereas others have failed to detect any effect on hatching success, but have reported significant effects on offspring quality (Simmons 1988; Sakaluk et al. 2002); one study has reported positive effects of polyandry on both hatching success and offspring quality (Fedorka and Mousseau 2002). In a previous study of *G. sigillatus*, Sakaluk et al. (2002) found that the mass of male offspring increased with increasing opportunities for female polyandry, but they detected no effect on offspring survival, contrary to the present study. Although the earlier study did not control for number of matings, there were also a number of differences with respect to the environmental conditions under which females were maintained (e.g., temperature, photoperiod). This seeming

inconsistency in treatment effects, both within and across species, raises interesting questions in terms of the general applicability of studies that seek to measure benefits to multiple mating in the laboratory. In nature, conditions are variable in time and space, and it is clear that seasonal variation in extrinsic factors, such as temperature and photoperiod, can greatly influence the mating behavior of insects (Ikeda 1976; Meats and Fay 2000; Walker and Cade 2003). However, although many studies of multiple mating have subjected experimental females to varying nutritional regimes, very few have varied extrinsic factors experienced by free-living individuals that might influence reproductive allocations and the costs and benefits associated with polyandry (but see Tregenza et al. 2003). Future studies should attempt to vary such conditions within the same experiment to better understand the circumstances that might lead to differences in the benefits afforded to females through multiple mating.

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