COPULATORY BEHAVIOR, GENITAL MORPHOLOGY, AND MALE FERTILIZATION SUCCESS IN WATER STRIDERS

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Abstract.——Recent theoretical and empirical interest in postmating processes have generated a need for increasing our understanding of the sources of variance in fertilization success among males. Of particular importance is whether such postmating sexual selection merely reinforces the effects of premating sexual selection or whether other types of male traits are involved. In the current study, we document large intraspecific variation in last male sperm precedence in the water strider *Gerris lateralis*. Male relative paternity success was repeatable across replicate females, showing that males differ consistently in their ability to achieve fertilizations. By analyzing shape variation in male genital morphology, we were able to demonstrate that the shape of male intromittent genitalia was related to relative paternity success. This is the first direct experimental support for the suggestion that male genitalia evolve by postmating sexual selection. A detailed analysis revealed that different components of male genitalia had different effects, some affecting male ability to achieve sperm precedence and others affecting male ability to avoid sperm precedence by subsequent males. Further, the effects of the shape of the male genitalia on paternity success was in part dependent on female morphology, suggesting that selection on male genitalia will depend on the frequency distribution of female phenotypes. We failed to find any effects of other morphological traits, such as male body size or the degree of asymmetry in leg length, on fertilization success. Although males differed consistently in their copulatory behavior, copulation duration was the only behavioral trait that had any significant effect on paternity.

Key words.——Copulatory courtship, evolution of genitalia, geometric morphometrics, sexual selection, sperm precedence.

In recent years, the theory of Darwinian sexual selection has been broadened to include not only male premating courtship characters, but also traits that may affect nonrandom postmating paternity success among males. This development has been boosted by observations of large variation in male postmating paternity success (Lewis and Austad 1990; Birkhead and Møller 1992; Eberhard 1996). The latter is of fundamental importance in evolutionary biology because most empirical studies of sexual selection reported so far analyze variance in male mating success (Andersson 1994) and therefore assume a simple and direct relationship between male mating frequency, or the number of females inseminated, and reproductive fitness. Although this may be true in some cases (Pemberton et al. 1992; Abell 1997; Wheatherhead and Boag 1997), there is typically enough residual variance in paternity success to significantly weaken the relationship (Lewis and Austad 1990, 1994; Birkhead and Møller 1992; Simmons and Parker 1992; Conner 1995; Bissoondath and Wiklund 1997; Gullberg et al. 1997; Wilson et al. 1997).

Our understanding of the causes of variation in postmating paternity success and of how male traits may become elaborated as a result of postmating sexual selection are, unfortunately, relatively poor and partly impeded by the shortage of empirical data on intraspecific variation in postmating paternity success. Some studies have shown that overall size (McLain 1980; Lewis and Austad 1990, 1994; Watson 1991a,b; Wedell 1991; LaMunyon and Eisner 1993, 1994; Ward 1993; Otronen 1994; Sakaluk and Eggert 1996; Simmons et al. 1996; Bissoondath and Wiklund 1997) and/or copulation duration (Siva-Jothy 1987; Rubenstein 1989; Siva-Jothy and Tsubaki 1989; Wolf et al. 1989; Thornhill and Sauer 1991; Simmons and Parker 1992; Arnqvist and Dan-

ielsson 1999) are positively related to relative male fertilization success. These effects could both be direct effects of sperm competition, mediated simply by a larger number of sperm inseminated by more successful males (Berrigan and Locke 1991; Parker and Simmons 1991; Eady 1995; Simmons et al. 1996). However, more subtle characters, such as relatedness or biochemical compatibility between mates, have also been shown to influence relative male success in several cases (Clark et al. 1995; Bishop et al. 1996; Olsson et al. 1996; Hughes 1997; Wilson et al. 1997). Of particular interest are two types of traits that cannot be explained by the actions of conventional premating sexual selection and thus have been suggested to evolve by postmating sexual selection alone: morphology of male genitalia and copulatory courtship characters (Eberhard 1985, 1994, 1996).

One of the most striking and general trends in the evolutionary radiation of animal taxa and one of the most poorly understood is the remarkable and rapid morphological diversification of the shape of male genitalia (Eberhard 1985; Arnqvist 1997a, 1998). Eberhard (1985, 1993, 1996) has suggested that male genitalia may evolve via postmating sexual selection, which is strongly supported by comparative data (Arnqvist 1998), but so far not directly supported by any experimental evidence. There are at least three different, but not mutually exclusive, ways by which a relationship between genital morphology (or any male phenotypic trait) and fertilization success could be generated: (1) competition between males or their gametes over fertilization, that is, sperm competition (Waage 1979; Gage 1992); (2) female processes that affect male fertilization success, that is, cryptic female choice (Lloyd 1979; Thornhill 1983; Eberhard 1985, 1996); or (3) coevolutionary arms races between males and females

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over the control of fertilization, that is, sexual conflict (Arnqvist and Rowe 1995; Rice 1996; Alexander et al. 1997).

In the majority of animal taxa, male courtship during and immediately following copulation is a conspicuous but neglected component of reproductive behavior (reviewed by Eberhard 1991, 1994, 1996). Because such copulatory courtship cannot be the direct result of classical premating sexual selection, it is thought to have evolved by postmating sexual selection. Again, estimations of the effect of male copulatory courtship behavior on relative fertilization success are very rare (Eberhard 1996). However, a few studies have indicated that males with more elaborate or intense copulatory courtship behavior indeed are favored in terms of relative paternity (Otronen 1990; Watson 1991a,b), although it is unclear how such a bias in paternity is brought about.

This study represents an explicit assessment of the relationship between phenotypic variation among males and relative fertilization success. Using water striders as model organisms, we estimate the effects of a series of morphological and behavioral traits on paternity in an extensive hierarchically structured double-mating experiment. We focus on effects due to genital morphology and copulatory behavior, but also evaluate the role of fluctuating asymmetry. To quantify complex morphological variation in shape of male genitalia, we apply some of the new tools offered by recent methodological development in the field of geometric morphometrics.

MATERIALS AND METHODS

The Organism

Water striders (Gerridae) inhabit water surfaces of various aquatic habitats both as juveniles and as adults. They are predators/scavengers that feed mainly on arthropods trapped at the water surface (Andersen 1982; Spence and Andersen 1994). Characteristics of male genitalia are very important both in grouping higher order taxa and in distinguishing among closely related congeneric species (Andersen 1982, 1993). The intromittent phallic organ, which is inflated/extended and inserted into the female genital tract during copulation, is mostly membranous, but carries an apical capsule (the vesica) which encloses an armature of genital sclerites (for illustrations see Andersen 1993).

This study was performed on *Gerris lateralis*, a member of the primarily Holarctic and relatively species-rich genus *Gerris* (42 species). In this genus, the sclerites in the apical part of the intromittent phallus have evolved particularly rapidly and divergently, and their morphology is a critical species character within the genus (Andersen 1993). Little is known of the functional morphology of these sclerites in *Gerris*, but they are thought to play an important part during insertion and/or positioning of the male phallus in the female genital tract during copulation (Andersen 1982; Heming-van Battum and Heming 1989). Although little classical premating courtship occurs in this group of insects (Rowe et al. 1994; Arnqvist 1997b), males exhibit distinct copulatory behaviors in the form of vibrations and various leg-tapping behaviors (Andersen 1982). These behaviors were originally interpreted as stimulatory courtship signals addressed to the female (Sattler 1957), but have more recently been suggested

to function primarily in terms of mate guarding by repelling other males (Wilcox and Di Stefano 1991).

Collection and Rearing

Males and virgin females were collected May 9–19, 1997, from the river Tavleån, 8 km SE of Umeå in northern Sweden. The water striders were kept in aerated tanks (1-m diameter, water depth 10–20 cm) in the laboratory with a simulated natural light regime and at 20°C (±2°). Females were held isolated from males, and males were held with nonexperimental females at a sex ratio of approximately 2:1. Both sexes were fed frozen *Gryllus* cricket larvae ad libitum and were provided with pieces of Styrofoam as resting sites. The water was changed weekly and surplus food was removed regularly. To ensure that only virgin females were used in the experiments, the fertilization status of individually isolated females were checked prior to the experiments over a minimum of four days. Females laying fertile eggs during the isolation period were discarded. Males were collected from the holding tanks while mating and isolated individually in cups (6 cm diameter) provided with water, food, and Styrofoam pieces during the day prior to the experiments.

Sterile Male Technique

Male fertilization success was estimated by the sterile male technique (Parker 1970). The relative fertilization success of a pair of males is estimated from the proportion of developing eggs from double matings of virgin females, mated with one normal and one sterile male. Males were sterilized by exposure to emission of high energy X-rays. Irradiation took place between 1600 and 1700 h the day before the mating experiment, and males had by then been isolated from females for about one hour. Males were placed in a petri dish in front of a linear electron accelerator with 6 MV photon beams. A 14-mm thick plexiglas disc was placed on top of the petri dish, and a water equivalent phantom for backscattering was placed underneath. Males received an absorbed dose of 130 ± 3% Gy with a dose rate of 10 Gy/min. Sperm of irradiated males are assumed to be able to fertilize eggs, but to carry lethal mutations that prevent normal embryonic development. The normal infertility and the degree of sterility in irradiated males were estimated from NN- and RR-matings (i.e., virgin females were mated successively with two normal [N] or two irradiated [R] males). Estimates of the relative fertilization success of the last male ($P2$) was subsequently estimated by the method presented by Boorman and Parker (1976; see Results below).

Mating Experiments

To allow an assessment of the repeatability of relative male fertilization success, while controlling for the irradiation treatment and allowing assessments of the effects of morphology and behavior on sperm precedence, we used a hierarchically structured experimental design in which each of a series of male pairs was mated with two different females. Males were randomly combined into pairs and one male in each pair was randomly assigned to the irradiation treatment. The male pairs ($n = 85$) were divided equally and randomly

between NR and RN mating orders to control for any differences in competitiveness of normal and irradiated sperm. Each pair of males was first allowed to mate with a randomly selected virgin female (A) with an intermating interval of 4–6 h. Each male pair was mated again with a second virgin female (B), the subsequent day, with the same mating order and intermating interval. Each male pair and the two females mated to these males collectively form a mating group. Males were kept isolated in cups and provided with food in between matings. The recovery period for males was thus 20–24 h. The control matings (NN- and RR-matings, $n = 24$) were conducted in the same way as the experimental matings. Matings were carried out in plastic jars (15×20 cm, water depth 5.5 cm) from June 20 through July 6. Each mating was continuously monitored, and the durations of the precopulatory struggle and the copulation were recorded. In the genus *Gerris*, copulation ends when the male withdraws his genitalia and is typically followed by a postcopulatory contact guarding phase (Arnqvist 1997b). Maximum copulation time was 60 min, whereafter the couple was gently separated if still attached.

Two components of male copulatory behavior were also recorded: leg-jerks and vibrations (cf. Sattler 1957). Leg-jerks are rapid vertical jerking movements and/or sudden horizontal twitches of one or both midlegs. The midlegs do not directly contact females during these movements. When performing copulatory vibrations, which are often a response to female reluctance behavior, males typically fold their midlegs around those of the female and cause the couple to rapidly oscillate vertically for 1–3 sec.

Egg Collection

Experimental females were isolated individually for a total of eight days in jars (15×20 cm) provided with Styrofoam and a piece of balsawood (2×1 cm). The latter served as oviposition substrate. Females were fed one frozen cricket (length about 1 cm) per day. The water and oviposition substrates were replaced after four days, and oviposition substrates were again collected after another four days. Eggs were allowed eight days of maturation in plastic cups with water (20°C), before recording the number of viable and nonviable eggs (egg age span was thus 8–12 days). Only eggs that showed normal development with red eyespots and legs clearly visible were regarded as viable. Partly developed and opaque eggs were considered as nonviable.

Morphometrics

All experimental individuals were frozen individually in 0.5 ml Eppendorf vials for subsequent morphometric analyses. Two-dimensional digital morphometric data were attained by placing a digitizing tablet (Summasketch® III, CalComp Technology, Inc., Anaheim, CA) under a side-mounted camera lucida attached to a dissecting microscope (Leica® MZ8, Leica AG, Heerbrugg, Switzerland). For each individual, we registered the following eight landmarks on the body in dorsal view: the anterior corners of the pronotum, the thoracic points of attachment of the mid- and hindleg coxae, and the tips of the connexival spines (Andersen 1993). The centroid size of the body (the square root of the sum of squared distances of the eight landmarks from their centroid) of each individual was subsequently used as an integrative measure of overall size (Rohlf and Marcus 1993). We also measured the length of both midlegs (femur plus tibia) of each individual twice, after first removing the legs from the body and flattening them between two microscopic slides, to allow assessment of asymmetry in leg length and its repeatability. We restricted our analysis to asymmetry in midleg length because this is the only asymmetry trait that has been found to be both repeatable and condition dependent in water striders (Arnqvist and Thornhill 1998).

We also measured variation in genital shape among males, as opposed to merely genital size (cf. Eberhard et al. 1998). Male genitalia were macerated in 10% KOH (17 h), cleared in 50% aqueous lactic acid solution (24 h) and placed in glycerine for examination. To describe variation in shape of the genital sclerites, we recorded, for each male, the outlines of the three intromittent vesical genital sclerites in lateral (dorsal and lateral sclerites) and ventral views (ventral sclerite) (for illustrations see Andersen 1993). These outlines were subsequently analyzed using elliptic Fourier analysis (Liu et al. 1996; Lestrel 1997; Arnqvist 1998). This method involves describing the outlines of the genitalia of each individual with a nonlinear function, and subsequently analyzing morphological shape variation among individuals as variance in the parameters of the fitted function (see Results). Geometric shape descriptors of the genital sclerites in water striders have previously been shown to be highly repeatable using this methodology (Arnqvist and Thornhill 1998).

RESULTS

Morphometrics

Our measures of midleg length and asymmetry in midleg length were both highly repeatable ($R = 0.992$ and $R = 0.741$, respectively). Therefore, we used the mean length and asymmetry of the two repeated measures for each individual. The distribution of midleg length asymmetry did not differ significantly from normality (Kolmogorov-Smirnov one-sample test, $P = 0.456$). Midleg length was not correlated with either asymmetry ($r = 0.007$) or the absolute value of asymmetry ($r = -0.078$). The average asymmetry was slightly, but significantly, different from zero (-0.048 mm, SD $= 0.088$, $t = 7.14$, df $= 170$, $P < 0.01$). Thus, our data indicated fluctuating asymmetry in leg length, in combination with directional asymmetry (Rowe et al. 1997). Because of the small magnitude of directionality, however, we used absolute value of leg asymmetry to characterize the asymmetry of each individual in the analyses reported below. Using the alternative measure in our models, that is, the absolute value of the difference between the observed asymmetry and the average asymmetry, did not qualitatively alter any of the results (see below).

For each of the three genital sclerites, the outlines of all individuals were included in a common elliptic Fourier analysis (Ferson et al. 1985; Rohlf 1992), using the software EFA-Win (Rohlf et al. 1995). The Fourier analyses were made invariant of size, position, and rotation, and all used 20 harmonics (yielding 80 Fourier coefficients). These functions provided a near perfect fit to all sclerite outlines. To reduce

TABLE 1. The results of nested analyses of variance of copulation behavior. Mating order refers to the order with which males were mated (NR or RN) and mating group refers to the quadruplet of individuals (two males and two females) involved in each replicate. Male and female factors, both crossed and nested within mating groups, express the extent to which variation in behavioral variables was due to differences between individual males and females.

Source	df	Precopulatory struggle duration		Copulation duration		Male copulatory leg-jerk rate		Male copulatory vibration rate	
		F	P	F	P	F	P	F	P
Mating order	1	0.352	0.555	0.149	0.700	0.833	0.364	2.616	0.109
Mating group [mating order]	83	0.977	0.543	4.882	<0.001	1.072	0.376	2.498	<0.001
Female [mating group]	85	1.024	0.456	1.362	0.078	0.726	0.928	1.868	0.002
Male [mating group]	85	1.000	0.500	2.476	<0.001	0.887	0.709	1.608	0.015

the dimensionality of our shape descriptors, the 80 Fourier coefficients for each sclerite were treated as variables in principal component analyses performed on the covariance matrix (Rohlf and Archie 1984; Liu et al. 1996). The first two principal components from each analysis were retained for subsequent analyses (see below). These two variables collectively described 57% of the shape variation in dorsal sclerites, 83% in lateral sclerites, and 84% in ventral sclerites. Measures of size of the sclerites were not included in subsequent analyses because they were closely correlated with our measure of overall size (see above) and thus caused inferential problems when included in our statistical models (due to multicollinearity).

Copulation Behavior

To assess the degree to which variation in copulation behavior was due to consistent differences among individual males and females, we analyzed the behavioral variables in a series of nested analyses of variance. Male and female identity were treated as two two-level crossed factors, both nested within mating group (i.e., quadruplet). Because these two factors are unreplicated within mating groups, this model does not allow an independent estimation of the interaction term (Sokal and Rohlf 1995). Mating groups, in turn, were nested in mating order (NR or RN).

We did not find any evidence of individual variation in reluctance to mate among females or in the propensity to elicit such female mate-rejection behavior among males, as indicated by our analysis of precopulatory struggle duration (Table 1). As has been suggested previously, copulation duration was largely determined by males (Rowe and Arnqvist 1996). The rate of male copulatory leg jerks was not influenced by individual variation in males or females. In contrast, males did differ in the rate at which they performed copulatory vibrations, and females differed in their ability to elicit such male vibrations (Table 1). However, female size was apparently not related to the tendency to trigger male vibrations. The average copulatory vibration rate elicited by a female in her two matings was not correlated with her overall size ($r = -0.096$, $P = 0.211$, $n = 170$), nor did the larger of the two females in a mating group in average elicit a higher rate of male vibrations than did the smaller female (paired t-test, $t = 1.078$, df $= 84$, $P = 0.284$).

Estimation of Relative Fertilization Success

The viability rates (arcsine transformed) of females mated with male control pairs (RR and NN, respectively) were an-

alyzed in a nested within-subjects (i.e., repeated measures) analysis of variance, with two within-subjects factors: female (first and second) and egg sampling period (days 1–4 and 5–8). With this design, male pairs were subjects nested in their treatment (R males or N males). This analysis verified that the irradiation treatment had an overwhelming effect on the viability rates (arcsine transformed, $F_{1,22} = 79.9$, $P < 0.001$), whereas neither female or egg sampling period, nor any of the interactions between the three factors, had any significant effect on viability rate (arcsine transformed, $F_{1,22} < 1.5$, $P > 0.25$, in all six cases). Therefore, the fertilization rates from both females and egg sampling periods were pooled to provide the most reliable overall estimate of the viability rate resulting from a mating with a normal male (0.922) and an irradiated male (0.249), respectively. These estimates represent the average rate of viable to total number of eggs across females, and were used to estimate the relative fertilization success of males in the double-mating experiment. The proportion of eggs fertilized by the R male, P_R in a given NR or RN mating is thus given by

$$P_R = \left(1 - \frac{x}{p}\right) + \left(\frac{z}{p} \times \frac{1 - (x/p)}{1 - (z/p)}\right), \tag{1}$$

where $z = 0.249$, $p = 0.922$, and x represents the observed viability rate (Boorman and Parker 1976). The relative fertilization success of the last male ($P2$) equals P_R in NR matings and $1 - P_R$ in RN matings.

Sources of Variance in Fertilization Success

To assess whether $P2$ differed between the first and second female in a mating group and between the first and second period of egg sampling among the experimental females, we performed a nested within-subjects analysis of variance. In this model, male pairs were subjects nested in mating order (NR or RN), and female mating sequence (1st or 2nd) and egg sampling period (days 1–4 or 5–8) were treated as two crossed within-subjects factors. There was no systematic difference in $P2$ between the first and second female ($F_{1,66} = 0.024$, $P = 0.877$), nor between the two egg sampling periods ($F_{1,66} = 0.198$, $P = 0.658$). Further, none of the interactions, neither that between these two factors nor those between these two factors and mating order, were significant ($F_{1,66} < 3.129$, $P > 0.05$ in all cases). Thus, data from the two periods were pooled to yield an overall $P2$ estimate for each female, which forms our measure of $P2$ used in subsequent analyses.

Our results revealed large intraspecific variation in the degree of sperm precedence. The average $P2$ value across all

TABLE 2. The effects of four morphological trait groups, each composed of two traits, on the relative fertilization success of the second male (*P2*). The effects of trait groups were tested separately for the first and second male by multiple partial *F*-tests in a nested within-subjects analysis of covariance (see Results). Between-subjects effects refer to effects across replicate male pairs, and within-subjects effects refer to interaction effects with female body size.

	First male		Second male	
	F	*P*	*F*	*P*
Between-subjects effects (df = 2, 75):				
Midleg length and asymmetry	1.692	0.191	0.471	0.626
Shape of dorsal genital sclerite	0.379	0.686	3.592	0.032
Shape of lateral genital sclerite	0.5373	0.007	1.887	0.159
Shape of ventral genital sclerite	0.102	0.903	3.377	0.039
Within-subjects effects (df = 2, 74):				
Midleg length and asymmetry	0.239	0.788	1.144	0.324
Shape of dorsal genital sclerite	1.352	0.265	3.252	0.044
Shape of lateral genital sclerite	2.908	0.061	0.114	0.892
Shape of ventral genital sclerite	0.477	0.623	0.180	0.835

females (*P2* = 0.57) was actually of a similar magnitude as the standard deviation (SD = 0.58), yielding a coefficient of variation of 101%. To estimate the repeatability of *P2* values between females within male pairs, the data on *P2* values were subjected to a two-level nested mixed-model analysis of variance where male pairs were nested in mating order. This analysis revealed a moderate but significant difference between mating orders in estimated *P2* ($F_{1,80}$ = 5.67, *P* = 0.019). The average *P2* values were 0.462 (SD = 0.58, *n* = 82) and 0.682 (SD = 0.55, *n* = 78) for NR and RN matings, respectively. Most importantly, however, there was a highly significant difference in *P2* between male pairs within mating order ($F_{83,80}$ = 2.87, *P* < 0.001). An extraction of variance components (Sokal and Rohlf 1995) from this analysis showed that male pair within-mating order accounted for 48% and female within-male pair for 52% of the variance in *P2*. Therefore, although the *P2* value of a given male pair was indeed significantly "repeatable" across replicate females, it also varied considerably between females within male pairs. However, the latter was not simply a result of temporal changes in the relative viability of sperm of irradiated males (see above).

Determinants of Fertilization Success

Our experiment, and thus our data on relative fertilization success, was structured in four hierarchical levels: mating order, male pair, female, and mating. Independent explanatory variables of interest were available at three of these levels: male morphology (male pair), relative female size, that is body size relative to the other replicate female in a given mating group (female), and copulatory behavior (mating). Because of the complexity of this design, our analysis proceeded in two steps. First, the effects of mating order, relative female size, and male morphology on *P2* were estimated in a nested within-subjects (i.e., repeated measures) analysis of covariance. In this analysis, male pairs were subjects nested in mating order, relative female size was a within-subjects factor, and measures of male morphology were treated as covariates at the subject level. Model assessment was made by inspection of residuals from these models. The residuals from the above analysis was also used to secondarily estimate the effects of copulatory behavior on *P2*, above and beyond

that accounted for by mating order, relative female size, and male morphology (see below).

We chose to include absolute values of the first and second male separately in our models, rather than using relative values (i.e., differences between the two males) for two reasons. First, there are theoretical reasons to expect that *P2* primarily relates to absolute characteristics of the last male to mate (see Parker et al. 1990; Parker and Simmons 1991; Eberhard 1996). Second, several empirical studies have verified that absolute values are more closely related to *P2* than are relative values (Parker and Simmons 1991; Watson 1991b; Otronen 1994; Ueno 1994; Eady 1995).

Because of the large number of morphological variables involved in our analysis, we applied a hierarchical inferential strategy. This involved testing the addition in model fit of groups of related variables above and beyond that of a basic reduced model. Most importantly, this strategy reduces the risk of inflated Type I error rates without deflating the statistical power by increasing the Type II error rate (Cohen 1988; Rice 1989; Arnqvist et al. 1997). Because body size, or traits correlated with body size, are known to influence relative fertilization success of males in several insect species (see introduction), our reduced model contained three components: mating order, male body size (between-subjects factors), and relative female size (within-subjects factor). We then tested the improvement in fit to data of the independent addition of four groups of morphological variables to this reduced model, for both the first and the second male, with multiple partial *F*-tests. These variable groups were midleg morphology (length and asymmetry), shape of the dorsal genital sclerite (PC1 and PC2), shape of the lateral genital sclerite (PC1 and PC2), and shape of the ventral genital sclerite (PC1 and PC2) (see Table 2). Neither body size nor length or asymmetry of midlegs had any significant impact on *P2*. However, the shape of the dorsal and ventral genital sclerites of the second male were both related to his relative fertilization success. The strength of the former effect was, at least in part, depending on the body size of the female (Table 2). Further, the shape of the lateral genital sclerite of the first male affected the relative fertilization success of the second male. A full model including these effects are presented, for illustration rather than statistical inference, in Table 3. It is evident that the second

TABLE 3. A nested within-subjects analysis of covariance of the effects of body, leg, and genital morphology on the relative fertilization success of the second male ($P2$) in the double-mating experiment (n = 80 male pairs).

Source	SS	df	MS	F	P
Between-subjects effects:					
Mating order	0.72	1	0.72	1.766	0.188
Body size of 1st male	0.30	1	0.30	0.732	0.395
Body size of 2nd male	0.05	1	0.05	0.134	0.715
Dorsal sclerite PC1 of 2nd male	0.09	1	0.09	0.228	0.635
Dorsal sclerite PC2 of 2nd male	1.54	1	1.54	3.770	0.056
Ventral sclerite PC1 of 2nd male	0.16	1	0.16	0.411	0.524
Ventral sclerite PC2 of 2nd male	2.10	1	2.10	5.142	0.026
Lateral sclerite PC1 of 1st male	0.46	1	0.46	1.130	0.291
Lateral sclerite PC2 of 1st male	2.05	1	2.05	5.013	0.028
Error	28.70	70	0.41		
Within-subjects effects:					
Female size[1]	0.06	1	0.06	0.382	0.539
Female size $\times$ mating order	0.54	1	0.54	3.484	0.066
Female size $\times$ body size of 1st male	0.01	1	0.01	0.057	0.812
Female size $\times$ body size of 2nd male	0.07	1	0.07	0.422	0.518
Female size $\times$ dorsal sclerite PC1 of 2nd male	0.87	1	0.87	5.554	0.021
Female size $\times$ dorsal sclerite PC2 of 2nd male	0.01	1	0.01	0.055	0.815
Female size $\times$ ventral sclerite PC1 of 2nd male	0.06	1	0.06	0.405	0.527
Female size $\times$ ventral sclerite PC2 of 2nd male	0.00	1	0.00	0.028	0.867
Female size $\times$ lateral sclerite PC1 of 1st male	0.45	1	0.45	2.855	0.096
Female size $\times$ lateral sclerite PC2 of 1st male	0.23	1	0.23	1.496	0.225
Error	10.96	70	0.16		

[1] Refers to the relative body size of each of the two females within each male pair (two replicate females per male pair).

principal components of the shape of the ventral and dorsal sclerites are most closely related to a given male's relative fertilization success. High $P2$ was associated with a relatively stout ventral sclerite and a dorsal sclerite with a relatively narrow and incurved apical part (see Fig. 1).

The distribution of residual $P2$ from the nested within-subjects analysis of covariance model presented in Table 3 was not different from normality (Kolmogorov-Smirnov one-sample test, P = 0.096) and did not exhibit any deviant cells when plotted against estimated and observed $P2$. Because hierarchical dependencies had been accounted for by the use of residuals from this model, we employed multiple regression analyses of the residuals to estimate the impact of copulatory behavior on fertilization success. Previous studies of *G. lateralis* have found a general positive relationship between copulation duration and fertilization success, but have also shown that copulations that are of extended duration (beyond 60 min) tend to result in reduced fertilization success (Arnqvist and Danielsson 1999). The latter was true also in the current study.

As expected, we found average residual $P2$ to be higher when first-male copulations were interrupted (at 60 min) compared to when they were not (0.06 $\pm$ 0.08 and $-0.03 \pm$ 0.04, respectively), and average residual $P2$ was lower when second-male copulations were interrupted ($-0.14 \pm$ 0.09 and 0.07 $\pm$ 0.04, respectively; two-way ANOVA of copulation duration $\geq$ 60 min: effects among first males $F_{1,156}$ = 3.33, P = 0.069; effects among second males $F_{1,156}$ = 8.91, P = 0.003). To avoid confounding effects of greatly extended copulation durations, and thus copulation interruptions, we restricted our analysis of the effects of copulatory behavior on $P2$ below to include only females where both copulations were shorter than 60 min. As expected, our analyses revealed a positive effect of second-male mating duration and indicated a negative effect of first-male mating duration, on residual $P2$ (Table 4). However, we failed to find any effects of male copulatory leg-jerk rate and male copulatory vibration rate on $P2$, either among first males ($F_{2,75}$ = 1.421, P = 0.248) or second males ($F_{2,75}$ = 0.857, P = 0.429).
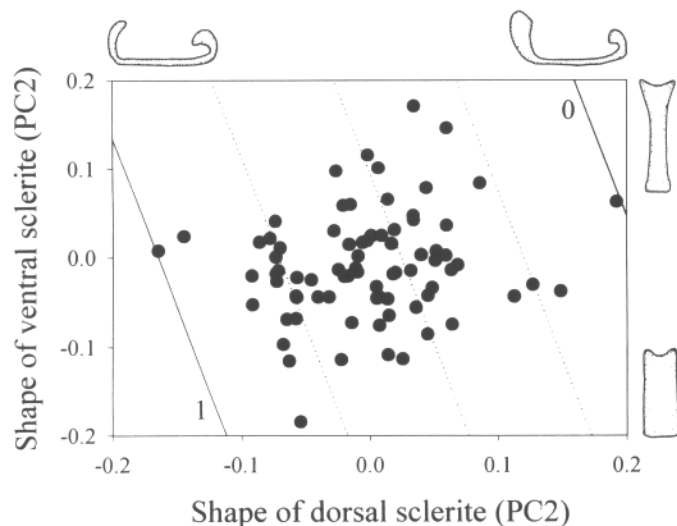

FIG. 1. The relationship between the shape of the genital sclerites of second males and their relative fertilization success, illustrated as an equal-height contour plot (Phillips and Arnold 1989) where the lines represent the linear topography of the relative fitness surface (0 and 1 refer to predicted $P2$). Inserted drawings illustrate the sclerite shape variants that result from positive and negative loadings along the shape axes. Males with high paternity success are equipped with stout ventral sclerites and narrow and apically incurved dorsal sclerites.

TABLE 4. Multiple regression analysis of the effects of copulation duration on residual fertilization success of the second male in the double-mating experiment (see Results). Overall test of regression model: $F_{2,77} = 2.663$, $P = 0.039$[1].

Effect	Estimate	SE	t	P
Intercept	−0.039	0.138	−0.285	0.388
Copulation duration of 1st male	−0.005	0.004	−1.345	0.091
Copulation duration of 2nd male	0.007	0.003	2.082	0.020

[1] One-tailed P.

DISCUSSION

Several authors have recently stressed the need of increasing our understanding of intraspecific variation in postmating paternity success (Lewis and Austad 1990; Eberhard 1996; Simmons et al. 1996; Cook et al. 1997). In theory, such variation could be due to more or less random factors or to determinate characteristics of individuals. The results of this study, as well as the few other studies that have directly examined among-male variation in sperm precedence via matings with replicate females (Lewis and Austad 1990; Otronen 1997; Wilson et al. 1997) not only document large variation in sperm precedence, but also show that a significant proportion of intraspecific variation in fertilization success is due to consistent differences among individual males. This repeatability in male sperm precedence demonstrates that males indeed differ in their ability to attain fertilizations and calls for studies examining which male traits are associated with high fertilization success. Although the overall level of sperm precedence is known to sometimes vary with the experimental setting (e.g., Boorman and Parker 1976; Zeh and Zeh 1994), associations between male traits and relative fertilization success should be less affected by such variation (e.g., Simmons et al. 1996). One should bear in mind, however, that our results are potentially limited to the experimental setting used and that future studies investigating the effects of experimental setting (female mating history, intermating interval, etc.) on the relationship between male traits and relative fertilization success are needed. Here, we discuss the importance of two types of male traits. First, we discuss the role of genital morphology in sperm precedence, and then we consider the influence of variance in male copulatory behavior.

Genital Morphology and Sperm Precedence

Several studies have investigated the effects of male body size, or traits that are correlated with body size, on the degree of sperm precedence (see introduction). Considering the generality of rapid and divergent evolution of male genitalia (Eberhard 1985; Arnqvist 1998) and the obvious role that these sexually dimorphic traits might have in determining relative fertilization success, it is remarkable that our study represents the first explicit assessment of the importance of male genital morphology in sperm precedence (but see Rodriguez 1995). Previous attempts might have been hampered by methodological problems with quantifying morphological shape variation in small complex traits, but recent developments in the area of geometric morphometrics have offered new tools to eliminate such problems (Rohlf and Marcus 1993). The results of the current study should encourage others to examine the role of male genital shape (as opposed to size; cf. Eberhard et al. 1998) in sperm precedence, especially because such information is critical to our understanding of genital evolution (Arnqvist 1997a, 1998).

We found the shape of male intromittent genitalia to be significantly related to the degree of sperm precedence, after controlling for potential allometric effects of body size. This is the first conclusive experimental support for the suggestion that male genitalia may evolve by sexual selection mediated by variance in fertilization success (Eberhard 1985, 1993, 1996; Arnqvist 1997a, 1998; Danielsson and Askenmo, unpubl. ms.). Three aspects of our results are particularly noteworthy.

First, we found no other morphological correlates of male fertilization success. Neither body size nor asymmetry in leg length had any detectable effects on our measures of P2. This indicates that variation in male genital morphology may be a major source of variance in sperm precedence in animals with internal fertilization and one that has been largely overlooked in empirical studies (Arnqvist 1997a). Interestingly, sperm precedence in the congeneric G. lacustris also seems to be determined to some extent by variation in similar components of male genital morphology (Danielsson and Askenmo, unpubl. ms.). Other studies of selection on male genitalia in water striders have exclusively analyzed variance in male mating success (e.g., Preziosi and Fairbairn 1996; Arnqvist et al. 1997).

Second, in theory, sperm competition generates two conflicting selective forces in males, forming an evolutionary arms race of antagonistic adaptations (Parker 1984): on one hand, selection will favor paternity assurance adaptations ("defensive" adaptations) and, on the other, selection will favor males that overcome the paternity assurance adaptations of other males ("offensive" adaptations). Interestingly, we found different components of male genitalia to be related to second-male sperm precedence (dorsal and ventral genital sclerites) and first-male ability to avoid sperm precedence (lateral genital sclerite). This implies that selection for both "offensive" and "defensive" functions may be important for the evolution of genitalia and that these conflicting selective forces may lead to morphological adaptations in different components of genital morphology. The fact that such fitness effects of genitalia may also be mediated by stimulation/titillation of females during copulation (Eberhard 1996) lends further support to the suggestion that the relative structural complexity of male genitalia may actually reflect a multifaceted functional complexity of these morphological structures (Lloyd 1979).

Third, we found the effects of the shape of male intromittent genitalia on paternity to be, in part, dependent on female morphology (body size). Similar interactions, but between male and female genotypes, have previously been

found to influence male fertilization success in seed beetles (Wilson et al. 1997) and fruit flies (Clark and Begun 1998). These interactions are very important because they suggest that the optimal genitalic conformation in males will be set by the frequency distribution of female phenotypes. If populations differ in the distribution of female phenotypes, selection on male genitalia may then vary considerably between populations. Such a scenario will not only tend to promote the maintenance of intraspecific variation in male genitalia in the face of postmating sexual selection (cf. Wilson et al. 1997; Clark and Begun 1998), but will also result in relatively rapid population differentiation, ultimately leading to allopatric speciation (Lande 1981, 1982; Andersson 1994; Barraclough et al. 1995; Iwasa and Pomiankowski 1995; Arnold et al. 1996; Parker and Partridge 1998). The shape of male genital sclerites has been shown to be as variable, both phenotypically and genetically, as nongenital traits in the congeneric *G. incognitus* (Arnqvist and Thornhill 1998)

Effects of genitalia on paternity success may be brought about in several different ways. Observations of genitalia that apparently function to remove sperm from previous mates (Waage 1979; Ono et al. 1989; Gage 1992; but see Siva-Jothy et al. 1996) suggest that direct sperm competition may be important. However, fitness effects of genital morphology could be the result of mechanisms that directly or indirectly render females to bias paternity in favor of males with certain genitalic conformations (i.e., cryptic female choice) (Eberhard 1985, 1996). The observation of Rodriguez (1995) that female sperm uptake is apparently related to male genital morphology in a beetle lends some support to this possibility. Although the current study was not designed to elucidate the mechanisms by which genitalic morphology affects sperm precedence, there are two facts that suggest that females play a key role in mediating such paternity effects in *G. lateralis*. First, females store sperm used for fertilizations in a narrow spermatheca, which is connected to the bursa copulatrix by a very narrow duct. Sperm are deposited in the bursa, are transported rapidly to the spermatheca, and male genitalia never reach or penetrate the sperm storage sites within females (Andersen 1982). Thus, it seems that males have little ability to affect the destiny of previous ejaculates with their genitalia by direct removal or manipulation. Such effects are more likely to be mediated by females. Second, we found that optimal male genitalic morphology in part depends on female morphology. This interaction reveals that the female "environment" in which genitalia of different males compete may affect the outcome in terms of relative paternity success. This strongly suggests that fitness effects of male genitalia are at least partly mediated by females.

Copulatory Courtship and Sperm Precedence

Vigorous male copulatory courtship behavior has been shown to be positively related to sperm precedence in the fly *Dryomyza anilis* (Otronen 1990) and in the spider *Neriene litigiosa* (Watson 1991a,b, 1998; Watson and Lighton 1994). Further, differences among males in the intensity of copulatory courtship behavior are known to be consistent in matings with replicate females in both of these species (Otronen 1997; P. J. Watson, pers. comm. 1998). Our findings also show that *G. lateralis* males differ consistently in their copulatory performance, as revealed by the significant repeatabilities of copulation duration and the rate at which copulatory vibrations were performed. However, we failed to find any influence on sperm precedence by any behavioral variable other than copulation duration. The most parsimonious explanation for the latter effect is numerical sperm competition because it is known that copulation duration is positively related to the number of sperm transferred in this species (Arnqvist and Danielsson 1999) as well as in other insects (see introduction), and because first-male copulation duration was negatively and second-male positively related to the degree of sperm precedence in the current study. Thus, this study provided no evidence that the function of male copulatory behavior is to increase sperm precedence by stimulating females (Sattler 1957). The vibratory signals generated by male water striders during copulation may instead serve to enhance mate guarding by repelling other males (see Wilcox and Di Stefano 1991) and to subdue reluctant females.

Previous studies of water striders have shown that female reluctance to mate, that is, the premating struggle behavior, generates nonrandom mating among males. Matings tend to be biased toward males that are better able to overcome female resistance (reviewed in Rowe et al. 1994; Arnqvist 1997b). The lack of consistent variation among females in the intensity of their reluctance documented in this study is in concordance with earlier findings of this being a condition-dependent and highly flexible behavior (Rowe et al. 1994; Lauer et al. 1996; Arnqvist 1997b).

The results of the current study demonstrate that the evolution of primary sexual traits, such as genitalia, may indeed be the result of sexual selection mediated via nonrandom paternity success (Eberhard 1996; Arnqvist 1998). In order to fully recognize the importance of such processes, we need many more studies where variation in primary sexual traits among males is related to relative paternity success. Such studies would obviously improve our understanding of the types of traits that are favored by postmating sexual selection, much in the same way that studies of variation in male mating success have been informative of the types of male traits that become elaborated as a result of classical premating sexual selection (Andersson 1994).

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