© 2016. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2016) 219, 1363-1368 doi:10.1242/jeb.133843

RESEARCH ARTICLE

Modification of sperm quality after sexual abstinence in Seba's short-tailed bat, *Carollia perspicillata*

Charlotte Wesseling[1,*], Nicolas Fasel[2,*,§], Heinz Richner[2,‡] and Fabrice Helfenstein[1,‡]

ABSTRACT

In polygynous mating systems, few males have stable access to sexual mates. With an expected higher copulation rate, harem males may deplete seminal fluids or increase epididymal sperm maturation, generating poor sperm quality. In a first study, we reported a higher sperm quality in sneaker males of *Carollia perspicillata*. To test whether the lower sperm quality observed in harem males was generated by an elevated copulation rate, we temporarily removed males of both social statuses from the colony. We thus assessed status-related changes of sperm quality resulting from sexual abstinence. Moreover, released from territory and female guarding, harem males were expected to show a reduction in somatic costs. On the basis of sperm competition models, we predicted a higher resource investment in the ejaculate with the reduction of pre-copulatory efforts. In line with our predictions, sperm quality of harem males improved significantly in contrast to sneaker males, whose sperm quality did not change. Without an increase in ejaculate lipid peroxidation, our results also provide evidence that the duration of sexual abstinence was not sufficient to generate sperm oxidative damage through senescence. Harem males did not show a reduction in blood lipid peroxidation or in the ratio of oxidized to reduced glutathione. In line with the maintenance of these somatic costs, harem males did not invest more superoxide dismutase to the ejaculate to maintain sperm quality. Our results suggest that a difference in copulation rate rather than an adaptation to sperm competition provides sneaker males with higher sperm quality in *C. perspicillata*.

KEY WORDS: Alternative reproductive tactics, Harem males, Sneaker males, Sperm competition, Copulation rate, Bats

INTRODUCTION

In polygynous mating systems, stable access to sexual mates is limited to a few males (Shuster and Wade, 2003). Harem males have to secure such a privileged position at a certain cost (Beaulieu et al., 2014; Corlatti et al., 2013). For the other males, the only way to reproduce is through sneaked copulations, and hence they face stronger sperm competition compared with harem males (Gross, 1996; Oliveira et al., 2008). Theory predicts that sneaker males may allocate a larger proportion of their resources into testicular and seminal functions to produce ejaculates of superior quality (Parker, 1990), and that harem males may reduce post-copulatory efforts as they generally invest more into pre-copulatory competition (e.g. courtship or guarding; Parker et al., 2013). Empirical evidence supporting theoretical models reveals adaptations in testicular production or accessory sexual glands by sneaker males (Firman et al., 2015; Simmons and Emlen, 2006; Stockley et al., 1994), but also an improvement of sperm quality including sperm velocity (Burness et al., 2004; Vladic and Jarvi, 2001) or motility (Kilgallon and Simmons, 2005). However, the mechanisms generating such intra-specific adaptations to sperm competition remain unclear. Sperm quality may be increased by modifications of sperm structure. Larger flagella or mid-pieces, as well as more flexible membranes, may allow spermatozoa to move faster (Blengini et al., 2014; Firman and Simmons, 2010; Safarinejad et al., 2010). Changes in sperm structure may, however, require the duration of a spermatogenesis cycle or of epididymal maturation period before they become conspicuous. Changes in the content of seminal fluid may provide the required nutrients and protection to sperm cells, improving sperm competitiveness on shorter notice (Perry et al., 2013; Poiani, 2006). Concurrently, sperm quality may be altered when sperm cells become damaged (e.g. by oxidative stress; Blount et al., 2001) or when they become senescent (Pizzari et al., 2008) before ejaculation. Sperm performance may also be reduced with shorter periods of sexual abstinence (Sukprasert et al., 2013), which may affect the efficiency of the seminal glands, the prostate or Cowper's glands (Dixson and Anderson, 2004), and thus the quality of seminal fluid.

Many bat species show some sort of polygyny, and harem males have stable and repetitive access to fertile females that allows them to mate at a higher frequency than sneaker males (Bradbury, 1977; Wilkinson, 1985). Although sperm cells of harem males are expected to face a low risk of senescence, they may have to swim within seminal fluid of poorer quality, with less protective and nutritive elements. A short period of sexual abstinence should allow sexual glands to recover and provide harem males with efficient seminal fluid, without detrimental effects caused by oxidative damage to sperm cells.

In a previous study (N.F., C.W., F.H. and H.R., unpublished data), we reported higher sperm quality in sneaker males than harem males of Seba's short-tailed bat [*Carollia perspicillata* (Linnaeus 1758)], a medium sized neotropical species. We further investigated three aspects of oxidative stress between social statuses: the allocation of the antioxidant superoxide dismutase (SOD) to the ejaculate (i.e. the ratio of ejaculate SOD capacity to blood SOD capacity), the relative ejaculate lipid peroxidation [malondialdehyde (MDA), a marker of oxidative damage; i.e. the ratio of ejaculate MDA concentration to blood MDA concentration], and the somatic redox balance [i.e. the ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) concentrations in the blood]. To test whether the lower ejaculate quality observed in harem males was generated by an elevated copulation rate, we imposed a period of sexual abstinence of 3 days to sneaker and harem males by removing them from the colony. In humans, a 3-day period of sexual abstinence provides optimal sperm quality (Levitas et al., 2005; Marshburn et al., 2014). Moreover, such a

[1]Department of Biology, University of Neuchâtel, 2000 Neuchâtel, Switzerland.
[2]Institute of Ecology and Evolution, University of Bern, 3012 Bern, Switzerland.
*Shared first authorship
‡Shared last authorship

§Author for correspondence (nicolas.fasel@iee.unibe.ch)

Received 27 October 2015; Accepted 23 February 2016

Journal of Experimental Biology (2016) 219, 1363-1368 doi:10.1242/jeb.133843

List of symbols and abbreviations

GSH	reduced glutathione
GSSH	oxidized glutathione
HPLC	high-performance liquid chromatography
MDA	malondialdehyde
RBC	red blood cells
SOD	superoxide dismutase
TBA	thiobarbituric acid
TBS	Tris-buffered saline
TEP	1,1,3,3-tetraethoxypropane
V_{CL}	curvilinear velocity

short abstinence period may not allow modifications of the sperm structure to take place (Morais et al., 2013). Under the assumptions that sneaker males copulate less frequently than harem males, and that 3 days of sexual abstinence is common for them, we predicted that sneaker males should not show changes in the SOD and MDA ratios, blood GSH ratio or sperm quality. Harem males should, however, show an improvement in sperm quality owing to the provision of richer seminal fluids, as long as the epididymial storage period of 3 days does not become detrimental to sperm cells (i.e. increase in MDA ratio due to an increase in absolute ejaculate MDA). In the absence of pre-copulatory expenditures (courtship and harem guarding) during the sexual abstinence period, we also predicted that the harem male blood MDA and GSH ratio would decrease. Moreover, based on the sperm competition models and the perceived lost of a privileged access to fertile females, we finally predicted an increase in the expenditure to the ejaculate with the reduction of pre-copulatory efforts in harem males. Indeed, isolated from the colony, males do not show any aggressive behaviour between them (N.F., unpublished data). Therefore, and provided that males can adjust the SOD capacity allocation within the period of sexual abstinence, the SOD ratio (SOD in sperm/SOD in blood) should increase significantly more in harem males than in sneaker males.

MATERIALS AND METHODS

Model species and studied population

The captive population of *C. perspicillata* was housed in a tropical zoo (Papilliorama, Kerzers FR, Switzerland). A colony of approximately 400 adults (sex ratio: 0.99 females to 1 male) was accommodated in a dome 40 m in diameter, which mimics a tropical habitat including an artificial cave where bats roost. The cave is open to visitors, and animals are used to human presence. A reversed light cycle enables researchers and visitors to observe the nocturnal activities of the bats from 09:30 to 21:30 h. Bats are fed twice a night with a fruit-based mixture.

Capture

Bats were caught with a harp-trap (Faunatech Austbat, Mount Taylor, Australia) mounted at the entrance of the cave, and a small fraction of focal individuals were captured with hand nets. Bats were ringed with a unique combination of three coloured plastic rings (A. C. Hughes, UK, size XB). Only males with functional, i.e. scrotal, testes (minimal volume 60 mm^3) were included in our study. Blood and ejaculates were sampled only of males of known social status. After blood collection, bats were placed in a cotton bag with food (a piece of apple) until ejaculate collection.

Social monitoring

The social environment of each male was monitored twice a month between 21:00 and 23:00 h at the beginning of the light period. Dawn is the time period where bats are still active but already cluster at their roosts. Videos were taken with camcorders under infrared light and then visualized on the computer to determine group sex ratio and the social status of males on the basis of spatial distribution of individuals. We defined social status using spot fidelity and access to females. Harem males were frequently observed on harem spots in the company of adult females (minimum 50% of observations, average observed was 81%). For the analysis, peripheral and bachelor males were merged and defined as sneaker males, in contrast with harem males, which have a privileged access to mates.

Blood collection

Blood samples were taken within 3 min of capture to avoid any potential influence of handling stress on measures of individual redox status. Blood was collected using microtainer tubes for capillary blood collection (Microvette CB 300, Sarstedt, Nümbrecht, Germany) by puncturing of the antebrachial vein. Samples were kept on ice until the blood was centrifuged at 21,913 ***g*** for 14 min at 4°C to allow the separation of red blood cells (RBC) from plasma, which were then stored in separate tubes (Eppendorf Tubes 3810×, Hamburg, Germany) and preserved at −80°C until analyses. In total, the blood of 27 harem, 19 peripheral and 27 bachelor males was collected.

Ejaculate collection

Ejaculates were collected by electro-ejaculation (Fasel et al., 2015). Males were first anaesthetized by a rodent nosecone non-rebreathing system (Rothacher Medical, Heitenried, Switzerland) of 0.8 l min^{-1} oxygen (Carbagas, Gümligen, Switzerland) mixed with 5% of isoflurane (Nicholas Piramal I, Mumbai, India) during approximately 5 s, and anaesthesia maintained with 1–2% isoflurane (v/v). After manipulation, pure oxygen was provided until bats regained awareness. For sperm collection, males were laid dorsally on a warming pad to maintain body temperature. Electric stimulations were generated with two electrodes situated at the end of a probe (ICSB, Sandy, OR, USA), which was inserted into the rectum with aqueous lubricant. The probe was linked to an audio amplifier (JVC A-X2, Yokohama, Japan) transmitting three series of regular and increasing electric stimulations (maximally 4 mA). The electrical current was continuously monitored with a milli-ampere-meter (Fluke 77 multimeter, Everett, WA, USA). In order to avoid desiccation, ejaculates were collected in 0.5 ml tubes (Eppendorf) holding 10 µl of pre-warmed (37°C) Tris-buffered saline (TBS) buffer. Once the complete ejaculate was collected, TBS volume was adjusted to obtain a 1:2 (v/v) dilution. From this mixture, 5 µl were transferred into 10 µl of pre-warmed Earle's balanced salt solution (SpermWash, Cryos, Aarhus, Denmark) buffer and kept warm for sperm mobility analysis. The remaining volume was stored on ice until it was placed into a −80°C freezer before further analyses.

Sperm quality analysis

Sperm motion was video-recorded for the first time within 10 min of ejaculation and then every 30 min until sperm had lost most of their motility (median: 90 min, max.: 210 min). Three microlitres of ejaculate mixed with Earle's balanced salt solution were placed in a 20-µm-deep chamber slide (SC 20-01-04-B, Leja, Nieuw-Vennep, Netherlands) under an Olympus XK41 microscope with dark-field conditions and mounted with a Kappa CF 8/5 camera at 200× magnification. Several 2 s videos (30 frames s^{-1}) were then analysed for each session using a CASA plug-in in ImageJ 1.47v

Journal of Experimental Biology (2016) 219, 1363-1368 doi:10.1242/jeb.133843

(National Institutes of Health, Bethesda, MD, USA) (Wilson-Leedy and Ingermann, 2007) to obtain estimates of sperm swimming parameters such as curvilinear velocity (V_{CL}, μm s^{-1}) and motility (proportion of moving spermatozoa). From those two measurements, we further estimated the decline in sperm velocity (sperm stamina) and motility (i.e. sperm survival; see 'Statistical analysis' below).

Blood and ejaculate cell homogenization

A volume of 20 μl of RBC was mixed with 20 μl of phosphate buffered saline, sonicated in an ice-cold water bath for 10 min and homogenized with four glass beads for 1 min at 30 Hz using an electric homogenizer. This homogenate was then aliquoted for analysis in 10 μl for MDA, 2 μl for GSH and 2 μl for the SOD assay, and stored at −80°C.

The ejaculate mixed with 1:2 (v/v) TBS was sonicated for 15 min and homogenized with two glass beads for 2 min at 30 Hz in a motorized homogenizer. This homogenate was then aliquoted for analysis in 10 μl for MDA and 1 μl for SOD assay, and stored at −80°C.

Lipid peroxidation

We assessed MDA (nmol ml^{-1}) by its reaction with thiobarbituric acid (TBA), which provides a pink pigment measurable by high performance liquid chromatography (HPLC) with fluorescence detection. MDA concentration estimation was assessed using a method adapted from Losdat et al. (2014).

All steps were conducted on ice to reduce oxidation. All chemicals were HPLC grade, and chemical solutions were prepared with ultra-pure water H_2OMQ (Milli-Q Synthesis; Millipore, Watford, UK). For calibration of the HPLC, a standard curve was prepared from a 1,1,3,3-tetraethoxypropane (TEP) stock solution (5 μmol l^{-1} in 40% ethanol) serially diluted using H_2OMQ, prepared freshly. A volume of 10 μl of homogenized samples or standards was first mixed with 40 μl of trichloroacetic acid 5% (v/v), allowing for the deproteinization of protein-bound MDA, and with 20 μl of 2-thiobarbituric acid TBA solution (42 mmol l^{-1}) for the acid-catalysed reaction. The TBA solution was prepared freshly by adding 30.89 mg of 98% TBA diluted with 5 ml of H_2OMQ, and dissolved on a stirring hot plate at 50°C. Mixtures of samples or standards, trichloroacetic acid and TBA were vortexed for 5 s, and 150 μl of H_2OMQ was added and then centrifuged for 14 min at 21,913 ***g*** at 4°C. The supernatant (205 μl) was transferred into screw-top tubes and incubated for exactly 60 min at 100°C in a dry bath, allowing the acid-catalyzed reaction for the formation of MDA–TBA adducts. Tubes were then cooled on ice for 5 min and vortexed for 10 s. Then, each tube was completed by adding 150 μl of butanol, vortexed and centrifuged for 10 min at 21,913 ***g*** at 4°C. The epiphase was transferred into Eppendorf tubes. Another 150 μl of butanol was added to each screw-top tube, and centrifuged again for 10 min at 21,913 ***g*** at 4°C. The second supernatant was collected and added to the first. Eppendorf tubes containing supernatant were evaporated in a Speedvac for 60 min at 35°C and then re-suspended in 90 μl of 30% (v/v) methanol, sonicated for 5 s and vortexed. Then, 70 μl were transferred into HPLC vial inserts (0.250 ml capacity) and stored at −80°C until HPLC analysis. Samples (5 μl) were injected into an Ultimate 3000 RSLC (Dionex, Thermo, Waltham, MA, USA) coupled to an Acquity UPLC BEH C18 column 1.7 μm, 2.1×50 mm, with temperature set at 30°C. Solution A was acetonitrile and solution B was ammonium acetate pH 6 (acetic acid 0.05% buffered at pH 6 with ammonium). The two solutions ran along a gradient with 5% to 100% solution A in 5 min, followed by 1.7 min with 100% solution A, then from 100% to 5% solution A in 0.8 min and 5% solution A until the end. Flow rate was 0.4 ml min^{-1} and total run time was 10 min. Retention time was 3.35 min and data were collected in a fluorescence detector (RF2000; Dionex) set at 515 nm (excitation) and 553 nm (emission). For calibration, a standard curve was prepared from a TEP stock solution [5 μmol l^{-1} in 40% (v/v) ethanol] serially diluted in 40% ethanol. TEP standards assayed in triplicate showed high repeatability (intra-class correlation coefficient=0.99, P<0.0001, n=12). Analyses were performed blindly with respect to the males' social status.

Antioxidant capacity

We assessed SOD activity (U ml^{-1}) using Cayman's SOD assay kit (Cayman Chemical, Ann Arbor, MI, USA), which is based on the detection of superoxide radicals generated by xanthine oxidase and neutralized by SOD. Homogenates were diluted in phosphate buffered saline 1:1000 (v:v) for the blood and 1:60 (v:v) for the ejaculate.

Sexual abstinence

After the first blood and sperm collection, bats were placed in a cage (100×100×200 cm) in groups of three to six males for 3 days. Social statuses were mixed and the number of males isolated in the cage was selected to limit the disturbance of the colony social structure. After this period of time, males were captured and a second set of blood and ejaculate samples was collected.

Statistical analysis

The analyses were performed with R (version 3.1.0; R Development Core Team 2008) using linear mixed models (function lme, package nlme). The significance level was set at 5%. Male identity was nested within cage number and both were considered as random factors.

With regard to our predictions, we first tested whether harem males showed stronger improvement in sperm quality traits [V_{CL}, sperm motility (proportion of spermatozoa moving; logit transformed), sperm stamina and sperm survival] after 3 days of sexual abstinence than sneaker males. Mobility traits were considered as response variables, and social status, collection order and their interaction as explanatory variables. Four univariate models were run.

Sperm survival and stamina were both defined by the linear (best data fit) slope of motility (logit transformed) and velocity over time. They were estimated with random slope and intercept linear mixed models, with ejaculate identity entered as a random factor (lme, package nlme).

Secondly, we tested whether several redox measures changed differently between social statuses, following a sexual abstinence period of 3 days. The absolute values of RBC and ejaculate SOD and MDA, the ratios of ejaculate SOD to RBC SOD and ejaculate MDA to RBC MDA, and the blood glutathione ratio (GSSG/GSH) were considered as response variables within univariate tests. Social status and collection order were considered as explanatory variables. The interactions including collection order were considered when the P-value was lower than 0.1 (Engqvist, 2005). In the case of a significant effect or a trend of the interaction term, both sessions were further analysed separately with a linear model and the effect of sexual abstinence was estimated without the social status factor with a linear mixed model. For those *post hoc* analyses, the significance level was reduced to 0.025. The absolute values of SOD and the SOD, MDA and GSH ratios were log transformed. The absolute values of MDA were square-root transformed. Variable transformations were performed to meet residual normality assumptions.

Journal of Experimental Biology (2016) 219, 1363-1368 doi:10.1242/jeb.133843

Animal welfare and ethics

Animal manipulation was performed as quickly after capture as possible to reduce stress. Post-anaesthesia recovery monitoring was systematically done by keeping bats for 1 to 3 h in individual cotton bags provided with food *ad libitum* (apple pieces). We recorded any injury or anomalous behaviour that could indicate excessive pain or stress and would require euthanizing the animal according to our guidelines. The veterinary office of the Canton Fribourg, after supervision of the Cantonal ethical committee, authorized the experimental setup and the detention conditions (FR_2013_46). Laboratory analyses were performed blindly with respect to sample identity.

RESULTS

Sperm quality traits

The effect of the interaction between collection order and social status on sperm velocity (V_{CL}, μm s^{-1}) was significant (22.35±9.28, $F_{1,41}$=4.70, P=0.021; Fig. 1). During the first collection, harem males had significantly slower spermatozoa (−16.45±6.06, $F_{1,34}$=7.24, P=0.011), but this was not the case after sexual abstinence (−2.73±7.65, $F_{1,33}$=0.13, P=0.723). The effect of sexual abstinence alone on sperm velocity was not significant (3.42±4.83, $F_{1,43}$=0.50, P=0.483).

The effect of the interaction between collection order and social status on sperm motility [proportion of motile sperm, logit(prop.)] was not significant (0.11±0.47, $F_{1,45}$=0.06, P=0.813). Motility did not differ significantly either between the two collections (−0.28±0.23, $F_{1,46}$=1.45, P=0.235) or between males of different social status (0.16±0.29, $F_{1,41}$=0.29, P=0.595).

Furthermore, the interaction effect of collection order and social status on sperm survival [i.e. the slope of decline in motility over time, logit(prop.) s^{-1}] was not significant (6.73×10^{-5}±3.83×10^{-5}, $F_{1,45}$=3.09, P=0.086). During the first collection, sperm survival of harem males was significantly lower than that of sneaker males (-6.95×10^{-5}±2.85×10^{-5}, $F_{1,37}$=5.95, P=0.020), but this was not the case after sexual abstinence (-1.05×10^{-6}±2.79×10^{-5}, $F_{1,37}$=0.14, P=0.708). Sexual abstinence alone did not significantly affect sperm survival (1.82×10^{-5}±1.92×10^{-5}, $F_{1,47}$=0.90, P=0.346).

Finally, the interaction effect of collection order and social status on sperm stamina (i.e. slope of the decline in sperm velocity over time, μm s^{-2}) was not significant (-3.12×10^{-4}±6.15×10^{-4}, $F_{1,41}$=0.26, P=0.615). Sperm stamina did not differ significantly either between the collections (-1.26×10^{-4}±3.07×10^{-4}, $F_{1,42}$=0.17, P=0.682) or between males of different social status (1.21×10^{-4}±3.31×10^{-4}, $F_{1,38}$=0.13, P=0.682).

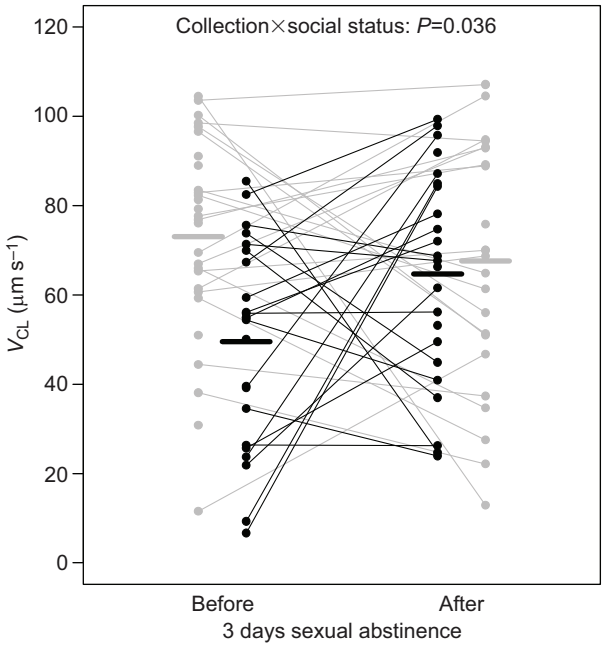

Fig. 1. Relationship between sperm velocity (curvilinear velocity, V_{CL}) and the interaction between collection order and social status in Seba's short-tailed bat, *Carollia perspicillata*. Harem males are represented with black points and lines, sneaker males with grey points and lines. The large horizontal bands represent the mean for each group.

Oxidative stress markers

The RBC SOD capacity [log(U ml^{-1})] did not significantly change with social status in interaction with collection order (−0.09±0.10, $F_{1,52}$=0.85, P=0.360). The RBC SOD was not significantly different either between males of different social status (0.01±0.05, $F_{1,52}$=0.01, P=0.905) or after sexual abstinence (0.06±0.05, $F_{1,53}$=1.22, P=0.274).

Secondly, the effect of the interaction between social status and collection order on ejaculate SOD capacity was not significant (0.29±0.28, $F_{1,41}$=1.05, P=0.311). The ejaculate SOD was not significantly different either between males of different social status (0.15±0.15, $F_{1,40}$=1.02, P=0.318) or after sexual abstinence (−0.12±0.14, $F_{1,42}$=0.73, P=0.399).

The RBC MDA concentration [sqrt(μmol l^{-1})] did not significantly change with social status in interaction with collection order (−0.06±0.10, $F_{1,55}$=0.32, P=0.574). Further, the RBC MDA was not significantly different either between males of different social status (−0.08±0.06, $F_{1,52}$=1.75, P=0.191) nor after sexual abstinence (−0.05±0.05, $F_{1,56}$=0.90, P=0.346).

The effect of the interaction between social status and collection order on the ejaculate MDA concentration [sqrt(μmol l^{-1})] was not significant (−0.04±0.11, $F_{1,49}$=0.12, P=0.734), and the ejaculate MDA did not differ significantly either between males of different social status (0.01±0.06, $F_{1,41}$=0.02, P=0.878) or after sexual abstinence (0.01±0.05, $F_{1,50}$=0.07, P=0.798).

For the ratio of ejaculate SOD capacity to RBC SOD capacity [log(ejac. SOD/RBC SOD)], the effect of social status in interaction with collection order was not significant (0.50±0.29, $F_{1,38}$=2.94, P=0.094). During the first and second collections, the SOD ratio did not differ significantly between males of different social status (−0.07±0.19, $F_{1,35}$=0.13, P=0.722) and (0.42±0.24, $F_{1,31}$=3.08, P=0.089). Sexual abstinence alone did not significantly affect the SOD ratio (−0.13±0.14, $F_{1,40}$=0.80, P=0.377).

The ratio of ejaculate MDA concentration to RBC MDA concentration [log(ejac. MDA/RBC MDA)] did not significantly change with social status in interaction with collection order (−0.04±0.40, $F_{1,42}$=0.01, P=0.923). The MDA ratio did not differ significantly either between males of different social status (0.13±0.25, $F_{1,40}$=0.25, P=0.619) or after sexual abstinence (0.12±0.19, $F_{1,43}$=0.37, P=0.546).

Finally, the effect of social status in interaction with collection order on the blood glutathione ratio [log(RBC GSSG/RBC GSH)] was not significant (−0.47±0.26, $F_{1,51}$=3.22, P=0.079). However, during the first and second collections, the blood glutathione ratio differed significantly between males of different social status (0.47±0.15, $F_{1,50}$=9.83, P=0.003) and (0.33±0.14, $F_{1,40}$=5.34, P=0.026). Sexual abstinence alone did not significantly affect the blood glutathione ratio (0.15±0.13, $F_{1,53}$=1.22, P=0.275).

Journal of Experimental Biology

DISCUSSION

Our results support the hypothesis that the copulation rate of harem males decreases sperm quality. Indeed, after 3 days of sexual abstinence, harem males showed a sperm velocity similar to that of sneaker males (Fig. 1). Moreover, the effect of the interaction between the social status and the collection order was not significant, sperm survival showed a similar pattern. This improvement of the sperm quality in harem males could be explained by the provision of richer seminal fluids, owing to sexual abstinence and the replenishment of seminal glands (Sukprasert et al., 2013). Furthermore, a longer storage period of the sperm cells in the cauda epididymis or in the vas deferens may influence the acquisition of specific membrane proteins (Dacheux and Dacheux, 2014) and the lipid remodelling of the membrane composition (Rejraji et al., 2006), which is expected to improve sperm performance (Ollero et al., 1996, 1994).

Harem males did not show a stronger increase in absolute (i.e. lipid peroxidation; MDA) or relative (i.e. ratio of ejaculate MDA to RBC MDA) ejaculate oxidative damage after a short period of sexual abstinence. This result indicates that lipid peroxidation in the ejaculate did not increase within 3 days due to ageing sperm cells. Similar results have also been found in humans (Marshburn et al., 2014). Despite a reduction of post-copulatory expenditures (e.g. harem guarding or female guarding), harem males showed neither a significant decrease in the glutathione ratio (GSSG/GSH) and RBC MDA, nor an increase in both relative and absolute SOD capacity in the ejaculate after the sexual abstinence period. The glutathione ratio did indeed stay higher for harem males than for sneaker males. This result reveals that the modification of the glutathione balance and the reduction in RBC MDA may require more than 3 days to adjust to a novel social environment. The maintenance of these somatic costs may explain the absence of an increase in the allocation of the antioxidant SOD to the ejaculate, predicted by the sperm competition model (Parker et al., 2013). Concurrently, such physiological adaptations to our treatment may involve antioxidants other than SOD (other endogenous enzymes or dietary antioxidants). These adaptations could additionally require an increased sperm competition level with the presence of mated females, a factor not included in our experiment.

In conclusion, after 3 days of abstinence, harem males produced spermatozoa of similar quality to that of sneaker males. This result suggests that a difference in copulation rate rather than an adaptation to sperm competition might explain the higher sperm quality of sneaker males in *C. perspicillata*.

Acknowledgements

We are thankful to the team of the Papiliorama for allowing us to work with their bat colony under excellent research conditions. We warmly thank Ophélie Gning, Gaëtan Glauser and Armelle Vallat for their help and advice in conducting biochemical analyses, and Ahana Fernandez, Felizia Koch, Alvaro Sobrino and Laura Panchione for their assistance in the social monitoring. We are also grateful to the world bat library (Geneva) for providing free literature on bat research.

Competing interests

The authors declare no competing or financial interests.

Author contributions

N.F., H.R. and F.H. designed the study. C.W. and N.F. carried out the experiment, analysed the data and wrote the manuscript. All authors gave final approval for publication.

Funding

This study was funded by the University of Bern and the Swiss National Science Foundation (grant no. 31003A_122566 to H.R., and grant no. PP00P3_139011/1 to F.H.).

Data availability

Data are available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.j71qr.

References

Beaulieu, M., Mboumba, S., Willaume, E., Kappeler, P. M. and Charpentier, M. J. E. (2014). The oxidative cost of unstable social dominance. *J. Exp. Biol.* **217**, 2629-2632.

Blengini, C. S., Sergio, N., Gabriela, C., Giojalas, L. C. and Margarita, C. (2014). Variability in sperm form and function in the context of sperm competition risk in two *Tupinambis* lizards. *Ecol. Evol.* **4**, 4080-4092.

Blount, J. D., Møller, A. P. and Houston, D. C. (2001). Antioxidants, showy males and sperm quality. *Ecol. Lett.* **4**, 393-396.

Bradbury, J. W. (1977). Lek mating behavior in the hammer-headed bat. *Z. Tierpsychol.* **45**, 225-255.

Burness, G., Casselman, S. J., Schulte-Hostedde, A. I., Moyes, C. D. and Montgomerie, R. (2004). Sperm swimming speed and energetics vary with sperm competition risk in bluegill (*Lepomis macrochirus*). *Behav. Ecol. Sociobiol.* **56**, 65-70.

Corlatti, L., Bassano, B. and Valencak, T. G. (2013). Foraging strategies associated with alternative reproductive tactics in a large mammal. *J. Zool.* **291**, 111-118.

Dacheux, J.-L. and Dacheux, F. (2014). New insights into epididymal function in relation to sperm maturation. *Reproduction* **147**, R27-R42.

Dixson, A. F. and Anderson, M. J. (2004). Sexual behavior, reproductive physiology and sperm competition in male mammals. *Physiol. Behav.* **83**, 361-371.

Engqvist, L. (2005). The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Anim. Behav.* **70**, 967-971.

Fasel, N. J., Helfenstein, F., Buff, S. and Richner, H. (2015). Electroejaculation and semen buffer evaluation in the microbat *Carollia perspicillata*. *Theriogenology* **83**, 904-910.

Firman, R. C. and Simmons, L. W. (2010). Sperm midpiece length predicts sperm swimming velocity in house mice. *Biol. Lett.* **6**, 513-516.

Firman, R. C., Garcia-Gonzalez, F., Thyer, E., Wheeler, S., Yamin, Z., Yuan, M. and Simmons, L. W. (2015). Evolutionary change in testes tissue composition among experimental populations of house mice. *Evolution* **69**, 848-855.

Gross, M. R. (1996). Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol. Evol.* **11**, 92-98.

Kilgallon, S. J. and Simmons, L. W. (2005). Image content influences men's semen quality. *Biol. Lett.* **1**, 253-255.

Levitas, E., Lunenfeld, E., Weiss, N., Friger, M., Har-Vardi, I., Koifman, A. and Potashnik, G. (2005). Relationship between the duration of sexual abstinence and semen quality: analysis of 9,489 semen samples. *Fertil. Steril.* **83**, 1680-1686.

Losdat, S., Helfenstein, F., Blount, J. D. and Richner, H. (2014). Resistance to oxidative stress shows low heritability and high common environmental variance in a wild bird. *J. Evol. Biol.* **27**, 1990-2000.

Marshburn, P. B., Giddings, A., Causby, S., Matthews, M. L., Usadi, R. S., Steuerwald, N. and Hurst, B. S. (2014). Influence of ejaculatory abstinence on seminal total antioxidant capacity and sperm membrane lipid peroxidation. *Fertil. Steril.* **102**, 705-710.

Morais, D. B., de Paula, T. A. R., Barros, M. S., Balarini, M. K., de Freitas, M. B. D. and da Matta, S. L. P. (2013). Stages and duration of the seminiferous epithelium cycle in the bat Sturnira lilium. *J. Anat.* **222**, 372-379.

Oliveira, R. F., Taborsky, M., Brockmann, H. J. (2008). *Alternative Reproductive Tactics: An Integrative Approach*. Cambridge: Cambridge University Press.

Ollero, M., Pascual, M. L., Muiñio-Blanco, T., Cebrián-Pérez, J. A. and López-Pérez, M. J. (1994). Revealing surface changes associated with maturation of ram spermatozoa by centrifugal counter-current distribution in an aqueous two-phase system. *J. Chromatogr. A* **668**, 173-178.

Ollero, M., Muiño-Blanco, T., López-Pérez, M. J. and Cebrián-Pérez, J. A. (1996). Viability of ram spermatozoa in relation to the abstinence period and successive ejaculations. *Int. J. Androl.* **19**, 287-292.

Parker, G. A. (1990). Sperm competition games: sneaks and extra-pair copulations. *Proc. R. Soc. B Biol. Sci.* **242**, 127-133.

Parker, G. A., Lessells, C. M. and Simmons, L. W. (2013). Sperm competition games: a general model for precopulatory male–male competition. *Evolution* **67**, 95-109.

Perry, J. C., Sirot, L. K. and Wigby, S. (2013). The seminal symphony: how to compose an ejaculate. *Trends Ecol. Evol.* **28**, 414-422.

Pizzari, T., Dean, R., Pacey, A., Moore, H. and Bonsall, M. B. (2008). The evolutionary ecology of pre- and post-meiotic sperm senescence. *Trends Ecol. Evol.* **23**, 131-140.

Poiani, A. (2006). Complexity of seminal fluid: a review. *Behav. Ecol. Sociobiol.* **60**, 289-310.

Rejraji, H., Sion, B., Prensier, G., Carreras, M., Motta, C., Frenoux, J.-M., Vericel, E., Grizard, G., Vernet, P. and Drevet, J. R. (2006). Lipid remodeling of murine

epididymosomes and spermatozoa during epididymal maturation. *Biol. Reprod.* **74**, 1104-1113.

Safarinejad, M. R., Hosseini, S. Y., Dadkhah, F. and Asgari, M. A. (2010). Relationship of omega-3 and omega-6 fatty acids with semen characteristics, and anti-oxidant status of seminal plasma: a comparison between fertile and infertile men. *Clin. Nutr.* **29**, 100-105.

Shuster, S. M. and Wade, M. J. (2003). *Mating Systems and Strategies*. Princeton, NJ: Princeton University Press.

Simmons, L. W. and Emlen, D. J. (2006). Evolutionary trade-off between weapons and testes. *Proc. Natl. Acad. Sci. USA* **103**, 16346-16351.

Stockley, P., Searle, J. B., Macdonald, D. W. and Jones, C. S. (1994). Alternative reproductive tactics in male common shrews: relationships between mate-searching behaviour, sperm production, and reproductive success as revealed by DNA fingerprinting. *Behav. Ecol. Sociobiol.* **34**, 71-78.

Sukprasert, M., Wongkularb, A. and Rattanasiri, S. (2013). The effects of short abstinence time on sperm motility, morphology, and DNA Damage. *Andrology* **2**, 1-5.

Vladic, T. V. and Jarvi, T. (2001). Sperm quality in the alternative reproductive tactics of Atlantic salmon: the importance of the loaded raffle mechanism. *Proc. R. Soc. B Biol. Sci.* **268**, 2375-2381.

Wilkinson, G. S. (1985). The social-organization of the common vampire bat. 1. Pattern and cause of association. *Behav. Ecol. Sociobiol.* **17**, 111-121.

Wilson-Leedy, J. G. and Ingermann, R. L. (2007). Development of a novel CASA system based on open source software for characterization of zebrafish sperm motility parameters. *Theriogenology* **67**, 661-672.