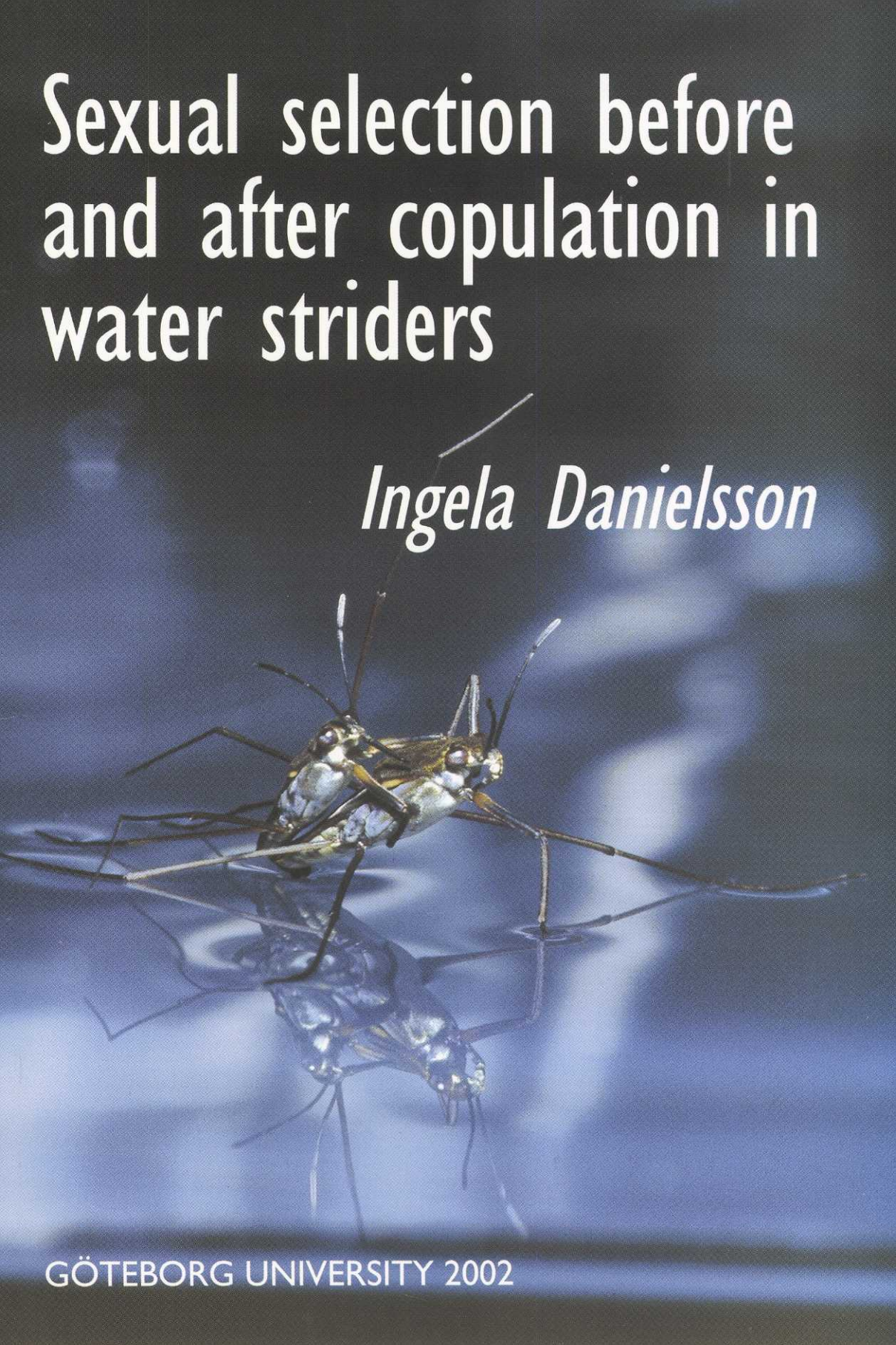
Sexual selection before and after copulation in water striders

Ingela Danielsson



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AKADEMISK AVHANDLING
som för avläggande av filosofie doktorsexamen i ekologisk zoologi,
enligt Naturvetenskapliga Fakultetens beslut, kommer att offentligen försvaras
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Avhandlingen försvaras på engelska. Examinator är Professor Malte Andersson.
Fakultetsopponent är Professor Leigh W. Simmons, Department of Zoology,
The University of Western Australia, Nedlands WA 6907, Australia.

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Abstract Post-copulatory sexual selection is now known to occur in most animal groups. Our knowledge of the causes and effects of variation in the relative fertilization success of the competing males is, however, still limited. Crucial questions concern which male traits are favoured by post-copulatory sexual selection, and whether such selection reinforces or counteracts pre-copulatory sexual selection. I address these questions by studying relative fertilization success among male water striders (*Gerris lacustris* and *G. lateralis*).

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By analysing shape variation in male genital morphology, a relationship was discovered between the shape of male intromittent genitalia and relative paternity success. This result provides experimental support for the hypothesis that male genitalia evolve via post-copulatory sexual selection. A detailed analysis revealed that different aspects of male genitalia affect different fitness components. Certain traits affected male ability to obtain fertilization success while others affected male ability to avoid sperm precedence by subsequent males. The effect of the male genitalia shape varied with female morphology, suggesting that selection on male genitalia depends on the frequency distribution of female phenotypes.

Male ejaculate size and fertilization success increased with copulation duration, suggesting that males are able to increase their relative fertilization success by transferring more sperm. Males appear to be sperm limited however, and transferred significantly less sperm after a short than after a long recovery period. Thus males apparently need some time to replenish sperm stores between matings, a constraint with implications for sexual selection of male body size. Mating is costly for females and they are generally reluctant to mate. This sexual conflict over mating generates directional pre-copulatory sexual selection for large males. When large and small males were allowed to compete for fertilizations, large males mated more often, but small males copulated longer and gained higher fertilization success per mating by transferring more sperm. Shorter copulations by large males were probably a result of a behavioural trade-off between mating frequency and ejaculate size caused by sperm limitation.

The result of this antagonistic effect of pre- and post-copulatory sexual selection was that large and small males had similar reproductive success. Although large males had a clear mating advantage they had no fertilization advantage, and there appears to be no net selection for increased male size.

Key words: sexual selection, sperm competition, cryptic female choice, fertilization success, mating success, evolution of genitalia, body size, copulation duration, ejaculate size, sperm transfer, Gerridae, *Gerris*, water striders

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Sexual selection before and after copulation in water striders

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Göteborg, April 2002

The oral defence of this thesis will take place Friday 26 April 2002,
at the Department of Zoology, Medicinaregatan 18 Göteborg, Sweden.
Faculty opponent is Professor Leigh W. Simmons, Department of Zoology,
The University of Western Australia, Nedlands WA 6907, Australia.

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List of papers

A doctoral thesis at a Swedish university is often presented as a collection of papers with a summarising introductory part, which constitute the formal thesis.

This thesis is based on the following papers:

1 Danielsson, I. (1998) *Mechanisms of sperm competition in insects*, Ann. Zool. Fennici 35: 241-257.

2 Danielsson, I. & Askenmo, C. (1999) *Male genital traits and mating interval affect male fertilization success in the water strider Gerris lacustris*, Behav. Ecol. Sociobiol. 46: 149-156.

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

4 Arnqvist, G. & Danielsson, I. (1999) *Postmating sexual selection: the effects of male body size and recovery period on paternity and female egg production rate in a water strider*, Behav. Ecol. 10: 358-365.

5 Danielsson, I. (2001) *Antagonistic pre- and post-copulatory sexual selection on male body size in a water strider (Gerris lacustris)*. Proc. R. Soc. Lond. B 268: 77-81.

6 Danielsson, I. & Askenmo, C. *Timing of sperm transfer in water striders*. Manuscript.

Contents

Introduction

Natural selection favours any trait that allows for the increased production of offspring as long as parental survival is not too severely jeopardized. Male reproductive success often depends largely on the number of mates, owing to the low cost of sperm and low or moderate male parental effort. Hence characters that improve the mating success are under strong selection. This form of selection arising from differences in reproductive success as a result of competition over mates is termed sexual selection (Andersson 1994). A male could be successful by either directly outcompeting other males (male-male contests) or by making females prefer him as a mate (female choice).

Successful reproduction is however not only about the number of mates. In most species insemination and fertilization are uncoupled both in space and time, allowing competition between males to continue inside the female through sperm competition. Parker (1970) defined sperm competition as "the competition within a single female between the sperm from two or more males for the fertilization of the ova". Sperm competition extends sexual selection up to the point of fertilization and can therefore be viewed as a post-copulatory form of male-male contests. Post-copulatory sexual selection also includes removal of rival sperm from the female, although in this case sperm from rival males may never coexist within the female. Post-copulatory female choice known as cryptic female choice (Thornhill 1983, Eberhard 1996) includes female control of sperm transfer, transport and storage, discrimination between sperm as well as control of offspring production.

As a result of these two competition components, a male's reproductive success is measured by the number of females he mates with together with the average number of offspring he sires per female. It is important to note that the concepts of pre- and post-copulatory sexual selection are not defined on basis of when a sexually selected character is recognised or used, but on basis of whether the trait affects either the mating success (pre) or, the relative fertilization success (post).

Aim of the thesis

Today there is good evidence that post-copulatory sexual selection occurs in most animal groups (Birkhead & Møller 1998). Large intraspecific variation in male relative fertilization success has been documented in many species (Lewis and Austad 1990, Simmons 2001), indicating the potential importance of post-copulatory sexual selection. Current knowledge concerning the causes and effects of this variation is however limited.

The aim of this thesis was to investigate variation in relative fertilization success in water striders (Gerridae). The main question being: Does paternity vary as a result of random factors, or do mating conditions and/or some specific male traits play a role? This question was addressed by assessing how fertilization success correlates to mating conditions, mating behaviour, and male morphology (see Table 1).

This thesis also investigates the interaction between selection acting before and after copulation, i.e. does post-copulatory sexual selection reinforce or counteract conventional pre-copulatory sexual selection? One would expect male traits important for both mating and fertilization success to evolve. A trait advantageous in competition over mates may however be disadvantageous with respect to relative fertilization

success. In this thesis I investigated the combined effect of pre- and post-copulatory sexual selection on male body size in water striders.

Table 1. *Potential determinants of fertilization success of water striders investigated in this thesis.*

Mating conditions	*Paper*
mating order	2
number of matings by the female	2
female mating interval	2
Male morphology	
male genitalia	2, 3
male size	2, 4, 5
Mating behaviour	
copulatory courtship	3
copulation duration	2, 3, 4
sperm transfer	6
guarding duration	2
male mating interval	4

Choice of study organism

A prerequisite for post-copulatory sexual selection is that a female mates with several males. Ejaculates from different males must also be simultaneously present within the female to allow for any sperm competition and female sperm selection (Simmons 2001). Insects are likely to display high levels of sperm competition since multiple matings are common and female insects have a specialised organ (spermathecae) for sperm storage (Parker 1970, Simmons 2001).

Water striders are rather typical insects in this respect. Both males and females mate repeatedly and females store sperm within a spermathecae for 10–30 days (Arnqvist 1997b). Since females often mate several times per day (Arnqvist 1997b, personal observations) the risk of sperm competition is high. Mating strategies within this group are unusually diverse (Arnqvist 1997b), with water striders having a flexible mating system, which allows for manipulations of mating behaviours (Rowe *et al.* 1994). Certain water strider species are common, allowing for the collection of large samples. They are also easily reared in the laboratory and feed willingly on a variety of fresh and frozen insects. These facts taken together makes the water strider an ideal animal for post-copulatory sexual selection studies.

Natural history of water striders (Andersen 1982, 1996)
Water striders (Heteroptera, Gerridae) can be found in almost all freshwater environments, with most species preferring stagnant waters such as ponds and pools where several species often coexist. Three water strider genera are found in Sweden, *Aquarius* (2 species), *Gerris* (7 species) and *Limnoporus* (1 species). In this thesis I

studied two commonly found species, *Gerris lacustris* (Figure 1) and *G. lateralis*, which often coexist.

Water striders are probably best known for the ability to walk on the water surface, as a result of hydrophobic hairs on their legs. Water striders move across the water surface using a form of rowing motion. The middle legs provide the stroke causing the water strider to slide forward supported by fore and hind legs. Water strides are both carnivores and scavengers feeding on various arthropods.

The life cycle consists of five nymphal instars, all living on the water surface. Adults diapause in a prereproductive state in winter with reproduction commencing a few weeks after emergence in early spring. The mating and egg-laying period is very long, lasting for two months or more in most species. Each female is capable of laying in excess of 250 eggs. Eggs (1–2 mm) hatch after 8–14 days and postembryonic development last 16–34 days. The nymphs are quite similar to the adults in their basic morphology only smaller.

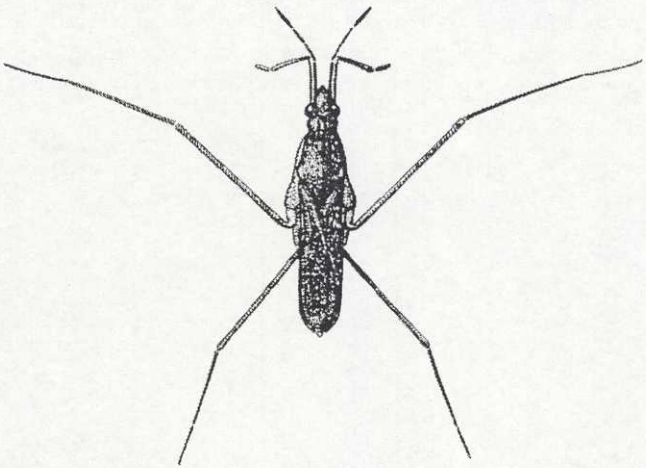

Figure 1. *Gerris lacustris (From Andersen 1982)*

Mating behaviour

Males in the genus *Gerris* actively search for females and initiate matings by lunging at the female without prior courtship (type I mating behaviour, *sensu* Arnqvist 1997b). Once contact is made the male grasps the female's thorax with his forelegs and rapidly attempts to insert his genitalia. During mating the female carries the male on her back, which exposes the female to higher mating costs; Females experience higher predation risks, reduced mobilities and also have higher energy requirements (Arnqvist 1997b, Watson *et al.* 1998). Females actively try to avoid superfluous matings and are generally reluctant to mate. They attempt to dislodge the male, typically by somersaulting backwards, jerking and try to loosen the male's grasp using their forelegs. If a male manages to withstand these dislodging attempts, copulation starts and usually

lasts for about 10–30 min, although time spent *in copula* varies greatly both between and within species (Table 8-1 Arnqvist 1997b, this thesis). After copulation, the male withdraws his genitalia but remains mounted on top of the female guarding her. Time spent mate guarding is highly variable with the pair separating usually after a struggle during which the female finally dislodges the male.

Mating system

Both males and females mate repeatedly and their mating system is often referred to as convenience polyandry (Thornhill and Alcock 1983, Rowe *et al.* 1994). Despite the associated mating costs females mate far more often than would be necessary to prevent sperm shortage. The reason seems to be that females mate repeatedly to avoid harassment by males. Rejecting males through elaborate struggles may be even more risky and expensive than mating itself, since mating females are less intensely harassed than single females. Using a flexible strategy females make the best of a bad job and balance their costs by varying their reluctance to mate (Rowe *et al.* 1994). When harassment levels are low females strongly resist mating, however they struggle less intensely when harassment levels are high, since females that dislodge a male will probably be harassed again. Harassment rates vary with local sex ratios. Several studies have shown that females alter their reluctance to mate according to the convenience polyandry hypothesis by manipulating the sex ratio (reviewed by Rowe *et al.* 1994).

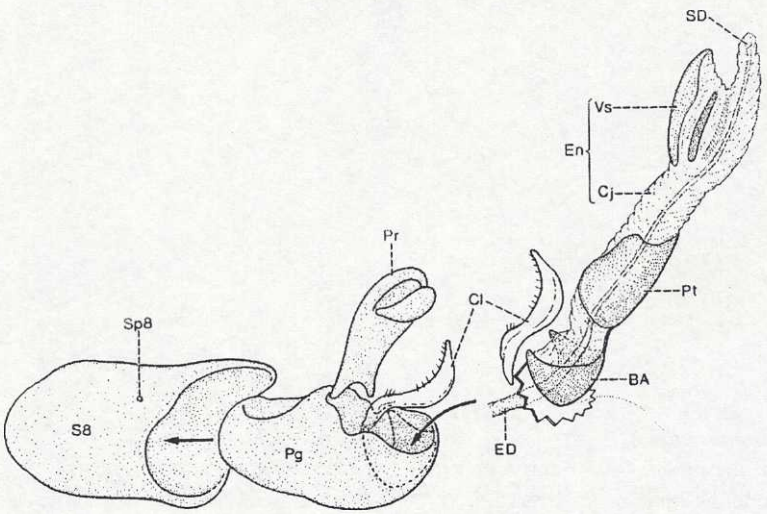

Figure 2. *Male genitalia of a generalized gerromorphan bug; pygophore (segment 9) pulled out of segment 8, and inflated phallic organ and left clasper detached from pygophore. Abbreviations: BA, basal articulatory apparatus of phallus; Cj, endosomal conjunctivum; Cl, clasper; ED ejaculatory duct; En, phallic endosoma; Pg, pygophore; Pr, proctiger; Pt, phallotheca; S 8, abdominal segment 8; SD, seminal duct; Sp 8 eighth abdominal spiracle; Vs, endosomal vesica. (From Andersen 1982)*

Male and female genitalia (Andersen 1982, 1993)
Water strider male genitalia are composed of the cylindrical abdominal segment 8, the boat-shaped segment 9 (pygophore) and the lid-shaped segment 10 (proctiger) (Figure 2). During non-mating periods the genital segments are telescoped into segment 8. The intromittent phallic organ hidden inside the pygophore is inserted into the female during copulation and can be inflated by pressure from the body fluid. The phallus is largely membranous, but consists of some sclerotized parts; a proximal phallotheca and an apical capsule (the vesica). The vesica encloses an armature of sclerites (Figure 5). These sclerites show rapid and divergent evolution within *Gerromorpha*, and are of taxonomic and phylogenetic importance.

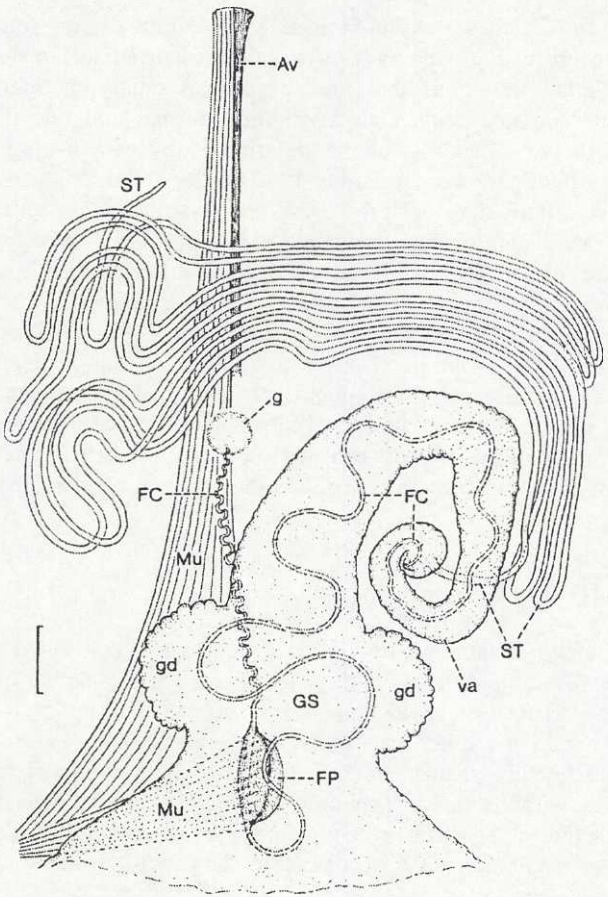

Figure 3. *Total preparation of female gynatrial complex (dorsal view) of Gerris lacustris (L.) with muscle fibres of right side removed. Abbreviations: Av, ventral apodeme of female abdomen; FC, fecundation canal; FP, fecundation pump; g, glandular tissue; gd, gynatrial gland; GS, gynatrial sac; Mu, muscle; ST, spermathecal tube; va, vermiform appendix. Scale 100μm (From Andersen 1982)*

In water striders, as in other animals with internal fertilization, there is a time lag between insemination and fertilization. During copulation males transfer sperm into the female gynatrial sac (bursa copulatrix), where sperm appear as a tangled mass (Figure 3, Andersen 1982, paper 6). The very long sperm, about 0,5–5 mm (Pollister 1930, Andersen 1982, personal observations), swim or are transported to the spermathecae. This single sperm storage organ is very long and narrow, sometimes longer than the female body (Andersen 1982). When an egg passes down the oviduct, sperm are moved (move) from the spermathecae or the bursa through the fecundity canal to the oviduct (Andersen 1982, personal observations).

The sterile-male technique

Paternity within egg batches was determined by labelling sperm; some males were sterilised by exposure to high-energy X-rays. Sperm of irradiated males still fertilize eggs but carry lethal mutations that prevent normal embryonic development. By allowing a female to mate sequentially with one normal and one irradiated male, paternity within the egg batches can be determined by establishing whether eggs develop normally or not (Boorman & Parker 1976). After 5–8 days normal eggs become brownish and have usually developed red eyespots. Non-viable eggs remain opaque and yellow and are easily identified. However, a small proportion of eggs fertilized by normal males are non-viable, while some eggs from irradiated matings develop normally. Estimates of paternity therefore must be corrected for both the average infertility rate of normal males and the average fertility rate of irradiated males (Boorman & Parker 1976). A problem associated with the sterile-male technique is that sperm from sterilised males may be competitively inferior to normal sperm. A way of checking for any differences in fertilizing ability between normal and irradiated sperm is to reverse the order of mating between the two males (Boorman and Parker 1976). Irradiation has not been found to affect male mating behaviour however (paper 2).

Variance in P_2

The proportion of eggs fathered by the last male in a controlled double-mating experiment is usually termed the P_2-value, P_3-value in a triple-mating experiment, and so on. Species have often been classified as showing either first or last-male sperm priority, i.e. either the first male or the last male mated in a sequence with a female gained most fertilizations. Consequently, mating order is thought to be a main determinant of male fertilization success, with the predominant opinion being that the last male advantage is the rule in insects. This view has shifted recently since species-specific P_2-value averages for most insects appear to fall around 0.5 (indicating mixed paternity without any order effect) or near 1 (indicating some sort of last male advantage) (Simmons 2001).

Available estimates of average P_2-values for water striders implies that mixed paternity occurs together with some degree of last-male advantage: 0.65 in *G. lateralis* (Arnqvist 1988), 0.80 in *A. remigis* (Rubenstein 1989) and 0.68 in *G. lacustris* (paper 2). Unfortunately, average P_2-values alone cannot be used to reveal mechanisms underlying non-random paternity (Simmons 2001). Species-specific averages conceal the huge intraspecific variation (Lewis and Austad 1990, Simmons 2001). In *G. lacustris* the

fertilization success of the last male ranged from 0 to 100% (paper 2), while in *G. lateralis* the variation was also large, with a coefficient of variation of 101% (paper 3). This variation is far more interesting than the mean values themselves and can provide information about sperm utilization processes and post-copulatory sexual selection (Simmons 2001).

Sperm utilization mechanisms

What factors that determine how many eggs each male fertilizes after a double mating are still unclear. In insects a number of mechanisms that may influence relative fertilization success have been identified (Simmons 2001). Sperm from different males may mix within the female resulting in sperm competition with fertilization success dependent on the relative number of sperm transferred by each male. Or sperm from the first male may be displaced, repositioned or in some other way precluded from fertilizing ova before the second male transfer his sperm. It is also possible that females choose among sperm from specific males. A review of mechanisms for sperm competition and adaptations that have evolved due to sperm competition are found in paper 1 and in a recent review (Simmons 2001).

Several aspects of the water strider mating situation correlate with males' fertilization success. These results together with the observed patterns of relative paternity allow for speculations about sperm utilization mechanisms. In double matings with short mating interval, we found a bimodal distribution of P_2-values in both *G. lacustris* (Figure 4, paper 2) and *G. lateralis* (unpubl. results). A bimodal fertilization success distribution may be a consequence of males producing mating plugs. A male gains full success if he removes the plug, but fails to fertilize any ova if he is unable to remove the plug. However, mating plugs have not been observed in water striders thus far. Insemination failure also seems unlikely since males rarely fail to transfer sperm during undisturbed copulations (paper 4, 6).

The observed bimodality of fertilization success suggests a low level of sperm mixing within the female. Since sperm is deposited into the bursa at copulation, and only later migrates to the spermathecae (paper 6), it is probable that some sperm remains behind in the bursa should the female remate within a short interval. Sperm removal or repositioning within the bursa may then occur; as a single ejaculate does not fill the bursa (personal observations). Such potential interference by the second mating male may explain why male genitalia form affects the fertilization success (paper 2, 3). It is also likely that such actions would result in an all or nothing pattern if sperm mixing is insignificant. However, some form of female influence over sperm utilization cannot be excluded, especially as sperm dumping after mating with an unpreferred male could generate the observed bimodality. Female water striders often dip their abdomen into the water after copulation, and this could be a possible way of dispose unwanted sperm. This has however not been verified.

As mentioned previously bimodality implies that sperm mixing does not occur. Absence of sperm mixing seems to prevent a direct proportional relationship between a male's fertilization success and the proportion of his sperm within the female (a fair raffle, *sensu* Parker *et al.* 1990). However, there are still indications of that the amount of sperm transferred by a male affects his relative fertilization success (see Copulation duration). Even though sperm number can affect paternity patterns without a raffle

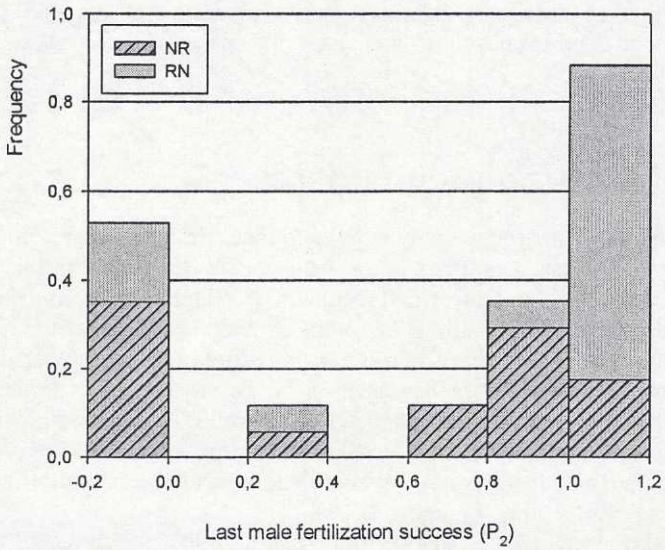

Figure 4. *The proportion of eggs fertilized by the second male in double matings with virgin females (P_2) in Gerris lacustris. Results from 17 NR and 17 RN matings (10-min interval). As pointed out by Boorman and Parker (1976), sampling error or individual variation may result in estimates of P_2 that exceed 1 or fall below 0*

taking place (see paper 6 for alternative mechanisms), it seems to be the most probable scenario in water striders. "Sloppy mixing" may account for this apparent contradiction (Harvey and Parker 2000). Instead of free-swimming sperm, a male's ejaculate may consist of a number of sperm packets, which mix randomly within the female. If ejaculates break into a small number of packets a bimodal P_2 distribution may arise (Harvey and Parker 2000). Males who copulate longer and transfer more sperm packets than other males may still receive higher fertilization success in average (Harvey and Parker 2000). In a *G. lacustris* study, the fertilization success of the last male was lower when the female had been previously mated twice instead of once, and the P_3-value did not differ significantly from the share predicted by a fair raffle (paper 2).

The fact that the P_2 value declined with increased mating interval between the first and the second male (paper 2) suggests that the sperm utilization mechanism is even more complex than initially assumed. Sperm priority changed from last-male to first-male when the mating interval was extended from 10 min to 24 h. Since the male's genitalia probably never reaches inside the very narrow spermathecae (Andersen 1982), possible removal or repositioning of rival sperm would be restricted to the bursa. A longer interval between matings might enable the first male's sperm to migrate to the spermathecae and thus be out of the subsequent mating male's reach. The change to first-male priority could also arise if the spermathecae has a restricted storage capacity. In this case a long mating interval may enable the first male's sperm to migrate to and fill the spermathecae,

leaving no room for rival sperm (Retnakaran 1974). However, sperm can directly enter the fecundity canal from the bursa (Campbell and Fairbairn 2001, personal observations), which suggests the sperm within the spermathecae may not have priority at fertilization. None the less it may still be important for sperm to reach the spermathecae, as females may not be able to maintain sperm in the bursa for longer periods.

The fact that contradictory conclusions may be drawn indicates that certain pieces of the puzzle are still missing. Too little is known about sperm transport and storage within the female to efficiently describe the mechanism of sperm utilization in water striders. It is important to note that several of the suggested mechanisms discussed above are not necessarily mutually exclusive.

Determinants of male fertilization success are also relevant in terms of the selection pressures on males. The following sections focus on male traits selected to achieve higher fertilization success.

Repeatability in male performance

In an experiment using *G. lateralis*, pairs of males were mated with two different females, to assess the repeatability (Lessels & Boag 1987) of a male's fertilization success, i.e. we tested whether the variation in fertilization success is due mainly to consistent differences amongst males. The P_2-value of a given pair of males was significantly repeatable across replicate females (paper 3). Thus males appeared to differ in their ability to gain fertilizations and would therefore be subject to post-copulatory sexual selection (Pitnick and Brown 2000). The next step was to ascertain which male traits are associated with high fertilization success as well as how these traits influence paternity.

Male genitalia

Rapid divergent evolution of intromittent genital morphology is a general trend in animals exhibiting internal fertilization (Eberhard 1985). There are three main hypotheses for intromittent genitalia evolution, a) the lock-and-key hypothesis, b) the pleiotropy hypothesis and c) the post-copulatory sexual selection hypothesis (Arnqvist 1997a). The lock-and-key hypothesis suggests that species-specific genitalia have been selected to prevent pre-insemination hybridisation. Male intromittent genitalia evolve to be a specific and unique "key" that fit into female genitalia ("the lock"). The pleiotropy hypothesis states that species-specific genital traits are selectively neutral so genitalia evolution is due to pleiotropic effects of selection acting on other morphological traits (Mayr 1963). The post-copulatory sexual selection hypothesis argues that variation in male genital traits results in non-random fertilization success amongst males. Three different though not mutually exclusive mechanisms of post-copulatory sexual selection have been suggested, a) male genitalia may function as internal, tactile courtship devices that stimulate the females resulting in cryptic female choice (Thornhill 1983, Eberhard 1985, 1996), b) males may use their genitalia to manipulate/coerce female into sperm usage (sexual conflict hypothesis (Lloyd 1979, Alexander *et al.* 1997), c) male genitalia may be used to remove or dislocate rival sperm within the female reproductive tract (sperm competition, Parker 1970, Smith 1984).

All these hypotheses make numerous predictions about the forms and mechanism of selection on genitalia traits in single species (Arnqvist 1997a). A way of testing these hypotheses involves assessing which fitness component is affected by male genitalia morphology. The lock-and-key hypothesis predicts a relationship between male genital traits and the number of achieved copulations (male mating success). Under the pleiotropism hypothesis, male genital traits should not correlate directly with any fitness component. Indirect selection on genitalia due to phenotypic correlation with other functional traits is however expected. Under the post-copulatory sexual selection hypothesis a relationship between male genital traits and male relative fertilizations success is predicted.

Current knowledge of intraspecific variation in genitalia morphology and their fitness effects are highly limited (Arnqvist 1997a). A few studies have shown that genitalia affect sperm transfer and sperm storage. In *Calopteryx haemorrhoidalis asturica* (Cordoba-Aguilar 1999) and *Chelymorpha alternans* (Rodriquez 1994 ref in Simmons 2001, Rodriguez 1995) genital width and length affected the degree of female ejection of rival sperm and consequently fertilization success. A novel relationship between male genital morphology and male relative fertilization success was found in water striders (paper 2, 3). In *G. lacustris* and *G. lateralis* we found that male fertilization success was dependent on the form of vesicle sclerites (Figure 5, 6). These results support the hypothesis that post-copulatory sexual selection is responsible for the evolution of male genitalia in water striders.

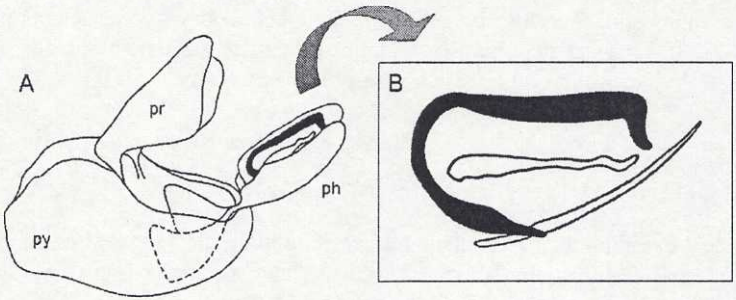

Figure 5. *A. The two posterior male genital segments, the phygophore (py) and the proctiger (pr) of Gerris lacustris. Attached to the proctiger is the phallus (ph), which contains an armament of sclerotized structures. B. Lateral view of the dorsal, lateral and ventral sclerites.*

The effect of male genitalia shape was partly dependent on female body size, which suggests that the optimal male genitalia form is dependent on the frequency distribution of female phenotypes within a population. Such an interaction may act to maintain heritable intraspecific variation in the male traits subject to sexual selection, thereby preventing the suppression of genetic variation due to directional selection. Variation in vesicle sclerite shape in *G. incognitus* was of a similar magnitude as found for nongenital traits (Arnqvist and Thornhill 1998). Interaction effects between sexes

may lead to rapid population differentiation, and ultimately speciation, if populations differ greatly in the distribution of the various female phenotypes.

In *G. lateralis* we also found that vesicle sclerites have different function. Shape of the dorsal and ventral sclerite affected the last male's ability to achieve fertilization success while lateral sclerite shape was correlated with the first male's ability to prevent sperm precedence of a subsequent male (paper 3). Sperm competition generates two opposing selective forces in males (Parker 1970). One force favours adaptations that improve the last-male priority (offensive adaptations), while the other favours males that are able to reduce subsequent competition from rival sperm (defensive adaptations). The dorsal and ventral sclerites of *G. lateralis* appear to be offensive adaptations, while lateral sclerites play a defensive role. These results imply that the structural complexity of male genitalia may reflect a multifaceted functional complex.

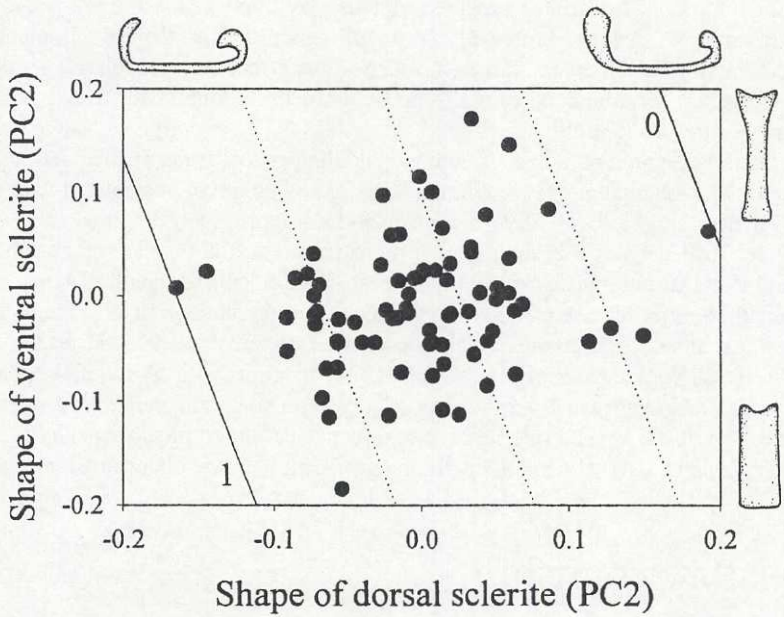

Figure 6. *The relationship between the shape of the genital sclerites of second males and their relative fertilization success for Gerris lateralis, illustrated as an equal-height contour plot (Phillips and Arnold 1989) where the lines represent the linear topography of the relative fitness surface (0 and 1 refer to predicted* P_2*). Inserted drawings illustrate the sclerite shape variants that result from positive and negative loading along the shape axes. Males with high paternity success are equipped with stout ventral sclerites and narrow and apically incurved dorsal sclerites.*

Genital morphology may affect paternity success in numerous ways. Observations that genital structures are apparently used to remove rival sperm (e.g. Waage 1979) suggest that they may have evolved to prevent sperm competition. Fitness effects of genital morphology could also arise via female mechanisms that produce a bias in favour of males

with specially shaped genitalia (i.e. cryptic female choice, Eberhard 1996). The spermathecae of female water striders is a very narrow long tubular structure which male genitalia probably never reaches or penetrates (Andersen 1982). Thus, direct removal or relocation of rival sperm within the spermathecae is unlikely. Additionally since the first male's lateral sclerite shape apparently affects the subsequent male's fertilization success (paper 3) sperm removal seems improbable. The vesical sclerites also appear to play a role in phallic organ extension during copulation (Andersen 1982), which is interesting because sperm positioning within the gynatrial complex may be important for sperm precedence. The interaction between male genitalia shape and female body size (paper 3) indicates that fitness effects of male genitalia are partly mediated by females. A variety of ways in which males could stimulate female processes has been put forward (Eberhard 1996). Recently Simmons (2001) suggested that genitalia could also function as a holdfast device during mating. This may be the case in water striders where there seems to be a sexual conflict over mating duration. The form of a male's genitalia may improve his ability to overcome a female's attempts to dislodge him while *in copula,* enabling him to prolong copulations. Increased copulation duration could then have a positive effect on male relative fertilization success. However, we found no relationship between the genitalia form and copulation duration in *G. lateralis* (unpubl. results).

Sexual selection on non-intromittent genitalia parts of water striders has also been investigated. The external genital length of *A. remigis* is positively correlated with mating success (Preziosi and Fairbairn 1996, Ferguson and Fairbairn 2000, Preziosi and Fairbairn 2000). Water strider males fold their genital segments under the female's abdomen during mating and Preziosi and Fairbairn (1996) suggest that longer male genitalia may make it more difficult for the female to dislodge the male during mating. In *G. incognitus* pre-copulatory sexual selection favoured a short external 1st genital segment instead (Arnqvist *et al.* 1997). This could possibly be an adaptation to cope with abdominal spines that females use to dislodge males. Arnqvist *et al.* (1997) also found indirect pre-copulatory sexual selection on the vesical sclerites due to direct selection on phenotypically correlated traits (body size and external genital length) in agreement with the pleiotropy hypothesis.

Copulatory courtship

Male courtship is a stereotyped behaviour performed during interactions with females to enhance reproductive success. The traditional view is that male courtship makes the female more willing to copulate, i.e. a trait under pre-copulatory sexual selection. However males often court the female during and immediately after the copulation and this copulatory courtship is more likely to influence post-copulatory female processes (Eberhard 1996).

Although males in the genus *Gerris* do not perform classical pre-copulatory courtships (type I mating behaviour, Arnqvist 1997b), they do perform species-specific behaviours during copulation and mate guarding (Andersen 1982). The function of these behaviours is unclear, but enhanced mate guarding due to the repulsion of other males has been suggested (Wilcox & Di Stefano 1991). Measures of leg-jerk frequency and vibration rate during copulation in *G. lateralis* did not show any correlation between these behaviours and fertilization success, despite the vibration rate differing consistently between males (paper 3). It is also possible that during mate guarding males provide stimuli that improve their fertilization success. However we found no correlation between P_2-values and guarding duration in *G. lacustris* (paper 2).

Copulation duration

The longer the copulation, the higher the fertilization success in water striders, irrespectively of the male's position in the mating sequence (Rubenstein 1989, paper 3, 4). This together with the fact that ejaculate size correlates with uninterrupted copulation duration (paper 4, 6) strongly suggests that fertilization success is proportional to the relative contribution of sperm (see also Sperm utilization mechanisms).

Interrupting copulating males is a standard way of manipulating copulation in many insect studies. It was thus surprising to find that the interruption of *G. lacustris* males *in copula* had such a drastic effect on fertilization success. Nearly all interrupted males failed to inseminate the female, regardless of copulation duration, and the correlation between ejaculate size and copulation duration disappeared (paper 6). Recently Campbell & Fairbairn (2001) found similar results in *A. remigis*. These results suggest that in water striders sperm deposition is not continuous, but only takes places at the end of the copulation.

These findings imply a possible sexual conflict and raise the question of which sex controls sperm transfer and copulation duration. If females can interrupt copulations before sperm transfer then it would be a case of cryptic female choice. Since such interruptions would almost eliminate a male's fertilization success, strong selection would be exerted on males to avoid being dislodged while *in copula*. Certain morphological adaptations in males (Arnqvist 1997b) might be a result of this selection. Several other authors have addressed the sexual conflict concerning mating frequency and mating duration in water striders (Rowe *et al.* 1994, Arnqvist 1997b). It is however difficult to assess which sex controls copulation duration. The fact that copulation duration changed when we altered the male's recovery time between matings implies male control (paper 4). An experiment by Väpsäläinen and Savolainen (1995) also suggested male control of copulation duration in *G. lacustris*. They manipulated the optimal duration of copulation separately for males and females by letting them experience different local sex ratios, thereby disentangling male and female effects. Other studies also support this view (paper 3, Rowe and Arnqvist 1996, but see Weigensberg and Fairbairn 1994, 1996 for different results on *A. remigis*).

Female influence

Earlier studies of post-copulatory sexual selection focused mainly on male strategies and sperm competition. However, in recent years the female's role has received increased attention. Cryptic female choice can be defined as non-random paternity biases that occurs after coupling as a result of female morphology, physiology or behaviour (Thornhill 1983, Eberhard 1996, Pitnick and Brown 2000). This definition however does not infer proximate control of either sex, nor any specific mechanism or evolutionary cause behind the bias in paternity. It only identifies female processes responsible for sexual selection. The bias in paternity could be caused by female benefits from sire discrimination, sexual conflict or male manipulation of pre-existing biases. It is clear that females are not passive arenas for sperm competition, and several possible mechanisms of cryptic female choice have been identified. For example females are able to bias paternity by influencing sperm transfer or storage of sperm in several insects (Eberhard 1996, Simmons 2001). However, there is still controversy

over the occurrence of female sperm selection, i.e. are females able to select sperm of a particular genotype from those available in their sperm stores (see discussion in Evolution (2000) 54: 1047-1060).

A common result from studies of paternity determinants is the large degree of unexplained variation in the P_2-value (e.g. Lewis and Austad 1990, Otronen 1997). In paper 4 experimental variables accounted for only 35% of the total variation in P_2. Some workers have attributed the large unexplained variation in P_2 to cryptic female choice. However it has been recently shown, that purely random effects related to the degree of sperm mixing can generate high intraspecific variation in P_2 (Harvey and Parker 2000). Thus the unexplained variation cannot be attributed to adaptive mechanisms without first estimating the amount of random variation in P_2.

A male x female interaction effect on P_2 strongly suggests female influence over paternity, and that females are able to bias paternity (Pinick and Brown 2000, Simmons 2001). It has been shown that fertilization success in *Drosophila melanogaster* depends on female genotype (Clark and Begun 1998, Clark *et al.* 1999). We found a similar phenotypic interaction between male genitalia shape and female body size for P_2-values in *G. lateralis* males (paper 3). This interaction suggests that the female influences the sperm utilization process (Pitnick and Brown 2000), although this may not necessarily be sexual selection since a male who successfully fertilizes one female may be unsuccessful with other females (Simmons 2001).

Males interrupted while in copula almost invariably failed to transfer sperm to the female, which may indicate female control of sperm transfer (see discussion paper 6). Female control of sperm transfer by contraction of the complex musculature of the gynatrial sac has been suggested for *Hebrus spp* (Heming-van Battum and Heming 1986). Similar structures are found in other gerromorphs.

Females may also bias paternity in favour of certain males by increasing offspring production after copulation with a preferred male. In *G. lateralis* we found females produced more eggs after mating with males that gained high fertilization success (paper 4). This pattern cannot be explained by male-male interactions alone, and must therefore be controlled by the female, i.e. these females exercised cryptic female choice. It is possible that males who fertilize more ova also provide more efficient stimuli for egg production. In this study we found that females seemed to disfavour small males only when males experienced a long recovery period (paper 4). These results are puzzling and add to the complexity of sexual selection on male body size (see Antagonistic sexual selection on male size).

Antagonistic sexual selection on male size

Water strider males seem to be able to increase their relative fertilization success by transferring more sperm (see Copulation duration). However, males appear to be sperm limited since males with short sperm recovery period, i.e. interval between matings, transferred significantly less sperm than males with a long recovery period (paper 4). Sperm recovery time affected ejaculate size independently of male body size (paper 4). Irrespectively of size males apparently need the same amount of time to replenish mature sperm stores. This constraint has interesting implications for sexual selection on male body size. Male-male fights and take-overs are rare in gerrids, while struggles between mates are quite frequent (Arnqvist 1997b, personal observations). Females are

generally reluctant to mate, and female resistance to mate places males under directional pre-copulatory sexual selection for an increased body size to better subdue females (see Mating system, Arnqvist 1997b). The reproductive success of different sized water strider males was experimentally investigated using pairs of males, one large and one small, that were allowed to compete over two females (paper 5). Large males mated more often, but small males gained higher relative fertilization success per mating. Small males copulated longer and probably gained higher fertilization success per mating by transferring more sperm. Short copulations by large males were probably a result of a trade-off between mating frequency and ejaculate size due to sperm limitation. The conclusion drawn was that large and small males gain similar reproductive success using somewhat different mating tactics. Despite the clear mating advantage in large males there did not seem to be any directional selection for larger males. The absence of any correlation between mating success and reproductive success (Figure 7) suggests that estimates of male fitness based solely on mating success should be viewed with caution, because of potentially counteracting post-copulatory sexual selection.

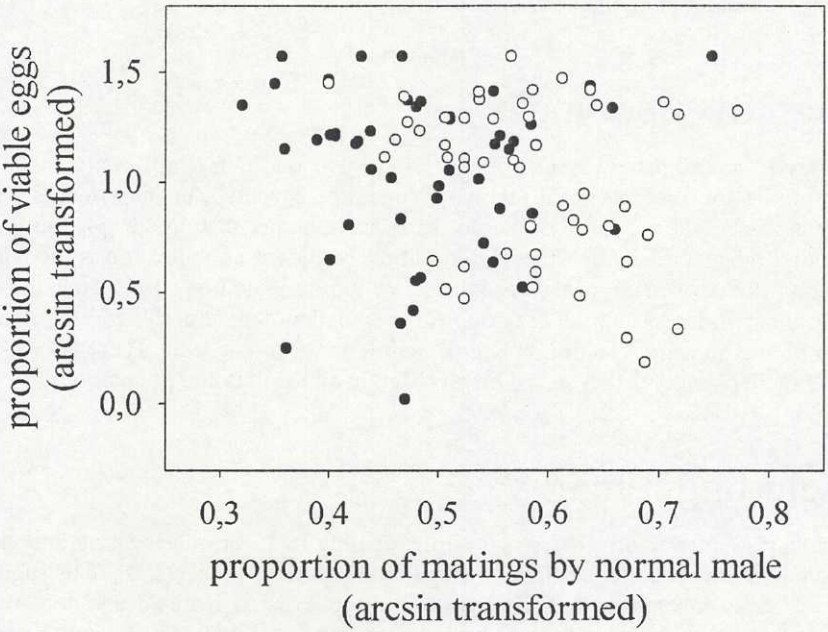

Figure 7. *The relationship between relative mating success (proportion of matings by normal male) and relative reproductive success (proportion of viable eggs) for Gerris lacustris. Closed symbols, small males; open symbols, large males.*

Variation in the strength of sexual selection

Since female reluctance to mate generates the mating advantage in large males, the intensity of pre-copulatory sexual selection depends on the local sex ratio (Rowe *et al.* 1994; Fairbairn & Preziosi 1996). The reason for this is that females seem to balance the cost of repelling harassing males against the cost of mating. Harassment rate is likely to increase with increased male local sex ratio, which would then favour reduced female resistance (convenience polyandry, Rowe *et al.* 1994). Other environmental factors like density, predation risk and food availability, may also affect female resistance (Rowe *et al.* 1994). Consequently, the strength of sexual selection on male body size is expected to vary between populations (c.f. Arnqvist 1992). I suggest that pre- and post-copulatory sexual selection counteracts each other and this can be investigated in more detail by manipulating the operational sex ratio. I would predict that as the intensity of pre-copulatory sexual selection is altered due to sex ratio manipulation, a corresponding shift in post-copulatory sexual selection is observed. When the sex ratio is male biased the difference in mating frequency between small and large males will decrease due to reduced female resistance; post-copulatory sexual selection for small males are then expected to disappear since small and large males will experience similar mating interval.

Design of super male

What does a "super" male water strider look like? The question is not easily answered. Male genitalia form affects male relative fertilization success, but the effect is partly dependent on female morphology. Large males have higher mating success, but small males gain higher fertilization success per mating. A further complication is the female reluctance to mate, which generates sexual selection on male body size. Thus the best male design will depend on the environment, population demography, and the relative strength of antagonistic selection pressures acting on a specific trait. There is probably no single male phenotype that would be favoured in all habitats and populations.

Concluding remarks

The results presented in this thesis suggest that mating frequency and mating interval of both male and female water striders affect paternity patterns (paper 2, 4). This suggests that results from laboratory studies would only be relevant if mating conditions in the laboratory fall within the natural range of conditions. Studies of mating behaviour in natural populations are therefore important. The results in paper 5 also underline the importance of considering behavioural differences between males. Large and small males displayed differing mating frequency and as a result differing relative fertilization success per mating. Such differences would pass undetected in traditional standardised double mating experiments.

The development of molecular methods to assess paternity in water striders would enable rapid progress within this field. One would then be able to shift the focus from a single male's fertilization success to several males involved in multiple mating

sequences. It would also be possible to analyse paternity in wild caught females, which would provide an important link between laboratory results and paternity patterns in natural populations.

Even though mating behaviour in water striders are well studied (Rowe *et al.* 1994, Arnqvist 1997b) and several determinants of relative fertilization success have been identified (this thesis), we still know very little about sperm utilization within this group. More studies of sperm transport and storage within the female are clearly needed. Quantitative measures of these parameters can be used to identify the relevant mechanisms for sperm utilization and sperm competition.

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