

Somatic production of reactive oxygen species does not predict its production in sperm cells across Drosophila melanogaster lines

Biz Turnell, Luisa Kumpitsch, Anne-Cécile Ribou, Klaus Reinhardt

▶ **To cite this version:**

Biz Turnell, Luisa Kumpitsch, Anne-Cécile Ribou, Klaus Reinhardt. Somatic production of reactive oxygen species does not predict its production in sperm cells across Drosophila melanogaster lines. BMC Research Notes, 2021, 14 (1), pp.131. 10.1186/s13104-021-05550-7 . hal-03241047

HAL Id: hal-03241047
https://hal.science/hal-03241047

Submitted on 19 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

BMC Research Notes

RESEARCH NOTE

Open Access

Somatic production of reactive oxygen species does not predict its production in sperm cells across *Drosophila melanogaster* lines

Biz R. Turnell[1*], Luisa Kumpitsch[1], Anne-Cécile Ribou[2] and Klaus Reinhardt[1]

Abstract

Objective: Sperm ageing has major evolutionary implications but has received comparatively little attention. Ageing in sperm and other cells is driven largely by oxidative damage from reactive oxygen species (ROS) generated by the mitochondria. Rates of organismal ageing differ across species and are theorized to be linked to somatic ROS levels. However, it is unknown whether sperm ageing rates are correlated with organismal ageing rates. Here, we investigate this question by comparing sperm ROS production in four lines of *Drosophila melanogaster* that have previously been shown to differ in somatic mitochondrial ROS production, including two commonly used wild-type lines and two lines with genetic modifications standardly used in ageing research.

Results: Somatic ROS production was previously shown to be lower in wild-type Oregon-R than in wild-type Dahomey flies; decreased by the expression of alternative oxidase (AOX), a protein that shortens the electron transport chain; and increased by a loss-of-function mutation in *dj-1*β, a gene involved in ROS scavenging. Contrary to predictions, we found no differences among these four lines in the rate of sperm ROS production. We discuss the implications of our results, the limitations of our study, and possible directions for future research.

Keywords: Alternative oxidase, *Dj-1*β, *DJ-1*, Oxygen radicals, Antioxidants, Sperm ageing, Sperm storage

Introduction

Like sexually reproducing organisms, sperm cells have a life cycle during which they develop, age, and die. As in organisms, the ageing process in sperm cells leads to declines in both performance and fitness outcomes. Older sperm cells have reduced motility [1], velocity [2, 3] and fertilization capacity [4], and produce fewer and less viable offspring [4–6]. Sperm ageing has major consequences for sexual selection. On the male side, it may account for some of the wide, largely unexplained variation in competitive fertilization success [7–9]. On the female side, sperm ageing may influence both pre- and post-copulatory behaviour, driving frequent mating to refresh old sperm stores [7] and providing a potential mechanism for cryptic choice [10]. As in somatic cells, ageing in sperm is driven in large part by reactive oxygen species (ROS). When more ROS are produced than are scavenged by antioxidants, they can damage cellular components like lipids, proteins, and DNA. Sperm cells are particularly vulnerable to this oxidative stress, both because of their limited antioxidant reserves and because of the high concentration of oxidation-prone polyunsaturated fatty acids in their membranes [1].

Most cellular ROS are produced by the mitochondria during aerobic respiration. Production rates vary widely across species and are often negatively correlated with lifespan, as predicted by the mitochondrial free radical theory of ageing [11]. ROS production also commonly varies with tissue type [12–14], and cross-species

*Correspondence: biz.turnell@tu-dresden.de
[1] Applied Zoology, Institute of Zoology, Technische Universität Dresden, Zellescher Weg 20b, Raum 258, 01069 Dresden, Germany
Full list of author information is available at the end of the article

© The Author(s) 2021. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

differences in ROS production can themselves be tissue-dependent; that is, production may be higher in one species than in another, but only in certain tissues [15]. These species by tissue interactions raise the question of whether the ageing rate of sperm is at all related to the ageing rate of the organism producing them [16]. Such a relationship would have significant implications for the intersection of life history patterns and sexual selection. To our knowledge, however, no studies have compared sperm ROS production rates within or across species.

Here, we measure rates of sperm ROS production and compare them to previously reported rates of somatic mitochondrial (mt) ROS production in four *Drosophila melanogaster* lines: (1) wild-type Oregon-R; (2) wild-type Dahomey; and two genetically modified lines expressing, in the Dahomey background, either (3) alternative oxidase (AOX), a protein that decreases ROS formation by bypassing part of the electron transport chain; or (4) or a loss-of-function mutation in *dj-1*β, a gene involved in ROS scavenging. Sanz et al. [17], as predicted, found a negative correlation between lifespan and whole-body mtROS production across the two wild-type lines, with Oregon-R flies living longer and producing less ROS than Dahomey flies. In contrast, lifespan did not differ between flies expressing AOX, which produced more mtROS, and mutant *dj-1*β flies, which produced less.

We predicted that sperm ROS production would mirror somatic mtROS production, being lowest in in Oregon-R and in AOX-expressing flies, moderate in Dahomey flies, and highest in mutant *dj-1*β flies.

Main text

Materials and methods

Fly lines and husbandry

The Oregon-R line (referred to hereafter as "OR") is a wild-type line widely used in *Drosophila* research. UAS-AOX F6 [18], *daughterless*-Gal4 driver (daGal4) (previously BL55849) [19], *dj-1*$\beta^{GE23381}$ [20], and *white* wild-type Dahomey [21] (wDAH, referred to hereafter as "DAH") flies were kindly provided by Dr. Alberto Sanz (then University of Newcastle). The UAS-AOX F6, daGal4, and *dj-1*β lines had been backcrossed into the DAH background for 11, 11, and 7 generations, respectively [17]. In the current study, AOX-expressing AOX/daGal4 flies (referred to hereafter as "AOX") were generated by mating UAS-AOX F6 males to virgin daGal4 females. Flies were maintained at 25 °C and 60% humidity on a 12:12 light:dark cycle and fed on a yeast-corn-sugar medium (40 g/l yeast, 90 g/l corn meal, 100 g/l sucrose, 12 g/l agar, 40 ml/l nipagin [10% in ethanol] and 3 ml/l propionic acid in water). Males were collected upon eclosion and kept in groups of three to eight individuals.

Rate of reactive oxygen species production measured by time-resolved microfluorimetry

The rate of ROS production in sperm was measured as described in [14]. Briefly, time-resolved microfluorimetry of the oxygen probe 1-pyrene butyric acid (PBA) was used to measure the in situ production rate of ROS. PBA's fluorescence lifetime decreases upon collision with small ROS molecules like superoxide, but not upon collision with the stable, accumulating ROS molecules like hydrogen peroxide into which they transform [22]. PBA lifetime is unaffected by probe concentration and cell number [23]. Seminal vesicles from three-to-six-day-old virgin males were dissected in PBS and punctured to release the sperm, which were then incubated in 20 µl of 1 µM PBA in 2% ethanol on a coverslip for four minutes and washed three times with PBS to remove the excess probe. Sperm were laser-excited as described previously to generate the fluorescence decay curve [14]. Eight readings per sample were taken, resulting in eight decay curves that were then averaged to obtain the mean fluorescence lifetime. Relative rates of ROS production were calculated using the Stern–Volmer equation [14, 24]. All statistics were performed in R version 3.6.1 [25].

Results

Sperm ROS production rates, being non-normally distributed (Shapiro–Wilk normality test, $p < 1e{-}7$), were compared using a Kruskal–Wallis rank sum test. There was no difference across lines in the rate of ROS production in sperm (Fig. 1; $\chi^2 = 0.10$, $p = 0.99$, $n = 21$ per line; see Additional file 1 for raw data).

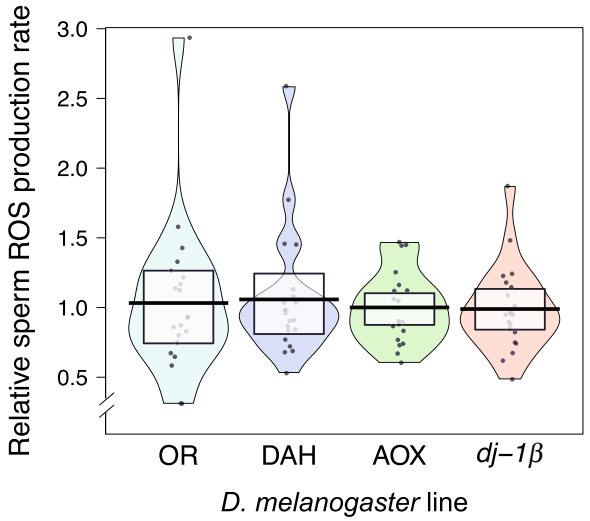


Fig. 1 Relative rate of production of reactive oxygen species in sperm from Oregon-R, Dahomey, AOX, and mutant *dj-1*β males, as measured by the fluorescence lifetime of the oxygen probe 1-pyrene butyric acid (PBA) and calculated by the Stern-Vomer equation (see text for details). Black line = mean, white box = 95% CI

Discussion

Sperm ageing, an underappreciated phenomenon, has the potential to drive selection on traits in males and females, pre- and post-mating, and at the gametic and organismal levels. Because sperm ageing shares a mechanism with somatic ageing—oxidative stress via ROS—a correlation between the rates of sperm and somatic ageing, whether across species or individuals, may be predicted. Here, we find no evidence for such a correlation. Contrary to expectations, sperm from wild-type Oregon-R (OR) flies, which have lower somatic mtROS production and longer lifespans [17], did not produce less ROS than sperm from high-mtROS, shorter-lived wild-type Dahomey (DAH) flies. More surprisingly, sperm ROS production did not differ between DAH and either of the two genetically modified lines with a DAH background: one expressing alternative oxidase (AOX) and producing less somatic mtROS, and one expressing a loss-of-function *dj-1*β mutation and producing more somatic mtROS.

Post-meiotic *D. melanogaster* sperm have been shown to undergo oxidative phosphorylation [14, 26], generating mtROS in the process [14, 27]. Sperm expressing AOX, which bypasses a major site of mtROS formation in the electron transport chain, complex III [28], would therefore be expected to show lower ROS production. Given our results, it seems likely that, while AOX is expressed in the testes and seminal vesicles of AOX males [29], it is not expressed in the germline itself. Although the daGal4 driver is supposedly ubiquitously expressed, it is not unusual for "ubiquitous" drivers to show no expression in germ cells [30]. Indeed, a different AOX construct driven by the also ubiquitous *α-tubulin* promoter was not expressed in the *D. melanogaster* male germline [29].

Unlike the AOX gene, the mutated *dj-1*β gene should, in theory, certainly be expressed in the germline. However, the dj-1*β* protein in *Drosophila* may chiefly promote the scavenging of hydrogen peroxide [31], a non-radical ROS whose production is not detected by our method. It is thus possible that mutant *dj-1*β sperm accumulates a greater amount of stable ROS than DAH sperm despite producing small radical ROS like superoxide at the same rate.

While sperm from the four lines did not differ in their rates of endogenous ROS production, they are likely to experience different levels of exogenous mtROS produced by the testes and seminal vesicles during spermatogenesis and storage. Sperm from AOX males may thus be expected to accumulate age-related cellular damage more slowly, despite producing normal amounts of ROS. Likewise, sperm from mutant *dj-1*β males may age more quickly due to decreased antioxidant production in the surrounding reproductive tissues, which would lead to less scavenging of ROS both exogenous and endogenous to the sperm.

The rate of sperm ageing may also be affected by the post-mating environment of the female reproductive tract. Sperm transferred to females in lines with low somatic mtROS production may survive longer in storage, regardless of their endogenous ROS production. Females may also mitigate the effects of mtROS, whether originating from the stored sperm itself or from the surrounding female tissues, by increasing antioxidant production. Antioxidant genes are upregulated in the sperm storage organs of mated females in *D. melanogaster* [32, 33] (reviewed in [34]), honeybees [35, 36], and ants [37], and high antioxidant levels have also been found in the female sperm storage organs of mammals and birds [38].

The rate of sperm ageing is likely affected by both male and female factors. Indeed, in a related study, we found indications of slower sperm ageing both in AOX sperm stored by control females and in control sperm stored by AOX females, as evidenced by the increased reproductive output of these crosses. Conversely, crosses involving mutant *dj-1*β males or females had decreased reproductive output, consistent with faster ageing in control-stored *dj-1*β sperm and in *dj-1*β-stored control sperm [39].

In conclusion, we predicted that sperm ROS production rates would correlate with somatic mtROS production rates across *D. melanogaster* lines. To our knowledge, this is the first study to compare sperm ROS production within or across species. While we found no between-line differences in the rates of sperm-endogenous ROS production, it is nonetheless possible that the lines differ in their rates of sperm ageing. Measuring cellular damage markers and antioxidant activity in male- and female-stored sperm, as well as the fitness outcomes of the individuals transferring and using that sperm, would help to shed further light on the factors influencing sperm ageing and on its evolutionary consequences.

Limitations

The fly lines used in this study were obtained directly from the authors of [17]. We did not independently verify either their genotypes or their rates of somatic mtROS production.

Abbreviations

AOX: Alternative oxidase; DAH: Wild-type Dahomey; mtROS: Mitochondrial reactive oxygen species; OR: Wild-type Oregon-R; PBA: 1-Pyrene butyric acid; ROS: Reactive oxygen species.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-021-05550-7.

Additional file 1. Fluorescence lifetime of PBA and relative rates of sperm ROS production.

Acknowledgements
Thanks to Alberto Sanz for providing fly lines.

Authors' contributions
BRT, LK, and KR conceived and designed the study. A-CR designed the instrumentation and software. LK collected the data. BRT analyzed the data and led the writing of the manuscript. All authors read and approved the final manuscript.

Funding
Open Access funding enabled and organized by Projekt DEAL. This work was supported by a National Science Foundation Postdoctoral Research Fellowship in Biology to BRT (1612234). KR was supported by the Deutsche Forschungsgemeinschaft through the Excellence Initiative Award to TU Dresden.

Availability of data and materials
All relevant data are included in additiona file.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1] Applied Zoology, Institute of Zoology, Technische Universität Dresden, Zellescher Weg 20b, Raum 258, 01069 Dresden, Germany. [2] Institut de Modélisation Et D'Analyse en Géo-Environnement Et Santé, Université de Perpignan Via Domitia, 52 Avenue Paul Alduy, 66860 Perpignan, France.

Received: 18 December 2020 Accepted: 30 March 2021
Published online: 07 April 2021

References

1. Aitken RJ, Gibb Z, Baker MA, Drevet J, Gharagozloo P. Causes and consequences of oxidative stress in spermatozoa. Reprod Fertil Dev. 2016;28:1–10.
2. Gasparini C, Dosselli R, Evans JP. Sperm storage by males causes changes in sperm phenotype and influences the reproductive fitness of males and their sons. Evol Lett. 2017;1:16–25.
3. Vega-Trejo R, Fox RJ, Iglesias-Carrasco M, Head ML, Jennions MD. The effects of male age, sperm age and mating history on ejaculate senescence. Funct Ecol. 2019;33:1267–79.
4. White J, Wagner RH, Helfenstein F, Hatch SA, Mulard H, Naves LC, et al. Multiple deleterious effects of experimentally aged sperm in a monogamous bird. Proc Natl Acad Sci USA. 2008;105:13947–52.
5. Tan CKW, Pizzari T, Wigby S. Parental age, gametic age, and inbreeding interact to modulate offspring viability in *Drosophila melanogaster*. Evolution. 2013;67:3043–51.
6. Tarín JJ, Pérez-Albalá S, Cano A. Consequences on offspring of abnormal function in ageing gametes. Hum Reprod Update. 2000;6:532–49. https://doi.org/10.1093/humupd/6.6.532.
7. Reinhardt K. Evolutionary consequences of sperm cell aging. Q Rev Biol. 2007;82:375–93.
8. Reinhardt K, Breunig HG, Uchugonova A, König K. Sperm metabolism is altered during storage by female insects: evidence from two-photon autofluorescence lifetime measurements in bedbugs. J R Soc Interface. 2015;12:20150609.
9. Evans JP, Wilson AJ, Pilastro A, Garcia-Gonzalez F. Ejaculate-mediated paternal effects: evidence, mechanisms and evolutionary implications. Reproduction. 2019;157:R109–26.
10. Firman RC, Gasparini C, Manier MK, Pizzari T. Postmating female control: 20 years of cryptic female choice. Trends Ecol Evol. 2017;32:368–82. https://doi.org/10.1016/j.tree.2017.02.010.
11. Barja G. Updating the mitochondrial free radical theory of aging: an integrated view, key aspects, and confounding concepts. Antioxid Redox Signal. 2013;19:1420–45. https://doi.org/10.1089/ars.2012.5148.
12. Tahara EB, Navarete FDT, Kowaltowski AJ. Tissue-, substrate-, and site-specific characteristics of mitochondrial reactive oxygen species generation. Free Radic Biol Med. 2009;46:1283–97.
13. Albrecht SC, Barata AG, Großhans J, Teleman AA, Dick TP. In vivo mapping of hydrogen peroxide and oxidized glutathione reveals chemical and regional specificity of redox homeostasis. Cell Metab. 2011;14:819–29.
14. Turnell BR, Reinhardt K. Metabolic rate and oxygen radical levels increase but radical generation rate decreases with male age in *Drosophila melanogaster* sperm. Journals Gerontol Ser A. 2020;75:2278–85.
15. Rodriguez KA, Wywial E, Perez VI, Lambert AJ, Edrey YH, Lewis KN, et al. Walking the oxidative stress tightrope: a perspective from the naked mole-rat, the longest-living rodent. Curr Pharm Des. 2011;17:2290–307.
16. Sepil I, Hopkins BR, Dean R, Bath E, Friedman S, Swanson B, et al. Male reproductive ageing arises via multifaceted mating-dependent sperm and seminal proteome declines, but is postponable in Drosophila. Proc Natl Acad Sci USA. 2020;117:17094–103.
17. Sanz A, Fernández-Ayala DJM, Stefanatos RKA, Jacobs HT. Mitochondrial ROS production correlates with, but does not directly regulate lifespan in Drosophila. Aging. 2010;2:200–23.
18. Fernández-Ayala DJM, Sanz A, Vartiainen S, Kemppainen KK, Babusiak M, Mustalahti E, et al. Expression of the Ciona intestinalis alternative oxidase (AOX) in Drosophila complements defects in mitochondrial oxidative phosphorylation. Cell Metab. 2009;9:449–60. https://doi.org/10.1016/j.cmet.2009.03.004.
19. Wodarz A, Hinz U, Engelbert M, Knust E. Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. Cell. 1995;82:67–76.
20. Park J, Sung YK, Cha GH, Sung BL, Kim S, Chung J. *Drosophila DJ-1* mutants show oxidative stress-sensitive locomotive dysfunction. Gene. 2005;361:133–9.
21. Broughton SJ, Piper MDW, Ikeya T, Bass TM, Jacobson J, Driege Y, et al. Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands. Proc Natl Acad Sci U S A. 2005;102:3105–10.
22. Oter O, Ribou A-C. Quenching of long lifetime emitting fluorophores with paramagnetic molecules. J Fluoresc. 2009;19:389–97.
23. Ribou A-C. Synthetic sensors for reactive oxygen species detection and quantification: a critical review of current methods. Antioxid Redox Signal. 2016;25:520–33.
24. Ribou A-C, Vigo J, Salmon J-M. Lifetime of fluorescent pyrene butyric acid probe in single living cells for measurement of oxygen fluctuation. Photochem Photobiol. 2004;80:274–80.
25. R Core Team. R: A language and environment for statistical computing. 2019.
26. Wetzker C, Reinhardt K. Distinct metabolic profiles in Drosophila sperm and somatic tissues revealed by two-photon NAD(P)H and FAD autofluorescence lifetime imaging. Sci Rep. 2019;9:19534. https://doi.org/10.1038/s41598-019-56067-w.
27. Guo R, Reinhardt K. Dietary polyunsaturated fatty acids affect volume and metabolism of *Drosophila melanogaster* sperm. J Evol Biol. 2020;1:1–7.
28. Miwa S, St-Pierre J, Partridge L, Brand MD. Superoxide and hydrogen peroxide production by *Drosophila* mitochondria. Free Radic Biol Med. 2003;35:938–48.
29. Saari S, Andjelković A, Garcia GS, Jacobs HT, Oliveira MT. Expression of Ciona intestinalis AOX causes male reproductive defects in *Drosophila melanogaster*. BMC Dev Biol. 2017;17:1–11.
30. White-Cooper H. Tissue, cell type and stage-specific ectopic gene expression and RNAi induction in the Drosophila testis. Spermatogenesis. 2012;2:1–12. https://doi.org/10.4161/spmg.19088.
31. Casani S, Gómez-Pastor R, Matallana E, Paricio N. Antioxidant compound supplementation prevents oxidative damage in a Drosophila model of

Parkinson's disease. Free Radic Biol Med. 2013;61:151–60. https://doi.org/10.1016/j.freeradbiomed.2013.03.021.
32. Mack PD, Kapelnikov A, Heifetz Y, Bender M. Mating-responsive genes in reproductive tissues of female *Drosophila melanogaster*. Proc Natl Acad Sci U S A. 2006;103:10358–63.
33. Prokupek AM, Kachman SD, Ladunga I, Harshman LG. Transcriptional profiling of the sperm storage organs of *Drosophila melanogaster*. Insect Mol Biol. 2009;18:465–75.
34. Heifetz Y, Rivlin PK. Beyond the mouse model: Using *Drosophila* as a model for sperm interaction with the female reproductive tract. Theriogenology. 2010;73:723–39. https://doi.org/10.1016/j.theriogenology.2009.11.001.
35. Weirich G, Collins A, Williams V. Antioxidant enzymes in the honey bee *Apis mellifera*. Apidologie. 2002;33:3–14.
36. Collins AM, Williams V, Evans JD. Sperm storage and antioxidative enzyme expression in the honey bee *Apis mellifera*. Insect Mol Biol. 2004;13:141–6. https://doi.org/10.1111/j.0962-1075.2004.00469.x.
37. Gotoh A, Shigenobu S, Yamaguchi K, Kobayashi S, Ito F, Tsuji K. Transcriptome profiling of the spermatheca identifies genes potentially involved in the long-term sperm storage of ant queens. Sci Rep. 2017;7:1–14. https://doi.org/10.1038/s41598-017-05818-8.
38. Holt WV, Fazeli A. Sperm storage in the female reproductive tract. Annu Rev Anim Biosci. 2016;4:291–310.
39. Turnell BR, Kumpitsch L, Reinhardt K. Production and scavenging of reactive oxygen species both affect reproductive success in male and female *Drosophila melanogaster*. Accepted to Biogerontology.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

