

ESSAY

Sperm, eggs, pollen, and gelato, oh my!

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On September 4, 2023, the sky cleared over Nynäshamn, Sweden, and researchers from across the globe gathered for the 16th Biology of Spermatozoa (BoS) meeting. What followed was a week fuelled by tasty food (find out below about the gelato!) and beautiful weather discussing sperm, eggs, reproductive fluids, fertility, and all things reproductive evolution. Held biennially from its inception in the early 1990s, BoS relocated from the Sheffield, UK area to Stockholm, Sweden in 2019 (Rowe & Rosengrave, 2020) and is now organised by Rhonda Snook and John Fitzpatrick. The winning formula for this meeting is simple: gather scientists passionate about reproduction in one conference centre for a few days, where they will live and eat together surrounded by beautiful nature, favouring fruitful exchanges and collaborations.

BoS16 felt particularly special as regular participants finally reunited after a 4-year pandemic-induced hiatus. On top of this, BoS16 welcomed many new attendees from across the globe (over 20% of delegates and 11 of the 22 contributed talks), providing the opportunity to discover new cutting-edge research and expand the community and collaboration. The topics were varied and covered a broad range of methods and study systems, highlighting the breadth of the field of evolutionary reproductive biology. Researchers used extensive field sampling, meta-analyses, mathematical modelling, experimental evolution, proteomics, lipidomics, single-cell transcriptomics, gene editing and more across the animal and plant kingdoms to tackle exciting topics such as the huge variation in gamete

morphology and reproductive tactics, the genetic basis of reproductive barriers, the influence of the female reproductive tract and external environment on fertilisation outcome, and nongenetic transgenerational inheritance.

The 2023 meeting was also a time to remember the late Professor Matthew (Matt) J.G. Gage (1967–2022), who was “the life and soul of Biology of Sperm meetings,” as highlighted by Dave Hosken in his dedicated talk. Matt’s contributions to the field of evolutionary biology and to promoting and supporting young researchers have been excellently summarized elsewhere (Chapman & Stockley, 2022; Hosken et al., 2022; Vasudeva et al., 2022). In memoriam, BoS16 introduced the Matt Gage Award for the best poster presented by an early career researcher. As decided by a panel of judges (Nina Wedell, Leigh Simmons, and Dave Hosken), the inaugural winners of this award were Lennart Winkler (TU Dresden) and Erin Macartney (Stockholm University) with their posters titled “Population density affects sexual selection in an insect model” and “Ejaculate traits and paternity share under sperm competition: a meta-analysis across species and fertilisation modes,” respectively. Winkler manipulated population density in red flour beetles (*Tribolium castaneum*) and showed that differences in group size affect both pre and postcopulatory sexual selection, but differently for females and males (Winkler et al., 2023). Macartney investigated correlations between multiple sperm and ejaculate traits and fertilisation success, revealing intriguing patterns when comparing internal and external

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fertilisers. These topics represent aspects of Matt's work that continue to reveal fascinating results on the evolution of sperm form and function.

After a short welcome presentation, BoS16 began with Andrea Pauli's (Vienna Biocenter) excellent opening plenary on the egg membrane protein Bouncer and the search for its interacting protein partners (Deneke et al., 2023), showcasing an impressive range of techniques, including a recently developed AI tool for protein structure prediction (AlphaFold, Jumper et al., 2021). This first plenary set the stage for one of the main themes of BoS meetings, which, despite its name, includes a diverse range of topics going well beyond spermatozoa. The non-sperm focus continued the following morning with Patricia (Patty) Brennan's (Mount Holyoke College) talk on intra-horn insemination in alpacas (*Lama pacos*) and the consequent wounding of the female reproductive tract. Her findings that alpaca sperm cells have low motility and high infertility led to a common theme and tagline for BoS16 - "If you have a great penis, you can have terrible sperm" and vice versa. In her poster presentation, Dara Orbach (Texas A&M University-Corpus Christi) highlighted the positive effects of mimicking accurate vagina morphology to sample ejaculates of high quality in marine mammals for cryopreservation.

Sperm cells do not swim in a sterile medium but generally need to navigate a female-produced fluid. This fluid may be inside the female reproductive tract or surrounding eggs in an external environment. Sperm behaviour and morphology cannot be understood without considering the characteristics of the environment in which they function (Yeates et al., 2013). Clelia Gasparini (University of Padova) presented work showing that the female reproductive fluid increases the opportunity for multiple paternity and postmating sexual selection by expanding the egg fertilisation window in zebrafish (*Danio rerio*) (Pinzoni et al., 2023). In her talk, Murielle Ålund (Uppsala University) used comparative proteomics of the female reproductive fluid to understand conspecific sperm precedence in hybridizing *Ficedula* flycatchers, showing some patterns of interspecific divergence that seem mirrored between female and male reproductive proteins. Beyond the molecular composition of reproductive fluid, there is a growing understanding that physical properties (e.g., viscosity) and fluid dynamics may be important in determining sperm swimming behaviour. Kamil Talar (University of Lincoln) and Adnan Morshed (Tulane University) presented posters on the role of biophysics and modelling fluid dynamics on insect sperm in the female reproductive tract. This is a growing field and seeing how it builds over future BoS meetings will be interesting.

Spermatozoa are one of the most diverse cell types, however many aspects of the causes and consequences of this diversity are still incompletely understood. Understandably, the morphological diversity of sperm was a prevalent topic at BoS16. John Fitzpatrick (Stockholm University) updated the audience on the current state of SpermTree, an excellent online resource for sperm morphology data (<https://spermtree.org/>), and asked how fertilisation mode might influence sperm length variance. On the theme of variance, Jan Lifjeld (University of Oslo) proposed the interplay between the female

reproductive tract and variance in sperm length may drive divergence between isolated populations. Dick Moberg (University of Stockholm) showed that covariation in sperm and female sperm storage organ length between *Drosophila montana* populations is condition-dependent and underlies reproductive isolation. There were also various talks assessing morphological diversity across species within taxonomic groups. For example, Emily (Becky) Cramer (University of Oslo) asked how sperm length (particularly the midpiece) has evolved across songbirds, Jason Gallant (Michigan State University) told us a "tale of no tail" with the unusual case of weakly electric mormyroid fish and their aflagellate sperm, the only known case among vertebrates. His group is using CRISPR gene editing to investigate the genomic features underlying this peculiar phenotype, as detailed in a poster by Alyssa Saunders. Jessica Frith (University of Lincoln) presented her work considering the diversity of sperm and reproductive tract morphology beyond length. She invited researchers to share samples of sperm and female reproductive tract to aid her in filling current gaps in the insect phylogeny.

The theme of sperm morphological diversity continued in the poster sessions, with Ariel Kahrl (Hamilton College) presenting diversity in sperm morphology and reproductive strategy in tropical frogs. Jonathan Cowart (University of Florida) and Melissa Rowe (Netherlands Institute of Ecology) showed us interesting sperm morphology in manatees (*Trichechus manatus*) and red-browed finch (*Neochmia temporalis*), respectively, and Laima Bagdonaite (University of Oslo) investigated the relationship between mitochondrial copy number and sperm midpiece length across birds.

Diversity in the ejaculate is not restricted to sperm morphology and, as represented at BoS16, the field's understanding of how ejaculate and reproductive strategies vary between and within males, populations and species is rapidly expanding. In his plenary talk, Noritake Hirohashi (Shimane University) detailed the alternative reproductive tactics of *Loligo* squids, covering differences in sperm morphology and energetics and even different insemination and storage sites in the female. Noriyosi Sato (Tokai University) gifted us with another talk full of rare and fascinating videos, this time on the strange mating habits of paper nautilus (*Argonauta argo*), while posters by Léa Daupagne (University of Pau), Yoko Iwata (University of Tokyo) and Tuba Rizvi (Bielefeld University) concerned sneaking, cheating and sperm removal behaviour in Arctic lamprey (*Lethenteron camtschaticum*), pygmy squid (*Idiosepiidae* sp.) and bushcrickets (*Metaplastes ornatus*), respectively. In his presentation, Antonio Gomez (Syracuse University) advocated for the importance and diversity of ejaculate structures. His work focuses on comparative proteomics of the spermatostyle, a dense structure in which sperm heads are embedded, across adaphagan beetles. Beyond the description of morphological diversity, Luisa Kumpitsch (Gothenburg University) dived into the function of parasperm, a non-fertilising sperm morph, in *Littorina* snails, and Matt Dean (University of Southern California) described the use of transgenic knock-out mice to understand the function of the baculum in mice.

The external environment and male condition can introduce diversity in sperm function and ejaculate composition. Charlotta

(Lotta) Kvarnemo (University of Gothenburg) presented work together with Leon Green, retracing the invasion history of the marine round goby (*Neogobius melanostomus*) from the Black Sea into brackish areas of the Baltic Sea and then out into the Atlantic. These invasive fish show a high capacity to acclimate to drastically different salinities (Green et al., 2023), but perhaps differences in sperm performance between salinities can curtail the spread of this invasive fish. There were several posters on the impact of climate (Ram Vasudeva, University of Leeds, and Graziella Iossa, University of Lincoln) and other external conditions (Sara Calhim, University of Jyväskylä's) on sperm function and fertility. Klaus Reinhardt (TU Dresden) investigated the effect of diet on sperm function in bedbugs (*Cimex lectularius*), while Irem Sepil and Krish Sanghvi (both University of Oxford) and Patrice Rosengrave (University of Otago) studied the effects of age and oxidative stress, respectively and Oliver Otti (TU Dresden) presented experimental work looking at interactions between male age and seminal fluid on sperm motility and viability.

The fundamental theories of sexual selection and the interplay with sperm competition and reproduction laid the ground for a field of research that continues to expand and flourish. In her plenary talk, Jeanne Tonnabel (CNRS, ISEM Montpellier) reminded us that anisogamy, and thus the key principles of sexual selection, is just as relevant to plants as to animals. However, both theoretical modelling and meta-analyses in plants are lagging behind the animal literature. The Tonnabel group is currently filling these gaps, on top of performing impressive large-scale experimental evolution studies to reveal the impacts of density and mating system on sexual selection in several angiosperm species. Timothée Chenin presented his work on pollen competition and cryptic female choice in *Brassica rapa*. Hanna Kokko (University of Mainz) turned to *Colias* butterflies to model the "sexual conflict load" resulting from a combination of sexual conflict over emergence time and environmental constraints (Ekrem & Kokko, 2023). Expanding on this, Kora Klein (also University of Mainz) explicitly modelled the maintenance of colour morph polymorphism and its link to spermatophore expenditure and fertility. Matthew Kustra (UC Santa Cruz) modelled the conditions under which cryptic female choice and conspecific sperm precedence evolve as barriers to interspecific reproduction. Surprisingly, he showed that codivergence between sperm traits and traits underlying cryptic female choice evolved even at low levels of sperm competition, highlighting the importance of considering cryptic female choice in theoretical models on the evolution of male traits (Kustra & Alonzo, 2023). Matilda (Tilly) Pembury-Smith (University of Stockholm) presented her work disentangling the male and female effects on a phenotype by measuring genotype x genotype interactions on sperm ejection rates across *Drosophila melanogaster* lines, while Mariana Wolfner's poster (Cornell University) focused on the components of the mating plug in this species. Mats Olsson (University of Gothenburg) used an extensive long-term data set on Swedish sand lizards (*Lacerta agilis*) and several classical measures to compare the strength of pre- and post-copulatory sexual selection in males and females. Estelle Barbot's (University of Montpellier) poster

looked at the pre- and post-pollination components of sexual selection in a flowering plant, refuting a long-assumed hypothesis on the relationship between male flower number and pollinator attraction, and showing that sexual selection acts on flower number in males by increasing pollen production and thus the opportunity to fertilise a higher number of female individuals (Barbot et al., 2022). Axel Wiberg (Stockholm University) investigated the impact of sexually antagonistic coevolution on remating rates in *D. melanogaster* and Caleb Ghione (University of Southern California) reviewed the literature for evidence of correlations between androgen response elements and sexual size dimorphism across mammals.

The primary function of spermatozoa is traditionally considered to be delivering the paternal haploid genome to the egg. However, it is now well recognised that sperm carry more than just DNA and that the environment experienced by a male can influence his offspring's phenotype. David Flores-Benitez (TU Dresden) characterised the lipidome of sperm and seminal fluid in bedbugs, suggesting that a male's diet may affect fertility, with potential carry-over effects to the next generation. Sperm cells are known to contain micro-RNA, whereas ribosomal and messenger RNA are assumed to be fragmented. In his talk, Xin Li (Zhejiang University) presented work detecting intact mRNA in mammalian sperm and asked if sperm RNAs can underlie transgenerational effects. Taking it a step further, Jukka Kekäläinen (University of Eastern Finland) contradicted the dogmatic belief that sperm cells are transcriptionally inert and presented evidence for translation and transcription in sperm. Considering ejaculate-mediated transgenerational effects, Rowan Lymberry (University of Western Australia) presented his work on paternal effects and the impact of diet quality on sperm function and fertilisation outcome. He asked if there is potential for genetic variance in the production of paternal effects between males. One of the mechanisms underlying paternal effects on sperm performance is selection on the Y chromosome. Yassi Hafezi (Cornell University) investigated the effects of Y-linked genes on sperm motility in *D. melanogaster*. Looking at the flip side of these mechanisms, Beverly Strassmann (University of Michigan) measured allele-specific expression in human placentas to detect biases in paternal versus maternal expression and its consequences over several generations of offspring.

One of the most popular and humorous traditions at BoS meetings is the poster synopses given by presenters before the start of each session. Our personal favourite was a beautiful poem written by ChatGPT at the prompt of Jessica Hadlow (University of Western Australia) on female reproductive fluid, sperm quality and fertilisation outcome in mussels (*Mytilus galloprovincialis*). Hadlow presented intriguing results showing that female reproductive fluid can rescue ageing-related declines in sperm motility in these broadcast spawners and select sperm within ejaculates (Hadlow et al., 2023a & 2023b). Other poster presenters used haikus, mysterious stories, videos and even 3D-printed genitals to convince the rest of the audience to visit their poster. At BoS16, the 33 presenters were given 30 s to

summarise and advertise their poster, a decrease from the full minute allowed at BoS15. Impressively everyone stayed on time and avoided the penalty for overrunning, which was a rendition of ABBA hits. This leads us to wonder what the organisers will do for BoS17.

Cutting-edge molecular techniques were well represented in the poster sessions, with Erica Leder (Göteborg University of Gothenburg) comparing spermatogenesis across bird species using transcriptomics, Daniel Marcu (University of East Anglia) showing the possibility for haploid selection using single-cell transcriptomics in zebrafish, Emma Whittington (University of Oslo) using proteomics to investigate the link between outer yolk membrane proteins and sperm morphology in passerine birds and Martin Garlovsky (TU Dresden) characterising the bedbug sperm proteome. Katerina Komrskova (Czech Academy of Sciences), Retha Kotze (University of the Western Cape) and Nalini Puniamorthy (National University of Singapore) presented work on the relationships between disease and fertility in humans, honeybees (*Apis mellifera*) and mosquitoes (*Aedes sp.*), respectively.

BoS meetings also have several nonscientific traditions, including a midweek walk. This year attendees were rewarded with a long walk in the beautiful Alhagen Wetlands Ecological Park followed by fantastic homemade gelato at Arlan restaurant in Nynäshamn. In the restaurant's first year selling gelato, attendees enjoyed a selection of no less than 12 different homemade gelatos, including the very popular and exotic cloudberry flavour, a new one for most attendees from outside of Scandinavia. The event, which contributed to serious queues in the owners' lovely garden, did not go unnoticed by the local news outlets (Anglerfjord, 2023). At its conclusion, BoS16 proved to be as full of exciting cutting-edge science and engaging discussions as its predecessors. BoS meetings continue to be attended by a supportive community of friends and colleagues. We hope to see many returning to or discovering this special atmosphere (and delicious food!) at the 17th Biology of Spermatozoa meeting, to be held 1, September 2025–5, September 2025, in Nynäshamn, Sweden. These meetings would not be possible without the hard work of Rhonda Snook and John Fitzpatrick and the members of their research groups at Stockholm University; Axel Wiberg, Dick Moberg, Matilda Pembury-Smith, Erin Macartney, Isabel Resende, and Ana Cristina Gomes.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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