BRIEF COMMUNICATION

doi:10.1111/j.1558-5646.2007.00107.x

MALE SCORPIONFLIES ASSESS THE AMOUNT OF RIVAL SPERM TRANSFERRED BY FEMALES' PREVIOUS MATES

Leif Engqvist

Department of Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, D-53121 Bonn, Germany
E-mail: lengqvist@evolution.uni-bonn.de

Received June 2, 2006
Accepted January 31, 2007

Theory predicts that when sperm compete numerically, selection will favor males who vary the number of sperm they transfer with the immediate level of sperm competition. In this study, I measured male mating investment in response to both female mating status (virgin vs. mated) and the number of foreign sperm stored by females in a previous mating in the scorpionfly *Panorpa cognata*. Female sperm storage was manipulated by interrupting copulations at different time points. Female mating status did not significantly influence male mating investment, but resource-limited males invested strategically in relation to the amount of sperm stored by females in a previous mating. I found continuously decreasing male investment in response to increasing amounts of competing sperm. These results demonstrate an unprecedented male ability to assess the number of sperm stored by females. As a result, males are capable of an extraordinarily fine-tuned reaction to the intensity of sperm competition.

KEY WORDS: Ejaculate size, Mecoptera, nuptial gifts, sperm competition, strategic sperm allocation.

Sperm competition is a strong selective force that will influence many aspects of both males' and females' reproductive behavior (Parker 1970; Eberhard 1996; Simmons 2001; Wedell et al. 2002; Arnqvist and Rowe 2005). When females copulate with two or more males, the sperm from those males will compete for fertilizations within the females' reproductive tract (Parker 1970; Birkhead and Møller 1998; Simmons 2001). Under numerical sperm competition, the more sperm a male inseminates, the larger proportion of offspring he is likely to sire (Dziuk 1996; Gage and Morrow 2003). It is thus well established that males in many species respond to a higher average risk of sperm competition by an increased allocation of resources to sperm production (see Parker et al. 1997; Engqvist and Reinhold 2005). Nevertheless, ejaculate production costs are nontrivial and males will have to conserve some resources for future matings (Dewsbury 1982; Nakatsuru and Kramer 1982; Pitnick and Markow 1994; Olsson et al. 1997), selecting for strategic male ejaculation (Parker 1998; Wedell et al. 2002). In many instances, the level of sperm competition is likely to differ between matings. Some females may be more likely to remate or have mated with other males than other females, and this will considerably affect the fertilization gain of male sperm investment. It is therefore predicted that males should tailor their investment in matings in response to the expected level of sperm competition in the subsequent raffle for fertilizations (Parker et al. 1996, 1997). Strategic ejaculation in relation to the risk of sperm competition have been widely documented (Wedell et al. 2002), and it has been shown that males are able to assess these differences in immediate sperm competition risk using visual (Gage 1991; Olsson 2001; Zbinden et al. 2003), olfactory (delBarco-Trillo and Ferkin 2004), or chemosensory (Siva-Jothy and Stutt 2003) cues.

Matings may differ not only in the probability that sperm competition will occur but also in the intensity defined as the amount of competing sperm or ejaculates. If males are certain of sperm competition, they should respond to an increased intensity of sperm competition by investing less sperm in a mating (Parker et al. 1996). The cause for this somewhat counterintuitive prediction is that the marginal fitness increase per additional sperm invested will be lower the more rival sperm is competing (Parker et al. 1996). In analogy with a lottery, the value of buying extra tickets in a raffle for a fixed prize becomes greater the fewer the competitors (Parker 1998). Thus, it should pay males to conserve sperm in raffles with high sperm competition intensity for future matings with less intense sperm competition, provided that such raffles are not too rare and the costs of searching and achieving further matings are relatively low. There are some evidence, although ambiguous (see Fuller 1998; Schaus and Sakaluk 2001; Pilastro et al. 2002; Wedell et al. 2002; Pizzarri et al. 2003; Engqvist and Reinhold 2005), that males decrease sperm investment in matings with increasing sperm competition intensity (Simmons and Kvarnemo 1997; Smith et al. 2003; delBarco-Trillo and Ferkin 2006), supporting this theoretical prediction. The intensity of sperm competition may be assessed by the number of potential competitors in the immediate vicinity (Fuller 1998; Schaus and Sakaluk 2001; Pilastro et al. 2002; Pizzarri et al. 2003; Smith et al. 2003), which may be a good estimation at least for species with external fertilizations (but see discussion in Engqvist and Reinhold 2005). For internal fertilizers, on the other hand, the number of sperm transferred by previous mates may provide a better estimation but few studies have considered male response to variation in female sperm storage. In studies of butterflies and moths, it has been shown that if differences in rival sperm amount are substantial, males react to these differences by differential sperm allocation (Cook and Gage 1995; Wedell and Cook 1999). However, both these studies found increased male ejaculate size in response to an increased amount of sperm stored by females.

In the present study, I determined whether male scorpionflies of the species *Panorpa cognata* (Insecta, Mecoptera) are able to assess and respond strategically both to the presence of competing sperm and differences in the amount of foreign sperm stored by females during an initial copulation. Females of this species often mate with several males (Engqvist and Sauer 2003b) and the sperm competition mechanism largely conforms to a “fair raffle” of sperm (Engqvist et al. 2007), which seem to be the general pattern of sperm precedence in *Panorpa* scorpionfly mating systems (see also Sauer et al. 1998, 1999; Kock et al. 2006). Before matings, males provide females with a nuptial gift, on which females feed on during copulation. Males occasionally may offer females a prey item but more frequently, a salivary secretion is provided as nuptial gift (Engqvist and Sauer 2003b). The duration of the copulation is to a large extent determined by the size of this salivary mass (Engqvist and Sauer 2001). Furthermore, there is a strong association between copulation duration and female sperm storage as sperm transfer is continuous during copulation (Sauer et al. 1997; Engqvist and Sauer 2003a; Engqvist et al. 2007). Individual males may thus influence copulation duration, sperm transfer, and fertilization success by changing the size of the offered salivary secretion. Males produce and store saliva in their salivary gland. Saliva production is strongly dependent on male food intake and, correspondingly, highly influenced by nutritional condition. Food-resource-limited males have thus a very limited amount of saliva in their glands and high marginal costs of sperm transfer (cf. Engqvist and Sauer 2001; Engels and Sauer 2006). Hence, this species offers some major benefits to study sperm allocation. First of all, sperm compete numerically (Engqvist et al. 2007), which is an assumption underlying most theoretical models concerning optimal sperm allocation (Parker 1990; Parker et al. 1996, 1997; Reinhold et al. 2002; Engqvist and Reinhold 2006). Second, male resources necessary for sperm transfer are highly limited, a prerequisite for male strategic mating investment. In addition, it is easy to quantify both mating investment and available resources by measuring the size of the salivary mass (investment) and the size of the salivary gland (resources), respectively. As a major benefit, this is an accurate estimate of male-intended mating investment (see also Engqvist and Sauer 2001), in contrast to measures of investment that are made during or after mating, which are potentially confounded by female actions during copulation. Finally, it is easy to manipulate female sperm storage, and thus competing sperm amount, by controlling copulation duration.

The aim of this study was to quantify male mating investment both in response to the mating status of females and in response to the amount of sperm stored by females in a previous mating.

Materials and Methods

All animals used were F_1 offspring from animals caught near Freiburg im Breisgau in southwestern Germany in May 2004. Larvae were reared using standard breeding protocols (Sauer 1970, 1977; Thornhill and Sauer 1992; Engqvist and Sauer 2003a) on a 18L:6D photoperiod enabling diapause-free development. Adult scorpionflies emerged in August 2004.

To measure male mating investment in relation to female mating status, males were randomly assigned to mate with an already mated female or with a virgin female. All females were held on identical standardized diets consisting of a one-segment piece of mealworm (*Tenebrio molitor*) every third day (Engqvist and Sauer 2001, 2003a). Between the age of 14 and 16 days females who were assigned to be used as mated females were mated to a first male. To estimate male mating investment in relation to female previous copulation duration and, thus, the amount of sperm stored by females, these copulations were randomly assigned to be uninterrupted or to be interrupted after 90, 120, 150, or 180 min. The mean ± SD copulation duration of uninterrupted matings amounted to 215.2 ± 68.3 min. Totally, 33 females were used as mated females. Twenty females were designated to interrupted copulations, five in each group, and the rest to uninterrupted copulations. In scorpionflies, there is strong evidence of a continuous sperm transfer during copulation (Sauer et al. 1997; Engqvist and Sauer 2003a; Engqvist et al. 2007). Interruption of copulations has no influence on the rate of sperm transfer (Engqvist and Sauer 2003a; Engqvist et al. 2007). Furthermore, the rate of sperm transfer does not seem to change during copulation, resulting in an approximate linear relationship between copulation duration and female sperm storage (Engqvist and Sauer 2003a). Copulation duration is therefore a good indicator of sperm transfer and the described actions will thus generate an experimentally controlled gradient in the amount of sperm stored by females, where sperm storage will be directly proportional to previous copulation duration. Males used in these initial matings were all well fed to guarantee that they are able to produce the amount of saliva required for the prescribed copulation duration. Only one male thus failed to do so (140 instead of 180 min). On the following day, females were allowed to mate again, this time with a male fed on a restricted diet (a one-segment piece of a mealworm every sixth day only), thus, with a highly limited amount of saliva available (Engqvist and Sauer 2001, 2002b). Just after salivary mass production but before the onset of copulation, pairs were interrupted and separated. Mating trials with virgin females were performed identically. The dry weight of the produced salivary mass was measured to the nearest 0.001 mg as described elsewhere (Engqvist and Sauer 2001). To estimate the amount of saliva available to males, their salivary glands were dissected out. Males were immediately killed under anesthesia and transferred to tubes containing 70% ethanol, in which they were held until preparation. The preparation and measurement of salivary glands followed standard protocols (Engqvist and Sauer 2001). The measurement of salivary masses and glands were both performed blindly. To estimate the amount of saliva in the salivary gland before copulation, I added the weight of the produced salivary mass to the weight of the dissected salivary gland as described elsewhere (Engqvist and Sauer 2001; Engels and Sauer 2006).

STATISTICAL ANALYSIS

Salivary mass weight of males mated to virgin and mated females was analyzed using an analysis of covariance (ANCOVA), whereas a multiple regression was used to analyze salivary mass weight in matings with females with different amount of sperm stored. The amount of saliva produced by males in a mating has previously been shown to be influenced by the amount of saliva stored in the salivary gland (Engqvist and Sauer 2001, 2002b). In addition, in matings with females in better condition, males provide larger salivary masses (Engqvist and Sauer 2001, 2002b). I therefore used both salivary gland size and female weight as covariates in all analyses.

Results

First, I measured male mating investment in relation to whether females had mated previously or not. I found that males produced slightly larger salivary masses prior to matings with mated females (0.826 ± 0.034 (SE), mg; $n = 33$) than with virgin females (0.778 ± 0.027 mg, $n = 52$), but this difference was not statistically significant (ANCOVA: $F_{1,81} = 1.85$; $P = 0.18$; Fig. 1A). However,

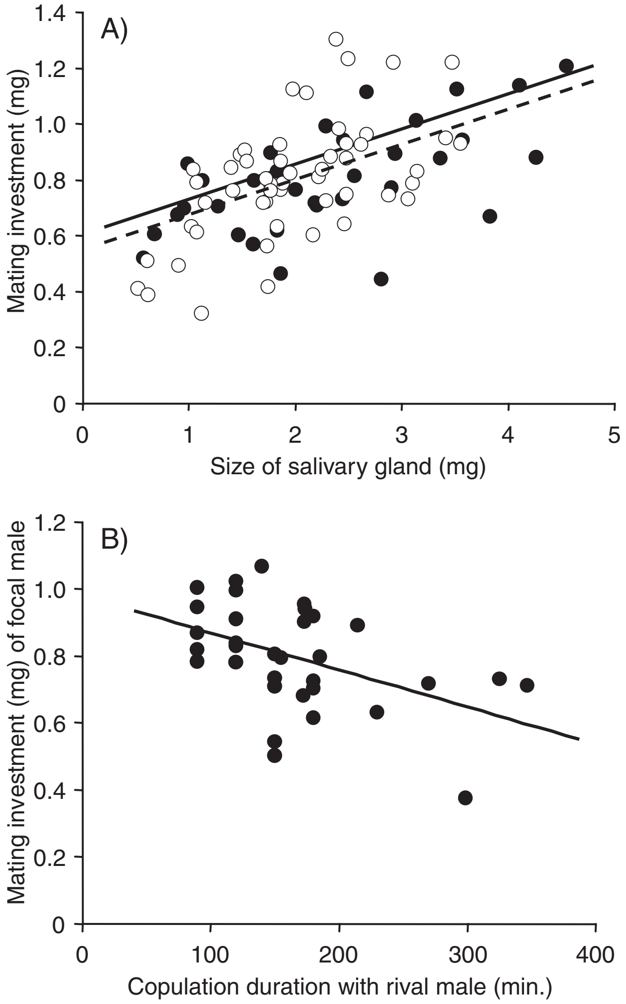

Figure 1. **(A) Mating investment in relation to female mating status (virgin: open circles, dashed line; mated: filled circles, solid line) and the salivary gland size of males. Gland size and the covariate female weight significantly affected male investment (salivary gland size: $F_{1,81} = 29.6$, $P < 0.001$; female weight: $F_{1,81} = 6.06$, $P = 0.016$), whereas female mating status did not ($P = 0.18$ see text; full model: $r^2 = 0.298$, $F_{3,81} = 11.5$, $P < 0.001$). Values give the expected investment for a male mating with a female of average weight (52.5 mg). For both statistics and plot homogeneous slopes were assumed ($F_{1,80} = 2.71$; $P > 0.1$). (B) A partial regression plot (salivary gland and female weight controlled) showing a negative relationship between a female's previous copulation duration and male mating investment ($P = 0.015$, see text). Values give the expected investment for a male with average salivary gland size (2.31 mg) mating with a female of average weight (52.5 mg). Both covariates significantly influenced male mating investment (salivary gland size: $F_{1,29} = 13.4$, $P = 0.001$; female weight: $F_{1,29} = 7.02$, $P = 0.013$; full model: $r^2 = 0.453$, $F_{3,29} = 8.00$, $P < 0.001$).**

there was a significant negative relation between the copulation duration of females in a previous mating and second males' mating investment (multiple regression: partial $r = -0.37$; $t_{29} = -2.59$; $P = 0.015$; Fig. 1B). Including a quadratic term of copulation duration did not significantly improve the model ($F_{1,28} = 0.25$, $P = 0.62$), indicating a constant slope ($b = -1.18 \pm 0.46$ μg/min). During the experiment, one of the females initially assigned to mate for 180 min actually only mated for 140 min. These data points were included in the multiple regression (as an uninterrupted mating with copulation duration 140 min). Excluding it would not change the results qualitatively (partial r for previous copulation duration on mating investment of focal male: -0.37; $t_{28} = -2.55$; $P = 0.017$).

Discussion

In summary, this study has demonstrated that male *P. cognata* scorpionflies respond to an increased number of competing sperm by a continuously decreasing mating investment. However, they did not change mating investment in relation to female mating status.

It may seem surprising that males would estimate and invest strategically in response to the amount of sperm stored but respond indifferently to whether females have mated or not. However, at least under seminatural conditions it has been shown that all *P. cognata* females mate with at least two males prior to oviposition (Engqvist and Sauer 2003b). Males may thus always anticipate sperm competition. Hence, in a promiscuous species, female mating status may tell males little about the ultimate level of sperm competition risk and it may therefore be inadequate to respond to it (Engqvist and Reinhold 2005). Consequently, the amount of sperm already stored by females give a better indication of sperm competition than female mating status. I therefore tested whether males are able to assess and respond to differences in the sperm amount stored by females in a previous copulation with rival males. For this purpose female sperm storage was experimentally manipulated by controlling copulation duration, as copulation duration is a good indicator of female sperm storage (Engqvist and Sauer 2003a). Focal males were not present at the initial mating and were therefore not able to assess copulation duration directly. Females did not differ in any other respect than previous copulation duration and thus the amount of sperm stored. Therefore, these results clearly reveal an unprecedented ability of males to quantify the number of rival sperm stored by females from a previous copulation.

These results complement several other studies demonstrating strategic male mating investment in relation to the risk and intensity of sperm competition (Wedell et al. 2002; Engqvist and Reinhold 2005). The risk of sperm competition have been demonstrated to be estimated by males through the presence of other males (e.g., Gage 1991; Zbinden et al. 2003; delBarco-Trillo and Ferkin 2004), the direct observation of females copulating (e.g., Nicholls et al. 2001; Olsson 2001), female mating status (e.g., Cook and Gage 1995; Wedell 1998; Wedell and Cook 1999; Martin and Hosken 2002; but see Engqvist and Reinhold 2006), or the direct detection of foreign sperm in the female genital tract (Siva-Jothy and Stutt 2003). In a number of studies, males have also been shown to modulate ejaculate size in response to the expected intensity of sperm competition (e.g., Simmons and Kvarnemo 1997; Schaus and Sakaluk 2001; Pilastro et al. 2002; Pizzarri et al. 2003; Smith et al. 2003; delBarco-Trillo and Ferkin 2006). In most of these studies, males respond to differences in the perception of number of males present nearby as a measure of expected immediate sperm competition intensity.

Only two other studies have examined male response to differences in the amount of sperm stored by females (Cook and Gage 1995; Wedell and Cook 1999). In these studies of sperm allocation in the Lepidopteran species *Plodia interpunctella* and *Pieris rapae*, differences in sperm storage were caused by manipulating the mating history of the males used as female initial mating partners (Cook and Gage 1995; Wedell and Cook 1999). Differences in sperm amount were thus relatively large (i.e., in comparison with the present study). In contrast to the present study, however, these studies revealed an increase in male sperm allocation in response to an increased number of competing sperm (Cook and Gage 1995; Wedell and Cook 1999). One reason for this discrepancy may be differences in the pattern of sperm precedence between the study species, which may have a huge impact on optimal sperm allocation (Parker et al. 1997; Engqvist and Reinhold 2006). The sperm precedence pattern of both *P. interpunctella* and *P. rapae* seems to follow a bimodal distribution with predominantly second male sperm precedence (Cook et al. 1997; Wedell and Cook 1998) in contrast to the fair raffle of sperm in the scorpionfly used in the present study (Engqvist et al. 2007). Another, perhaps more intriguing explanation, could be that the range of sperm competition intensity faced by males possibly differed between the studies. In Parker's (1996) original model, sperm competition intensity varied in discrete units between zero and several competing ejaculates, which is a reasonable assumption for external fertilizers. This model predicts that males should maximize sperm allocation in raffles with exactly one competing ejaculate and spend a decreasing amount of sperm with increasing sperm competition intensity above this level (Parker et al. 1996). The situation in the present and similar studies (i.e., Cook and Gage 1995; Wedell and Cook 1999) is slightly different, as males faced sperm competition with one other ejaculate of varying size. In a recent theoretical study, Engqvist and Reinhold (2007) found that when males compete against ejaculates of different size, the optimal male response will strongly depend on the size of the male's own sperm reserve. Thus, depending on the range of sperm competition intensity faced in competing ejaculates, one may expect both a positive and a negative relationship between the size of competing ejaculates and sperm investment. Therefore the decrease in investment with increasing sperm competition intensity found here and the increase found in other studies (Cook and Gage 1995; Wedell and Cook 1999) may both well be compatible with predicted optimal sperm allocation (Engqvist and Reinhold 2007). A negative relation between male sperm allocation and the size of competing ejaculates will be expected when competing ejaculates are relatively large compared to the focal males' own ability to produce large ejaculates (Engqvist and Reinhold 2007). In the experiment presented here, well-fed males were used to generate competing sperm amount. The long copulations of these males are equivalent to a saliva amount well beyond what the resource-limited males had available in their salivary glands during the test. Therefore, these situations are likely to correspond to a high intensity situation causing a reduction in male mating investment.

The present study does not reveal how males may determine female sperm storage. Extraordinary pairing preludes, lasting for up to seven hours, have been reported for this species (Engqvist and Sauer 2002a). During this time, males often repeatedly obtain genital contact with the females, and copulation initiations without any such contacts are extremely rare. At least it seems highly imaginable that males use the prolonged pairing preludes to gather information about female sperm storage. In bedbugs, sensillae on males' intromittent organ give information on the presence of competing sperm (Siva-Jothy and Stutt 2003). However, male scorpionflies do not possess an intromittent organ that will enable such an access to females' sperm storage organ for direct sperm assessment. Nevertheless, previous female mating extent may modify several other female traits including olfactory changes (Simmons et al. 2003; Carazo et al. 2004), behavioral changes (Dickinson and Rutowski 1989), and even internal morphological changes (Crudgington and Siva-Jothy 2000) are possible. For instance mating is widely known to affect female receptivity (see, e.g., Wedell 2005), and sperm amount received in previous matings have been shown to affect female willingness to mate (e.g., Cook and Gage 1995; Proshold 1995; Aisenberg and Costa 2005). Possibly, males are able to use these potential signals revealed during male courtship as indicators of female previous mating history and sperm storage (see for instance Friberg 2006; Carazo et al. 2007).

This work highlights two important issues. First, it demonstrates that sperm resource-limited males respond to an increased number of competing sperm by a decreased investment in matings. To be able to perform this prudent response, however, males must be capable of a more precise assessment of competing sperm amount than previously assumed. Thus theoretical models allowing a more precise estimation of sperm competition intensity may be required. Also in the field of sensory biology, future research may focus on the type of information males use to assess sperm competition intensity.

ACKNOWLEDGMENTS

The project was supported by grants from the Deutsche Forschungsgemeinschaft (EN 469/1-1). J. Leven helped me with the rearing of scorpionflies and C. Fritsche assisted me during the experiments. J. Frommen, T. Bakker, K. Reinhold, M. Noor, N. Wedell, and two anonymous reviewers gave helpful criticism on a previous version of this manuscript.

LITERATURE CITED

Aisenberg, A., and F. G. Costa. 2005. Females mated without sperm transfer maintain high sexual receptivity in the wolf spider Schizocosa malitiosa. Ethology 111:545–558.

Arnqvist, G., and L. Rowe. 2005. Sexual conflict. Princeton Univ. Press, Princeton, NJ.

Birkhead, T. R., and A. P. Møller. 1998. Sperm competition and sexual selection. Academic Press, San Diego, CA.

Carazo, P., E. Sanchez, E. Font, and E. Desfilis. 2004. Chemosensory cues allow male *Tenebrio molitor* beetles to assess the reproductive status of potential mates. Anim. Behav. 68:123–129.

Carazo, P., E. Font, and B. Alfthan. 2007. Chemosensory assessment of sperm competition levels and the evolution of internal spermatophore guarding. Proc. R. Soc. Lond. B 274:261–267.

Cook, P. A., and M. J. G. Gage. 1995. Effects of risks of sperm competition on the numbers of eupyrene and apyrene sperm ejaculated by the moth *Plodia interpunctella* (Lepidoptera, Pyralidae). Behav. Ecol. Sociobiol. 36:261–268.

Cook, P. A., I. F. Harvey, and G. A. Parker. 1997. Predicting variation in sperm precedence. Philos. Trans. R. Soc. Lond. B 352:771–780.

Crudgington, H. S., and M. T. Siva-Jothy. 2000. Genital damage, kicking and early death—the battle of the sexes takes a sinister turn in the bean weevil. Nature 407:855–856.

delBarco-Trillo, J., and M. H. Ferkin. 2004. Male mammals respond to a risk of sperm competition conveyed by odours of conspecific males. Nature 431:446–449.

———. 2006. Male meadow voles respond differently to risk and intensity of sperm competition. Behav. Ecol. 17:581–585.

Dewsbury, D. A. 1982. Ejaculate cost and male choice. Am. Nat. 119:601–610.

Dickinson, J. L., and R. L. Rutowski. 1989. The function of the mating plug in the chalcedon checkerspot butterfly. Anim. Behav. 38:154–162.

Dziuk, P. J. 1996. Factors that influence the proportion of offspring sired by a male following heterospermic insemination. Anim. Reprod. Sci. 43:65–88.

Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton Univ. Press, Princeton, NJ.

Engels, S., and K. P. Sauer. 2006. Resource dependent nuptial feeding in *Panorpa vulgaris*: an honest signal of male quality. Behav. Ecol. 17:628–632.

Engqvist, L., and K. P. Sauer. 2001. Strategic male mating effort and cryptic male choice in a scorpionfly. Proc. R. Soc. Lond. B 268:729–735.

———. 2002a. Amorous scorpionflies: causes and consequences of the long pairing prelude of *Panorpa cognata*. Anim. Behav. 63:667–675.

———. 2002b. A life history perspective on strategic mating effort in male scorpionflies. Behav. Ecol. 13:632–636.

———. 2003a. Determinants of sperm transfer in the scorpionfly *Panorpa cognata*: male variation, female condition and copulation duration. J. Evol. Biol. 16:1196–1204.

———. 2003b. Influence of nutrition on courtship and mating in the scorpionfly *Panorpa cognata*. Ethology 109:911–928.

Engqvist, L., and K. Reinhold. 2005. Pitfalls in experiments testing predictions from sperm competition theory. J. Evol. Biol. 18:116–123.

———. 2006. Theoretical influence of female mating status and remating propensity on male sperm allocation patterns. J. Evol. Biol. 19:1448–1458.

———. 2007. Sperm competition games: optimal sperm allocation in response to the size of competing ejaculates. Proc. R. Soc. Lond. B 274:209–217.

Engqvist, L., G. Dekomien, T. Lippmann, J. T. Epplen, and K. P. Sauer. 2007. Sperm transfer and paternity in the scorpionfly *Panorpa cognata*: large variance in traits favoured by postcopulatory episodes of sexual selection. Evol. Ecol. doi:10.1007/s10682-006-9152-6.

Friberg, U. 2006. Male perception of female mating status: its effect on copulation duration, sperm defence and female fitness. Anim. Behav. 72:1259–1268.

Fuller, R. C. 1998. Sperm competition affects male behaviour and sperm output in the rainbow darter. Proc. R. Soc. Lond. B 265:2365–2371.

Gage, M. J. G. 1991. Risk of sperm competition directly affects ejaculate size in the Mediterranean fruit fly. Anim. Behav. 42:1036–1037.

Gage, M. J. G., and E. H. Morrow. 2003. Experimental evidence for the evolution of numerous, tiny sperm via sperm competition. Curr. Biol. 13:754–757.

Kock, D., C. Hardt, J. T. Epplen, and K. P. Sauer. 2006. Patterns of sperm use in the scorpionfly *Panorpa germanica*, L. (Mecoptera: Panorpidae). Behav. Ecol. Sociobiol. 60:528–535.

Martin, O. Y., and D. J. Hosken. 2002. Strategic ejaculation in the common dung fly *Sepsis cynipsea*. Anim. Behav. 63:541–546.

Nakatsuru, K., and D. L. Kramer. 1982. Is sperm cheap? Limited male fertility and female choice in the Lemon tetra (Pisces, Characidae). Science 216:753–755.

Nicholls, E. H., T. Burke, and T. R. Birkhead. 2001. Ejaculate allocation by male sand martins, *Riparia riparia*. Proc. R. Soc. Lond. B 268:1265–1270.

Olsson, M. 2001. 'Voyeurism' prolongs copulation in the dragon lizard *Ctenophorus fordi*. Behav. Ecol. Sociobiol. 50:378–381.

Olsson, M., T. Madsen, and R. Shine. 1997. Is sperm really so cheap? Costs of reproduction in male adders, *Vipera berus*. Proc. R. Soc. Lond. B 264:455–459.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45:525–567.

———. 1990. Sperm competition games: sneaks and extra-pair copulations. Proc. R. Soc. Lond. B 242:127–133.

———. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. Pp. 3–54 *in* T. R. Birkhead and A. P. Møller, eds. Sperm competition and sexual selection. Academic Press, San Diego, CA.

Parker, G. A., M. A. Ball, P. Stockley, and M. J. G. Gage. 1996. Sperm competition games: individual assessment of sperm competition intensity by group spawners. Proc. R. Soc. Lond. B 263:1291–1297.

———. 1997. Sperm competition games: a prospective analysis of risk assessment. Proc. R. Soc. Lond. B 264:1793–1802.

Pilastro, A., M. Scaggiante, and M. B. Rasotto. 2002. Individual adjustment of sperm expenditure accords with sperm competition theory. Proc. Natl. Acad. Sci. USA 99:9913–9915.

Pitnick, S., and T. A. Markow. 1994. Large-male advantages associated with costs of sperm production in *Drosophila hydei*, a species with giant sperm. Proc. Natl. Acad. Sci. USA 91:9277–9281.

Pizzari, T., C. K. Cornwallis, H. Løvlie, S. Jakobsson, and T. B. Birkhead. 2003. Sophisticated sperm allocation in male fowl. Nature 426:70–74.

Proshold, F. I. 1995. Remating by gypsy moths (Lepidoptera, Lymantriidae) mated with F-1-sterile males as a function of sperm within the spermatheca. J. Econ. Entomol. 88:644–648.

Reinhold, K., J. Kurtz, and L. Engqvist. 2002. Cryptic male choice: sperm allocation strategies when female quality varies. J. Evol. Biol. 15:201–209.

Sauer, K. P. 1970. Zur Monotopbindung einheimischer Arten der Gattung *Panorpa* (Mecoptera) nach Untersuchungen im Freiland und im Laboratorium. Zool. Jahrb. Syst. 97:201–284.

———. 1977. The adaptive significance of genetic variability of photoperiodic response in *Panorpa vulgaris*. Zool. Jahrb. Syst. 104:489–538.

Sauer, K. P., J. Sindern, and N. Kall. 1997. Nutritional status of males and sperm transfer in the scorpionfly *Panorpa vulgaris* (Mecoptera: Panorpidae). Entomol. Gen. 21:189–204.

Sauer, K. P., T. Lubjuhn, J. Sindern, H. Kullmann, J. Kurtz, C. Epplen, and J. T. Epplen. 1998. Mating system and sexual selection in the scorpionfly *Panorpa vulgaris* (Mecoptera: Panorpidae). Naturwissenschaften 85:219–228.

Sauer, K. P., C. Epplen, I. Over, T. Lubjuhn, A. Schmidt, T. Gerken, and J. T. Epplen. 1999. Molecular genetic analysis of remating frequencies and sperm competition in the scorpionfly *Panorpa vulgaris* (Imhoff and Labram). Behaviour 136:1107–1121.

Schaus, J. M., and S. K. Sakaluk. 2001. Ejaculate expenditures of male crickets in response to varying risk and intensity of sperm competition: not all species play games. Behav. Ecol. 12:740–745.

Simmons, L. W. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton Univ. Press, Princeton, NJ.

Simmons, L. W., and C. Kvarnemo. 1997. Ejaculate expenditure by male bushcrickets decreases with sperm competition intensity. Proc. R. Soc. Lond. B 264:1203–1208.

Simmons, L. W., J. Alcock, and A. Reeder. 2003. The role of cuticular hydrocarbons in male attraction and repulsion by female Dawson's burrowing bee, *Amegilla dawsoni*. Anim. Behav. 66:677–685.

Siva-Jothy, M. T., and A. D. Stutt. 2003. A matter of taste: direct detection of female mating status in the bedbug. Proc. R. Soc. Lond. B 270:649–652.

Smith, C., M. Reichard, and P. Jurajda. 2003. Assessment of sperm competition by European bitterling, *Rhodeus sericeus*. Behav. Ecol. Sociobiol. 53:206–213.

Thornhill, R., and K. P. Sauer. 1992. Genetic sire effects on the fighting ability of sons and daughters and mating success of sons in a scorpionfly. Anim. Behav. 43:255–264.

Wedell, N. 1998. Sperm protection and mate assessment in the bushcricket *Coptaspis* sp. 2. Anim. Behav. 56:357–363.

———. 2005. Female receptivity in butterflies and moths. J. exp. Biol. 208:3433–3440.

Wedell, N., and P. A. Cook. 1998. Determinants of paternity in a butterfly. Proc. R. Soc. Lond. B 265:625–630.

———. 1999. Butterflies tailor their ejaculate in response to sperm competition risk and intensity. Proc. R. Soc. Lond. B 266:1033–1039.

Wedell, N., M. J. G. Gage, and G. A. Parker. 2002. Sperm competition, male prudence and sperm-limited females. Trends Ecol. Evol. 17:313–320.

Zbinden, M., D. Mazzi, R. Künzler, C. R. Largiadèr, and T. C. M. Bakker. 2003. Courting virtual rivals increase ejaculate size in sticklebacks (*Gasterosteus aculeatus*). Behav. Ecol. Sociobiol. 54:205–209.

Associate Editor: N. Wedell