

Research



Cite this article: Simmons LW, Lovegrove M. 2017 Socially cued seminal fluid gene expression mediates responses in ejaculate quality to sperm competition risk. *Proc. R. Soc. B* **284**: 20171486.
<http://dx.doi.org/10.1098/rsob.2017.1486>

Received: 2 July 2017

Accepted: 20 July 2017

Subject Category:

Evolution

Subject Areas:

evolution, behaviour

Keywords:

strategic ejaculation, seminal fluid, *Teleogryllus oceanicus*

Author for correspondence:

Leigh W. Simmons

e-mail: leigh.simmons@uwa.edu.au

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.3844774>.

Socially cued seminal fluid gene expression mediates responses in ejaculate quality to sperm competition risk

Leigh W. Simmons and Maxine Lovegrove

Centre for Evolutionary Biology, School of Biological Sciences (M092), The University of Western Australia, Crawley 6009, Australia

LWS, 0000-0003-0562-1474

There is considerable evidence that males will increase the number of sperm ejaculated in response to sperm competition risk. However, whether they have the capacity to adjust seminal fluid components of the ejaculate has received less attention. Male crickets (*Teleogryllus oceanicus*) have been shown to adjust the viability of sperm in their ejaculate in response to sperm competition risk. Here we show that socially mediated plasticity in sperm viability is probably due, at least in part, to male adjustments in the protein composition of the seminal fluid. Seven seminal fluid protein genes were found to have an increased expression in males exposed to rival calls. Increased expression of these genes was correlated with increased sperm viability in whole ejaculates, and gene knockdown confirmed that at least one of these proteins promotes sperm viability. Our results lend support for recent theoretical models that predict complex responses in male allocation to seminal fluid composition in response to sperm competition risk.

1. Introduction

It is now widely appreciated that females, like males, can increase their reproductive success by accepting more than one mating partner [1–3]. Multiple mating by females (polyandry) has wide-ranging evolutionary consequences [4,5], not least the selection imposed on males through sperm competition [6] and cryptic female choice [7].

When females mate with more than one male, the sperm from these males must compete to fertilize a limited supply of ova. Among species, increased risk (the probability that females will mate with one other male) and intensity (the average number of males mated) of sperm competition are both predicted to favour the evolution of increased expenditure on the ejaculate [8]. Within species, however, males are predicted to increase their immediate allocation of ejaculate reserves to individual females as the risk of sperm competition increases, but to decrease their allocation with increasing intensity of sperm competition because the payoff per unit allocation will decline as the number of competing males increases [8]. Considerable research effort has been made to test these theoretical predictions. Thus, there is taxonomically widespread evidence of evolutionary associations between the strength of selection from sperm competition and testes mass, productivity, and sperm form and function (reviewed in [9,10]). Within species, males have been found to allocate greater numbers of sperm to females when they perceive the presence of sperm competition rivals in their environment [11,12]. Empirical tests of sperm competition theory have thus focused primarily on the production and allocation of sperm. However, sperm represent only one component of the ejaculate.

Sperm are delivered to the female bathed in a cocktail of seminal fluid proteins (sfps) derived from the male accessory glands. Seminal fluid proteins have significant effects on sperm function, including the promotion of motility,

capacitation and fertilization efficacy [13]. They also protect sperm within the female tract, and can profoundly affect female reproductive behaviour and physiology [9,13–15]. Given their role in promoting male fertilization success we might expect that sfps, like sperm, should be subject to selection from sperm competition [9]. Indeed, seminal fluid proteins include the most rapidly diverging proteins known, and comparative studies suggest that sfp divergence is correlated with the strength of selection from sperm competition [16–20]. Within species, studies of fish with alternative reproductive tactics have reported greater velocity of sperm produced by sneaker males, with differences in sperm performance in at least one species being due to differences in seminal fluid [21]. A handful of studies have reported phenotypic plasticity in sperm velocity in response to social cues to sperm competition [22,23] or female quality [24,25], which might also be due to plasticity in seminal fluid composition [26,27].

Theoretical models are beginning to be developed in order to predict how males should allocate their seminal fluids in response to sperm competition [28–30]. The broad predictions arising from these models make intuitive sense, in that males are predicted to increase their allocation to sfps that enhance fertilization success and/or decrease their allocation to sfps that enhance female fecundity when they face sperm competition. However, tests of these predictions are currently limited to just two species, *Drosophila melanogaster* and house mice *Mus musculus*. Male *D. melanogaster* transfer a sfp (ovulin) that stimulates females to manufacture and lay eggs, and a second sfp (sex peptide) that inhibits female remating. When males mate with previously mated females they reduce their allocation of ovulin, though not sex peptide, because reproduction is already triggered in these females by their previous mates [31]. Social manipulations have also found that males exposed to rivals during copulation transfer more sex peptide and ovulin to females than males copulating in isolation [32], but exposure to rivals prior to mating results in a decreased expression of *Acp26a*, the gene responsible for ovulin production, and a third sfp gene, *Acp62f*, implicated in defensive sperm competition success [33]. In house mice, exposure to rival male scent during sexual development was found to increase the abundance of three sfps in the seminal vesicles (SVS6, SVS5 and CEACAM 10), supporting the idea that sfp production can exhibit socially cued phenotypic plasticity [34]. Though the effect of these sfps on competitive fertilization success is yet to be fully understood, CEACAM has been reported to enhance sperm motility [35]. These studies have been possible because *Drosophila* and house mice are both well characterized genomically. Functionally characterizing individual seminal fluid proteins in non-model organisms remains challenging [27]. Nonetheless, here we document socially cued phenotypic plasticity in sfp gene expression, and identify those sfps that contribute to sperm fertilization competency in a non-model organism, the Australian field cricket *Teleogryllus oceanicus*.

When exposed to the sexual advertisement calls of rival males during their development, male *T. oceanicus* produce ejaculates with a greater proportion of viable sperm compared with males reared in acoustic isolation [36]. Moreover, manipulating male perceptions of sperm competition intensity, by the perfuming of females with cuticular hydrocarbons from increasing numbers of rival males, has the effect of reducing the proportion of viable sperm in a male's ejaculate [37]. These studies show that males can make finely tuned

adjustments to the quality of their ejaculates in response to the risk and intensity of sperm competition (see also [38,39]). Interestingly, sperm viability has a greater impact on a male's competitive fertilization success [40] than does the number of sperm [41], and the reciprocal transfer of sperm between the seminal fluid fractions of rival ejaculates suggests that variation in seminal fluid composition might underlie the strategic adjustments in sperm viability seen in this species [42]. Proteomic analyses have identified at least 21 different proteins in the seminal fluid of *T. oceanicus* [43], and eleven of these proteins were found to increase in abundance in ejaculates as males mature sexually [44]. Both sperm viability [40] and competitive fertilization success [45] also increase with male age, suggesting these eleven sfps as candidate proteins for the strategic adjustment of seminal fluid composition, and thus sperm viability, in response to sperm competition. Here we examined sperm viability and quantified sfp gene expression for males exposed to the calls of rival males during their development, and compared these with males reared in acoustic isolation. We show that the expression of seven sfp genes vary in response to sperm competition risk and use RNA interference to identify which of these sfps are responsible for the strategic adjustments in sperm viability found in this cricket.

2. Material and methods

(a) Social manipulation

Animals used in this study were taken from a large outbred stock derived originally from a fruit plantation in Carnarvon Western Australia, and seeded annually with newly caught individuals. First instar nymphs were taken at hatching and assigned randomly to one of two treatments. The first treatment group was exposed throughout their development to the calls of conspecifics via five boxes containing approximately 100 adult sexually active crickets, placed within a 2 m radius of the experimental crickets. Crickets were thus exposed continually to both the calling and courtship calls of conspecifics at a sound intensity of 70–80 dB. The second treatment group was raised in acoustic isolation (silence) [36].

Crickets were initially raised en masse at 29°C on a 12:12 h light:dark cycle with constant access to cat chow and water. At the seventh nymphal instar, when sex can first be determined, females were discarded and males housed individually in boxes (7 × 7 × 5 cm) separated from each other by cardboard divisions that prevented visual contact. Crickets were then monitored daily until they emerged as adults. Upon emergence the sound-producing forewings of males in both treatments were removed to prevent them from producing their own calls.

(b) Sperm viability

Fourteen days after the adult molt, a spermatophore was removed from the genital pouch of 32 males from each treatment and assessed for sperm viability. The spermatophore was placed on a slide with 20 µl of Beadle saline (128.3 mM NaCl, 4.7 mM KCl and 23 mM CaCl₂). The evacuating tube was severed with fine scissors and the sperm were allowed to completely evacuate over a period of 10 min. The ejaculate was mixed gently with the saline on the slide and 5 µl transferred to a second slide. Five microlitres of SYBR 14 (1 mM), diluted 1:50 with Beadle saline, was added and the sample incubated in the dark for 10 min. Two microlitres of propidium iodide (2.4 mM) was then added and incubated for a further 10 min. The sperm were then viewed using a fluorescence microscope, and the number of live (stained green with SYBR 14) and dead (stained

red with propidium iodide) sperm in the first 500 sperm counted was used to estimate sperm viability (the proportion of live sperm in the sample). After assessment of ejaculate quality crickets were frozen at -20°C . The proportion of viable sperm in an ejaculate was square-root-arcsine transformed for statistical analysis.

(c) Gene expression

Each cricket was thawed and the accessory gland removed, weighed and placed in RNALater (Life Technologies), stored overnight at 4°C and then placed at -20°C . Using the PureLink RNA mini kit (Life Technologies), RNA was extracted from the entire accessory gland following the manufacturer's instructions. The tissue was disrupted using a micropestle (Interpath) and then a homogenizer (Life Technologies). RNA was removed using an on-column PureLink DNase treatment (Life Technologies). The RNA yield was quantified using a Qubit 2.0 fluorometer (Life Technologies). The elution volume for the RNA was $100\ \mu\text{l}$ and yields ranged from 366 to $1200\ \mu\text{g}\ \text{ml}^{-1}$ per sample. Two micrograms of each RNA sample were converted to cDNA in a $20\ \mu\text{l}$ reaction volume using the high capacity RNA-to-cDNA kit (Life Technologies), following the manufacturer's instructions.

Ten seminal fluid proteins and one sperm protein were selected for gene expression assays based on their observed changes in abundance in the seminal fluid with age [44]. We used *actin* as the reference gene (Isotig01761 from the *T. oceanicus* sequence database lodged with Genbank under the project number SRA044883.2 [46]). The six TaqMan gene expression custom assays (Life Technologies) designed previously [44] were used, and in addition, a further six assays were designed using the online Custom TaqMan assay design tool. Forward, reverse and reporter sequences for the 12 assays can be found in electronic supplementary material, table S1. In order to determine a suitable starting concentration of cDNA for the new gene expression assays, a standard curve of cDNA was prepared from one of the acoustic isolation samples, to include 100, 10, 1 and $0.1\ \text{ng}\ \mu\text{l}^{-1}$. Gene expression assays were then set up in triplicate for each of the standards and for each of the assays as follows: $1\ \mu\text{l}$ cDNA, $5\ \mu\text{l}$ $2\times$ TaqMan Gene expression master mix (Life Technologies) and $0.5\ \mu\text{l}$ $20\times$ TaqMan custom assay mix in a $10\ \mu\text{l}$ reaction volume. The assays were run in compatible 96-well plates on a StepOne Plus Real-Time PCR system (Life Technologies) using the following cycling conditions: 50°C for 2 min, 95°C for 10 min, and then 40 cycles of 95°C for 15 s and 60°C for 1 min. The efficiency of each assay was assessed with all R^2 values greater than 0.994 and slopes from -3.772 to -3.282 corresponding to efficiencies varying from 84% to 102%. As the efficiency for the assay corresponding to *ToSfp005* (isotig01832) was low at 84%, the standard curve was repeated with the following concentrations of cDNA: 100, 50, $25\ \mu\text{l}^{-1}$, 12.5, 6.25 and $3.125\ \text{ng}\ \mu\text{l}^{-1}$. The R^2 value for this assay was 0.994, slope -3.58 and the efficiency 90%, all within acceptable limits. For 10 of the genes, 10 ng cDNA gave average C_T values varying from 19.97 to 26.93, so this was the chosen input amount for all further assays. For *ToSfp026* (isotig06304), 10 ng cDNA gave a C_T value of 29.36, which is unacceptably high, so this assay required 100 ng of input cDNA to bring the C_T value within acceptable limits.

Gene expression assays were carried out on all samples using the cDNA amounts determined above, each assay run in triplicate for each candidate gene and reference gene, and with negative controls (without cDNA) on each plate. Results were analysed by STEPONE software v. 2.3 (Life Technologies) and the results exported into DATAASSIST v. 3.0 software (Life Technologies) for sample comparison using the comparative C_T ($\Delta\Delta C_T$) method [47]. The expression of *actin* did not differ between treatment groups (CT: male calls, 22.74 ± 0.09 ; silence 22.62 ± 0.10 ; $t_{61} = 0.94$, $p = 0.350$) so was an appropriate gene to use as a reference gene.

(d) Interference RNA

We explored the function of seven seminal fluid genes that exhibited socially mediated changes in expression using RNAi. Double-stranded RNA was prepared using the Megascript RNAi kit (Thermo Fisher Scientific). The manufacturer recommends dsRNA 400 bp or larger so PCR primers were designed with GENEIOUS v. R6 [48] to give a product of approximately 500 bp (see electronic supplementary material, table S2) using the RNA sequence data for the genes previously identified. The T7 promoter sequence was added to the 5' end of each primer. Template DNA was then prepared in four identical polymerase chain reactions (PCRs), each $50\ \mu\text{l}$ reaction containing $1\times$ PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), 1.5 mM MgCl_2 , 200 μM of each dNTP, 250 nM of forward primer, 250 nM of reverse primer, 2.5 units of Platinum Taq polymerase and 100 ng cDNA. PCR amplification was performed with cycling conditions as follows: 94°C for 3 min, then 35 cycles of 94°C for 30 s, 60°C for 60 s and 72°C for 60 s, and finally 72°C for 15 min. For genes *ToSfp011* (Isotig1709) and *ToSfp022* (Isotig444), a two-step PCR reaction was performed as follows: the same concentrations of reagents as above were used with the addition of 10% DMSO and an increase in MgCl_2 concentration from 1.5 to 3 mM and cycling conditions of 94°C for 3 min, then 35 cycles of 94°C for 30 s and 68°C for 60 s, and finally 72°C for 5 min (all reagents were from Thermo Fisher Scientific). The four PCR reactions were pooled and purified using the Favorgen PCR clean-up kit (Fisher Biotech) with elution into $40\ \mu\text{l}$. The DNA was then ethanol precipitated using 1/10 volume of 3 M sodium acetate pH 5.2 and 2 volumes of 100% ethanol. The DNA pellet was resuspended in $20\ \mu\text{l}$ elution buffer and the concentration determined using a Nanodrop 1000. The Megascript RNAi kit was then used to generate dsRNA via T7 RNA transcription using the manufacturer's protocol. Each transcription reaction contained $1\ \mu\text{g}$ template DNA and the transcription time was 4 h. The excess DNA and ssRNA were removed, and the dsRNA purified following the manufacturer's instructions with the final elution in $100\ \mu\text{l}$. A small amount was diluted 1 in 10 and run on a 3% agarose gel to check the size and integrity of the dsRNA. The concentration was also checked using the Nanodrop 1000. The volume was adjusted to a final concentration of $1\ \mu\text{g}\ \mu\text{l}^{-1}$ in elution buffer.

Eighth instar male nymphs were collected from laboratory stock. The nymphs were housed individually in boxes ($7 \times 7 \times 5\ \text{cm}$), and provided with cat chow and water ad libitum. On the first day of the final (ninth) nymphal instar, each cricket was injected with $2\ \mu\text{g}$ dsRNA and checked daily for adult emergence. An average of 15 males (10–18) were injected for each gene. Upon adult emergence, males were reinjected with a further $2\ \mu\text{g}$ dsRNA and left for 4 days. At 4 days of adult age the male was assayed for sperm viability following the protocol outlined above. We were able to obtain spermatophores from 9 to 15 males per gene (mean \pm s.e. 12 ± 1). A group of 30 males acted as controls, and were injected with the elution buffer from the Megascript kit. To confirm that gene expression was reduced via the RNAi, we conducted gene expression assays for a subsample of males ($n = 5$ per gene and control) following the protocols described above.

3. Results

Replicating previous work [36], we found that male *T. oceanicus* exposed to the calls of rivals during development produced ejaculates containing a higher proportion of viable sperm than males reared in silence ($t_{61} = 2.58$, $p < 0.012$; figure 1a). Increased sperm viability in response to rival calls was accompanied by increased expression of seminal fluid protein genes in the male accessory glands.

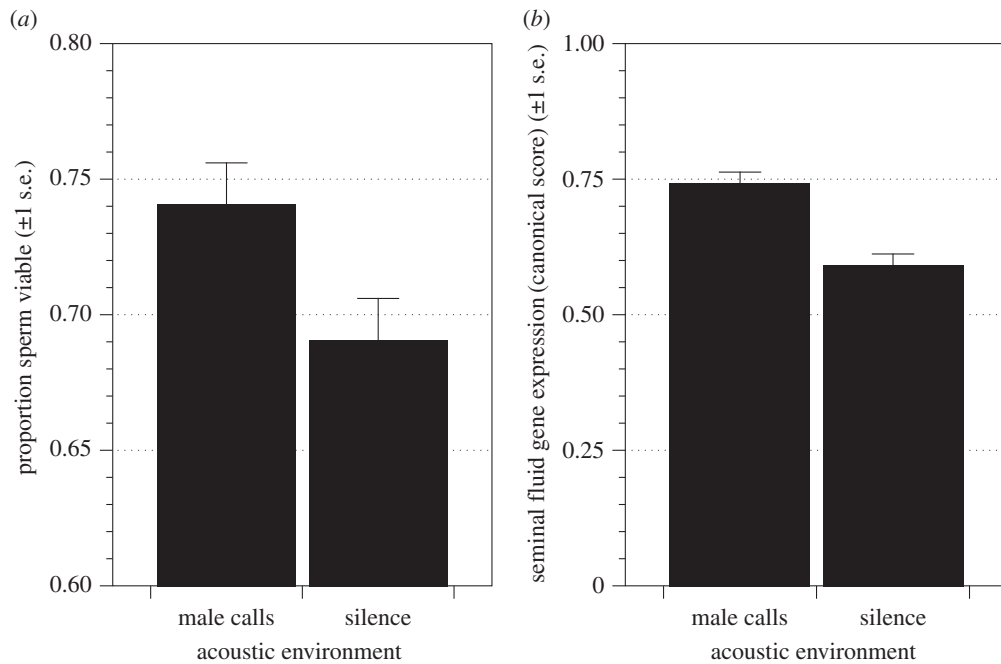


Figure 1. The effects of exposure to rival male calls during development on (a) the mean \pm s.e. proportion of live sperm in an ejaculate (sperm viability) and (b) the expression of seminal fluid protein genes. In (b) the mean \pm s.e. canonical scores extracted from a MANOVA describe the summed expression of 10 seminal fluid and 1 sperm protein gene.

Table 1. Differences in seminal fluid gene expression between males exposed to rival calls during their development and those raised in silence.

gene	transcript reference	$\Delta\Delta C_T \pm$ s.e.		univariate effect	
		male calls	silence	t_{61}	p
<i>ToSfp001</i>	isotig01262	0.185 \pm 0.009	0.156 \pm 0.009	2.232	0.029
<i>ToSfp005</i>	isotig01832	0.489 \pm 0.081	0.209 \pm 0.083	2.415	0.019
<i>ToSfp007</i>	isotig00263	0.054 \pm 0.021	0.050 \pm 0.013	0.755	0.453
<i>ToSfp011</i>	isotig01709	0.923 \pm 0.054	0.758 \pm 0.055	2.137	0.037
<i>ToSfp017</i>	isotig05129	5.359 \pm 0.207	4.371 \pm 0.211	3.343	0.001
<i>ToSfp022</i>	isotig00444	0.141 \pm 0.033	0.046 \pm 0.034	2.020	0.047
<i>ToSfp023</i>	isotig00811	0.521 \pm 0.019	0.464 \pm 0.020	2.090	0.041
<i>ToSfp024</i>	isotig02293	0.302 \pm 0.013	0.291 \pm 0.014	0.566	0.573
<i>ToSfp026</i>	isotig06304	0.019 \pm 0.004	0.023 \pm 0.004	0.725	0.471
<i>ToSfp027</i>	isotig00169	0.896 \pm 0.066	0.678 \pm 0.067	2.313	0.024
<i>ToSp009</i>	isotig00560	0.048 \pm 0.002	0.047 \pm 0.002	0.435	0.665

Expression levels for each of the 11 candidate genes were entered into a single MANOVA to test for differences in gene expression between social environments. Accessory gland weight was entered as a covariate. There was a significant effect of social environment on seminal fluid protein gene expression ($F_{1,60} = 19.49$, $p < 0.001$), but the effect of accessory gland weight was not significant ($F_{1,60} = 3.83$, $p = 0.060$). Males exposed to the calls of rivals had higher levels of seminal fluid gene expression than males reared in silence (figure 1b). Univariate tests revealed that 7 of the 11 genes assayed had higher expression in males exposed to rival calls (table 1). To explore how these genes might affect sperm viability we entered their expression levels into a principal components analysis that returned two axes of variation which explained 77% and 11% of the variation in gene

expression, respectively (electronic supplementary material, table S3). The first axis was loaded most strongly by the expression of *ToSfp017* and to a lesser extent by *ToSfp011*, and predicted variation in sperm viability in whole ejaculates (effect estimate 0.022 ± 0.010 ; $F_{1,60} = 4.46$, $p = 0.039$). The second axis was loaded most strongly by the expression of *ToSfp005* and to a lesser extent *ToSfp027*, but this axis did not predict sperm viability (effect estimate 0.004 ± 0.028 ; $F_{1,60} = 0.02$, $p = 0.885$). We used interference RNA to further explore the function of these seven genes.

RNAi was successful in knocking down the expression of target genes (table 2). There was significant variation in sperm viability across RNAi treatment groups ($F_{7,70} = 3.35$, $p = 0.004$; figure 2). Knockdown of *ToSfp011* significantly reduced sperm viability relative to controls, while knockdown

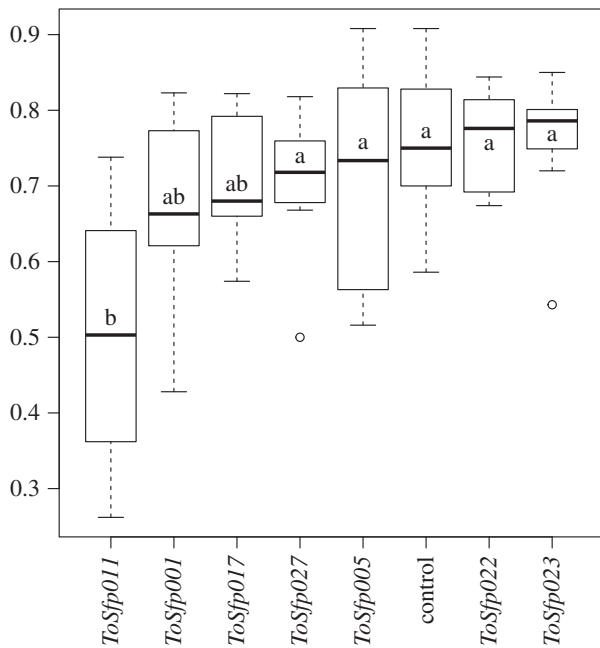


Figure 2. Box plots showing the proportion of sperm alive (sperm viability) in ejaculates from males in which seminal fluid gene expression was knocked down using RNAi or control males injected with elution buffer. Statistical analysis conducted on square-root-arc sine transformed proportions. Groups not connected by the same letter (a,b) are significantly different using post hoc Tukey HSD.

Table 2. Seminal fluid gene expression following RNA interference relative to controls.

gene	$\Delta\Delta C_T \pm \text{s.e.}$	
	control	RNAi
<i>ToSfp001</i>	0.232 ± 0.045	0.005 ± 0.001
<i>ToSfp005</i>	0.734 ± 0.425	0.017 ± 0.006
<i>ToSfp011</i>	0.484 ± 0.080	0.007 ± 0.001
<i>ToSfp017</i>	4.472 ± 0.306	0.033 ± 0.005
<i>ToSfp022</i>	0.105 ± 0.056	0.003 ± 0.003
<i>ToSfp023</i>	0.567 ± 0.054	0.075 ± 0.017
<i>ToSfp027</i>	0.096 ± 0.006	0.037 ± 0.007

of *ToSfp001* and *ToSfp0017* resulted in males with sperm viabilities intermediate between *ToSfp011* knockdowns and controls. Knockdown of the remaining genes had no discernible effect on sperm viability relative to controls (figure 2).

4. Discussion

We found socially mediated increases in seminal fluid protein gene expression in the accessory glands of male crickets, *T. oceanicus*. Socially mediated changes in gene expression have been reported previously, in the context of pre-mating sexual selection. Rapid increases in the expression of genes in various regions of the female brain have been documented in response to social exposure to courting males in fish [49,50], birds [51–53] and insects [54,55], with these changes

in gene expression often being of greater magnitude when females experience more attractive males. For males, changes in gene expression in the brain have been found following territory intrusion by rivals in both birds [56] and fish [57]. In male *Drosophila melanogaster*, the social environment was found to affect the expression of over 500 genes [58–60], half of which change only in response to rival males [60]. Socially mediated plasticity in gene expression was recently reported in the closely related cricket *T. commodus* using a similar experimental paradigm to that used here [61].

Male *T. commodus* exposed to rival male calls tend to show accelerated development and a reduced lifespan, but invest less in calling effort than males reared in silence [62–64]. These socially cued changes in life-history strategy were found to be associated with changes in the expression of genes that affect courtship, muscle development and the storage of energy reserves [61]. Interestingly, Kasumovic *et al.* [61] found changes in the expression of five genes associated with mating and spermatogenesis in male *T. commodus* exposed to rival calls. Two of these genes, *Four wheel drive* and *spargel*, appear to be involved in spermatogenesis and mitochondrial metabolism [65–67], and their increased expression in males exposed to rival male calls might be associated with the general finding that males increase their expenditure on sperm production in response to social cues to sperm competition risk [11,12]. Like *T. commodus*, male *T. oceanicus* also show socially mediated plasticity in a host of life-history traits, including immune function [68], cuticular hydrocarbon expression [69], mating behaviour [70] and sperm viability [36]. Our targeted approach examined the expression of 11 sfp genes that were candidates for the socially mediated changes in sperm viability.

We found an increased expression of seven sfp genes in response to rival calls. One of these genes, *ToSfp011*, showed a 20% increase in expression compared with males reared in silence. This sfp has a close match to a hypothetical accessory gland protein in the field cricket *Gryllus pennsylvanicus*, but was previously of unknown function [71,72]. When *ToSfp011* was knocked down using interference RNA, males produced ejaculates with a 30% reduction in the proportion of sperm that were viable. While increased gene expression is indicative of an increase in protein production, we cannot directly infer that the amount of protein allocated to individual ejaculates is increased. Moreover, if the targeted gene is also expressed in tissues beyond the accessory gland, its knockdown could affect other aspects of male physiology that indirectly influence sperm viability. Nevertheless, increased *ToSfp011* and *ToSfp017* expression were also associated with increased sperm viability in whole ejaculates when males were exposed to rival calls. We conclude, therefore, that adjustments in the production of this protein may underlie adjustments in sperm viability found in this cricket as males respond to the risk and intensity of sperm competition [36–39].

Knockdown of two additional sfp genes, *ToSfp001* and *ToSfp017*, resulted in sperm viability intermediate between *ToSfp011* knockdowns and controls, suggesting that these proteins may also contribute to strategic adjustments in sperm viability. One of these proteins, *ToSfp001*, is an apyrase with a predicted function in nucleotide metabolism [43]. Apyrases have been shown to play important roles in sperm motility and viability, and to bind tightly to sperm [43]. Indeed *ToSfp001* is the same protein as *ToSp027* identified from the *T. oceanicus* sperm proteome [43]. The second

protein, ToSfp017, is a hypothetical protein of previously unknown function [43].

For the remaining four *sfp* genes that showed increased expression on exposure to rival calls, interference RNA had no detectable effect on sperm viability. However, these *sfp*s may serve other functions in postcopulatory male fitness. Two of these proteins, ToSfp022 and ToSfp023, remain of unknown function [44], but the third, ToSfp005, is a dipeptidase [43]. Dipeptidases are involved in the biosynthesis of prostaglandin [73,74]. Studies of *T. commodus* have found that males transfer prostaglandin synthesizing compounds in the ejaculate, and that prostaglandin induces egg laying and oviposition [75–79]. Fecundity-enhancing seminal fluid proteins are well known in insects [80], with this function being served by ovulin in *D. melanogaster* [31]. However, in contrast to our finding of increased expression in *ToSfp005* in males exposed to rivals, in *D. melanogaster* exposure to rivals was found to result in a reduced expression of the gene responsible for ovulin production [33].

Four *sfp* genes that show increased expression with male age were unaffected by the social manipulation. It may be that these genes lack phenotypic plasticity because they encode proteins essential for minimal reproductive function. Alternatively, the seven genes that did show phenotypic plasticity may share some regulatory signal or cell type

expression that the remaining four genes do not. Further work is required to fully understand the function of seminal fluid proteins in this and other species [9,27].

In conclusion, our data provide evidence that males will increase their expenditure on *sfp*s that enhance the fertilization capacity of their sperm when faced with an increased risk of sperm competition. Studies of *Drosophila* have found that males will reduce their allocation of fecundity stimulating *sfp*s in response to rival males, while male house mice exposed to rivals have an increased abundance of several seminal fluid proteins in their seminal vesicles, some of which may enhance sperm motility. Collectively these studies lend growing support for recent theoretical models that predict complex responses in male allocation to seminal fluid composition, responses that depend on the roles that individual seminal fluid proteins play in fecundity enhancement and competitive fertilization success [28–30].

Data accessibility. Data are available at the Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.8rq18>) [81].

Authors' contributions. L.W.S. designed the study, analysed the data and wrote the manuscript. M.L. conducted the molecular work. Both authors approved the final version of the manuscript.

Competing interests. The authors declare no competing interests.

Funding. This work was funded by the Australian Research Council (DP110104594).

References

1. Arnqvist G, Nilsson T. 2000 The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* **60**, 145–164. (doi:10.1006/anbe.2000.1446)
2. Simmons LW. 2005 The evolution of polyandry: sperm competition, sperm selection, and offspring viability. *Ann. Rev. Ecol. Evol. Syst.* **36**, 125–146. (doi:10.1146/annurev.ecolsys.36.102403.112501)
3. Slatyer RA, Mautz BS, Backwell PRY, Jennions MD. 2012 Estimating genetic benefits of polyandry from experimental studies: a meta-analysis. *Biol. Rev.* **87**, 1–33. (doi:10.1111/j.1469-185X.2011.00182.x)
4. Pizzari T, Wedell N. 2013 The polyandry revolution. *Phil. Trans. R. Soc. B* **368**, 20120041. (doi:10.1098/rstb.2012.0041)
5. Kvarnemo C, Simmons LW. 2013 Polyandry as a mediator of sexual selection before and after mating. *Phil. Trans. R. Soc. B* **368**, 20120042. (doi:10.1098/rstb.2012.0042)
6. Parker GA. 1970 Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525–567. (doi:10.1111/j.1469-185X.1970.tb01176.x)
7. Eberhard WG. 1996 *Female control: sexual selection by cryptic female choice*. Princeton, NJ: Princeton University Press.
8. Parker GA, Pizzari T. 2010 Sperm competition and ejaculate economics. *Biol. Rev.* **85**, 897–934. (doi:10.1086/656840)
9. Simmons LW, Fitzpatrick JL. 2012 Sperm wars and the evolution of male fertility. *Reproduction* **144**, 519–534. (doi:10.1530/REP-12-0285)
10. Parker GA. 2016 The evolution of expenditure on testes. *J. Zool.* **298**, 3–19. (doi:10.1111/jzo.12297)
11. delBarco-Trillo J. 2011 Adjustment of sperm allocation under high risk of sperm competition across taxa: a meta-analysis. *J. Evol. Biol.* **24**, 1706–1714 (doi:10.1111/j.1420-9101.2011.02293.x)
12. Kelly CD, Jennions MD. 2011 Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. *Biol. Rev.* **86**, 863–884. (doi:10.1111/j.1469-185X.2011.00175.x)
13. Poiani A. 2006 Complexity of seminal fluid: a review. *Behav. Ecol. Sociobiol.* **60**, 289–310. (doi:10.1007/s00265-006-0178-0)
14. Wolfner MF. 2002 The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* **88**, 85–93. (doi:10.1038/sj.hdy.6800017)
15. McGraw LA, Suarez SS, Wolfner MF. 2015 On a matter of seminal importance. *Bioessays* **37**, 142–147. (doi:10.1002/bies.201400117)
16. Haerty W *et al.* 2007 Evolution in the fast lane: rapidly evolving sex-related genes in *Drosophila*. *Genetics* **177**, 1321–1335. (doi:10.1534/genetics.107.078865)
17. Karn RC, Clark NL, Nguyen ED, Swanson WJ. 2008 Adaptive evolution in rodent seminal vesicle secretion proteins. *Mol. Biol. Evol.* **25**, 2301–2310. (doi:10.1093/molbev/msn182)
18. Ramm SA, McDonald L, Hurst JL, Beynon RJ, Stockley P. 2009 Comparative proteomics reveals evidence for evolutionary diversification of rodent seminal fluid and its functional significance in sperm competition. *Mol. Biol. Evol.* **26**, 189–198. (doi:10.1093/molbev/msn237)
19. Dorus S, Evans PD, Wyckoff GJ, Choi SS, Lahn BT. 2004 Rate of molecular evolution of the seminal protein gene *SEMG2* correlates with levels of female promiscuity. *Nat. Genet.* **36**, 1326–1329. (doi:10.1038/ng1471)
20. Wagstaff BJ, Begun DJ. 2007 Adaptive evolution of recently duplicated accessory gland protein genes in desert drosophila. *Genetics* **177**, 1023–1030. (doi:10.1534/genetics.107.077503)
21. Locatello L, Poli F, Rasotto MB. 2013 Tactic-specific differences in seminal fluid influence sperm performance. *Proc. R. Soc. B* **280**, 20122891. (doi:10.1098/rspb.2012.2891)
22. Kilgallon SJ, Simmons LW. 2005 Image content influences men's semen quality. *Biol. Lett.* **1**, 235–255. (doi:10.1098/rsbl.2005.0324)
23. Smith C.C., Ryan M.J. 2011 Tactic-dependent plasticity in ejaculate traits in the swordtail *Xiphophorus nigrensis*. *Biol. Lett.* **7**, 733–735. (doi:10.1098/rsbl.2011.0286)
24. Cornwallis CK, Birkhead TR. 2007 Changes in sperm quality and numbers in response to experimental manipulation of male social status and female attractiveness. *Am. Nat.* **170**, 758–770. (doi:10.1086/521955)
25. Leivers S, Rhodes G, Simmons LW. 2014 Context-dependent relationship between a composite measure of men's mate value and ejaculate quality. *Behav. Ecol.* **25**, 1115–1122. (doi:10.1093/beheco/aru093)
26. Cornwallis CK, O'Conner EA. 2009 Sperm: seminal fluid interactions and the adjustment of sperm

- quality in relation to female attractiveness. *Proc. R. Soc. B* **276**, 3467–3475. (doi:10.1098/rspb.2009.0807)
27. Perry JC, Sirot L, Wigby S. 2013 The seminal symphony: how to compose an ejaculate. *Trends Ecol. Evol.* **28**, 414–422. (doi:10.1016/j.tree.2013.03.005)
28. Cameron E, Day T, Rowe L. 2007 Sperm competition and the evolution of ejaculate composition. *Am. Nat.* **169**, E158–E172. (doi:10.1086/516718)
29. Alonzo SH, Pizzari T. 2010 Male fecundity stimulation: conflict and cooperation within and between the sexes: model analyses and coevolutionary dynamics. *Am. Nat.* **175**, 174–185. (doi:10.1086/649596)
30. Dhole S, Servedio MR. 2014 Sperm competition and the evolution of seminal fluid composition. *Evolution* **68**, 3008–3019. (doi:10.1111/evo.12477)
31. Sirot LK, Wolfner MF, Wigby S. 2011 Protein-specific manipulation of ejaculate composition in response to female mating status in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **108**, 9922–9926. (doi:10.1073/pnas.1100905108)
32. Wigby S, Sirot LK, Linklater JR, Buehner N, Calboli FCF, Bretman A, Wolfner MF, Chapman T. 2009 Seminal fluid protein allocation and male reproductive success. *Curr. Biol.* **19**, 751–757. (doi:10.1016/j.cub.2009.03.036)
33. Fedorka KM, Winterhalter WE, Ware B. 2011 Perceived sperm competition intensity influences seminal fluid protein production prior to courtship and mating. *Evolution* **65**, 584–590. (doi:10.1111/j.1558-5646.2010.01141.x)
34. Ramm S, Edward D, Claydon A, Hammond D, Brownridge P, Hurst J, Beynon R, Stockley P. 2015 Sperm competition risk drives plasticity in seminal fluid composition. *BMC Biol.* **13**, 87. (doi:10.1186/s12915-015-0197-2)
35. Li S-H, Lee RK-K, Hsiao Y-L, Chen Y-H. 2005 Demonstration of a glycoprotein derived from the Ceacam10 gene in mouse seminal vesicle secretions. *Biol. Reprod.* **73**, 546–553. (doi:10.1095/biolreprod.105.039651)
36. Gray B, Simmons LW. 2013 Acoustic cues alter perceived sperm competition risk in the field cricket *Teleogryllus oceanicus*. *Behav. Ecol.* **24**, 982–986. (doi:10.1093/beheco/art009)
37. Thomas ML, Simmons LW. 2008 Male-derived cuticular hydrocarbons signal sperm competition intensity and affect ejaculate expenditure in crickets. *Proc. R. Soc. B* **276**, 383–388. (doi:10.1098/rspb.2008.1206)
38. Thomas ML, Simmons LW. 2007 Male crickets adjust the viability of their sperm in response to female mating status. *Am. Nat.* **170**, 190–195. (doi:10.1086/519404)
39. Simmons LW, Denholm A, Jackson C, Levy E, Madon E. 2007 Male crickets adjust ejaculate quality with both risk and intensity of sperm competition. *Biol. Lett.* **3**, 520–522. (doi:10.1098/rsbl.2007.0328)
40. García-González F, Simmons LW. 2005 Sperm viability matters in insect sperm competition. *Curr. Biol.* **15**, 271–275. (doi:10.1016/j.cub.2005.01.032)
41. Simmons LW, Wernham J, García-González F, Kamien D. 2003 Variation in paternity in the field cricket *Teleogryllus oceanicus*: no detectable influence of sperm numbers or sperm length. *Behav. Ecol.* **14**, 539–545. (doi:10.1093/beheco/arg038)
42. Simmons LW, Beveridge M. 2011 Seminal fluid affects sperm viability in a cricket. *PLoS ONE* **6**, e17975. (doi:10.1371/journal.pone.0017975)
43. Simmons LW, Tan Y-F, Millar AH. 2013 Sperm and seminal fluid proteomes of the field cricket *Teleogryllus oceanicus*: identification of novel proteins transferred to females at mating. *Insect. Mol. Biol.* **22**, 115–130. (doi:10.1111/imb.12007)
44. Simmons LW, Beveridge M, Li L, Tan Y-F, Millar AH. 2014 Ontogenetic changes in seminal fluid gene expression and the protein composition of cricket seminal fluid. *Evol. Dev.* **16**, 101–109. (doi:10.1111/ede.12068)
45. Dowling DK, Nystrand M, Simmons LW. 2010 Maternal effects, but no good or compatible genes for sperm competitiveness in Australian crickets. *Evolution* **64**, 1257–1266. (doi:10.1111/j.1558-5646.2009.00912.x)
46. Bailey NW, Veltsos P, Tan Y-F, Millar AH, Ritchie MG, Simmons LW. 2013 Tissue-specific transcriptomics in the field cricket *Teleogryllus oceanicus*. *G3: Genes|Genomes|Genetics* **3**, 225–230. (doi:10.1534/g3.112.004341)
47. Schmittgen TD, Livak KJ. 2008 Analysing real-time PCR data by the comparative CT method. *Nat. Prot.* **3**, 1101–1108. (doi:10.1038/nprot.2008.73)
48. Kearse M *et al.* 2012 Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649. (doi:10.1093/bioinformatics/bts199)
49. Cummings ME, Larkins-Ford J, Reilly CRL, Wong RY, Ramsey M, Hofmann HA. 2008 Sexual and social stimuli elicit rapid and contrasting genomic responses. *Proc. R. Soc. B* **275**, 393–402. (doi:10.1098/rspb.2007.1454)
50. Ramsey M, Maginnis T, Wong R, Brock C, Cummings M. 2012 Identifying context-specific gene profiles of social, reproductive, and mate preference behavior in a fish species with female mate choice. *Front. Neurosci.* **6**, 62. (doi:10.3389/fnins.2012.00062)
51. Mello CV, Vicario DS, Clayton DF. 1992 Song presentation induces gene expression in the songbird forebrain. *Proc. Natl Acad. Sci. USA* **89**, 6818–6822. (doi:10.1073/pnas.89.15.6818)
52. Sockman KW, Gentner TQ, Ball GF. 2002 Recent experience modulates forebrain gene-expression in response to mate-choice cues in European starlings. *Proc. R. Soc. Lond. B* **269**, 2479–2485. (doi:10.1098/rspb.2002.2180)
53. Avey MT, Phillmore LS, MacDougall-Shackleton SA. 2005 Immediate early gene expression following exposure to acoustic and visual components of courtship in zebra finches. *Behav. Brain Res.* **165**, 247–253. (doi:10.1016/j.bbr.2005.07.002)
54. Lawniczak MKN, Begun DJ. 2004 A genome-wide analysis of courting and mating responses in *Drosophila melanogaster* females. *Genome* **47**, 900–910. (doi:10.1139/g04-050)
55. Immonen E, Ritchie MG. 2012 The genomic response to courtship song stimulation in female *Drosophila melanogaster*. *Proc. R. Soc. B* **279**, 1359–1365. (doi:10.1098/rspb.2011.1644)
56. Mukai M, Replogle K, Drnevich J, Wang G, Wacker D, Band M, Clayton DF, Wingfield JC. 2009 Seasonal differences of gene expression profiles in song sparrow (*Melospiza melodia*) hypothalamus in relation to territorial aggression. *PLoS ONE* **4**, e8182. (doi:10.1371/journal.pone.0008182)
57. Sanogo YO, Band M, Blatti C, Sinha S, Bell AM. 2012 Transcriptional regulation of brain gene expression in response to a territorial intrusion. *Proc. R. Soc. B* **279**, 4929–4938. (doi:10.1098/rspb.2012.2087)
58. Carney G. 2007 A rapid genome-wide response to *Drosophila melanogaster* social interactions. *BMC Genomics* **8**, 288. (doi:10.1186/1471-2164-8-288)
59. Ellis LL, Carney GE. 2009 *Drosophila melanogaster* males respond differently at the behavioural and genome-wide levels to *Drosophila melanogaster* and *Drosophila simulans* females. *J. Evol. Biol.* **22**, 2183–2191. (doi:10.1111/j.1420-9101.2009.01834.x)
60. Ellis LL, Carney GE. 2011 Socially-responsive gene expression in male *Drosophila melanogaster* is influenced by the sex of the interaction partner. *Genetics* **187**, 157–169. (doi:10.1534/genetics.110.122754)
61. Kasumovic MM, Chen Z, Wilkins MR. 2016 Australian black field crickets show changes in neural gene expression associated with socially-induced morphological, life-history, and behavioral plasticity. *BMC Genomics* **17**, 827. (doi:10.1186/s12864-016-3119-y)
62. Kasumovic MM, Hall MD, Try H, Brooks RC. 2011 The importance of listening: juvenile allocation shifts in response to acoustic cues of the social environment. *J. Evol. Biol.* **24**, 1325–1334. (doi:10.1111/j.1420-9101.2011.02267.x)
63. Kasumovic MM, Hall MD, Brooks RC. 2012 The juvenile social environment introduces variation in the choice and expression of sexually selected traits. *Ecol. Evol.* **2**, 1036–1047. (doi:10.1002/ece3.230)
64. Kasumovic MM, Hall MD, Try H, Brooks RC. 2013 Socially cued developmental plasticity affects condition-dependent trait expression. *Behav. Ecol.* **24**, 429–434. (doi:10.1093/beheco/ars180)
65. Giansanti MG, Belloni G, Gatti M. 2007 Rab11 is required for membrane trafficking and actomyosin ring constriction in meiotic cytokinesis of *Drosophila* males. *Mol. Biol. Cell* **18**, 5034–5047. (doi:10.1091/mbc.E07-05-0415)
66. Polevoy G, Wei H-C, Wong R, Szentpetery Z, Kim YJ, Goldbach P, Steinbach SK, Balla T, Brill JA. 2009 Dual roles for the *Drosophila* PI 4-kinase Four wheel drive in localizing Rab11 during cytokinesis. *J. Cell Biol.* **187**, 847. (doi:10.1083/jcb.200908107)
67. Tiefenböck SK, Baltzer C, Egli NA, Frei C. 2009 The *Drosophila* PGC-1 homologue Spargel coordinates mitochondrial activity to insulin signalling. *EMBO J.* **29**, 171. (doi:10.1038/emboj.2009.330)

68. Bailey NW, Gray B, Zuk M. 2011 Exposure to sexual signals during rearing increases immune defence in adult field crickets. *Biol. Lett.* **7**, 217–220. (doi:10.1098/rsbl.2010.0659)
69. Thomas ML, Gray B, Simmons LW. 2011 Male crickets alter the relative expression of cuticular hydrocarbons when exposed to different acoustic environments. *Anim. Behav.* **82**, 49–53. (doi:10.1016/j.anbehav.2011.03.023)
70. Bailey NW, Gray B, Zuk M. 2010 Acoustic experience shapes alternative mating tactics and reproductive investment in male field crickets. *Curr. Biol.* **20**, 845–849. (doi:10.1016/j.cub.2010.02.063)
71. Andrés JA, Maroja LS, Bogdanowicz SM, Swanson WJ, Harrison RG. 2006 Molecular evolution of seminal proteins in field crickets. *Mol. Biol. Evol.* **23**, 1574–1584. (doi:10.1093/molbev/msl020)
72. Andrés JA, Maroja LS, Harrison RG. 2008 Searching for candidate speciation genes using a proteomic approach: seminal proteins in field crickets. *Proc. R. Soc. B* **275**, 1975–1983. (doi:10.1098/rspb.2008.0423)
73. Trachte GJ, Meixner K, Ferrario CM, Khosla MC. 1990 Prostaglandin production in response to angiotensin-(1–7) in rabbit isolated vasa deferentia. *Prostaglandins* **39**, 385–394. (doi:10.1016/0090-6980(90)90120-k)
74. Jaiswal N, Diz DI, Chappell MC, Khosla MC, Ferrario CM. 1992 Stimulation of endothelial cell prostaglandin production by angiotensin peptides: characterization of receptors. *Hypertension* **19**(2 Suppl.), II49–II55. (doi:10.1161/01.HYP.19.2_Suppl.II49)
75. Loher W, Edson K. 1973 The effect of mating on egg production and release in the cricket *Teleogryllus commodus*. *Entomol. Exp. Appl.* **16**, 483–490. (doi:10.1111/j.1570-7458.1973.tb00300.x)
76. Loher W. 1979 The influence of prostaglandin E2 on oviposition in *Teleogryllus commodus*. *Ent. Exp. Appl.* **25**, 107–119. (doi:10.1111/j.1570-7458.1979.tb02853.x)
77. Stanley-Samuelson DW, Loher W. 1983 Arachidonic and other long-chain polyunsaturated fatty acids in spermatophores and spermathecae of *Teleogryllus commodus*: significance in prostaglandin-mediated reproductive behaviour. *J. Insect. Physiol.* **29**, 41–45. (doi:10.1016/0022-1910(83)90104-X)
78. Stanley-Samuelson DW, Peloquin JJ, Loher W. 1986 Egg-laying in response to prostaglandin injections in the Australian field cricket, *Teleogryllus commodus*. *Physiol. Entomol.* **11**, 213–219. (doi:10.1111/j.1365-3032.1986.tb00408.x)
79. Stanley-Samuelson DW, Jurenka RA, Blomquist GJ, Loher W. 1987 Sexual transfer of prostaglandin precursor in the field cricket *Teleogryllus commodus*. *Physiol. Entomol.* **12**, 347–354. (doi:10.1111/j.1365-3032.1987.tb00760.x)
80. Simmons LW. 2001 *Sperm competition and its evolutionary consequences in the insects*. Princeton, NJ: Princeton University Press.
81. Simmons LW, Lovegrove M. 2017 Data from: Socially cued seminal fluid gene expression mediates responses in ejaculate quality to sperm competition risk. Dryad Digital Repository. (<http://dx.doi.org/10.5061/dryad.8rq18>)