THE ROYAL SOCIETY biology letters

Extreme repeated mating as a counter-adaptation to sexual conflict?

G. Laird[1,2], D. T. Gwynne[1*] and M. C. B. Andrade[2]

[1]*Biology Department, University of Toronto at Mississauga, Mississauga, Ontario L5L 1C6, Canada*
[2]*Integrative Behaviour and Neuroscience Group, University of Toronto at Scarborough, Scarborough, Ontario M1C 1A4, Canada* (*mandrade@utsc.utoronto.ca*)
[*] *Author for correspondence* (*dgwynne@utm.utoronto.ca*).

Recd 19.01.04; *Accptd* 09.03.04; *Published online* 06.05.04

The Australian scaly cricket, *Ornebius aperta*, can copulate over 50 times with the same partner; the benefits of such extreme repeated copulation are unclear. We support the hypothesis that repeated copulation increases insemination success, as the number of sperm transferred increases with each spermatophore. This probably increases paternity for males, as on average a female mates with over 40 males. Despite intense sperm competition each ejaculate has only a few hundred sperm, orders of magnitude less than in related crickets. We show that all sperm are transferred from each spermatophore in the few seconds before a female removes and eats it. Repeated copulation increases effective copulation duration while a small ejaculate ensures that this strategy is not excessively costly. Thus repeated copulation in these crickets may have arisen as a counter-adaptation to female-imposed limits on copulation.

Keywords: repeated mating; scaly cricket; sexual conflict; sperm

1. INTRODUCTION

Males typically mate multiply (with different females) because male reproductive output increases with each new mate (Bateman 1948). Less common, and more puzzling, is repeated mating with the same female because it is unclear how this benefits males. Benefits for females are also unclear because frequent mating can be costly for females (e.g. in terms of time, energy or predation risk; Hunter *et al.* 1993). An extreme example of repeated copulation is in Australian scaly crickets, *Ornebius aperta* (Orthoptera: Mogoplistidae), where a male can transfer over 50 spermatophores (sperm-filled packages) to his mate in the absence of outside disturbances (Andrade & Mason 2000). Because females completely consume each spermatophore after transfer, repeated copulation by male *O. aperta* may function as a male nutrient investment. However, this is less likely than in other cricket species where spermatophores have specialized nutritional structures (Gwynne 1983; Vahed 1998; Shaw & Khine 2004). Second, repeated mating insures against failed spermatophore attachment (Sakaluk & Cade 1983). Third, repeated mating may increase paternity by increasing the number of sperm inseminated (Dewsbury 1982; Thornhill & Alcock 1983; Simmons 1987), particularly if males are constrained from optimizing the number of sperm transferred in a single copulation (Wedell *et al.* 2002). Increasing insemination by repeated mating may indicate a history of sexual conflict (Chapman *et al.* 2003) if female *O. aperta* interrupt sperm transfer when eating the small spermatophore (e.g. Boldyrev 1915). This is suggested by the rapid (*ca.* 3 s) spermatophore removal (Andrade & Mason 2000), an exceptionally brief period when compared with other insects with externally attached spermatophores (Alexander & Otte 1967) including another mogoplistid cricket (Dambach & Beck 1990).

Here, we support the hypothesis that repeated mating by males has arisen as a counter-adaptation to rapid spermatophore consumption by females. We found that, as expected given the cost of sperm (Wedell *et al.* 2002), a spermatophore contains only as much sperm as can be transferred during the brief attachment period. In support of the paternity assurance hypothesis, the number of sperm inseminated increases with each spermatophore transferred.

2. MATERIAL AND METHODS

We first examined whether rapid spermatophore removal by females interferes with sperm transfer. Last-instar females from colonies at the University of Toronto (Mississauga, Canada) were reared in isolation until sexual maturity to ensure virginity. Recently caught (less than one week) adult males from gardens of the University of Western Australia (Perth, Australia) were similarly kept in isolation in the laboratory for at least 5 days prior to use in mating trials to ensure previous matings had not depleted sperm. The crickets were housed in individual 5.7 cm × 4.0 cm × 4.0 cm clear plastic boxes on a 12 D (20 °C) : 12 L (25 °C) cycle at 65% relative humidity (see Andrade & Mason 2000). Crickets had *ad libitum* access to fresh apple, fish flakes, rolled oats and bee pollen; and moist cardboard for oviposition.

Males and females were paired haphazardly and randomly assigned to a control or lengthened spermatophore attachment treatment. Each cricket was used only once. We compared the number of sperm transferred to the female's sperm storage organ (spermatheca) in these treatments to the number of sperm in the spermatophores that males carry in their genitalia just before mating. Control pairs ($n = 12$) were allowed to mate once (one spermatophore transferred), and females were allowed to remove the spermatophore immediately after attachment, as in natural matings. This allowed us to quantify the average number of sperm transferred during a typical first mating. To determine how many sperm would be transferred if the spermatophore was not rapidly removed, females were prevented from removing the spermatophore for 10 min following initial attachment (200 times longer than in natural matings) ($n = 11$). We distracted females from bending to eat the spermatophore by lightly stroking them with a paintbrush. Control females were similarly stroked for 10 min following spermatophore consumption.

We assessed the number of sperm in the spermatophore prior to mating by using forceps to remove the first spermatophore appearing in the male's genitalia. Males were paired with virgin females then anaesthetized with carbon dioxide after spermatophore production but prior to mating ($n = 18$). The spermatophore or spermatheca was dissected, placed on a glass slide, shredded with a sharp pin and mixed with a drop of water to create a uniformly dilute sperm solution (technique developed from L. W. Simmons, personal communication and Simmons & Achmann (2000)). After the solution dried, the total number of sperm heads on the slide was counted under a microscope

We next examined sperm transfer as a function of the number of copulations completed by a male. It was not possible to measure the number of sperm transferred with successive copulations by a single male because experimental spermatophore removal interrupted the repeated mating sequence. Instead, we manipulated the number of repeated copulations completed by pairs, and measured the total number of sperm subsequently transferred to a female.

Last-instar male and female *O. aperta* from our Australian field site were laboratory reared in isolation until sexual maturity. They were housed and fed as described above. Randomly chosen adult virgin females were each allowed 0, 1, 2, 4, 8 or 16 copulations with a virgin male of the same post-moult age. Each cricket was used only once. Matings were staged in 8.3 cm × 8.3 cm × 15.5 cm plastic boxes with *ca.* 4.0 cm of moist sand. A leaf of *Philodendron scandens* anchored in

DOI 10.1098/rsbl.2004.0198

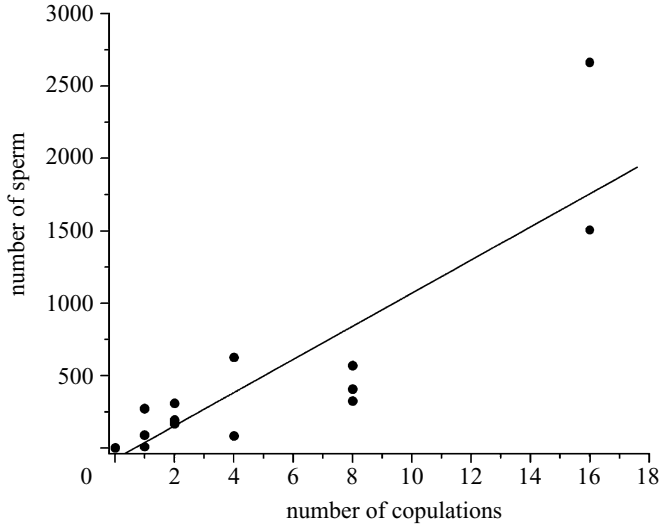

Figure 1. The number of sperm transferred to the spermathecae of virgin female *Ornebius aperta* crickets in relation to the number of repeated matings males were allowed to complete ($y = 114.5x - 75.6$). Overlapping points are indicated by numbers.

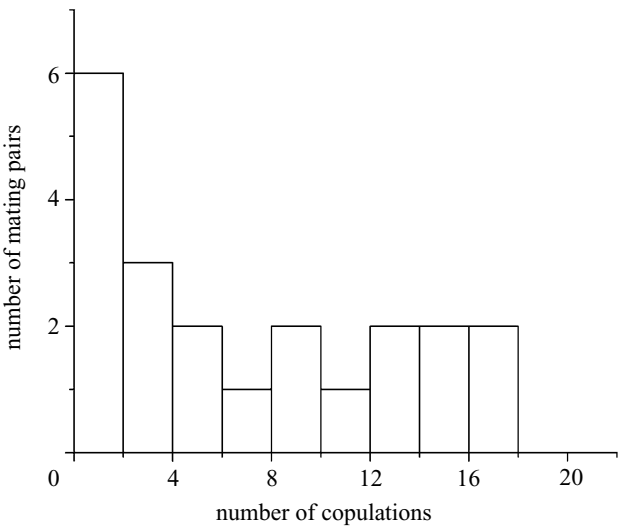

Figure 2. Frequency distribution of the number of repeated matings by pairs of *Ornebius aperta* in nature ($n = 21$). Each mating took a mean 8.7 ± 2.1 min (mean ± s.d.; $n = 12$).

the sand provided a mating platform on which the crickets could interact, as in nature. The box was cleaned, and the sand and leaf replaced before each mating. One hour after the final copulation in a trial, the female's spermatheca was dissected and the number of sperm counted as above.

Finally, to estimate the potential level of sperm competition in nature, we estimated female mating frequency by comparing the number of sperm stored by field-caught adult females, the number stored by experimental females in our manipulated mating experiment (above) and the mean number of repeated matings with a single male. Adult females ($n = 10$) were collected at our field site in December 2001, their spermathecae dissected, and sperm counted (mature breeding *O. aperta* can be observed year-round). To estimate natural repeated mating rates, we observed undisturbed complete copulations at our field site ($n = 21$).

3. RESULTS

The number of sperm transferred to females during a single normal (control) copulation ranged from 5 to 225 (mean ± s.d.; 95% confidence limits: 100.8 ± 72.1; 54.9–146.6; $n = 12$) and did not differ significantly from the number of sperm transferred when females were prevented from removing the spermatophore for 10 additional minutes after mating (115.1 ± 92.6; 52.9–177.3; $n = 11$) or from the total number of sperm available within spermatophores immediately prior to mating (106.6 ± 88.4; 62.7–150.6; $n = 18$) ($F = 0.08$, $p = 0.921$). Thus, males apparently include only as many sperm per spermatophore as can be transferred during the few seconds of attachment and ejaculate, a remarkably low number of sperm with each copulation. Other crickets typically transfer thousands or tens of thousands of sperm in a single spermatophore (e.g. Simmons 1986; Schaus & Sakaluk 2001).

The total number of sperm transferred to a female was positively related to the number of spermatophores (repeat matings) received (figure 1; $r^2 = 0.82$, $p < 0.0001$, $n = 20$: analysis conducted on $\log(x + 1)$-transformed variables to restore homogeneity of variances). This strong linear relationship between sperm transfer and number of repeated copulations suggests that the number of sperm transferred per copulation remains constant. Thus, failure to inseminate is rare and insurance against failed copulations (Sakaluk & Cade 1983) cannot explain male repeated mating. Moreover, these data confirm that males transfer only about 100 sperm in every copulation (not just the first).

The number of copulations (spermatophores transferred) by a male is expected to have a strong influence on his paternity given that mature females ($n = 10$) store a mean (± s.d.) of 23 804 ± 10 223 sperm. This corresponds to *ca*. 240 copulations per female. Our field observations, including the fact that population density is very high, suggest that these copulations may be with as many as 48 different males, because in nature females receive a median five spermatophores from each mate ($n = 21$; figure 2). (This is lower than the average observed in laboratory matings (Andrade & Mason 2000) because most field matings are interrupted by rival males or other animals, whereas longer laboratory pairings are generally ended by females.)

4. DISCUSSION

Our results support the hypothesis that repeated mating increases insemination success and thus paternity for *O. aperta* males, assuming that sperm mix while stored by females, as in other crickets (Simmons 1987). Moreover, a history of selection via sexual conflict is suggested because male repeated mating is associated with very small ejaculate size and rapid termination of insemination by spermatophore-removing females. Removal probably occurred because females foraged by eating spermatophore capsules. In another cricket, females eat several sperm-less microspermatophores before receiving a larger sperm-filled spermatophore (Shaw & Khine 2004; see also Simmons 1988).

Virtually no sperm remain in the *O. aperta* spermatophore when it is removed and eaten by the female. If a significant number of sperm did remain, this would suggest either that the conflict is not in equilibrium (with

females gaining the upper hand; see Arnqvist & Rowe 2002) or that the sperm in spermatophores is a nutrient gift.

Polyandry (multiple mating) and thus the high risk of sperm competition in *O. aperta* predict that males should inseminate as many sperm as possible in a given female to maximize fertilization (Parker 1998). Because females remove the spermatophore rapidly and the ejaculate is so small (Simmons 1986; Schaus & Sakaluk 2001), males achieve this through repeated mating. Because males should be prudent with sperm allocation (Wedell *et al.* 2002), *O. aperta* males may pass multiple spermatophores with few sperm to avoid wasting the sperm that would be consumed by females if they transferred a single larger spermatophore.

Studies of sexual conflict support the idea that costs increase with each additional copulation by a female (Chapman *et al.* 2003). Many of these costs will increase whether females mate repeatedly (i.e. with a single male) or multiply (polyandry). We conclude by noting that in *O. aperta* a female behaviour—rapid spermatophore removal—has favoured small ejaculates coupled with repeated mating by males, and this in turn appears to require that females suffer the costs of many repeated and multiple matings to avoid sperm limitation. Thus our results suggest the intriguing idea that sexual conflict over mating duration could drive the evolution of very frequent copulation.

Acknowledgements

This work was supported by NSERC grants to D.T.G. and M.C.B.A., and a PREA to M.C.B.A. We appreciate comments on this study and the manuscript from L. Rowe, A. C. Mason and R. Laird. Work in Australia was greatly facilitated by L. Simmons, I. Dadour and C. House; L. Simmons and K. Shaw provided methodological help and access to unpublished manuscripts.

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