

Sperm competition in the water strider, *Gerris remigis*

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Abstract. Sperm competition occurs in the giant water strider. In serial mating experiments using normal and sterilized males, the last male to mate was able to displace, on average, 65% of the sperm of the previous male. Success of second males was, however, highly variable but can be partially explained by relative copulation lengths of the two males. Although there was a tendency for long copulations by the first male to be followed by long copulations by the second, second males could only obtain the 'last male' advantage if their copulations were long relative to those of first males. A long latency period occurred between genital contact and the effective transfer of sperm as no viable eggs were produced until copulations lasted at least 15 min. Once sperm transfer began, however, virtually the entire sperm load was delivered instantaneously to the female. Females continued to lay fertile eggs for 24 days after a single copulation, and although this period increased to 30 days after two matings, the difference was not statistically significant. Although the incompleteness of sperm displacement suggests that alternative copulatory strategies among males should be common, the existence of a long latency period before sperm transfer should not only reduce this tendency, but also increase female choosiness.

Reproductive competition among males can take many forms. Usually, males attempt to maximize their reproductive success by searching for and acquiring a large number of mates. If, however, females are able to mate many times and store sperm for long periods, then a copulation does not necessarily guarantee male reproductive success. Only if a male can minimize having his sperm displaced by either behavioural, chemical or mechanical means will he maximize his chances of fathering offspring from a given mate. As a consequence, a balance will be reached between mating with as many females as possible and protecting sperm already invested in a mated female (Parker 1984).

Once conflicts among males for sperm precedence evolve, selection enhancing paternity assurance will operate in two different ways. One force will favour mechanisms that increase the effectiveness of sperm displacement. In some damselfly and dragonfly species, for example, copulatory structures either scoop out, or pound into inaccessible places the sperm of previous matings (Waage 1979, 1984). In other species, such as *Drosophila* (Lefevre & Johnson 1962), giant waterbugs, *Abedus lerberti* (Smith 1979), locusts, *Locusta migratoria* (Parker & Smith 1975), and dungflies, *Scatophaga stercoraria* (Parker 1970), sperm are either displaced volumetrically, or pre-empted by dilution due to the mixing effects of repeated

copulations. A second force, however, will augment techniques by which males can protect their investment. In some insect species, such as dungflies (Parker 1970), males simply guard females by driving away rivals both before and after mating. In others species, males block female spermathecae with plugs (locusts: Parker & Smith 1975, some lepidoptera: Parker 1970) or even anoint females with scents, as occurs in *Drosophila* (Tompkins & Hall 1981) and mosquitoes, *Aedes* spp. (Craig 1967), that make them both less receptive and unattractive to subsequent males. In general, these traits should co-evolve since any increase in the ability to displace the sperm of the last male should lead to enhancements in his ability to protect his investment.

The giant water strider is a species in which females often mate more than once before laying a batch of eggs. Mating also lasts for extended periods in the field averaging just over 1.5 h, but sometimes lasting as long as 9 h (personal observation). Such long copulatory periods limit a male's ability to acquire additional matings, and suggest that sperm displacement is a potential problem that males must cope with if they are to obtain reproductive success. The purpose of this study was to determine the magnitude of this problem and to discern how the male struggle for sperm precedence affects female behaviour and reproductive success. Moreover, recent research on water striders shows

that inter-male competition is often intense and that alternative mating strategies co-exist within populations (Rubenstein 1984; Wilcox 1984; Hayashi 1985; Vepsäläinen & Nummelin 1985; Spence & Wilcox 1986). Until patterns of sperm precedence and displacement are understood, however, the evolutionary significance of male mating behaviour will remain obscure.

MATERIALS AND METHODS

The giant water strider lives on the surface of streams feeding on drift. Both adult males and females defend feeding positions in the current, whereas juveniles, which pass through five instars, spend most of their development in quieter waters near stream edges. In New Jersey, populations are bivoltine with the year's first generation emerging from diapause as adults towards the end of February. They begin mating immediately, and the young they produce mature during June and continue breeding until mid-August. Young from this second generation were collected from a small stream near Princeton, New Jersey and each individual was reared in the laboratory in isolation in a small plastic dish measuring 10 × 10 cm on excess quantities of fruit flies. Isolation ensured that when males and females matured sexually, they were virgins.

In the first experiment single males and single females were placed in 40-litre aquaria and were allowed to mate for a specified length of time. Times for successive pairs were assigned at random and ranged from 1 to 60 min. Copulations were terminated by prodding males to dismount and females were returned to their plastic dishes and fed *ad libitum* on fruit flies. Small pieces of styrofoam were provided and females laid eggs on them. The appearance of new eggs and their subsequent development were recorded daily. Eggs were judged to be fertile if they darkened in colour and red eye-spots appeared. From these experiments it was possible to determine the course of sperm transfer by monitoring how copulation length affected the percentage of fertile eggs laid by females. They also provided information on the number of days that a female lays viable eggs as the result of a single copulation.

To determine if sperm displacement occurs in these water striders, a second set of matings was performed using serial pairings by males either

having normal sperm, or having sperm whose DNA had been fragmented by gamma irradiation. By irradiating males with doses of 12 Krads (from gamma-emitting cobalt chloride), fertility could be completely eliminated. Four types of matings were performed. Two were experimental, in which 10 females were mated first by a normal male followed by an irradiated male, or in which 10 females were mated first by an irradiated male and then a normal male. Two were controls, in which five females were mated in sequence to either two normal or two irradiated males. To ensure that irradiated males were once fertile, all males to be irradiated were mated once as normal males. The length of each copulation, as well as the duration of the interval between copulations, was recorded. From these data it was possible to determine if the amount of time a male spent in copula affected the number of eggs he fathered, and if the interval between matings influenced the effectiveness of sperm displacement. These data also provided information on the period during which two loads of sperm enabled females to lay fertile eggs.

After the serial matings, females were returned to their plastic dishes and given styrofoam pieces measuring approximately 2 × 1 cm upon which to lay eggs. Since the development of every egg was monitored daily, the magnitude of sperm displacement could be determined by measuring the difference between the viabilities of eggs laid by females mated to two normal males and the viabilities of eggs of females mated to irradiated and then to normal males. As a check on the accuracy of the results, the complimentary comparison of the difference in egg viabilities between females mated to two irradiated males and those mated to normal and then to irradiated males was also made. Since the fertility of normal matings is never 100%, the degree of sperm displacement, x , can be estimated by the formula

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

where a is the mean proportion of fertile eggs from irradiated-normal mating, b is the mean proportion of fertile eggs from normal-normal matings and c is the mean proportion of fertile eggs from irradiated-irradiated matings. Or alternatively, a can be the mean proportion of infertile eggs from normal-irradiated matings and b and c can represent the average proportions of inviable eggs from irradiated-irradiated and normal-normal matings, respectively.

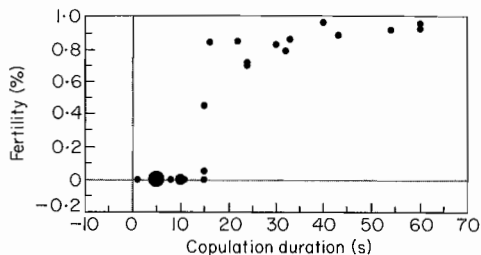


Figure 1. Copulation duration and proportion of viable eggs laid. Circle size is proportional to the number of replicates. Small circle represents one mating ($N=23$).

Copulation duration was measured from the beginning to the end of genital contact. In water striders, males simply pounce on females and females immediately hop and roll often dislodging males. If, however, males can clasp females tightly around their thoraxes, they can obtain genital contact. In this population of water striders, the pre-copulatory phase lasts on average (\pm SE) 0.62 ± 0.46 min. Pairs remained in genital contact until mating was terminated either by females throwing off males, or males voluntarily hopping off females. Only matings in which both males mated for at least 15 min were used in the analysis of sperm priority (see below).

RESULTS

Course of Sperm Transfer

There is a long period before any sperm is transferred. Judging by fertility rates, it took at least 15 min before sperm entered the female after genital contact was made (Fig. 1), and even then, there was tremendous variability in the amount transferred. Sometimes few sperm appeared to enter, whereas at other times, a complete sperm load seemed to be transferred. For copulations lasting longer than 20 min, fertility improved dramatically, but more importantly from a male's perspective, the variability in fertility diminishes. Not until copulations lasted longer than 30 min, however, did fertility reach its maximum of about 93%.

Load Size

The amount of sperm transferred during a single copulation did not appear to limit a female's ability

to fertilize eggs. Females copulating once for at least 20 min continued to lay batches of viable eggs on average (\pm SE) for 24.2 ± 1.4 days, whereas those mating in sequence with two normal males continued to do so on average for 30.2 ± 4.6 days. During these periods the average yields were 72.7 ± 10.8 and 84.6 ± 16.6 eggs, respectively. Although from a female's perspective acquiring a slightly larger volume of sperm appears to be a small advantage that can be derived from multiple matings, the difference was too small to be statistically significant (days: $t_{14} = 1.65$, $P < 0.13$; eggs: $t_{14} = 0.6$, $P < 0.6$).

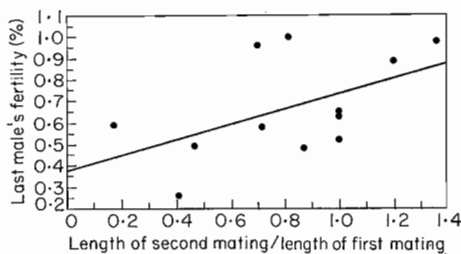
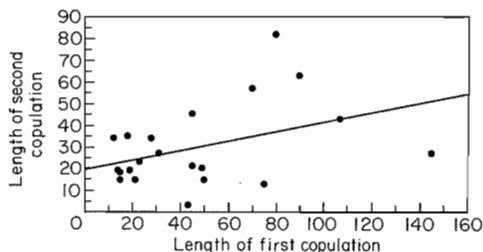
Sperm Priority

The last male to mate had a significant reproductive advantage. About 65% of the eggs derived from irradiated-normal matings were viable (Table I). But since 7% of the eggs produced by females mated serially to two normal males also failed to develop, the actual magnitude of sperm displacement in irradiated-normal matings was 70%. In normal-irradiated matings the estimate was 62%, which was not significantly different ($t_{13} = 0.98$, $P < 0.35$). Thus, on average, the last male to mate fertilized 65% of the eggs a female laid. This proportion of actual eggs fertilized by the second male differs significantly from 0.5, which would be expected if the sperm from two males was completely mixed and used randomly by the female to fertilize eggs ($t_{13} = 2.25$, $P < 0.05$).

Although the last male to mate can, on average, displace the sperm of previous matings, his ability to do so is highly variable (Table I). Some of this variation (27%) appears to be accounted for by the relative length of each male's copulation (Fig. 2). When the length of the first male's copulation was held constant, a significant partial correlation occurs ($r_{10} = 0.61$, $F = 5.23$, $P < 0.05$) between the second male's fertility and the length of his copulation. Thus, relatively long first copulations tend to reduce the second male's advantage, whereas relatively long second copulations increased the second male's advantage. Interestingly, there was a weak positive correlation between the length of each male's copulation ($r = 0.41$, $N = 21$, $P < 0.07$; Fig. 3). The effect of inter-copulatory interval (range 0.1–6.3 h), however, had no effect on the magnitude of the last male's advantage ($r = 0.02$, $N = 12$, $P = 0.95$).

Table I. Proportion of fertile eggs laid by females mated twice to normal and irradiated males in various combinations

Mating sequence	Number of mated females	Proportion of viable eggs	SE	Coefficient of variation
Normal-normal	5	0.93	0.01	2.4
Irradiated-irradiated	5	0.00	0.00	0.0
Normal-irradiated	8	0.36	0.09	70.4
Irradiated-normal	7	0.65	0.15	58.2

**Figure 2.** Relationship between the ratio of copulation lengths of second mated male to the first mated male and fertility of the last male. Regression equation: $y = 0.36x + 0.38$, R -squared = 0.27 ($N = 12$).**Figure 3.** Relationship between lengths of first and second copulations. Regression equation: $y = 0.22x + 19.5$, R -squared = 0.17 ($N = 21$).

DISCUSSION

In the giant water strider, sperm precedence occurs and the last male to mate fertilizes approximately 65% of the eggs a female subsequently lays. This degree of sperm precedence by the last male is similar to that shown by many other insect species having tubular shaped spermathecae (for review, see Walker 1980; Gwynne 1984). Nevertheless, the

fact that the last male's advantage is incomplete means that even if a male is displaced, or voluntarily terminates a mating quickly, he still has a chance of fertilizing approximately $(\frac{1}{3})^N$ eggs, where N is the number of males with whom a female subsequently mates.

This raises the interesting possibility that some males might be favouring a 'mate and run' strategy over one of prolonging copulation. For this to be the case, however, the efficiency of sperm displacement must be very low, or males must differ in their ability to acquire matings. As long as finding mates is fairly easy, the existence of complete sperm mixing means that defending females that are going to re-mate anyway is not as cost effective as simply locating new females (Parker 1984). Thus, if escalated fights are generally avoided because asymmetries, such as resource value, are used to settle disputes conventionally, then mating males should cease mating with, or guarding, females as soon as they are challenged and a mate and run strategy will spread. If, however, the costs of mating are high, or mating males are of superior fighting ability, then these factors could offset the resource value asymmetry and tend to favour long copulations and 'guarding' by superior mating males even when sperm precedence is low. Interestingly, in populations with moderate levels of sperm precedence and where copulations that are just long enough to be moderately costly, a mixture of long and short copulation strategies could coexist. At least in the Princeton population of the giant water strider, a moderately long latency period occurs before sperm can be effectively transferred, and some males, those with large forelegs, have a significant fighting advantage (Rubenstein 1984). Such individuals would be selected to copulate for prolonged periods. Others, such as the small or

subordinate mating males, however, may not always have these offsetting factors operating in their favour. Conversely, they would be selected to dismount as soon as they are challenged. As a result, copulations by small or subordinate males should be, on average, shorter than those of larger or more dominant males and thus generate the variability in copulation length observed within natural populations.

Even though the sperm of the last male to mate has priority, this advantage appears to be highly variable. While some second males achieve almost total fertility, others obtain virtually none. The relative length of each copulation helps account for some of this variation. Relatively long copulation by the first male can limit the second male's advantage. Yet, the fact that copulations tend to be followed by copulations of similar length (long by long and short by short) suggests that a second male's response is affected by the behaviour of the first male. Somehow a long first copulation seems to require that a subsequent male must copulate for a disproportionately long time before he can obtain the 'last male' advantage. There appears to be pressure on all males, both first and last, to remain in copula for long periods if maximizing fertility from an individual female is their 'goal'. Interestingly, the slope of the relationship between the two copulations is less than 1, suggesting that the second male need not mate as long as the first male to get his advantage. With only a 65% mating advantage, it may be the savings in mating duration that really enhance the second male's reproductive success.

The giant water strider exhibits a long period of genital contact before any sperm is transferred. But once transfer begins, virtually all of the effective sperm is received instantaneously by the female. The function of this long pause is unknown. Possibly the male uses this time to assess whether or not the female has already mated, or possibly to displace the sperm of previous males. Since this latency period occurs when matings involve only virgin females, these possibilities cannot be the only explanations; two others may apply. First, this latency period may give females the opportunity to assess males. Eberhardt (1985) has suggested that male penises are ideal structures for sexual selection to operate upon since their elaboration via arbitrary female preferences will rarely be opposed by natural selection. Female water striders ordinarily exhibit a large degree of selectivity prior to

genital contact (Rubenstein 1984). They initiate wrestling matches before mating that are often vigorous and prolonged; males with large claspers are more able to hold on. Assessment and choice by females could presumably continue, however, even after genital contact has been made by allowing only those males deemed suitable to transfer sperm. Villavaso (1975) has demonstrated that boll weevil, *Anthonomus grandis*, females can, by using their spermathecal muscle, limit the extent to which males can displace another male's sperm. Moreover, in closely related semi-aquatic herbivorous bugs, *Herbrus pusillus* and *H. ruficeps*, females draw sperm from the male's seminal duct and pack it into the spermathecae by contraction of dilator muscles (Henning-van Battum & Henning 1986). These examples suggest that the use of anatomical mechanisms by females to control sperm placement and use may be more widespread in insects. Thus, they could account for the existence of a long latency period preceding sperm transfer. Second, the existence of a long latency period may enable females to ensure that males will copulate and guard them for long periods, thus permitting females to accrue large material benefits as occurs in damselflies (Waage 1979), dung flies (Parker 1970; Borgia 1981) and water striders (see below). And, according to Parker's (1984) model, elevating the cost of mating is one of the surest ways of allowing males to profit by prolonging copulation.

Long copulations and extended periods of post-copulatory guarding are usually interpreted as mechanisms for ensuring paternity or providing benefits to females and offspring. In the water strider, long copulations serve both of these functions. They clearly can lead to large amounts of sperm displacement, but they can also provide females with feeding benefits. While mating, females are able to spend more time feeding (Rubenstein 1984) and they are actually able to acquire more food per unit time (Wilcox 1984). It is unlikely, however, that these benefits will ultimately favour a male's own offspring. By the time these benefits are used in provisioning eggs, it is likely that the sperm of the beneficent male will only, at best, be fertilizing a minority of the eggs. Since this foraging benefit is largest when females are paired with the most dominant males, pressure is presumably put on females to test a male's strength and tenacity. Moreover, receipt of such a material benefit could explain why females mate repeatedly even though they acquire enough sperm

from a single mating to fertilize the eggs they will lay for approximately 3 weeks. At least in the giant water strider it appears that the 'battle of the sexes' has been somewhat reduced since males and females both seem to benefit by long copulations, a behaviour which most probably evolved as a response to both females being able to control sperm transfer, and males being able to displace effectively the sperm of previous matings.

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