INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

The Adaptive Significance of Multiple Mating by Males in the Polygynandrous Waterstrider *Aquarius remigis* (Heteroptera: Gerridae)

Richard Vermette

A thesis

in

The Department

of

Biology

Presented in Partial Fulfilment of the Requirements

for the Degree of Master of Science

Concordia University

Montreal, Quebec, Canada

May 2001

ABSTRACT

The Adaptive Significance of Multiple Mating by Males of the Polygynandrous Waterstrider *Aquarius remigis*

Richard Vermette

In polygynandrous species, sperm competition and female post-insemination processes may influence male paternity success. Hence, insemination (i.e. successful sperm transfer) may not guarantee reproductive success for the male. Using a variation of Parker's (1970) sterile (irradiated) male technique, I examine the relationships among paternity success, mating success (proportion of matings resulting in sperm transfer) and duration of copulation for males of the polygynandrous waterstrider *Aquarius remigis*. Prolonged copulation is characteristic of this species and has been interpreted as a paternity assurance strategy, analogous to post-copulatory contact guarding. Mating success was a significant predictor of fertilization success for males. The results of this study lend strength to previous studies of sexual selection in *A. remigis* that have used mating success as an estimate of reproductive fitness. However, the presence of considerable unexplained variance in paternity success suggests that post-insemination processes may also be important. Copulation duration correlated negatively with mating success, suggesting a trade-off between mating frequency and duration. Contrary to expectations, copulation duration also tended to have a negative effect on paternity success. These results suggest that prolonged copulation is not analogous to post-copulatory contact guarding and does not function as a paternity assurance strategy in *A. remigis*.

ACKNOWLEDGMENTS

I would like to thank Dr. Daphne Fairbairn for her patient guidance and enthusiasm throughout this study. The suggestions of committee members Dr. J. Grant and Dr. P. Albert served to improve the quality of my project and were greatly appreciated. Thanks to Dr. R. Roy for his help in using the X-ray apparatus and to the staff at Procrea for their helpful suggestions. Many thanks to Ian Ferguson, Jeff Reeve, Matthias Foellmer and Cheryl Johnson for their input, and to Angela Wilby, Genevieve Ring and Véronique Campbell for their technical assistance. Finally, I wish to express my gratitude to Mylen Carpentier and my family for their unwavering support. Dissections in Experiment 2 were performed by V. Campbell.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Sexual selection can be defined as differential reproduction owing to variation in the ability to obtain mates (Darwin 1871). Hence, many studies continue to focus on the effects of particular traits on mating success, assuming a simple and direct relationship between the frequency or number of matings and reproductive fitness (*e.g.* Houck *et al.* 1985; Harvey 1990; Arnqvist 1992a, 1992b; Sih and Krupa 1992; Krupa and Sih 1993; Andersson 1994; Petrie and Halliday 1994; Preziosi and Fairbairn 1996; Weigensberg and Fairbairn 1996; Arnqvist *et al.* 1997; Abell *et al.* 1999; Goulson *et al.* 1999; Savalli and Fox 1999; Shine *et al.* 2000). However, recent studies have reported large variances in post-mating paternity success in a variety of animal species (e.g. Rubenstein 1989; Lewis and Austad 1990; Schwagmeyer and Foltz 1990; Simmons and Parker 1992; Birkhead *et al.* 1995; Conner 1995; Sakaluk and Eggert 1996; Dzuik 1996; Radwan and Siva-Jothy 1996; Eberhard 1996; Cook *et al.* 1997; Wirtz 1997; Arnqvist and Danielsson 1999a, 1999b) suggesting that successful copulation does not guarantee reproductive success for the male. When females are capable of obtaining sperm from two or more males, competition between males for female gametes may extend beyond insemination (sperm competition: Parker 1970). Thornhill (1983) and Eberhard (1991, 1994, 1996, 1998) have also suggested that female post-insemination processes may play an important role in determining which sperm fertilizes her eggs. It is therefore necessary to investigate how successful copulation translates into offspring production when using mating success as an estimate of male reproductive fitness. Referred to as the sexual selection gradient by Arnold and Duvall (1994), the relationship between

mating success and fertilization success defines the single common path to reproductive fitness for all sexually selected traits.

Prolonged copulation, where males remain *in copula* beyond the time necessary for complete sperm transfer, has been observed in many insect species (Parker 1970; Thornhill and Alcock 1983; Alcock 1994). Several hypotheses been proposed to explain the adaptive significance of prolonged copulation, most of which have focussed on the benefits for the male. In polygynandrous species, benefits of prolonged copulation for the male may include increased time for 1) the transfer of greater sperm volumes (Arnqvist and Danielsson 1999b); 2) the transfer of non-gametic components of the ejaculate (Parker 1970, Wing 1985, Gwynne 1986); 3) strategic positioning of gametes (Siva-Jothy and Tsubaki 1989, Waage 1979), or 4) copulatory courtship (Eberhard 1991, 1994). Another hypothesis is that the male remains *in copula* to prevent his mates from acquiring additional sperm from rival males (copulatory guarding: Parker 1970, Sillen-Tullberg 1981, Thornhill and Alcock 1983; Wing 1985; Clark 1988; McLain 1989; Alcock 1994; Danielsson 1998). Hence, the duration of copulation may have profound effects on post-insemination paternity success. However, males that engage in prolonged copulation may incur costs in the form of lost mating opportunities with additional mates (Parker 1970, Arnqvist 1988), increased risk of predation (Fairbairn 1993, Stockley 1997) and lost foraging opportunities (Blanckenhorn *et al* 1995). For prolonged copulation to evolve and persist, the benefits must outweigh these costs.

The waterstrider *Aquarius remigis* is a common heteropteran insect found in lotic habitats throughout most of temperate North America (Gallant and Fairbairn 1997). It is a polygynandrous species; both sexes generally mate at least once per day over a two to

three month period (Blanckenhorn *et al.* 1995, Preziosi and Fairbairn 1997). Females can maintain full fertility and fecundity for at least 15 days (Fairbairn unpublished) and continue to lay viable eggs for up to 24 days following a single mating (Rubenstein 1989). Therefore, overlapping of ejaculates from different males should be quite common in this species. Rubenstein's (1989) study of sperm competition in *A. remigis* revealed that an average of 65% of eggs were fertilized by the last male to mate prior to oviposition. However, the last male advantage was highly variable as some 'last' males achieved almost complete fertilization success while others obtained virtually none (Rubenstein 1989). These results are based on artificially ordered, staged mating assays involving only two males. This is not very representative of natural conditions in polygynandrous species (Zeh and Zeh 1994). Nevertheless, Rubenstein's (1989) results suggest that the relationship between mating and fertilization success may be highly variable in *A. remigis*.

Prolonged copulation is characteristic of *A. remigis* (Wilcox 1984, Clark 1988, Rubenstein 1989). Males require matings of at least 15 minutes to complete sperm transfer (Rubenstein 1989, Campbell and Fairbairn unpublished). The average duration of copulation is two to three hours (Fairbairn 1988) but matings of over 12 hours have been observed (Wilcox 1984; Weigensberg and Fairbairn 1994). Copulation is generally terminated with a struggle which is indicative of sexual conflict over the duration of copulation (Weigensberg and Fairbairn 1994, Rowe *et al.* 1994, Gentile 1998). Rubenstein (1989) found that males increased their fertilization success if they increased their relative copulation duration. Given the last male sperm precedence in *A. remigis*, prolonged copulation in waterstriders is generally considered a mate guarding tactic

(Rubenstein 1989). However, Rubenstein's (1989) study did not consider lost mating opportunities associated with prolonged copulation.

The primary objective of this thesis is to determine whether male mating success is a significant predictor of male reproductive fitness in polygynandrous species, using *A. remigis* as a model. To do so, I used a variation of Parker's (1970) sterile male technique for paternity analysis. Since the technique involves the irradiation of males, I have included an appendix summarizing my preliminary investigation of the effects of irradiation on male behaviour and sperm transfer. As a secondary objective, I investigated the effects of prolonged copulation on the relationship between fertilization success and mating success. Considering the costs associated with prolonged copulation, the copulatory guarding hypothesis predicts that 1) for an equal number of matings, males that engage in longer copulations will fertilize a greater proportion of eggs (Rubenstein 1989), and 2) males will regulate copulation duration in order to maximize their fertilization rate (Clark 1988).

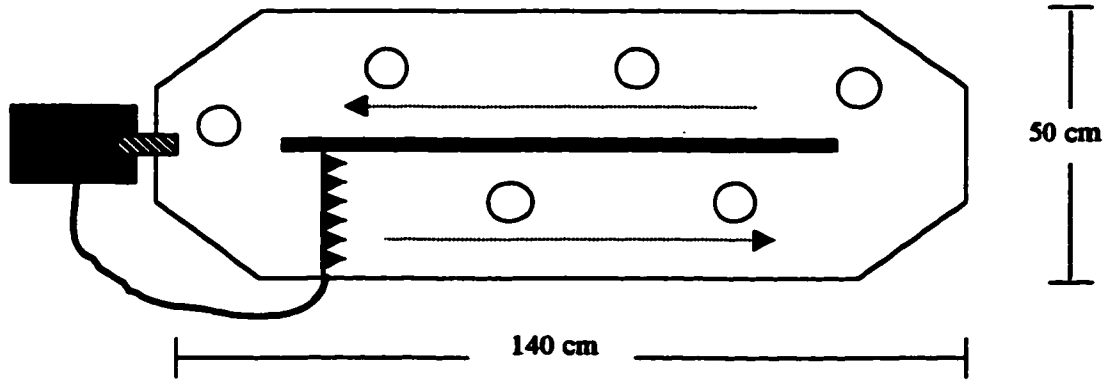
METHODS

Several hundred pre-reproductive waterstriders of both sexes were collected from three streams in southwestern Quebec (Morin-Heights, Mont Orford, and Mont Tremblant) in September and early October of 1999. The waterstriders were brought to the laboratory where they were placed in an environmental chamber (10L:14D, 10°C ± 2°C,). Once diapause was induced, I moved the striders to sphagnum-filled plastic containers at 4°C where they remained until early February 2000. In February, I transferred 32 waterstriders of each sex to aerated tanks (50 x 40 x 20 cm, water depth 10cm) and fed them one frozen *Acheta* cricket each day. Inverted Styrofoam cups were provided as resting and oviposition sites. Previous experiments have established these as a suitable oviposition substrate (Gentile 1998). The waterstriders were allowed to acclimate to laboratory conditions (14L:10D, 22°C ± 2°C) over a three day period. Males and females were held in separate tanks to ensure the virginity of females.

To estimate male fertilization success, I used a variation of Parker's (1970) sterile male technique (Jablonski and Kaczanowski 1994). Prior to the sterilization, I marked all males with numbers from 1-32 using non-toxic yellow enamel paint (Fairbairn 1985, Krupa and Sih 1993). Once marked, I randomly selected 16 males for sterilization. Selected waterstriders were irradiated with 12 Krad X-ray doses using a Mueller MG300, Mod 301/4 X-ray tube. Preliminary experiments (Appendix 1) have established that this dose does not affect behaviour and results in sperm transfer, but not fertilization success.

Figure 1

Schematic of the artificial streams used for the mating assay



aquarium pump and outlet

water intake

water outlet

inverted Styrofoam cups

Table 1

Distribution of fertile and sterile males among streams and tanks.

	Focal male	Background males
3 streams	1 sterile	3 fertile
3 streams	1 fertile	3 sterile
fertile "control"	none	4 fertile
sterile "control"	none	4 sterile

Following irradiation, I assigned each male at random to one of two treatments in six artificial streams (Fig 1). Irradiated males were focal males in three streams and "background" males in three streams (Table 1). The eight remaining males were placed in one of two reference tanks (50 x 40 x 20 cm; water depth: 10cm). One tank contained four sterile males and served to estimate the degree of sterility achieved by irradiation. The other tank contained four fertile males and served to estimate levels of fertility of non-irradiated males. Both tanks were equipped with inverted Styrofoam cups and an air diffuser to simulate a current. All males were fed one cricket each and allowed to recover overnight at 22 ± 2°C.

At 9h55 on the following day, I introduced four marked virgin females to all streams and tanks and allowed them to mate freely with the males. The females were marked on their mid- and hind- femora so identification was possible when mating (Gentile 1998). Once the females were introduced, I scanned each stream every 15 minutes between 10h00 and 20h00 for four consecutive days and recorded the number of the female mating with each male. The 15 minute time interval corresponds to the minimum amount of time necessary for sperm transfer to occur in *A. remigis* (Rubenstein 1989, Campbell and Fairbairn, unpublished). I estimated copulation duration by counting the number of consecutive times a male was seen copulating with the same female. Thus, if a mating pair was seen six consecutive times, the duration of copulation was scored as 90 ± 15 minutes. If the mating pair was not seen on two consecutive scans (i.e. mating did not last at least 15 min) the mating was scored as unsuccessful. This criterion has been used in previous assays of mating success in *A. remigis* (e.g. Blanckenhorn *et al.* 1995; Preziosi and Fairbairn 1996). I analysed the relationships among mating success,

copulation duration and paternity success using both total matings and successful matings only.

At 20h00 on day 4 of the experiment, each group of females was transferred from their respective artificial streams to corresponding, aerated water tanks (40 x 27 x 13 cm, water depth: 7.5cm) within an environmental chamber set to 22 ± 2°C, 14L:10D. The females were fed one frozen cricket per day and provided with two new Styrofoam cups for resting and oviposition. The eggs laid on Styrofoam cups between days 1-4 were transferred to separate water tanks (32 x 17 x 9 cm , water depth: 7.5 cm) in the environmental chamber where they were allowed to develop for four days. On the fifth day, the eggs were assessed for viability under a dissecting microscope. Eggs were considered viable if they darkened in colour and red eye-spots appeared (Rubenstein 1989, Gallant and Fairbairn 1997).

I continued to collect eggs from the isolated groups of females every three or four days for 18 days. This provided fertility estimates for a total of 22 days from the beginning of the mating assay (collections on days 4, 8, 11, 15, 18, 22). Estimates of fertility were divided into two periods; 1) fertility prior to isolation of females (days 1-4), and 2) fertility after isolation of females (days 5-11). Eggs collected on days 8 and 11 were pooled to provide us with a sufficient sample size for a reliable estimate of fertility. Viability estimates for eggs collected on days 15, 18 and 22 were unreliable because female fertility declined and females began dying. These estimates were excluded from my analysis. The experiment was repeated seven times under identical environmental conditions for a total of eight replicates. One fertile focal male died on the second day of

the mating assay. Hence, I collected data for a total of 47 focal males (23 fertile and 24 sterile).

When fertile and sterile focal males were compared, I estimated the proportion of offspring attributable to focal males (x) from each tank by using the following formula, which corrects for possibility of incomplete fertilization success of fertile males, and incomplete sterility of irradiated males (Rubenstein 1989; Ueno and Ito 1992):

$$x = (a-c)/(b-c)$$

In tanks where the focal male was fertile:

a = mean proportion of viable eggs from the focal males tank

b = mean proportion of viable eggs from the fertile control tank

c = mean proportion of viable eggs from the sterile control tank.

For tanks with sterile focal males, a = the proportion of non-viable eggs from the focal male's tanks; b = the proportion of non-viable eggs from the sterile control tank, and c = the proportion of non-viable eggs from the fertile control tank.

When fertile and sterile males were not compared, there was no need to apply the correction factor as I deal exclusively with variance in the proportion of eggs attributable to males of the same treatment. In addition, corrected data violated the assumption of normality and required ranking. Hence, I follow Arnqvist and Danielsson (1999b) and used uncorrected data. The use of uncorrected data eliminates the need for ranking.

RESULTS

Mating success, copulation duration and the proportion of eggs attributable to focal males showed considerable variation among focal males (Table 2). As expected (Appendix 1- experiment 1), the behavioural variables (mating success and copulation duration) were very similar for fertile and sterile focal males. However, sterile focal males were generally attributed a lower proportion of eggs than fertile focal males. A two-way analysis of variance (Table 3) indicated no significant effect of week (replicate) on the proportion of eggs attributable to focal males, but irradiation had a highly significant effect (Table 3). This is consistent with the finding that irradiated males experience a reduction in their long-term ability to transfer sperm (Appendix 1- experiment 3). Hence, data for fertile and sterile focal males were analyzed separately.

Fertile focal males: Covariance analysis was performed to determine whether the relationship between proportion of eggs attributable to fertile focal males and the proportion of matings acquired or mean duration of copulation (expressed as the deviation from the mean of the focal male's group) remained constant across the eight weeks of experimentation. Neither the main effect of week or the interaction had any effect on the proportion of eggs attributable to fertile focal males (all p values > 0.11). Therefore, I pooled the data for all eight weeks.

The proportion of matings acquired was a significant univariate predictor of the proportion of eggs fertilized by fertile focal males on days 1-4 (Fig 2 – solid line) and on days 5-11 (Fig 2 – dashed line).

Table 2

Mean and range of mating success, copulation duration and the proportion of eggs attributable to focal males. Male behavioural traits are summed over four days.

	Fertile focal males		Sterile focal males	
	Mean	Range	Mean	Range
Number of successful matings (>15 min)	6.08	3 - 10	6.21	3 - 11
Proportion of successful matings	0.25	0.13 - 0.43	0.27	0.10 - 0.44
Proportion of total matings	0.26	0.11 - 0.36	0.27	0.08 - 0.48
Duration of successful matings (min.)	241.3	99 - 559	280.2	70 - 589
Proportion of eggs attributable (days 1-4)	0.55	0 - 1	0.14	0 - 0.4
Proportion of eggs attributable (days 5-11)	0.61	0 - 0.93	0.10	0 - 0.26

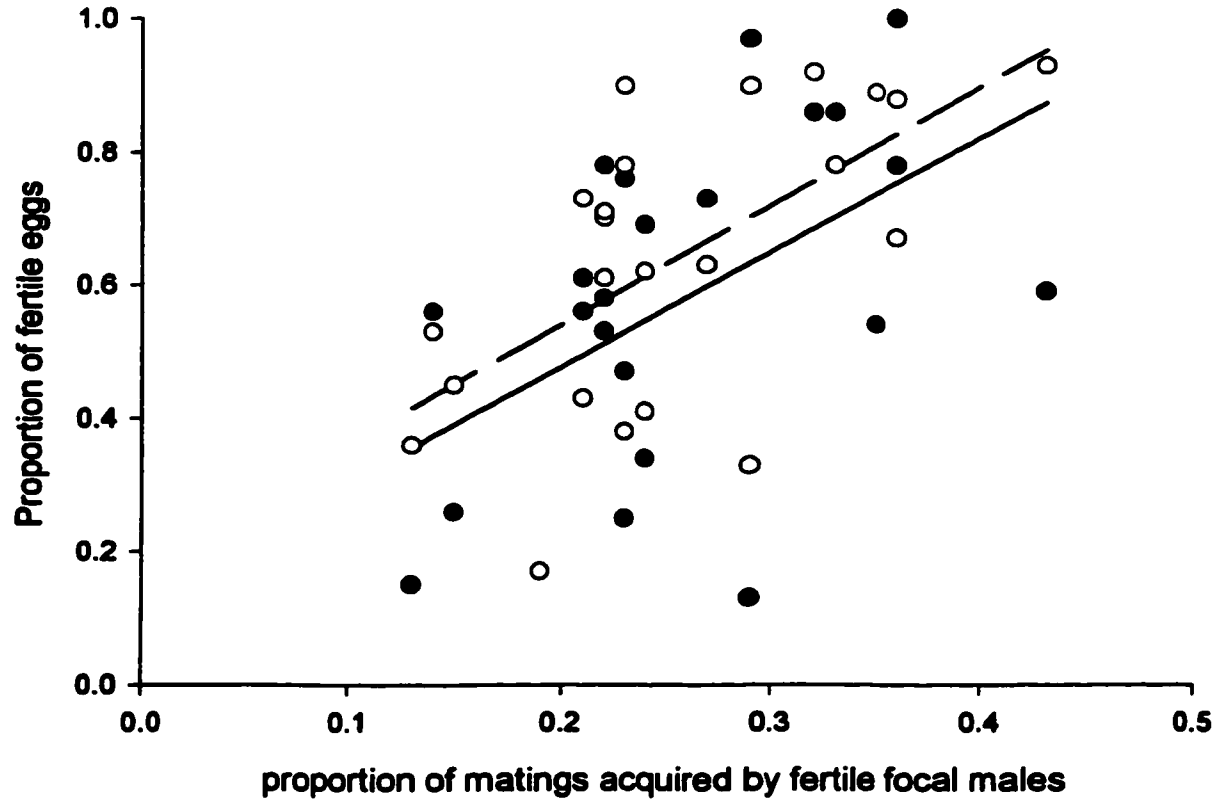
Table 3

Results of the two-way analysis of variance testing for the effect of week (i.e. replicate) and irradiation treatment on the proportion of eggs attributable to focal males, for eggs laid on days 1-4 and 5-11

	Days 1-4			Days 5-11		
Source of variation	F	df	p	F	df	p
Week	1.949	7	0.094	0.965	7	0.472
Treatment (sterile/fertile)	70.717	1	0.000	98.107	1	0.000
Interaction	0.644	7	0.700	0.495	7	0.831

Figure 2

Relationship between the proportion of fertile eggs and the proportion of matings acquired by fertile focal males. The solid line represents the least square best fit line for eggs laid during the first four days of the experiment (filled circles) ($F_{1,21} = 6.305$, $p = 0.020$, $r^2 = 0.231$). The dashed line represents the least square best fit line for eggs laid during days 5 to 11 (empty circles) ($F_{1,21} = 12.029$, $p = 0.002$, $r^2 = 0.364$).


1.0
0.8
0.6
0.4
0.2
0.0
Proportion of fertile eggs
0.0
0.1
0.2
0.3
0.4
0.5
proportion of matings acquired by fertile focal males

Based on the copulatory guarding hypothesis, I predicted that for an equal number of matings, males that remained *in copula* for longer periods would enjoy greater fertilization success. To test this prediction, I used a multiple regression model incorporating mating success and mean duration of copulation (expressed as the deviation from the mean of the focal male's group) (Table 4). Adding copulation duration plus the interaction term did not significantly improve the model for days 1-4 (partial $F = 0.310$, partial $r^2 = 0.024$, $p = 0.737$) or days 5-11 (partial $F = 2.483$, partial $r^2 = 0.132$, $p = 0.110$). Hence, there was no evidence that prolonged copulation enhanced fertilization success for fertile focal males. In contrast to the results reported by Rubenstein (1989), there was a tendency towards a negative effect of mean copulation duration on the proportion of eggs attributable to fertile focal males, particularly for eggs collected on days 5-11 (Table 4).

As expected, a negative correlation was found between the number of matings acquired by focal males and mean mating duration ($n = 23$, $r = -0.483$, $p = 0.010$, 1-tailed), suggesting a trade-off between mating frequency and duration. Therefore, under the copulatory guarding hypothesis, I expected males to regulate copulation duration in order to maximize fertilization success with their current partner while minimizing the number of lost mating opportunities. Thus, quadratic regression was expected to reveal a curvilinear relationship between fertilization success and mean copulation duration, with maximal fitness benefits at an intermediate duration. However, no significant curvilinear trend was found (quadratic terms - days 1-4: $B = 2.05 \times 10^{-6}$; partial t significance, $p = 0.633$; days 5-11: $B = -2.96 \times 10^{-6}$, partial t significance $p = 0.387$).

Table 4

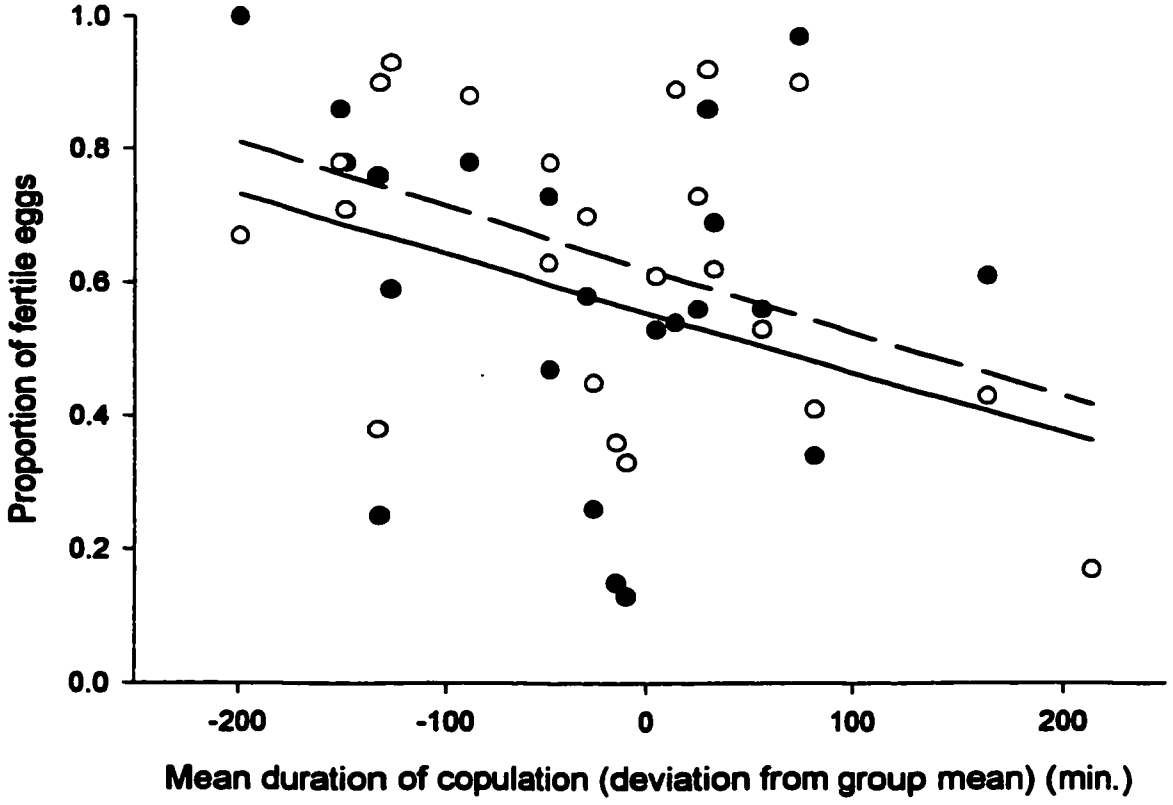
Multiple regression analysis of the effects of proportion of matings acquired and copulation duration (expressed as the deviation from the mean of the focal male's group) on proportion of eggs attributable to fertile focal males. (Full model days 1-4: $F_{3,19} = 2.170$, $p = 0.125$, $r^2 = 0.255$; days 5-11: $F_{3,19} = 6.232$, $p = 0.004$, $r^2 = 0.496$)

	Days 1-4			Days 5-11		
	B	t	p	B	t	p
Constant	0.188	0.921	0.369	0.166	1.170	0.257
P(matings)	1.516	1.807	0.087	1.952	3.344	0.003
Mean duration	-0.001	-0.494	0.627	-0.003	-2.084	0.051
interaction	0.003	0.308	0.761	0.010	1.790	0.089

Hence, there was no evidence to support the hypothesis that males optimize copulation duration. There was a tendency towards a negative linear relationship between the proportion of eggs attributable to fertile focal males and mean copulation duration, although the relationship for days 1-4 was not statistically significant (Fig 3). This suggests that matings of short duration yield the greatest fitness benefits to males.

Figure 3

Relationship between the proportion of eggs attributable to fertile focal males and the mean duration of copulation (expressed as the deviation from the mean of each group). The solid line represents the least square best fit line for eggs laid on days 1-4 (filled circles) ($F_{1,21} = 2.548$, $p = 0.125$, $r^2 = 0.125$). The dashed line represents the least square best fit line for eggs laid during days 5 to 11 after the start of the experiment (empty circles) ($F_{1,21} = 5.216$, $p = 0.033$, $r^2 = 0.199$).


1.0
0.8
0.6
0.4
0.2
0.0
Proportion of fertile eggs
-200
-100
0
100
200
Mean duration of copulation (deviation from group mean) (min.)

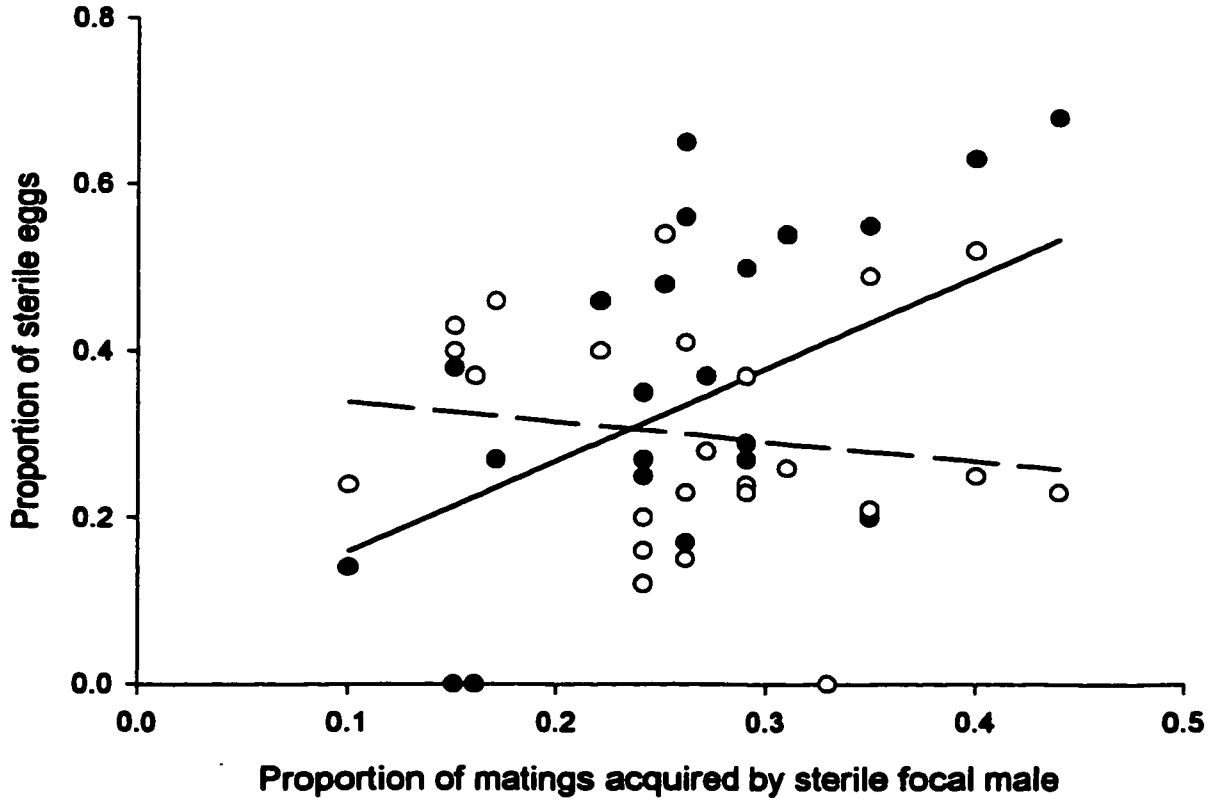
Sterile focal males: Covariance analysis revealed that the week of experimentation and the interaction between the week and the proportion of matings acquired had a significant effect on the proportion of eggs attributable to sterile focal males on days 1-4 (week: $F = 4.009$, $df = 7$, $p = 0.035$; week x proportion of matings: $F = 4.084$, $df = 7$, $p = 0.033$). The interaction between the week and the mean copulation duration also had a significant effect on the proportion of eggs attributable to focal males on days 1-4 (week x mean copulation duration: $F = 4.182$, $df = 7$, $p = 0.031$). However, once I removed a statistical outlier from the data (Criterion for outliers: residual value > 2 standard deviations from mean) these effects disappeared (week: all p-values > 0.285, interaction terms: all p-values > 0.253). Neither the week or the interaction between the week and the proportion of matings acquired or the mean duration of copulation had an effect on the proportion of eggs attributable to sterile focal males on days 5-11 prior to the removal of the outlier (all p –values > 0.326). Therefore, I combined the data for all eight weeks of experimentation. Further analysis included the statistical outlier because it had no qualitative impact on the results.

As for the fertile focal males, the proportion of eggs attributable to sterile focal males on days 1-4 increased significantly with the proportion of matings acquired (Fig. 4 – solid line). However, no significant relationship was found for days 5-11 (Fig. 4 – dashed line).

A multiple regression model incorporating the proportion of matings acquired and mean duration of copulation (expressed as the deviation from the mean of the focal male's

Figure 4

Relationships between the proportion of eggs attributable to sterile focal males and the proportion of matings acquired. The proportion of eggs attributable to sterile focal males was arcsine-square root transformed to satisfy the assumption of normality. The solid line represents the least square best fit line for eggs laid during the first four days of the experiment (filled circles) ($F_{1,22} = 6.197$, $p = 0.021$, $r^2 = 0.220$). The dashed line represents the least square best fit line for eggs laid during days 5 to 11 after the start of the experiment (empty circles) ($F_{1,22} = 0.684$, $p = 0.483$, $r^2 = 0.030$).


0.8
0.6
0.4
0.2
0.0
Proportion of sterile eggs
0.0
0.1
0.2
0.3
0.4
0.5
Proportion of matings acquired by sterile focal male

group) (Table 5) revealed that mean copulation duration did not increase the proportion of eggs attributable to sterile focal males when the proportion of matings was held constant (Days 1-4: F change = 2.180, p = 0.707, r^2 change = 0.027; days 5-11: F change = 0.109, p = 0.897, r^2 change = 0.010). Hence, there was no evidence that prolonged copulation enhanced fertilization success for sterile focal males.

The number of matings acquired by sterile focal males was negatively correlated with the mean duration of copulation (n = 24, r = -0.704, p = 0.0001). Thus, as for fertile focal males, there is evidence for a trade-off between mating frequency and duration. I used quadratic regression to test the prediction that males regulate copulation duration to maximize their fertilization rate. Quadratic regression did not reveal a curvilinear relationship between the proportion of eggs attributable to sterile focal males and mean copulation duration (quadratic terms days 1-4: B = 5.8×10^{-7}, partial t significance = 0.758; days 5-11: B = 3.9×10^{-7}, partial t significance = 0.758). Thus, as for the fertile focal males, there is no evidence to support the hypothesis that males regulate copulation duration. No linear trend was found in the relationship between the proportion of eggs attributable to sterile focal males and mean copulation duration (Fig. 5).

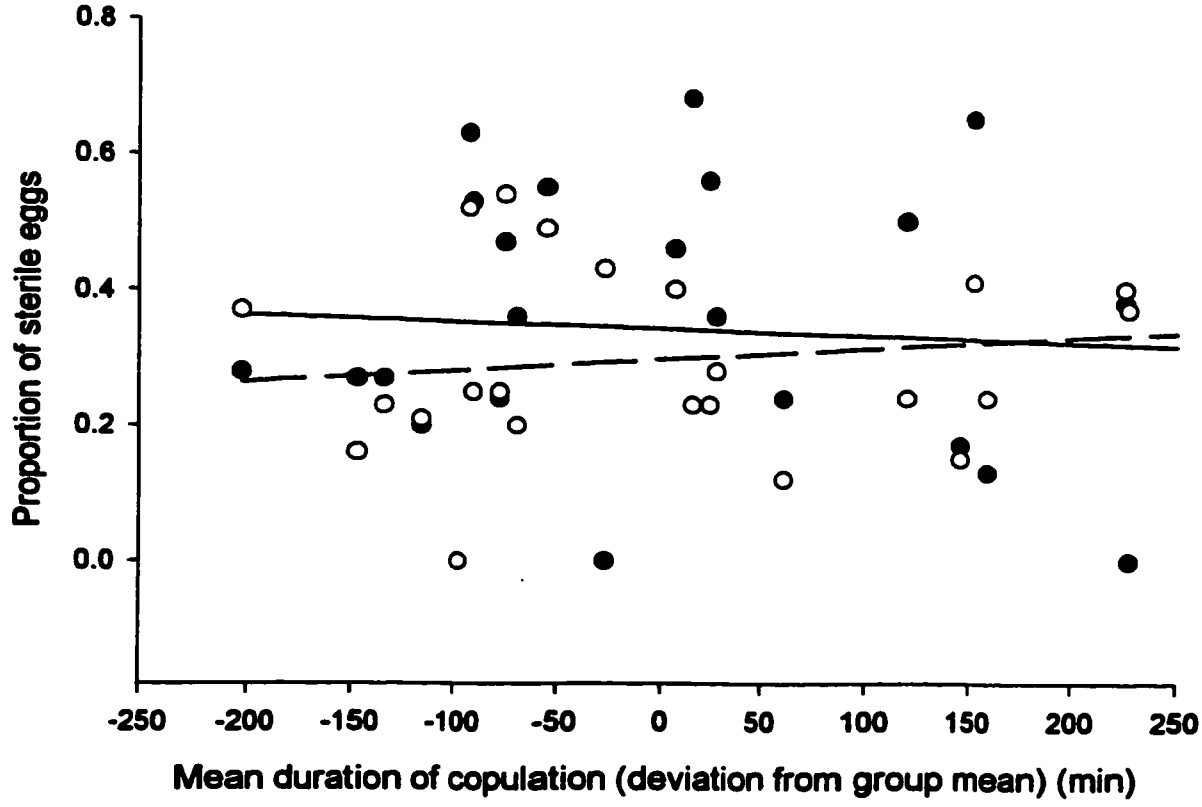
Table 5

Multiple regression analysis of the effects of proportion of matings acquired and copulation duration (expressed as the deviation from the mean of the focal male's group) on proportion of eggs attributable to sterile focal males. (Full model days 1-4: $F_{3,20} = 2.180$, $p = 0.122$, $r^2 = 0.246$; days 5-11: $F_{3,20} = 0.282$, $p = 0.838$, $r^2 = 0.041$). The proportion of eggs attributable to sterile focal males was arcsine-square root transformed to satisfy the assumption of normality.

	Days 1-4			Days 5-11		
	B	t	p	B	t	p
Constant	-0.059	-0.329	0.746	0.339	2.507	0.021
P(matings)	1.481	2.262	0.035	-0.164	-0.333	0.742
Mean duration	0.0004	0.494	0.627	0.0003	0.426	0.675
interaction	-0.0004	-0.131	0.897	-0.0007	-0.293	0.773

Figure 5

The relationship between the proportion of eggs attributable to sterile focal males and mean duration of copulation (expressed as the deviation from the mean of each group). The proportion of eggs attributable to sterile focal males was arcsine-square root transformed to satisfy the assumption of normality. The solid line represents the least square best fit line for eggs laid during the first four days of the experiment (filled circles) ($F_{1,22} = 1.103$, $p = 0.305$, $r^2 = 0.048$). The dashed lines represent the least square best fit line for eggs laid during days 5 to 11 after the start of the experiment (empty circles) ($F_{1,22} = 0.730$, $p = 0.402$, $r^2 = 0.032$).


0.8
0.6
0.4
0.2
0.0
Proportion of sterile eggs
-250
-200
-150
-100
-50
0
50
100
150
200
250
Mean duration of copulation (deviation from group mean) (min)

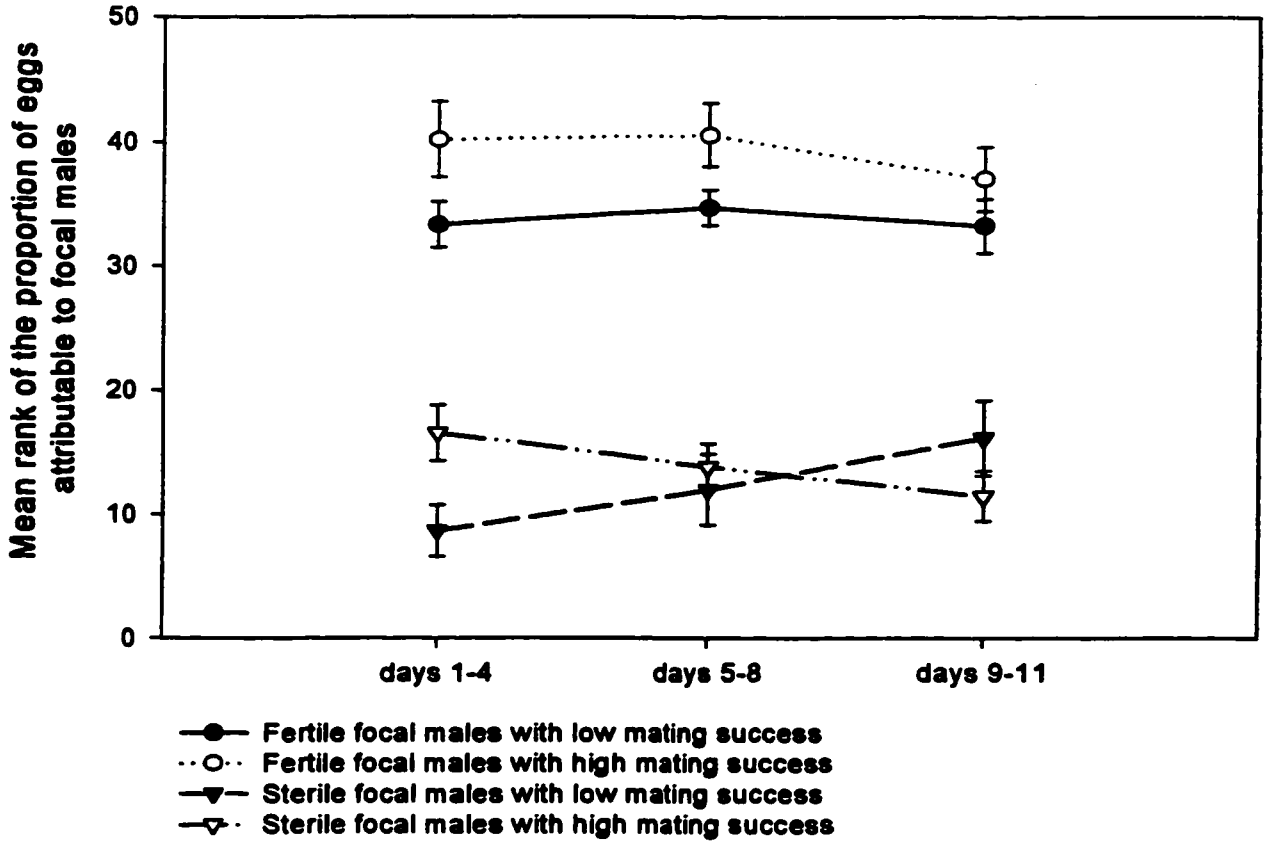
The reduction in long term sperm transfer by sterile males may explain the loss of a relationship between fertilization and mating success for sterile focal males on days 5-11. As irradiated males lose the ability to transfer sperm over time, the proportion of the sterile focal male's sperm present within the female's reproductive tract diminishes due to continued sperm contributions by fertile background males and sperm use by females. Hence, the difference between focal males with high and low mating success will diminish because matings occurring after males stop transferring sperm do not contribute to fitness.

To test this hypothesis, I conducted a two-way analysis of variance to determine the effect of egg laying period (days 1-4, 5-8 and 9-11) and mating success (high ≥ 25% of matings in focal male's group, low < 25%) on the proportion of eggs attributable to focal males. As expected, fertile males with high mating success were attributed a greater proportion of eggs for all three periods (mating success: $F = 9.127$, $df = 1$, $p = 0.004$; egg laying period: $F = 0.665$, $df = 2$, $p = 0.518$; mating success x egg laying period: $F = 0.239$, $df = 2$, $p = 0.788$). In contrast, the main effects of egg laying period and mating success were not significant for sterile focal males (egg laying period: $F = 0.159$, $df = 2$, $p = 0.853$; mating success: $F = 537$, $df = 1$, $p = 0.466$). However, as expected, a significant interaction was detected (mating success x egg laying period: $F = 3.285$, $df = 2$, $p = 0.044$). The mean rank of the proportion of eggs attributable to sterile males with high mating success diminished over time while the opposite is true for males with low mating success (Fig. 6). This pattern is not simply a function of the ranking procedure. Two-way analysis of variance for sterile males only, based on arcsine-square root transformed proportion of eggs revealed the same pattern (mating success: F =

0.397, df = 1, p = 0.531; egg laying period: F = 0.923, df = 2, p = 0.402; mating success x egg laying period: F = 4.084, df = 2, p = 0.021). As for the ranked data, males with high mating success had higher paternity success on days 1-4 (mean proportion of eggs ± standard deviation; high mating success: 0.403 ± 0.167; low mating success: 0.219 ± 0.199), while the opposite was true on days 9-11 (high mating success: 0.286 ± 0.195; low mating success: 0.405 ± 0.203).

Figure 6

Mean rank (± standard error) of the proportion of eggs attributable to fertile (*circles*) and sterile (*triangles*) focal males in relation to the egg laying period. Filled symbols represent focal males that acquired 25% or fewer of all matings within each group of four competing males (low mating success). Empty symbols represent focal males that acquired more than 25% of all matings within each group (high mating success). Proportions were ranked to satisfy the assumption of normality.


Mean rank of the proportion of eggs attributable to focal males
50
40
30
20
10
0
days 1-4
days 5-8
days 9-11
Fertile focal males with low mating success
Fertile focal males with high mating success
Sterile focal males with low mating success
Sterile focal males with high mating success

DISCUSSION

Numerous studies have shown that male mating success is positively related to male body size, or components of total size in waterstriders (e.g. Arnqvist 1992, Sih and Krupa 1992, Fairbairn and Preziosi 1994, Preziosi and Fairbairn 1996, Ferguson and Fairbairn 2000). However, considering the polygynandrous mating system of *A. remigis*, it was not known how male mating success translated into offspring production. The results of this study demonstrate that mating frequency can predict fertilization success for a polygynandrous species under natural conditions of multiple matings by both sexes. This lends strength to previous studies of sexual selection that have used mating success as an estimate of reproductive fitness in polygynandrous species (e.g. Houck *et al.* 1985; Arnqvist 1992a, 1992b; Sih and Krupa 1992; Krupa and Sih 1993; Andersson 1994; Preziosi and Fairbairn 1996; Weigensberg and Fairbairn 1996; Arnqvist *et al.* 1997; Goulson *et al.* 1999; Savalli and Fox 1999). Considering the difficulty in obtaining estimates of paternity in natural environments, the results of this study are promising for future field studies of sexual selection in polygynandrous species.

However, the relationship between fertilization success and mating success is imperfect. Mating success accounts for 22 – 36% of the variation in fertilization success. Some of the unexplained variance in the relationship between fertilization and mating success may be due to missed copulations, since my observations included only copulations occurring between 10h00 and 20h00. Although most copulations begin in the afternoon (mean start time between 14h00 and 15h00; Gentile 1998), some copulations may have may have occurred between 20h00 and 10h00.

In agreement with previous studies on sperm competition in insects (e.g. Simmons 1987; Gage and Baker 1991; Birkhead and Parker 1997; Villavaso *et al.* 1998), the amount of sperm transferred appears to be an important determinant of paternity success in *A. remigis*, and may explain some of the variance in the relationship between fertilization and mating success. The difference in the number of eggs attributable to fertile and sterile focal males may be seen as evidence for this. Fertile males were expected to benefit from a numerical advantage in sperm competition with sterile males (Appendix 1- experiment 3). The difference in paternity success of fertile and sterile focal males reflects the fertile male advantage as sterile focal males were consistently attributed fewer eggs.

Variance in female mating and oviposition behaviour may also account for some of the variance in the relationship between male fertilization and mating success. Evidence of a relationship between female reproductive effort and male characteristics has been reported in *Harpobittacus nigriceps* (Thornhill 1983) and in *Erythemis simplicicolis* (McVey 1988), and may be interpreted as a form of postcopulatory female choice (Eberhard 1996, 1997, 1998). On average, female *A. remigis* lay two to five eggs each day for a total of 100 to 300 eggs over their reproductive lifetimes (Blanckenhorn 1991, Blanckenhorn and Fairbairn 1995, Preziosi and Fairbairn 1997). Females generally lay one or two eggs during each oviposition bout and may mate again between bouts (Gentile 1998). Given that, on average, sperm precedence favours the last male to mate before oviposition in this species (Rubenstein 1989), female mating and oviposition behaviour may affect male reproductive fitness in two ways. Firstly, variance in female reproductive effort per oviposition bout may influence the absolute fertilization success

of the last male without reducing the proportional advantage of the last male. Secondly, additional mating by females prior to or between oviposition bouts may significantly reduce the proportion of eggs attributable to a previous male.

Given the last male sperm precedence in *A. remigis*, Rubenstein (1989) suggested that prolonged copulation functions as a mate guarding strategy. However, Jablonski and Kaczanowski (1994) found no correlation between copulation duration and paternity success in a related waterstrider *Gerris lacustris*. In this species, as in many temperate waterstriders, copulation is followed by a period of contact guarding during which the male remains mounted on the female. Jablonski and Kaczanowski (1994) found a positive correlation between the duration of this post-copulatory contact guarding and paternity success. Thus, guarding duration, but not copulation duration, enhances paternity in *G. lacustris*. *Aquarius remigis* are atypical in that males do not remain mounted on the female's back beyond the end of copulation. Successful copulations (i.e. > 15 min) end with a struggle that results in separation of the couple (Weigensberg and Fairbairn 1994, Gentile 1998). Females therefore effectively prevent males from post-copulatory contact guarding (Gentile 1998; Campbell and Fairbairn, unpublished). If the copulation itself serves as a mate guarding tactic, as proposed by Rubenstein (1989), we would expect a positive correlation between guarding duration and male fertilization success. If anything, my results show the opposite effect. For an equal number of matings, males that engaged in shorter matings tended to fertilize a greater proportion of eggs. The negative effect of prolonged copulation on male fertilization success suggests that prolonged copulation in *A. remigis* is not analogous to post-copulatory guarding in other temperate waterstriders.

Rubenstein (1989) also suggested that male *A. remigis* have two, alternative reproductive strategies. Some males may maximize fitness through copulatory guarding, having relatively few, prolonged matings, while others maximize their fertilization rate by inseminating as many females as possible with relatively little guarding. This hypothesis is predicated on the assumption that prolonged copulation serves as a mate guarding tactic. Since there is no evidence to support this assumption and since no curvilinear trend was found in the relationship between fertilization and mating success, neither the optimal duration hypothesis or the alternative mating strategies hypothesis were supported by my results. The only successful mating strategy appears to be the 'mate-and-run' tactic. Therefore, from the male perspective, the lack of a positive relationship between fertilization success and copulation duration, coupled with the costs in the form of lost mating opportunities, makes it difficult to understand why prolonged copulation persists in this species.

In a recent study, Campbell and Fairbairn (unpublished) found that insemination occurs towards the end of copulation. Similar results were found in *Hebrus sp.* (Heming-Van Battum and Heming 1986). The structure of the female reproductive tract led them to hypothesize that insemination is induced by contractions of the muscles in the wall of the gynatrial sac. The similarities in the morphology of the reproductive tracts of *A. remigis* and *Hebrus sp.* have led Campbell and Fairbairn (unpublished) to suggest the same hypothesis. This suggests that females may influence the timing of insemination. Because males gain no fitness benefits from a given mating if insemination does not occur, they may remain *in copula* until the female allows sperm transfer. Hence, females may exert a strong influence on the duration of copulation. The finding that prolonged

copulation does not enhance male fertilization success is consistent with this hypothesis. Female influence over copulation duration may also explain why no evidence was found to support the hypothesis that males regulate the duration of copulation to maximize their fertilization rate.

Despite the increased risk of predation and reduced mobility experienced when *in copula* (Fairbairn 1993), mating females are subjected to lower levels of harassment by males than are solitary females. They are therefore able to spend more time foraging as opposed to resisting mating attempts (Wilcox 1984, Rowe 1992). Thus, selection may favour females that remain *in copula* for prolonged periods under high levels of harassment. Females cannot prevent males from ending copulation. However, since males do not transfer sperm if they disengage prematurely (Campbell and Fairbairn, unpublished), male termination of copulation may prove costly. Hence, the pre-insemination latency period may have evolved as part of a general strategy of convenience polyandry (Weigensberg and Fairbairn 1994; Rowe et al. 1996).

This study reveals that mating success is a reliable predictor of reproductive fitness in a polygynandrous species under the conditions of natural polygynandry. The positive relationship between fertilization and mating success strengthens previous studies of sexual selection that have used mating success as an estimate of reproductive fitness in polygynandrous species (e.g. Houck *et al.* 1985; Arnqvist 1992a, 1992b; Sih and Krupa 1992; Krupa and Sih 1993; Andersson 1994; Preziosi and Fairbairn 1996; Weigensberg and Fairbairn 1996; Arnqvist *et al.* 1997; Goulson *et al.* 1999; Savalli and Fox 1999). However, my results also highlight the potential influence of post-insemination sexual selection on male reproductive success through sperm competition

and female choice. In contrast to Rubenstein's (1989) results, copulation duration tended to have a negative effect on paternity success. This suggests that prolonged copulation does not function as a paternity assurance mechanism analogous to the post-copulatory contact guarding that is typical of temperate waterstriders. A full understanding of the adaptive significance of multiple mating and prolonged copulation will require a wider view that includes both male and female interests.

REFERENCES

Abell, A.J., Cole, B.J., Reyes, R. and Wiernasz, D.C. (1999) Sexual selection on body size and shape in the western harvester ant, *Pogonomyrmex occidentalis* Cresson, Evolution 53(2) pp. 535-545.

Alcock, J. (1994) Postinsemination associations between males and females in insects: The mate guarding hypothesis. Annual Review of Entomology 39:1-21

Andersson, M. (1994) Sexual selection. Princeton University Press, Princeton, New Jersey.

Arnold. S.J. and Duvall, D. (1994) Animal mating systems: A synthesis based on selection theory. American Naturalist 143: 317-348.

Arnqvist, G. (1988) Mate guarding and sperm displacement in the waterstrider *Gerris lateralis* Schumm. (Heteroptera: Gerridae). Freshwater biology 19:269-274

Arnqvist, G. (1992a) Male phenotypes in the water strider *Gerris odontogaster* (Zett.) (Heteroptera; Gerridae). Animal Behaviour 44: 681-683

Arnqvist G. (1992b) Spatial variation in selective regimes: sexual selection in the water strider, *Gerris odontogaster*. Evolution 46: 914-929

Arnqvist, G., Thornhill, R. and Rowe, L. (1997) Evolution of animal genitalia: morphological correlates of fitness components in a water strider. Journal of Evolutionary Biology. 10: 613-640.

Arnqvist G., and Danielsson, I. (1999a) Copulatory behaviour, genital morphology and male fertilization success in water striders. Evolution 53: 147-156.

Arnqvist G., and Danielsson, I. (1999b) Postmating sexual selection: the effects of male body size and recovery period on paternity and egg production rate in a water strider. Behavioral Ecology 10:358-365.

Birkhead, T.R., Fletcher, F., Pellat, E.J., and Staples, A. (1995) Ejaculate quality and the success of extra-pair copulations in the zebra finch. Nature 377:422-423.

Birkhead, T.R. and Parker, G.A. (1997) Sperm competition and mating systems in Krebs, J.R. and Davies, N.B. (eds.) Behavioural ecology: An evolutionary approach. Fourth edition. Blackwell science, London. Pp 121-145.

Blanckenhorn, W.U. (1991) Fitness consequences of foraging success in water striders (*Gerris remigis*; Heteroptera: Gerridae). Behavioural Ecology 2: 46-55.

Blanckenhorn, W.U. and Fairbairn, D.J. (1995) Life history adaptation along a latitudinal cline in the water strider Aquarius remigis (Heteroptera: Gerridae). Journal of Evolutionary Biology 8:21-41.

Blanckenhorn, W.U., Preziosi, R.F. and Fairbairn, D.J. (1995) Time and energy constraints and the evolution of sexual size dimorphism – to eat or to mate? Evolutionary Ecology 9: 369-381.

Blanckenhorn, W.U., Grant, J.W.A. and Fairbairn, D.J. (1998) Monopolization in a resource queue: water striders competing for food and mates. Behavioural Ecology and Sociobiology 42:63-70

Boorman, E. and Parker G.A. (1976) Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. Ecological Entomology 1:145-155

Clark. S.J. (1988) The effects of operational sex ratio and food deprivation on copulation duration in the water strider (*Gerris remigis* Say). Behavioural Ecology and Sociobiology. 23:317-322.

Conner, J.K. (1995) Extreme variability in sperm precedence in the fungus beetle, *Bolitotherus cornutus* (Coleoptera Tenebrionidae). Ethology Ecology and Evolution 7:277-280

Cook, P.A., Harvey, I.F. and Parker, G.A. (1997) Predicting variation in sperm precedence. Philosophical Transactions of the Royal Society - series B. 352: 771-780.

Danielsson, I. (1998) Mechanisms of sperm competition in insects. Annales Zoologici Fennici 35:241-257

Danielsson I., and Askenmo, C. (1999) Male genital traits and mating interval affect male fertilization success in the water strider Gerris lacustris. Behavioural Ecology and Sociobiology 46:149-156.

Darwin, C. (1871) The descent of man and selection in relation to sex. Appelton inc.; New York, 688 pages.

Dzuik, P.J. (1996) Factors that influence the proportion of offspring sired by a male following heterospermic insemination. Animal Reproduction Science 43:65-88

Eberhard. W.G. (1991) Copulatory courtship and cryptic female choice in insects. Biological Review 66: 1-31

Eberhard, W.G. (1994) Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. Evolution 48:711-733.

Eberhard, W.G. (1996) *Female control: sexual selection by female cryptic choice.* Princeton University Press.

Eberhard, W.G. (1997) Sexual selection by cryptic female choice in insects and arachnids. *In Social competition and Cooperation in Insects and Arachnids. I. Evolution of Mating Systems.* J. Choe and B. Crespi (eds) , pp. 32-57. Cambridge University Press, Cambridge, U.K.

Eberhard, W.G. (1998) Female roles in sperm competition. In *Sperm competition and sexual selection.* T.R. Birkhead and A.P. Moller (eds) pp. 91-111. Academic Press. London, UK.

Fairbairn, D.J. (1985) Comparative ecology of *Gerris remigis* (Hemiptera: Gerridae) in two habitats: a paradox of habitat choice. Canadian Journal of Zoology 63: 2594-2603.

Fairbairn, D.J. (1988) Adaptive significance of wing dimorphism in the absence of dispersal: a comparative study of wing morphs in the waterstrider *Gerris remigis.* Ecological Entomology 13:273-281

Fairbairn, D.J. (1993) Costs of loading associated with mate-carrying in the waterstrider, *Aquarius remigis.* Behavioral Ecology 4: 224-231

Fairbairn, D.J., and Brassard, J. (1988) Dispersion and spatial orientation of *Gerris remigis* in response to water current: a comparison of pre-and post-diapause adults. Physiological Entomology 13:153-164

Fairbairn, D.J. and Preziosi, R.F. (1994) Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, *Aquarius remigis.* American Naturalist 144: 101-118.

Fairbairn, D.J. and Preziosi, R.F. (1996) Sexual selection and the evolution of sexual size-dimorphism in the water strider *Aquarius remigis*. Evolution 50: 1549-1559.

Ferguson, I.M. and Fairbairn, D. J. (2000) Sex-specific selection and sexual size dimorphism in the waterstrider *Aquarius remigis.* Journal of Evolutionary Biology 13:160-170

Gage, M.J.G. and Baker, R.R. Ejaculate size varies with socio-sexual situation in an insect. Ecological Entomology 16:331-337.

Gallant, S.L. and Fairbairn, D.J. (1997) Patterns of postmating reproductive isolation in a newly discovered species pair, *Aquarius remigis* and *A. remigoides* (Hemiptera: Gerridae) Heredity 78: 571-577.

Gentile, G. (1998) Daily patterns of oviposition mediate sexual conflict in a polygynandrous mating system. Unpublished M.Sc. thesis. Concordia University, Montreal.

Gilchrist, A.S. and Partridge, L. (2000) Why it is difficult to model sperm displacement in *Drosophila melanogaster*: the relationship between sperm transfer and copulation duration. Evolution 54:534-542.

Goulson, D., Bristow, L., Elderfield, E., Brinklow, K., Parry-Jones, B. and Chapman J.W. (1999) Size, symmetry, and sexual selection in the housefly *Musca domestica.* Evolution 53: 527-534

Gwynne, D.T. (1986) Courtship feeding in katydids (Orthoptera: Tettigoniidae): Investment in offspring or in obtaining fertilizations. American Naturalist 128:342-352

Harvey, A.W.(1990) Sexual differences in contemporary selection acting on size in the hermit crab *Clibanarius digueti*. American Naturalist 136: 292-304.

Heming-Van Battum, K.E. and Heming, B.S. (1986) Structure, function and evolution of the reproductive system in the females of *Hebrus pusillus* and *H. ruficeps* (Hemiptera, Gerromorpha, Hebridae). Journal of Morphology 190:121-167.

Henneberry, T.J., and McGovern, W.L. (1963) Effects of gamma radiation on mating competitiveness and behaviour of *Drosophila melanogaster* males. Journal of Economic Entomology 56: 739–741.

Houck, L.D., Arnold, S.J. and Thisted, R.A. (1985) A statistical study of mate choice: sexual selection in a plethodontid salamander. Evolution 39:370-386

Hurpin, B. (1968) Application aux Oryctes (Coleopteres: Scarabaeidea) de la methode du lacher de males steriles. *in* Isotopes and radiation in entomology, Proceedings of a symposium, International Atomic Energy Agency, Vienna 428 pages.

Jablonski, P. and Kaczanowski, S. (1994) Influence of mate-guarding duration on male reproductive success: An experiment with irradiated water strider (*Gerris lacustris*) males. Ethology 98: 312-320.

Krupa, J.J. and Sih, A. (1993) Experimental studies on water strider mating dynamics: spatial variation in density and sex ratio. Behavioural Ecology and Sociobiology 33:107-120.

Lee, K.B., Hwang, U.K. and Lee, K.S. (1968) Exploratory studies on the eradication of the Korean pine caterpillar *Dendrolimus spectabilis* Butler by means of radiation *in* Isotopes and radiation in entomology, Proceedings of a symposium, International atomic energy agency, Vienna 428 pages.

Lewis, S.M., and Austad, S.N. (1990) Sources of intraspecific variation in sperm precedence in red flour beetles. American Naturalist 135: 351-359.

McLain, D.K. (1989) Prolonged copulation as a post-insemination guarding tactic in a natural population of the ragwort seed bug. Animal Behaviour 38:659-664.

McVey, M.E. (1988) The opportunity for sexual selection in a territorial dragonfly, *Erythemis simplicicolis*. In *Reproductive-Success – Studies of Individual Variation in Contrasting Breeding Systems*. T.H. Clutton-Brock (ed.), pp. 44-58. University of Chicago Press, Chicago, USA.

Otronen, M. (1994) Repeated copulations as a strategy to maximize fertilization in the fly, *Dryomyza anilis* (Dryomyzidae). Behavioral Ecology 5: 51-56.

Parker, G.A. (1970) Sperm competition and its evolutionary consequences in the insects. Biological Review 45: 525-567.

Petrie, M. and Halliday, T. (1994) Experimental and natural changes in the peacock's (*Pavo cristatus*) train can affect mating success. Behavioural Ecology and Sociobiology 35:213-217.

Pollister, A.W. (1930) Cytoplasmic phenomena in the spermatogenesis of *Gerris*. Journal of Morphology and Physiology 49:455-494.

Preziosi, R.F. and Fairbairn, D.J. (1996) Sexual size dimorphism and selection in the wild in the waterstrider *Aquarius remigis*: Body size, components of body size and male mating success. Journal of Evolutionary Biology 9: 317–336.

Preziosi, R.F. and Fairbairn, D.J. (1997) Sexual size dimorphism and selection in the wild in the waterstrider *Aquarius remigis*: Lifetime fecundity selection on female total length and its components. Evolution 51:467-474

Radwan, J. and Siva-Jothy, M.T. (1996) The function of post-insemination mate association in the bulb mite, *Rhizoglyphus robini*. Animal Behaviour 52: 651-657

Rowe, L. (1992) Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. Animal Behaviour 44:189-202

Rowe, L., Arnqvist, G., Sih, A., Krupa, J. (1994) Sexual conflict and the evolutionary ecology of mating patterns; water striders as a model system. Trends in Ecology and Evolution 9:289-293.

Rubenstein, D.I. (1989) Sperm competition in the water strider, *Gerris remigis*. Animal Behaviour 38: 631-636.

Sakaluk, S. and Eggert, A.-K. (1996) Female control of sperm transfer and intraspecific variation in sperm precedence: Antecedents to the evolution of a courtship food gift. Evolution 50:694-703

Savalli, U.M. and Fox, C.W. (1999) The effect of male size, age, and mating behavior on sexual selection in the seed beetle *Callosobruchus maculatus*. Ethology Ecology and Evolution 11:49-60.

Schwagmeyer, P.L. and Foltz, D.W. (1990) Factors affecting the outcome of sperm competition in thirteen-lined ground squirrels. Animal Behaviour 39: 817-824.

Seo, S.T., Vargas, R.I., Gilmore, J.E., Hurashima, R.S., and Fujimoto, M.S. (1990) Sperm transfer in normal and gamma-irradiated, laboratory reared Mediterranean fruit flies (Diptera: Tephritidae). Journal of Economic Entomology 85: 1949-1953.

Shine, R., Olsson, M.M., Moore, I.T., Lemaster, M.P., Greene, M. and Mason, R.T. (2000) Body size enhances mating success in male garter snakes, Animal Behaviour 59, pp. F4-F11

Sih, A. and Krupa, J.J. (1992) Predation risk, food deprivation and non-random mating by size in the stream water strider, *Aquarius remigis.* Behavioural Ecology and Sociobiology 31: 51-56

Sillen-Tullberg, B. (1981) Prolonged copulation: A male 'postcopulatory' strategy in a promiscuous species, *Lygaeus equestris* (Heteroptera: Lygaeidae). Behavioural Ecology and Sociobiology. 9: 283-289.

Simmons, L.W. (1987) Sperm competition as a mechanism of female choice in the field cricket, *Gryllus bimaculatus.* Behavioural Ecology and Sociobiology 21:197-202

Simmons, L.W. and Parker, G.A. (1992) Individual variation in sperm competition success of yellow dungflies *Scatophaga stercoraria.* Evolution 46: 366-375

Simmons, L.W., Stockley P. Jackson, R.L. and Parker , G.A. (1996) Sperm competition or sperm selection: no evidence for female influence over paternity in yellow dung flies *Scatophaga stercoraria.* Behavioural Ecology and Sociobiology 38:199-206.

Simon, J.E. (1968) Cria masal de *Dysdercus peruvianus* G. y su esterilizacion mediante rayos gamma *in* Isotopes and radiation in entomology; Proceedings of a symposium, International Atomic Energy Agency, Vienna 428 pages.

Siva-Jothy, M.T. and Tsubaki, Y. (1989) Variation in copulation duration in *Mnais pruinosa pruinosa* Selys (Odonata:Calopteryigidae). I. alternative mate-securing tactics and sperm precedence. Behavioural Ecology and Sociobiology 24:39-46.

Smith, P.H., Barton Browne, L. and van Gerwen, A.C.M. (1988) Sperm storage and utilization and egg fertility in the sheep blowfly, *Lucilia cuprina*. Journal of Insect Physiology 34:125-129

Snow, J.W., Jones, R.L., North, D.T., Holt, G.G. (1972) Effects of irradiation on ability of adult male corn earworms to transfer sperm and field attractiveness of females mated to irradiated males. Journal of Economic Entomology 65:906-908

Sternberg, S., Peleg, B.A., and Galun, R. (1973) Effect of irradiation on mating competitiveness of the male tick *Argas persicus* (Oken). Journal of Medical Entomology 10: 137-142

Stockley, P. (1997) Sexual conflict resulting from adaptations to sperm competition. Trends in Ecology and Evolution 12: 154-159

Tandler, B. and Moriber, L.G. (1966) Microtubular structures associated with the acrosome during spermiogenesis in the water strider *Gerris remigis* (Say). Journal of Ultrastructure Research, 14: 391-404.

Thornhill, R (1983) Cryptic female choice and its implications in the scorpion fly, *Harpobittacus nigriceps*. American Naturalist 122: 765-788.

Thornhill, R., and Alcock, J. (1983) The evolution of insect matings systems. Cambridge: Harvard University press

Ueno. H., and Ito, Y. (1992) Sperm precedence in *Eysarcoris lewisi* Distant (Heteroptera: Pentatomidae) in relation to duration between oviposition and the last copulation. Applied Entomology and Zoology 27:421-426

Villavaso, E.J., McGovern, W.L., Wagner, T.L., and Willers, J.L. (1998) Components of competitiveness in sterile male boll weevils (Coleoptera: Curculionidae). Journal of Economic Entomology 91:631-636.

Waage, J.K. (1979) Dual function of the dragonfly penis: sperm removal and transfer. Science 203:916-918.

Ward P.I. (1993) Females influence sperm storage and use in the yellow dung fly *Scatophaga stercoraria* (L.). Behavioural Ecology and Sociobiology 32:313-319

Weigensberg, I. and Fairbairn, D.J. (1994) Conflicts of interest between the sexes: a study of mating interactions in a semiaquatic bug. Animal Behaviour 48: 893-901.

Weigensberg, I. and Fairbairn D.J. (1996) The sexual arms race and phenotypic correlates of mating success in the waterstrider, *Aquarius remigis* (Hemiptera: Gerridae). Journal of Insect Behaviour 9: 307-319.

Wilcox, R.S. (1984) Male copulatory guarding enhances female foraging in a water strider. Behavioural Ecology and Sociobiology 15: 171-174.

Wing, S.R. (1985) Prolonged copulation in *Photinus macdermotti* with comparative notes on *Photinus collustrans* (Coleoptera: Lampyridae). The Florida Entomologist 68:627-634

Wirtz, P. (1997) Sperm selection by females. Trends in Ecology and Evolution. 12: 172-173

Zeh, J.A., and Zeh, D.W. (1994) Last male sperm precedence breaks down when females mate with three males. Proceedings of the Royal Society of London, series B. 257:

Appendix 1: investigation of the effects of irradiation of males for paternity analysis.

Parker's (1970) sterile male technique in which a number of males are irradiated to induce sterility, has commonly been used as a means of determining levels of sperm competition in arthropods (Parker 1970, Boorman and Parker 1976, Rubenstein 1989, Simmons and Parker 1992, Ueno and Ito 1992; Radwan and Siva-Jothy 1996, Arnqvist and Danielsson 1999a, 1999b, Danielsson and Askenmo 1999). Sperm from irradiated males is assumed to induce dominant lethality in fertilized ova resulting in developmental failure of eggs (Parker 1970, Rubenstein 1989, Arnqvist and Danielsson 1999a, 1999b). Hence, the proportion of non-developing eggs produced by females mated to both irradiated and non-irradiated males permits the estimation of the proportion of eggs attributable to both the fertile and sterile males.

Sterilization doses of radiation are known to have little effect on mating behaviour in arthropods (e.g. *Drosophila melanogaster*: Henneberry and McGovern 1963, Boorman and Parker 1976; *Dendrolimus spectabilis*: Lee *et al.* 1968; *Dysdercus peruvianus*: Simon 1968; *Argas persicus*: Sternberg *et al.* 1973). However in some species of insects, irradiation has resulted in a reduction of sexual vigour (e.g. *Oryctes rhinoceros* and *O. monoceros*: Hurpin 1968) or mating frequency (e.g. *Gerris lacustris*: Jablonski and Kaczanowski 1994). Sterilization doses of radiation have also led to reduced sperm production in *Ceratitus capitata* (Seo *et al* 1990) and aspermia in *Drosophila melanogaster* (Gilchrist and Partridge 2000). In *C. capitata*, 15 Krad doses of irradiation destroy nearly all the germinal tissue so that the numbers of sperm that

can be transferred are limited to those that were already produced prior to irradiation (Seo *et al* 1990).

Most studies using the sterile male technique require that males mate only once (e.g. Parker 1970; Boorman and Parker 1976; Rubenstein 1989; Simmons and Parker 1992; Arnqvist and Danielsson 1999a, 1999b; Danielsson and Askenmo 1999). Hence, effects of irradiation on male behaviour and sperm transfer have been considered negligible. However, under natural conditions of polygynandry, males mate repeatedly. Since the amount of sperm transferred is likely to be an important factor in determining a male's competitiveness (Simmons 1987, Gage and Baker 1991, Birkhead and Parker 1997, Villavaso *et al.* 1998), estimates of relative fertilization success of non-irradiated males may be inflated if irradiated males copulate less frequently or cease sperm production. Therefore, I conducted three experiments to determine the effects of sterilizing doses of radiation on male behaviour and ability to transfer sperm over four days under mating conditions mimicking natural polygynandry.

Experiment 1 – Effect of irradiation on male behaviour

Methods – Male and female waterstriders were collected from streams in Morin Heights and Ste-Marguerite-du-Lac-Masson (Quebec, Canada) on May 12 ,1999 and were brought to the laboratory at Concordia University, Montreal. On May 31, I marked 36 males using Testor model airplane paint (Fairbairn 1985, Krupa and Sih 1993), and randomly selected 6 of the 36 males for irradiation treatment. I placed the males in a shallow, 15cm x 15cm plastic dish and covered the dish with cheese cloth. After 64 minutes of X-ray exposure at dose rate of 78 Rads/min using a Mueller MG300 (Mod

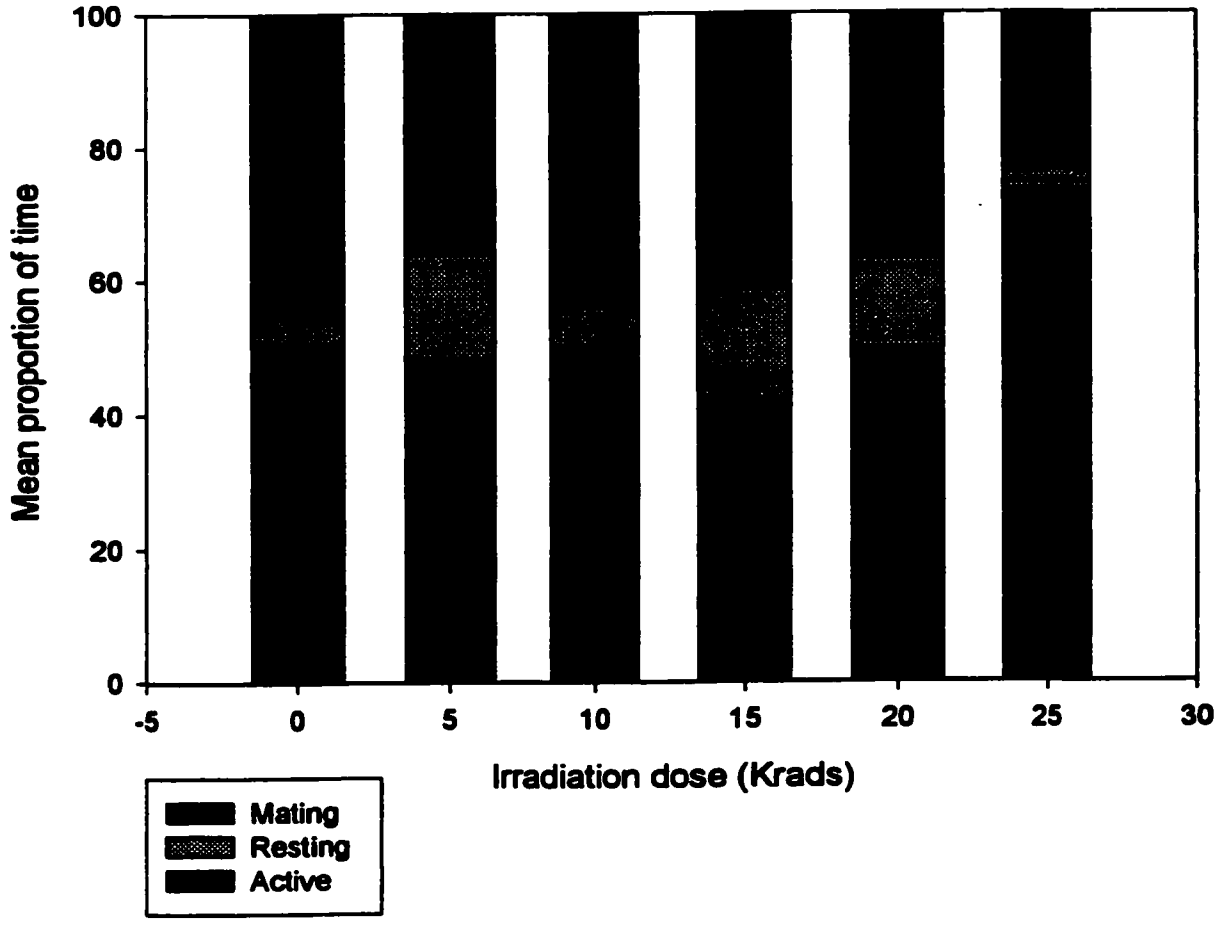
301/4) X-ray tube, I added six more males to the dish. This was repeated every 64 minutes until each consecutive group of six males respectively received a dose of 25, 20, 15, 10 and 5 Krads ± 10%. The 6 remaining males were not sterilized. The X-ray doses used in this assay are representative of the range of doses used in previous insect studies (*e.g.* Henneberry and McGovern 1963, Snow *et al.* 1972, Sternberg *et al.* 1973, Boorman and Parker 1976, Rubenstein 1989, Seo *et al.* 1990, Ueno and Ito 1992, Jablonski and Kaczanowski 1994, Otronen 1994, Simmons *et al.* 1996; Arnqvist and Danielsson 1999). Once the sterilization process was complete, one male from each radiation dose treatment was haphazardly assigned to each of six 50cm x 40cm x 20cm plastic water tanks so that each tank contained one male from each treatment. I provided the waterstriders with inverted Styrofoam cups and rocks for resting and oviposition, as well as one frozen cricket each. The tanks were covered with cheese cloth and the males were allowed to recover over night.

At 9h55 the following morning, I added six females to each tank. Starting at 10h00, I conducted the first of three 90 min scan-sampling sessions. The two other sessions began at 13h00 and 16h00 respectively. During each session, I scanned each tank for three periods of 5 min duration on a rotational basis, spaced 35 minutes apart (Blanckenhorn 1991). Each minute, I scored the six males in the tank as mating, resting or active on the water surface. A male was assumed to be mating if he was in genital contact with a female, resting if at least one leg was touching the provided substrate or the sides of the tank; or active if he was on the water's surface and not touching a substrate (includes feeding) (Blanckenhorn *et al.* 1995). This was repeated for 3 more days. A total of 180 observations were made for each male.

Results - Male's spent an average of 54.78% ± 26.65% of their time mating, 9.72% ± 13.89% resting, and 37.8% ± 18.63% active on the water (Fig. A1). Covariance analysis (P(behaviour) = c + dose + tank + dose x tank) revealed no effect of the different tanks and no interaction between the tank and the dose for any of the behaviours (tank: all p-values ≥ 0.199; tank x dose: all p-values ≥ 0.389). Although the males exposed to the 25 Krad dose appear to spend more time mating at the expense of other activities (Fig. A1), multiple analysis of variance revealed that the amount of time allocated to each behaviour did not vary significantly with the irradiation dose after we combined data from all tanks (MANOVA: df = 5, 30, all p-values ≥ 0.301). Hence, sterilization doses of irradiation do not appear to have any detectable effects on male activity and mating behaviour.

Figure A1

Results of the assay to determine the effects of irradiation on male behaviour. The proportion of time spent mating, resting and active on the water surface is expressed as a function of irradiation dose.


100
80
60
40
20
0
Mean proportion of time
-5
0
5
10
15
20
25
30
Irradiation dose (Krads)
Mating
Resting
Active

Experiment 2 – Immediate effect of irradiation on sperm transfer

Methods - Non-reproductive adult *A. remigis* were collected from streams near Mont-Tremblant and Morin-Heights, (Quebec) in late September and early October 1999. I separated the males from the females to ensure female virginity and placed them in sphagnum-filled containers. The containers were placed in the laboratory refrigerator where the waterstriders remained in diapause until late January 2000 (cf. Fairbairn 1988). On January 28, I transferred 5 males to a plastic tank (50 x 40 x 20cm) at 22°C and a 14L:12D light cycle. Twenty-five females were placed in another tank under identical conditions. The tanks were half filled with dechlorinated water and air diffusers were provided to simulate a current. I provided rocks for resting and fed the waterstriders daily with frozen crickets.

After a 24h acclimation period, each male was placed in a separate tank with one randomly selected virgin female. I monitored the tanks for matings in excess of 30 minutes to ensure that males had enough time to transfer sperm (Rubenstein 1989). When copulation ended, females were removed and anaesthetized in vials containing chloroform. The females were dissected in Ringer's solution. The gynatrial complex was transferred to a microscopic slide and examined using a Nikon Optishot microscope equipped with differential interference contrast optics. The gynatrial sac and the spermathecal tube were examined for the presence of sperm.

Once it was determined that all 5 males were capable of transferring motile sperm, I irradiated them with a 12 Krad X-ray dose. I placed the males in a shallow, 15cm x 15cm plastic dish and covered the dish with cheese cloth. The dish was placed under the X-ray tube (Mueller MG300, Mod 301/4) for 154 minutes. Males absorbed a dose of 12

Krads at a rate of 78 rads/min. As established in the previous experiment, a 12 Krad dose had no significant effect on male behaviour and is sufficient to eliminate male fertility (Rubenstein 1989). Following irradiation, I placed the males in a common water tank where they were provided with one frozen cricket each, and allowed to recover until the following morning. At 10h00 on each of the four following days, I placed each irradiated male in a separate plastic tank (50 x 40 x 20cm) with one virgin female, and allowed them to mate. Matings were allowed to continue for their full duration. After mating, females were dissected in Ringer's solution. The gynatrial complex was transferred to a slide for microscopic examination. We examined the gynatrial sac, vermiform appendix and spermathecal tube to determine whether sperm transfer was successful. Any sperm found was examined for motility.

Results - All five males successfully transferred motile sperm to each mate over the first three days following irradiation. On the fourth day, only one male failed to transfer sperm. Where sperm transfer occurred, sperm quantities were sufficient to fill the gynatrial sac. In comparison, Campbell and Fairbairn (unpublished) found that 25 out of 27 matings between females and non-irradiated males resulted in successful sperm transfer to the gynatrial sac. All but one of these matings resulted in complete filling of the gynatrial sac.

In *A. remigis*, individual sperm are 5 mm long (Pollister 1930, Tandler and Moriber 1966). They are transferred in tangled masses from which individual sperm are difficult to extract. Hence, attempts to produce a uniform solution of sperm for direct counts (e.g. Smith *et al.* 1988, Ward 1993) failed. However, since successful

inseminations generally resulted in complete filling of the gynatrial sac, irradiated and non-irradiated males appear to transfer similar amounts of sperm. Therefore, irradiation of males with a 12 Krad X-ray dose does not appear to have any short term effects on their ability to transfer sperm.

Experiment 3 – Long term effects of irradiation on sperm transfer

Under natural conditions males may mate more than four times over a four day period (Blanckenhorn *et al* 1998). Exhaustion of sperm reserves occurs after five to eight matings in irradiated male *C. capitata* (Seo *et al.* 1990) and after fewer than nine matings in irradiated *Drosophila melanogaster* (Gilchrist and Partridge 2000). If irradiated males lose the ability to transfer sperm over time, matings occurring after exhaustion of sperm reserves will not contribute to fitness. Therefore, I conducted an experiment to determine whether males lost the ability to transfer sperm over a four day period during which they were allowed to mate freely.

Methods - For this experiment, non-reproductive waterstriders of both sexes were collected from populations at Mt. Mansfield, Vt. and Ste-Marguerite-du-Lac-Masson, Qc. on September 30 and October 1, 2000. Males were separated from the females to ensure female virginity and overwintered in the laboratory refrigerator (cf. Fairbairn 1988). On February 8, I transferred 10 males and 10 females from the refrigerator to two separate plastic tanks (50 x 40 x 20cm) at 22°C. I provided rocks for resting and fed the waterstriders daily with frozen crickets.

On the following day, I marked all males with a pattern of dots and lines using Testors model airplane paint, and randomly selected five males for a 12 Krad X-ray treatment. Once irradiation was complete, I placed irradiated and normal males in separate artificial streams. The artificial streams consisted of plastic or wooden tanks (150 x 50 x 25cm) equipped with aquarium pumps and six inverted Styrofoam cups (Fig 1). The pump outflow was suspended above the water surface to create a continuous current and an area of riffles which simulated the lotic environment where natural populations of *A. remigis* are found (Fairbairn and Brassard 1988). Males were fed frozen crickets and were allowed to recover over night.

The following day, I added five females to each artificial stream and allowed the waterstriders to mate freely over a four day period in order to deplete the sperm produced prior to irradiation. The waterstriders were fed daily with one frozen cricket each. On the fifth day, I transferred each male to a randomly selected water tank (50 x 40 x 20cm) and provided them with one frozen cricket each. Water tanks were equipped with air diffusers to simulate a current. Four inverted Styrofoam cups were placed in each tank for resting and oviposition. I placed one virgin female with each male. I monitored the tanks for matings and recorded mating duration for each mating pair. Once all mating pairs had separated, the males were removed from their respective tanks. On the following morning, I anaesthetized the females in vials containing chloroform and dissected them in insect Ringer's solution. The gynatrial complex was transferred intact to a slide for microscopic examination (40x). I looked for sperm in the gynatrial sac and the spermathecal tube and compared the sperm complements between females mated with normal and irradiated males. On February 21 (7 days after being separated from the

males), I dissected the 10 original females used to deplete male sperm stores and transferred their reproductive tracts to slides for examination under a light microscope (40x).

Results – On the fifth day of the assay, all irradiated males mated with virgin females for more than 30 minutes, which is enough time for a male to complete sperm transfer (Rubenstein 1989). Only two males successfully transferred any sperm during this time. No sperm was seen in the vermiform appendix or in the spermathecal tube. In contrast, four of the five non-irradiated males successfully transferred large masses of sperm. Sperm from these males were observed within the gynatrial sac, the vermiform appendix and the spermathecal tube. One non-irradiated male failed to transfer any sperm.

As mentioned previously, attempts to obtain uniform dilutions of sperm from dissected females have failed. Hence, sperm counts were not possible. Nonetheless, differences in the quantities of sperm transferred by irradiated and non-irradiated males were obvious. Non-irradiated males transferred approximately ten-fold greater volumes of sperm than their irradiated counterparts. These results suggest that irradiation with a 12 Krad X-ray dose leads to a marked reduction in the quantity of sperm transferred within four days under natural conditions of polygynandry.

Differences in sperm complements transferred to the females used to deplete sperm reserves were also seen. Of the five females that mated with irradiated males, only one contained any sperm after seven days of isolation from males. The sperm was only found within the spermathecal tube, which was 80% full. In comparison, sperm from non-irradiated males was still abundant after seven days. Sperm was seen within the

gynatrial sac and the vermiform appendix of all of these females, and in the spermathecal tube of three of these five females. Therefore, the results of this experiment suggest that under natural conditions of polygynandry, irradiation results in reduced levels of sperm transfer over four days.

This investigation demonstrates that irradiation does not affect male behaviour or ability to transfer sperm immediately after irradiation. However, under natural conditions of polygynandry, irradiation results in reduced levels of sperm transfer. Therefore, non-irradiated males will benefit from a numerical advantage in sperm competition with irradiated males.