

Zeitschrift: Alpine entomology : the journal of the Swiss Entomological Society
Herausgeber: Swiss Entomological Society
Band: 5 (2021)

Artikel: Female accessory gland fluid promotes sperm survival in yellow dung flies
Autor: Thüler, Karin / Blanckenhorn, Wolf U. / Ward, Paul I.
DOI: <https://doi.org/10.5169/seals-966045>

Nutzungsbedingungen

Die ETH-Bibliothek ist die Anbieterin der digitalisierten Zeitschriften auf E-Periodica. Sie besitzt keine Urheberrechte an den Zeitschriften und ist nicht verantwortlich für deren Inhalte. Die Rechte liegen in der Regel bei den Herausgebern beziehungsweise den externen Rechteinhabern. Das Veröffentlichen von Bildern in Print- und Online-Publikationen sowie auf Social Media-Kanälen oder Webseiten ist nur mit vorheriger Genehmigung der Rechteinhaber erlaubt. [Mehr erfahren](#)

Conditions d'utilisation

L'ETH Library est le fournisseur des revues numérisées. Elle ne détient aucun droit d'auteur sur les revues et n'est pas responsable de leur contenu. En règle générale, les droits sont détenus par les éditeurs ou les détenteurs de droits externes. La reproduction d'images dans des publications imprimées ou en ligne ainsi que sur des canaux de médias sociaux ou des sites web n'est autorisée qu'avec l'accord préalable des détenteurs des droits. [En savoir plus](#)

Terms of use

The ETH Library is the provider of the digitised journals. It does not own any copyrights to the journals and is not responsible for their content. The rights usually lie with the publishers or the external rights holders. Publishing images in print and online publications, as well as on social media channels or websites, is only permitted with the prior consent of the rights holders. [Find out more](#)

Download PDF: 21.08.2025

ETH-Bibliothek Zürich, E-Periodica, <https://www.e-periodica.ch>

Female accessory gland fluid promotes sperm survival in yellow dung flies

Karin Thüler¹, Wolf U. Blanckenhorn¹, Paul I. Ward¹, Stefan Lüpold¹, Luc F. Bussière^{1,2}

¹ Zoological Museum, Department of Evolutionary Biology and Environmental Studies, University of Zurich, 8057 Zurich, Switzerland

² Biology and Environmental Sciences, University of Gothenburg, Box 451, Gothenburg 40530, Sweden

<http://zoobank.org/A0444AB9-0452-4894-A572-EEDDF000AF33>

Corresponding author: Wolf U. Blanckenhorn (Wolf.Blanckenhorn@ieu.uzh.ch)

Academic editor: Claudia Buser ♦ Received 10 May 2021 ♦ Accepted 16 August 2021 ♦ Published 10 September 2021

Abstract

Female and male reproductive traits co-evolve through pre- and post-copulatory sexual selection and sexual conflict. Although males typically transfer many sperm during copulation, only a small proportion reach the fertilization site because females often actively or passively reduce sperm number in their reproductive tract. Males may transfer accessory substances to protect their ejaculates against female selective processes, which benefits males but can harm females. In turn, females may use accessory gland fluids to control paternity or sperm storage. Female yellow dung flies (*Scathophaga stercoraria*) have paired accessory glands that produce fluids involved in fertilization and egg laying. One proposed function for these fluids is spermicide. Alternatively, female accessory gland fluid may help keep sperm alive to avoid fertilization failure or encourage sperm competition. Using yellow dung flies, we investigated the interaction of female accessory gland fluid with sperm *in vitro*. Significantly more sperm remained alive when exposed to accessory gland fluid compared to buffer only (63% vs. 44%). We conclude that female accessory gland fluid in yellow dung flies can help nourish rather than kill male sperm, although selective nourishment of sperm is as consistent with cryptic female choice as is selective spermicide.

Key Words

Reproduction, sexual conflict, *Scathophaga stercoraria*, sexual selection, sperm competition, spermicide

Introduction

In species with internal fertilization, only a fraction of the vast numbers of sperm transferred by males tend to ever reach the fertilization site (Chang 1951; Hartman 1957; Bedford 1970; Austin 1975; Suarez 1987; Williams et al. 1993; Suarez and Pacey 2006). This sperm loss can result from physiological or biochemical challenges within the female reproductive tract (Birkhead et al. 1993) and, if their tract is large, from additional dilution effects (Immler et al. 2011; Lüpold and Fitzpatrick 2015). Further, females of a diversity of species actively reduce sperm numbers in their reproductive tract by extrusion, dissolution or degradation (Davey 1985; house flies: Degrugillier 1985; bruchid beetles: Eady 1994; spiders: Peretti and Eberhard 2010; *Drosophila* fruit flies: Snook and Hosken

2004; Holman and Snook 2008; Lüpold et al. 2013; Manier et al. 2013; fowl: Pizzari and Birkhead 2000; Dean et al. 2011), implying female influences on sperm storage and paternity that may or may not be adaptive. Therefore, sperm are often short-lived within the female reproductive tract, although there are exceptions such as bees, ants, or bats (Hosken 1997; den Boer et al. 2008; King et al. 2011). In response, males of several insects produce and transfer various accessory substances to protect their ejaculates against female enzymatic attack and digestion (Leopold et al. 1971; Merritt 1989; Duvoisin et al. 1999; Chapman et al. 2001; Lung et al. 2002; Poiani 2006; Holman and Snook 2008; den Boer et al. 2008; King et al. 2011; Avila 2011). These male substances may benefit the males even if to the detriment of the females (i.e., indicating potential sexual conflict; Chapman et al. 2003; Arnqvist and Rowe

2005), for example by decreasing female receptivity to further mating, by accelerating egg laying, storage and use of sperm, or by reducing female life span (Chen 1996; Wolfner 2002; Chapman et al. 1995, 2001).

The female reproductive tract of insects typically includes a pair of ovaries from which the oviducts emanate to further join and form a common oviduct, one to several spermathecae (i.e., sperm storing organs), and paired accessory glands (Wigglesworth 1967; Gillott 1988; Chapman 1998). Davey (1985) documented various functions of female accessory glands: lubrication during copula and oviposition for faster mating and egg laying, production of oviposition pheromones, or protective secretions to coat the eggs. In *Musca domestica*, accessory gland fluid moves along with the spermatozoa to the fertilization chambers and is used to dissolve the cap of the mature egg to allow fertilization (Leopold and Degrugillier 1973; Leopold et al. 1978). Female accessory gland fluid may also facilitate cryptic female choice by creating a selective insemination site (Birkhead et al. 1993; Eberhard 1996; Hellriegel and Ward 1998; Hosken et al. 2001). Several studies have hypothesized or shown that female accessory gland fluids can selectively kill sperm, thus acting as a spermicide (Greeff and Parker 2000; Bernasconi et al. 2002; Holman and Snook 2008). A female may benefit from such sperm killing by promoting male competition (Birkhead et al. 1993; Bernasconi and Keller 2001), countering antagonistic male adaptations (Chapman et al. 1995; Rice 1996; Andrés and Arnqvist 2001), or simply by biasing paternity in favour of males of high genetic quality (Birkhead et al. 1993; Greeff and Parker 2000). However, while the number of sperm stored is generally lower than the number transferred (Hosken et al. 2001; Bernasconi et al. 2002), this does not necessarily mean that females actively kill or eject sperm; sperm may simply get lost in the female reproductive tract (Arthur et al. 2008).

The yellow dung fly *Scathophaga stercoraria* L. (Diptera: Scathophagidae) is a cool-climate species that is common around livestock (especially cattle) pastures in cold-temperate regions of the northern hemisphere (Blanckenhorn et al. 2010). In Switzerland, this species abounds up to high altitudes beyond the treeline (Kraushaar et al. 2002). Females lay their eggs into vertebrate dung, which the larvae consume. Consequently, males are usually found in large numbers on and around cow dung pats, waiting for females to mate with (Parker 1970). Due to the pioneering work of Geoff Parker and colleagues, the yellow dung fly has become the classic species for studies of sexual selection, sexual conflict, and sperm competition (Parker 1970, 1978; Simmons et al. 2020). Following copulation with a male, some sperm are stored and partitioned among typically three storage organs (spermathecae) within the female reproductive tract, potentially allowing some level of sperm choice by sorting (Otronen et al. 1997; Ward 2000; Bussière et al. 2010; Demont et al. 2021). Although sperm viability varies in different parts of the female reproductive tract, Bernasconi et al. (2002) found no evidence for female acces-

sory gland fluid affecting sperm viability. One limitation of their approach, however, was that they used previously frozen accessory gland fluid for their *in vitro* experiments. Thus, there is no direct evidence yet that accessory gland products can debilitate sperm in this species (cf. Holman and Snook 2008). To the contrary, it is also conceivable that female accessory gland fluid may actually promote sperm survival (King et al. 2011). Killing sperm by degradation or keeping sperm alive are thus two contrasting functions of female accessory gland products that are both consistent with a female influence on paternity.

Here, we revisited the potential role of accessory gland fluid of female yellow dung fly in sperm viability by using fresh accessory gland fluid to circumvent the possibility of inactivating some important substances by freezing. We predicted two contrasting observations for sperm viability depending on which, if any, of the alternative hypothetical functions for accessory gland fluids is true: compared to a control treatment, we should find more (rather than fewer) live sperm after exposure to accessory gland fluid if accessory glands nourish sperm. In contrast, if the glands promote spermicide, fewer sperm should be alive after exposure to accessory glands than in controls.

Materials and methods

We collected flies from a pasture in Fehraltorf, Switzerland (47°23'N, 8°44'E) and maintained them for multiple generations in the laboratory using standard conditions (Ward 2000; Blanckenhorn et al. 2010). For our experiment we used offspring of the 6th laboratory generation, dissecting a total of 50 females (of which three had to be discarded as dissection was unsuccessful) and 30 males after flies reached sexual maturity (>10 d after adult emergence for females, >4 d for males: Blanckenhorn and Henseler 2005). We performed these dissections in five temporal blocks no more than 12 minutes apart to provide equally fresh sperm, with 10 females and 6 males per block. The fluid of both female accessory glands of each female was extracted by rupturing each gland in a micro-centrifuge tube containing 20 µl buffer (Schneider's *Drosophila* medium; this solution is henceforth referred to as "accessory gland fluid suspension"). We then mixed the extracted accessory gland fluid from all 10 females (i.e., 20 accessory glands) per block so that all sperm samples of the 6 males within a block received the same accessory gland product. To obtain live sperm samples, we dissected individual males and extracted sperm from the proximal end (adjacent to the ejaculatory duct) of one of their testes by piercing the testis and pressing it lightly with a needle until approximately one third of the testis content was released into 100 µl buffer onto a glass slide (Schneider's *Drosophila* medium plus 10% heat-inactivated fetal calf serum: see Bernasconi et al. 2002; this solution is henceforth referred to as "sperm suspension"). All dissections were performed after flies had been anesthetized with CO₂.

To ascertain whether the female accessory gland fluid affects sperm viability, we exposed sperm samples from 30 individual males to both fresh accessory gland fluid mixed with buffer or to buffer alone in a paired design. We incubated 15 μ l of the (male) sperm suspension with 30 μ l of buffer plus 15 μ l of the female accessory gland fluid suspension (or 45 μ l of buffer in the control treatment) for a total of 60 μ l for 11 ± 2 min at room temperature. Subsequently, we released 30 μ l of these mixtures on a glass slide and examined them under a fluorescent microscope. We assessed sperm viability using the LIVE/DEAD Sperm Viability Kit (L-7011, Molecular Probes), which consists of a green membrane-permeant (live) nucleic acid stain (SYBR14, 1 mM in DMSO, diluted 1:50; emission max. 516 nm) and a red stain that penetrates only the damaged membranes of dead sperm (propidium iodide, 2.4 mM in water; emission max. 617 nm). After incubation, we added 5 μ l of each stain, vortexed lightly, and incubated the suspension in the dark for 5 min before viewing the sample under the fluorescent microscope. In the rare cases that cells took on both stains, we scored them as dead (Bernasconi et al. 2002). The fluorescent microscope contains three filter sets, allowing the viewing and recording of digital photographs under green light only, red light only, and green plus red light to clearly distinguish dead from live sperm.

We calculated sperm viability as the proportion of live sperm among all sperm counted in the sample (for plot-

ting), based on 20 randomly taken images (frames) per male at $20\times$ magnification within 20 ± 2 min of dissection (corresponding to 9 ± 1 min after adding the stains). Data were analysed with a binomial generalized linear mixed model with logit-link, implemented in the lme4 package (Bates et al. 2015) of R version 4.04 (R Core Team 2021): the two-vector response variable was the absolute number of sperm that were alive versus dead (summed across the 20 frames taken for each male), and the lone fixed predictor was the experimental treatment. We fitted a random effect for male identity in recognition of the paired nature of the design, and an additional observation level random effect to account for overdispersion in the response. We used parametric bootstrapping (implemented in the pbkrtest package for R: Halekoh and Højsgaard 2014) to assess the significance of treatment.

Results

We counted a mean of 258 ± 53 (SD) sperm per male (sums by treatment: buffer dead: 1819; buffer alive: 1453; accessory gland dead: 1520; accessory gland alive: 2955). The proportion of live sperm was higher when sperm were exposed to accessory gland fluid (mean [\pm 95%CI]: 0.63 [0.57, 0.68]) than in plain buffer (0.44 [0.38, 0.50]; parametric bootstrap P-value = 0.001; Fig. 1).

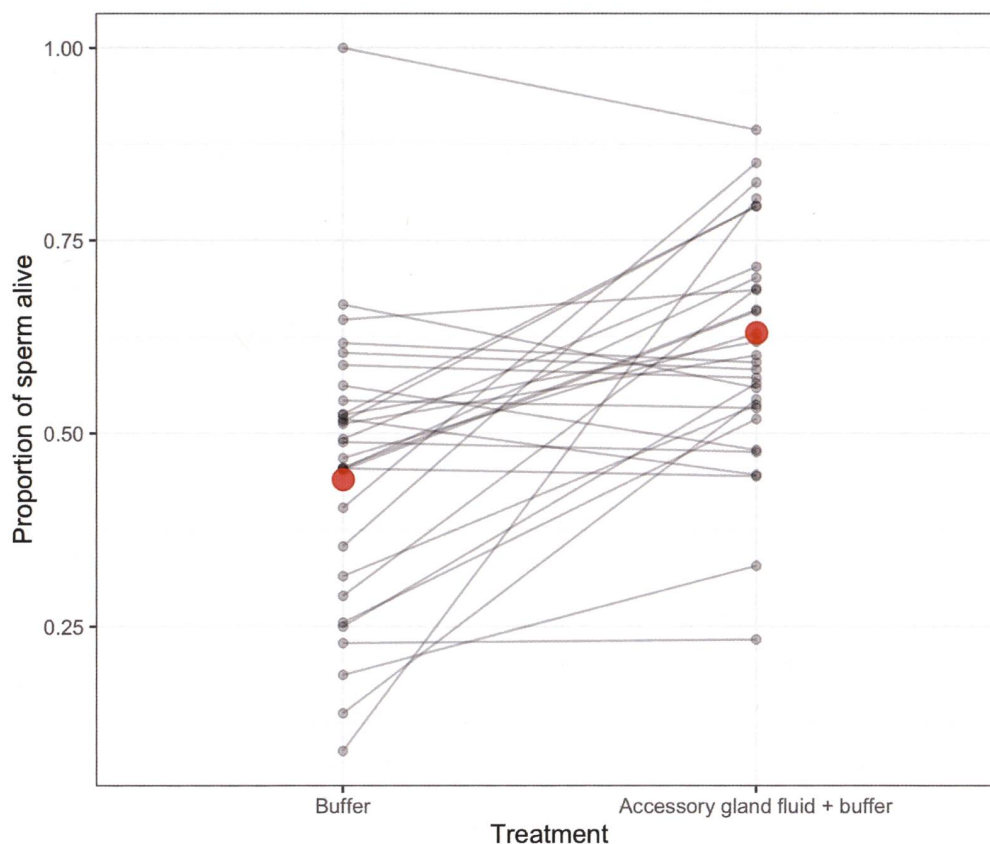


Figure 1. Proportion of sperm from 30 random *Scathophaga stercoraria* males remaining alive after *in vitro* paired treatment with female accessory gland fluid vs. buffer control (red dot = overall mean). Proportions were based on absolute counts of live and dead sperm, which could be distinguished by stain colour, across 20 equal-sized images per male.

Discussion

Bernasconi et al. (2002) had previously shown for yellow dung flies that after mating sperm viability was significantly reduced in spermathecae compared to a male's testes. Nevertheless, *in vitro* exposure of sperm to several parts of the female reproductive tract, including the accessory glands, showed no sperm degradation (Bernasconi et al. 2002). Here, using fresh rather than frozen female accessory gland fluid, we found that this fluid contains substances that apparently increase sperm survival, at least *in vitro*. However, we cannot exclude the possibility that other organs or their secretions may additionally affect sperm viability either inside the spermathecae or in other parts of the female reproductive tract.

Unless there is strict monogamy, which is rare (Birkhead and Møller 1998), the sexes have different reproductive interests, potentially leading to sexual conflict (Chapman et al. 2003). A selective female environment could kill or absorb some incoming sperm before (actively or passively) transferring the remaining sperm to her spermathecae (Hellriegel and Ward 1998). Selective spermicide could be a mechanism to reduce adverse effects of genetic incompatibility (Bishop 1996; Stockley 1999), but could also provide females with a mechanism to bias paternity through cryptic female choice (Birkhead 1998). It is thus possible that accessory gland fluid components with spermicidal functions are female adaptations that arose in the context of sexual conflict, but we found no evidence for this process here.

Sperm are often short-lived within the female reproductive tract. Sperm survival is primarily a function of sperm quality, motility and longevity, and secondarily depends on the female environment such as her accessory gland fluids. Our experiment supports the latter mechanism (without addressing the first). Studies of bees and ants have shown that male accessory fluids can also prolong sperm viability in the female reproductive tract (den Boer et al. 2008; King et al. 2011). The number of sperm transferred by male yellow dung flies during copulation increases with copula duration (Parker and Simmons 1994), and in principle any effect of accessory gland fluid could change with the ratio of sperm-to-fluid. As we diluted the accessory gland fluid in buffer following Bernasconi et al. (2002), we may also have diluted important effects of accessory gland fluid on sperm. However, it seems highly unlikely that the direction of the effect of accessory gland fluid on sperm reverses depending on the concentration of the fluid.

Other studies of insects have found positive effects of female accessory gland fluids on sperm viability and fertilization success. Hosken et al. (2002) showed for *S. stercoraria* that gland extract does not inhibit bacterial growth, suggesting that accessory gland fluid is more likely involved in fertilization functions rather than antimicrobial immunological processes. We stress, however, that selective provisioning of sperm could in principle serve the same function as selective spermicide by creating conditions of sperm storage that favour some males over others. But whether a sperm nourishing function of accessory gland fluid can actively favour certain ejaculates over others remains unclear.

Because there is almost always a surfeit of males at the dung, females might not need to keep sperm alive unless they want to impose competition on males they are forced to mate with at the oviposition site. Nevertheless, because keeping sperm alive for weeks inside the reproductive tract may be energetically costly, females may benefit from selective nutrient provisioning of sperm. Females should store only as many sperm as are needed in the short term, and kill or absorb any unnecessary or disfavoured sperm (Birkhead 2000). In yellow dung flies, far fewer sperm are released from the spermathecae during fertilization of individual eggs than was previously thought based on theory (Sbilordo et al. 2009). Along with the abundance of available males willing to mate, this makes sperm limitation unlikely in this species (Simmons et al. 2020).

While our study has clarified one aspect of the function of female accessory glands in yellow dung flies, more work on the physiological and biochemical interactions involved in sperm storage and use, as well as on the reproductive consequences of sperm mortality for male fertilization success and female fitness, is clearly needed to elucidate the multiple facets of sexual conflict and postmating sexual selection in insects.

Acknowledgements

We thank Ursula Briegel for help in the lab, and Andy Pemberton, Barbara Hellriegel and Giorgia Bernasconi for useful comments on the protocol and methods. This paper is in memory of our colleague and mentor Paul I. Ward, who sadly died during the course of this study. After completion of her PhD Karin Thüler has left the academic circles for an administrative position as a biologist.

References

- Andrés JA, Arnqvist G (2001) Genetic divergence of the seminal signal-receptor system in houseflies: the footprints of sexually antagonistic co-evolution? *Proceedings of the Royal Society B* 268: 399–405. <https://doi.org/10.1098/rspb.2000.1392>
- Arnqvist G, Rowe L (2005) *Sexual Conflict*. Princeton University Press, Princeton, NJ. <https://doi.org/10.1515/9781400850600>
- Arthur Jr BI, Sbilordo SH, Pemberton AJ, Ward PI (2008) The anatomy of fertilization in the yellow dung fly *Scathophaga stercoraria*. *Journal of Morphology* 269: 630–637. <https://doi.org/10.1002/jmor.10617>
- Austin CR (1975) Sperm fertility, viability and persistence in the female tract. *Journal of Reproductive Fertility Supplement* 22: 75–89.
- Avila FW, Sirot LK, LaFlamme BA, Rubinstein CD, Wolfner MF (2011) Insect seminal fluid proteins: identification and function. *Annual Review of Entomology* 56: 21–40. <https://doi.org/10.1146/annurev-ento-120709-144823>
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67: 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bedford JM (1970) Sperm capacitation and fertilization in mammals. *Biological Reproduction Supplement* 2: 128–158. https://doi.org/10.1095/biolreprod2.Supplement_2.128

- Bernasconi G, Keller L (2001) Female polyandry affects their sons' reproductive success in the red flour beetle *Tribolium castaneum*. *Journal of Evolutionary Biology* 14: 186–193. <https://doi.org/10.1046/j.1420-9101.2001.00247.x>
- Bernasconi G, Hellriegel B, Heyland A, Ward PI (2002) Sperm survival in the female reproductive tract in the fly *Scathophaga stercoraria*. *Journal of Insect Physiology* 48: 197–203. [https://doi.org/10.1016/S0022-1910\(01\)00164-0](https://doi.org/10.1016/S0022-1910(01)00164-0)
- Birkhead TR (1998) Cryptic female choice: criteria for establishing female sperm choice. *Evolution* 52: 1212–1218. <https://doi.org/10.1111/j.1558-5646.1998.tb01848.x>
- Birkhead TR (2000) Promiscuity: An Evolutionary History of Sperm Competition. Harvard University Press, Cambridge, MA.
- Birkhead TR, Møller AP (1998) Sperm Competition and Sexual Selection. Academic Press, San Diego. <https://doi.org/10.1016/B978-012100543-6/50027-1>
- Birkhead TR, Møller AP, Sutherland WJ (1993) Why do females make it so difficult for males to fertilize their eggs? *Journal of Theoretical Biology* 161: 51–60. <https://doi.org/10.1006/jtbi.1993.1039>
- Bishop JDD (1996) Female control of paternity in the internally fertilizing compound Ascidian *Diplosoma listerianum*. I. Autoradiographic investigation of sperm movements in the female reproductive tract. *Proceedings of the Royal Society B* 263: 369–376. <https://doi.org/10.1098/rspb.1996.0057>
- Blanckenhorn WU, Henseler C (2005) Temperature-dependent ovariole and testis maturation in the yellow dung fly. *Entomologia Experimentalis et Applicata* 116: 159–165. <https://doi.org/10.1111/j.1570-7458.2005.00316.x>
- Blanckenhorn WU, Pemberton AJ, Bussière LF, Roembke J, Floate KD (2010) Natural history and laboratory culture of the yellow dung fly, *Scathophaga stercoraria* (L.; Diptera: Scathophagidae). *Journal of Insect Science* 10: 1–17. <https://doi.org/10.1673/031.010.1101>
- Bussière LF, Demont M, Pemberton AJ, Hall MD, Ward PI (2010) The assessment of insemination success in yellow dung flies using competitive PCR. *Molecular Ecology Resources* 10: 292–303. <https://doi.org/10.1111/j.1755-0998.2009.02754.x>
- Chang MC (1951) Fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature* 168: 697–698. <https://doi.org/10.1038/168697b0>
- Chapman RF (1998) The Insects. Structure and Function. Cambridge University Press, Cambridge, MA.
- Chapman T, Arnqvist G, Bangham J, Rowe L (2003) Sexual conflict. *Trends in Ecology and Evolution* 18: 41–47. [https://doi.org/10.1016/S0169-5347\(02\)00004-6](https://doi.org/10.1016/S0169-5347(02)00004-6)
- Chapman T, Liddle L, Kalb J, Wolfner MF, Partridge L (1995) Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* 373: 241–244. <https://doi.org/10.1038/373241a0>
- Chapman T, Herndon LA, Heifetz Y, Partridge L, Wolfner MF (2001) The Acp26Aa seminal fluid protein is a modulator of early egg-hatchability in *Drosophila melanogaster*. *Proceedings of the Royal Society B* 268: 1647–1654. <https://doi.org/10.1098/rspb.2001.1684>
- Chen PS (1996) The accessory gland proteins in male *Drosophila*: structural, reproductive, and evolutionary aspects. *Experientia* 52: 503–510. <https://doi.org/10.1007/BF01969718>
- Davey KG (1985) The female reproductive tract. In: Kerkut GA, Gilbert LI (Eds) *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 1. Pergamon Press, Oxford, UK, 15–36.
- Dean R, Nakagawa S, Pizzari T (2011) The risk and intensity of sperm ejection in female birds. *American Naturalist* 178: 343–354. <https://doi.org/10.1086/661244>
- Degrugillier ME (1985) In vitro release of housefly *Musca domestica* L. (Diptera: Muscidae) acrosomal material after treatments with secretions of female accessory-gland and micropyle cap substance. *International Journal of Insect Morphology and Embryology* 14: 381–391. [https://doi.org/10.1016/0020-7322\(85\)90017-0](https://doi.org/10.1016/0020-7322(85)90017-0)
- Demont M, Ward PI, Blanckenhorn WU, Lüpold S, Martin OY, Bussière LF (2021) How biases in sperm storage relate to sperm use during oviposition in female yellow dung flies. *Behavioral Ecology* 32: 756–768. <https://doi.org/10.1093/beheco/arab026>
- den Boer SPA, Boomsma JJ, Baer B (2008) Seminal fluid enhances sperm viability in the leafcutter ant *Atta colombica*. *Behavioural Ecology and Sociobiology* 62: 1843–1849. <https://doi.org/10.1007/s00265-008-0613-5>
- Duvoisin N, Baer B, Schmid-Hempel P (1999) Sperm transfer and male competition in a bumblebee. *Animal Behaviour* 58: 743–749. <https://doi.org/10.1006/anbe.1999.1196>
- Eady PE (1994) Sperm transfer and storage in relation to sperm competition in *Callosobruchus maculatus*. *Behavioural Ecology and Sociobiology* 35: 123–129. <https://doi.org/10.1007/BF00171502>
- Eberhard WG (1996) Female control: Sexual selection by cryptic female choice. Princeton University Press, Princeton, NJ. <https://doi.org/10.1515/9780691207209>
- Gillott C (1988) Accessory sex glands in arthropoda – insecta. In: Adiyodi KG, Adiyodi RG (Eds) *Reproductive Biology of Invertebrates. III. Accessory Sex Glands*. John Wiley and Sons, New York, 319–473.
- Greeff JM, Parker GA (2000) Spermicide by females: what should males do? *Proceedings of the Royal Society B* 267: 1759–1763. <https://doi.org/10.1098/rspb.2000.1207>
- Halekoh U, Højsgaard S (2014) A Kenward-Roger Approximation and Parametric Bootstrap Methods for Tests in Linear Mixed Models - The R Package pbkrtest. *Journal of Statistical Software* 59(9): 1–30. <https://doi.org/10.18637/jss.v059.i09>
- Hartman CG (1957) How do sperm get into the uterus? *Fertility and Sterility* 8: 403–427. [https://doi.org/10.1016/S0015-0282\(16\)32820-5](https://doi.org/10.1016/S0015-0282(16)32820-5)
- Hellriegel B, Ward PI (1998) Complex female reproductive tract morphology: its possible use in post-copulatory female choice. *Journal of Theoretical Biology* 190: 179–186. <https://doi.org/10.1006/jtbi.1997.0546>
- Holman L, Snook RR (2008) A sterile sperm caste protects brother fertile sperm from female-mediated death in *Drosophila pseudoobscura*. *Current Biology* 18: 292–296. <https://doi.org/10.1016/j.cub.2008.01.048>
- Hosken DJ (1997) Sperm competition in bats. *Proceedings of the Royal Society B* 264: 385–392. <https://doi.org/10.1098/rspb.1997.0055>
- Hosken DJ, Uhía E, Ward PI (2002) The function of female accessory reproductive gland secretion and a cost to polyandry in the yellow dung fly. *Physiological Entomology* 27: 87–91. <https://doi.org/10.1046/j.1365-3032.2002.00271.x>
- Hosken DJ, Garner TWJ, Ward PI (2001) Sexual conflict selects for male and female reproductive characters. *Current Biology* 11: 489–493. [https://doi.org/10.1016/S0960-9822\(01\)00146-4](https://doi.org/10.1016/S0960-9822(01)00146-4)
- Immler SS, Pitnick S, Parker GA, Durrant KL, Lüpold S, Calhim S, Birkhead TR (2011) Resolving variation in the reproductive tradeoff between sperm size and number. *Proceedings of the National Academy of Sciences of the United States of America* 108: 5325–5330. <https://doi.org/10.1073/pnas.1009059108>
- King M, Eubel H, Millar AH, Baer B (2011) Proteins with the seminal fluid are crucial to keep sperm viable in the honeybee *Apis mellifera*. *Journal of Insect Physiology* 57: 409–414. <https://doi.org/10.1016/j.jinsphys.2010.12.011>

- Kraushaar U, Goudet J, Blanckenhorn WU (2002) Geographical and altitudinal population genetic structure of two dung fly species with contrasting mobility and temperature preference. *Heredity* 89: 99–106. <https://doi.org/10.1038/sj.hdy.6800097>
- Leopold RA, Degrugillier ME (1973) Sperm penetration of house fly eggs: Evidence for involvement of a female accessory secretion. *Science* 181: 555–557. <https://doi.org/10.1126/science.181.4099.555>
- Leopold RA, Terranova AC, Swilley AC (1971) Mating refusal in *Musca domestica*: effects of repeated mating and decerebration upon frequency and duration of copulation. *Journal of Experimental Zoology* 176: 353–359. <https://doi.org/10.1002/jez.1401760310>
- Leopold RA, Meola S, Degrugillier ME (1978) The egg fertilization site within the house fly, *Musca domestica* (L.) (Diptera: Muscidae). *International Journal of Insect Morphology and Embryology* 7: 111–120. [https://doi.org/10.1016/0020-7322\(78\)90011-9](https://doi.org/10.1016/0020-7322(78)90011-9)
- Lüpold S, Fitzpatrick JL (2015) Sperm number trumps sperm size in mammalian ejaculate evolution. *Proceedings of the Royal Society B* 282: 2015–2122. <https://doi.org/10.1098/rspb.2015.2122>
- Lüpold S, Pitnick S, Berben KS, Blengini CS, Belote JM, Manier MK (2013) Female mediation of competitive fertilization success in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* 110: 10693–10698. <https://doi.org/10.1073/pnas.1300954110>
- Lung O, Tram U, Finnerty CM, Eipper-Mains MA, Kalb JM, Wolfner MF (2002) The *Drosophila melanogaster* seminal fluid protein Acp62F is a protease inhibitor that is toxic upon ectopic expression. *Genetics* 160: 211–224. <https://doi.org/10.1093/genetics/160.1.211>
- Manier MK, Belote JM, Berben KS, Lüpold S, Ala-Honkola A, Collins WF, Pitnick S (2013) Rapid diversification of sperm precedence traits and processes among three sibling *Drosophila* species. *Evolution* 67: 2348–2362. <https://doi.org/10.1111/evo.12117>
- Merritt DJ (1989) The morphology of the phallosome and accessory gland material transfer during copulation in the blowfly, *Lucilia cuprina* (Insecta, Diptera). *Zoomorphology* 108: 359–366. <https://doi.org/10.1007/BF00312276>
- Otronen M, Reguera P, Ward PI (1997) Sperm storage in the yellow dung fly *Scathophaga stercoraria*: Identifying the sperm of competing males in separate female spermathecae. *Ethology* 103: 844–854. <https://doi.org/10.1111/j.1439-0310.1997.tb00125.x>
- Parker GA (1970) The reproductive behaviour and the nature of sexual selection in *Scathophaga stercoraria* L. (Diptera: Scathophagidae) I. Diurnal and seasonal changes in population density around the site of mating and oviposition. *Journal of Animal Ecology* 39: 185–204. <https://doi.org/10.2307/2895>
- Parker GA (1978) Searching for mates. In: Krebs JR, Davies NB (Eds) *Behavioural ecology*, 1st edn. Blackwell, Oxford, 214–244.
- Parker GA, Simmons LW (1994) Evolution of phenotypic optima and copula duration in dung flies. *Nature* 370: 53–56. <https://doi.org/10.1038/370053a0>
- Peretti AV, Eberhard WG (2010) Cryptic female choice via sperm dumping favours male copulatory courtship in a spider. *Journal of Evolutionary Biology* 23: 271–2781. <https://doi.org/10.1111/j.1420-9101.2009.01900.x>
- Pizzari T, Birkhead TR (2000) Female feral fowl eject sperm of subordinate males. *Nature* 405: 787–789. <https://doi.org/10.1038/35015558>
- Poiani A (2006) Complexity of seminal fluid. *Behavioural Ecology & Sociobiology* 60: 289–310. <https://doi.org/10.1007/s00265-006-0178-0>
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/>
- Rice WR (1996) Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* 381: 232–234. <https://doi.org/10.1038/381232a0>
- Sbilordo SH, Schäfer MA, Ward PI (2009) Sperm release and use at fertilization by yellow dung fly females (*Scathophaga stercoraria*). *Biological Journal of the Linnean Society* 98: 511–518. <https://doi.org/10.1111/j.1095-8312.2009.01296.x>
- Simmons LW, Parker GA, Hosken DJ (2020) Evolutionary insight from a humble fly: sperm competition and the yellow dung fly. *Philosophical Transactions of the Royal Society B* 375: 20200062. <https://doi.org/10.1098/rstb.2020.0062>
- Snook RR, Hosken DJ (2004) Sperm death and dumping in *Drosophila*. *Nature* 428: 939–941. <https://doi.org/10.1038/nature02455>
- Stockley P (1999) Sperm selection and genetic incompatibility: does relatedness of mates affect male success in sperm competition? *Proceedings of the Royal Society B* 266: 1663–1669. <https://doi.org/10.1098/rspb.1999.0829>
- Suarez SS, Pacey AA (2006) Sperm transport in the female reproductive tract. *Human Reproduction Update* 12: 23–37. <https://doi.org/10.1093/humupd/dmi047>
- Ward PI (2000) Cryptic female choice in the yellow dung fly *Scathophaga stercoraria*. *Evolution* 54: 1680–1686. <https://doi.org/10.1111/j.0014-3820.2000.tb00712.x>
- Wigglesworth VB (1967) *The Principles of Insect Physiology*. Methuen and Co, London.
- Williams M, Hill CJ, Scudamore I, Dunphy B, Cooke ID, Barratt CL (1993) Sperm numbers and distribution within the human fallopian tube around ovulation. *Human Reproduction* 8: 2019–2026. <https://doi.org/10.1093/oxfordjournals.humrep.a137975>
- Wolfner MF (2002) The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* 88: 85–93. <https://doi.org/10.1038/sj.hdy.6800017>

Supplementary material 1

Table S1

Authors: Karin Thüler, Wolf U. Blanckenhorn, Paul I. Ward, Stefan Lüpold, Luc F. Bussière

Data type: excel table

Explanation note: Sperm counts for 30 males under two conditions, buffer (control) & accessory gland suspension (AG), generated by this study.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/alpento.5.68501.suppl1>