JOURNAL OF Evolutionary Biology

doi: 10.1111/j.1420-9101.2012.02511.x

Sperm competition roles and ejaculate investment in a promiscuous mammal

J.-F. LEMAÎTRE[1], S. A. RAMM[2], J. L. HURST & P. STOCKLEY

Mammalian Behaviour & Evolution Group, Institute of Integrative Biology, Faculty of Health and Life Sciences, University of Liverpool, Neston, UK

Keywords:

bank vole;
copulatory behaviour;
copulatory plug;
Myodes glareolus;
sexual selection;
social dominance;
sperm allocation;
sperm velocity.

Abstract

Theoretical models of sperm competition predict how males should allocate sperm and seminal fluid components to ejaculates according to their mating role (dominant vs. subordinate). Here, we present a detailed analysis of ejaculate expenditure according to male roles in the bank vole (*Myodes glareolus*). Sperm competition occurs regularly in this species, and dominant males typically achieve higher fertilization success than subordinates. Contrary to theoretical predictions, we found that dominant male bank voles invest more sperm per ejaculate than subordinates, both absolutely and relative to body and testes mass. The testes of dominant males were also absolutely (although not relatively) larger than those of subordinates. However, we found no evidence that subordinate males compensate for lower sperm numbers per ejaculate by increasing ejaculation frequency or sperm velocity. Similarly, we found no evidence for differential investment in copulatory plug size according to male roles in sperm competition, although dominant males had significantly larger seminal vesicles (both absolutely and relative to body mass) compared with subordinates. We conclude that sperm competition roles can have significant but unexpected influences on ejaculate investment in mammals with clearly defined differences in male social status.

Introduction

Sperm competition occurs when the ejaculates from two or more males compete to fertilize a given set of ova (Parker, 1970, 1998) and is a powerful selective force in the evolution of male reproductive traits (Birkhead & Møller, 1998). Our understanding of how males attempt to maximize reproductive success under sperm competition owes much to an expanding theoretical framework in which optimal ejaculate investment decisions are predicted within constraints imposed by costs of sperm production (review in Parker & Pizzari, 2010). As the outcome of sperm competition often depends on the relative number of sperm ejaculated by competing males, the main focus of such theory has been investment in sperm production and/or sperm allocation per ejaculate (e.g. Ball & Parker, 2000; Parker & Ball, 2005; Williams *et al.*, 2005; Engqvist & Reinhold, 2006). In addition, there is also increasing interest in optimal investment decisions with respect to seminal fluid components of the ejaculate (Cameron *et al.*, 2007; Alonzo & Pizzari, 2010), which can also influence sperm competition outcomes (Fricke *et al.*, 2009; Wigby *et al.*, 2009).

Optimal sperm investment within species is expected to vary predictably with respect to male roles, as defined within theoretical models known as sperm competition games (Parker, 1990, 1998; Parker & Pizzari, 2010). In this context, male roles are defined as either favoured or disfavoured, depending on whether the male mating in the role is likely to be advantaged or disadvantaged in a loaded sperm competition 'raffle', all else being equal. For example, mating first with a female may provide an

Correspondence: Jean-François Lemaître, Mammalian Behaviour & Evolution Group, Institute of Integrative Biology, University of Liverpool, Leahurst Campus, Neston CH64 7TE, UK.
Tel.: +44 1517 946007; fax: +44 1517 946107;
e-mail: jean-francois.lemaitre@univ-lyon1.fr
[1]Present address: UMR CNRS 5558, Biométrie et Biologie Evolutive, Université Claude Bernard Lyon 1, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France.
[2]Present address: Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland.

advantage in sperm competition (e.g. Schwagmeyer & Foltz, 1990; Raveh *et al.*, 2010), in which case the first male to mate has a favoured role and the second male a disfavoured role (Parker, 1990, 1998). In some species, sperm competition roles are often consistently associated with particular male phenotypes – for example, dominant males may be more likely to ejaculate at an optimal time relative to ovulation (i.e. a favoured role), whereas subordinate males are more likely to mate at a less optimal time (i.e. a disfavoured role) (Parker, 1990; Drews, 1993; Stockley & Purvis, 1993; Preston *et al.*, 2003). Male roles in this case are thus non-random with respect to male phenotype, and theory predicts greater ejaculate expenditure in the disfavoured role (Parker, 1990, 1998; Tazzyman *et al.*, 2009). In other words, where sperm numbers are the main determinant of fertilization success, subordinate males should transfer more sperm per mating than dominant males (Parker, 1990).

Although current theory offers clear predictions with respect to expected sperm investment under sperm competition roles, it is important to consider species-specific behaviours and adaptations when attempting to test such predictions (Stockley & Preston, 2004; Engqvist & Reinhold, 2005). For example, if the copulatory behaviour of a study species potentially involves repeated ejaculations with the same female, then a thorough quantification of ejaculate investment requires consideration of the total number of ejaculations performed as well as the number of sperm per ejaculate. To date, however, relatively few studies have achieved this (e.g. delBarco-Trillo & Ferkin, 2004), and to our knowledge, no previous studies have included ejaculation number per female to quantify ejaculate investment in the context of sperm competition roles, although differences in copulatory behaviour according to male social status have been reported in some cases (deCatanzaro & Ngan, 1983; Shapiro & Dewsbury, 1986). Following predictions of sperm competition games (Parker, 1990, 1998), males consistently mating in a disfavoured role may thus perform more ejaculations per female, potentially facilitated by a reduced number of intromissions prior to ejaculation (Stockley & Preston, 2004; Preston & Stockley, 2006).

Although less well advanced than theory relating to sperm numbers, predictions concerning optimal investment strategies in seminal fluid proteins have recently been developed in relation to sperm competition roles as defined above (Cameron *et al.*, 2007). Here, depending on how seminal fluid proteins affect male fertilization success, ejaculate composition (relative investment in sperm and non-sperm components of the ejaculate) is predicted to differ according to male roles. Seminal fluid products such as a copulatory plug are likely to play an important role in mammalian sperm competition (Ramm *et al.*, 2005, 2008, 2009). For example, investment in producing a larger plug may promote sperm transport and/or delay female remating (Ramm *et al.*, 2005; Poiani, 2006) and thus increase paternity success under sperm competition (Polak *et al.*, 2001). Where increased investment in seminal fluid products increases the fertilization bias, the model predicts that disfavoured males will spend more on sperm than favoured males but favoured males should invest more energy in both seminal products and total ejaculate production than disfavoured males (Cameron *et al.*, 2007). However, the extent to which relative investment in the copulatory plug differs according to male roles in sperm competition has yet to be investigated.

In the present study, we conduct a detailed analysis of ejaculate expenditure according to male roles in sperm competition for a promiscuous rodent, the bank vole *Myodes glareolus*. This species has a promiscuous mating system in which sperm competition occurs regularly (Ratkiewicz & Borkowska, 2000; Klemme *et al.*, 2008) and dominant males typically achieve higher fertilization success than subordinates (Klemme *et al.*, 2006; Kruczek & Zatorska, 2008). A fertilization bias for dominant males may be explained in part by favourable timing of copulation, because they have an advantage over subordinates in aggressive contests (Kruczek, 1997) and are preferred by females (Horne & Ylönen, 1996; Kruczek, 1997). Fertilization outcomes are also likely to be biased in favour of dominant males due to the higher motility of their sperm (Kruczek & Styrna, 2009). Dominant male bank voles can thus be assumed to have a favoured role under sperm competition and subordinates a disfavoured role. Importantly, previous studies have also established that dominant male bank voles have larger testes than subordinates (Kruczek & Zatorska, 2008; Kruczek & Styrna, 2009) and sometimes larger body mass (Kruczek & Styrna, 2009; but see Klemme *et al.*, 2006). Hence, it appears that dominant males may be capable of producing more sperm than subordinate males. As yet however, it is unknown whether ejaculate allocation strategies differ for dominant and subordinate males as predicted by sperm competition theory. That is, although dominant males appear capable of greater overall sperm production rates compared to subordinates, they also have higher expected mating rates (see Parker & Ball, 2005) and may still invest relatively less in each ejaculate (or per female) than subordinates while achieving higher fertilization success in sperm competition (Parker, 1990). In the present study, to test predictions of sperm competition theory as outlined above, we quantify differences between males of known social status in (i) sperm number per ejaculate, (ii) total number of ejaculates per copulatory series (copulatory behaviour that occurs from the first intromission to the point of male satiety) and (iii) the size of the copulatory plug. Our findings enable us to compare relative investment in sperm and non-sperm components of the ejaculate and provide the first thorough investigation of ejaculate investment for a promiscuous mammal in which contrasting sperm

competition roles are well established according to male social status.

Materials and methods

Subjects and housing

Subjects were the adult F1 and F2 offspring of 29 wild-caught bank voles (15 males and 14 females) trapped in Cheshire (UK) and bred under laboratory conditions using a breeding strategy designed to maintain genetic diversity within the captive population. After weaning, animals were housed with siblings of the same sex in MB1 cages (45 × 28 × 13 cm, North Kent Plastic Cages Ltd., Rochester, UK) containing substrate (Corn Cob Absorb 10/14 substrate) and paper-wool nest material. Hence, at the start of the experiments, all subjects had equivalent social experience and none had previously mated. Food and water were provided *ad libitum* (LabDiet 5002 Certified Rodent Diet; Purina Mills, St Louis, MO, USA). Animals were maintained on a reversed photoperiod (light: 16 h, dark: 8 h, lights on at 17:00 h) and at a temperature of 21 ± 1 °C. All experiments were conducted during the dark phase. For purposes of identification, male subjects were PIT-tagged (tag inserted under the skin). Variation in sample sizes in the analyses occurs because certain male subjects did not copulate (two dominants and three subordinates) and hence could not be included in all analyses or died before completion of the study.

Establishing dominance relationships

We used an established approach (Horne & Ylönen, 1996; Klemme *et al.*, 2006; Lemaître *et al.*, 2012) to assess male dominance status, by pairing unrelated males in MB1 cages divided into two by a mesh barrier, with one male of each pair housed in each half of the cage (i.e. in a 45 × 14 × 13 cm area) to allow continuous olfactory, visual and auditory contact. Males were then kept paired over a period of 3–5 weeks. Soiled nest material (approximately 13 g) from an unrelated female was added once to each compartment of the divided cages, 1 day prior to the first collection of scent marks from each male pair, to increase male competitiveness (Kruczek, 1997). To identify scent-marking behaviour and assign social status to subjects, both males from each pair were transferred to either side of a clean divided Benchkote-lined MB1 cage and left for 30 min during the dark phase. Scent marks were scanned using a Bio-rad Fluor-S™ MultiImager (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK; QuantityOne software: 12-s exposure, 530DF60 Filter, UV light source Epi illumination, high-resolution mode). Social status was assessed following Rozenfeld & Rasmont (1991) (see also Horne & Ylönen, 1996). Specifically, thin streaks of urine deposited throughout the arena are characteristic of dominant males. By contrast, subordinates either deposit large pools of urine, especially in corners of the arena (Rozenfeld *et al.*, 1987; Rozenfeld & Rasmont, 1991; Klemme *et al.*, 2006), or deposit no urine marks (Rozenfeld & Rasmont, 1991). We started scent-marking assays after the two males had been in the divided cages for 1 week. Male roles as either dominant or subordinate were assigned when a clear and consistent difference in the pattern of scent marks within a pair was apparent for three successive scent-marking assays (each conducted at least 24 h apart). If no clear dominant–subordinate pattern could be identified within a pair, males were rehoused in their original cages or paired with a new unrelated male. Only pairs with an unambiguous dominant–subordinate relationship (N = 26 pairs) were used in our experiments. In support of our method for determining dominance relationships between male pairs, we subsequently confirmed that males classed as dominant on the basis of scent-marking behaviour also had significantly heavier preputial glands (dominants: $\overline{X}$ = 46.65 ± 4.78 mg; subordinates: $\overline{X}$ = 23.34 ± 2.18 mg; t_{44} = 4.75; $P < 0.001$), consistent with previous evidence that high social rank is associated with large preputial glands in this and other species (e.g. Kruczek, 1997; Pohorecky *et al.*, 2008). Dominant and subordinate males did not differ significantly in age (age on the day of the first mating opportunity: dominants: $\overline{X}$ = 229.6 ± 13.8 days; subordinates: $\overline{X}$ = 210.3 ± 16.6 days; t_{50} = 0.90; P = 0.37) or body mass prior to pairing (dominants: $\overline{X}$ = 25.24 ± 0.82 g; subordinates: $\overline{X}$ = 24.56 ± 0.65 g; t_{44} = 0.52; P = 0.61).

Experiment 1

Our first experiment was designed to test for differences in total ejaculate investment per female according to male social status. Here, we quantified the total number of ejaculations performed by dominant (N = 14) and subordinate (N = 11) males when allowed to mate to satiety with a female.

As females were initially sexually naïve, nest material (15 g) from unrelated males to those used in this experiment was introduced into female cages for 2 days preceding their first mating opportunity to provide experience of male odour. Females were weighed using an electronic balance before each mating opportunity. The timing of ovulation in relation to copulation was equivalent for each of our subject males, because ovulation in bank voles is induced by the act of copulation, and in each case, the female was mating for the first time within a given reproductive cycle (Clarke *et al.*, 1970). Mating took place in a neutral arena (70 × 60 × 50 cm), within which females were initially restrained for 10 min (using a Perspex tube closed at one end by a mesh barrier) to permit acclimatization. If no mating behaviour occurred (mounts or intromissions) during the first 30 min following release of the female or if persistent aggression occurred, the female was removed and replaced by a new unrelated female (see Klemme

et al., 2006; Borkowska, 2010). Dominant and subordinate males did not differ significantly in the number of females they encountered before achieving a successful copulation (data from experiments 1 and 2 combined: dominant: $\overline{X} = 4.56 \pm 0.97$; subordinate: $\overline{X} = 5.53 \pm 6.11$; $t_{42} = -0.58$; $P = 0.56$) and females that mated with dominant and subordinate males did not differ in their body mass (data from experiments 1 and 2 combined: females mated with dominants: $\overline{X} = 23.16 \pm 0.75$; females mated with subordinates: $\overline{X} = 22.00 \pm 0.74$; $t_{42} = 0.98$; $P = 0.33$). All copulations were recorded under red light on a DVD using a CCTV video stream relayed to an adjoining room, allowing rapid intervention in case of aggressive behaviour. We separated mating pairs 30 min after the male's last ejaculation, assuming that the male had then mated to satiety (Dewsbury, 1975; Stockley & Preston, 2004). Typically male bank voles topple on one side when they ejaculate, so this behaviour is easily identifiable. DVD recordings were used to quantify the total number of ejaculations and the number of intromissions performed by subject males during the first ejaculatory series. At the end of the experiment, females were rehoused individually in M3 cages (48 × 11.5 × 2 cm, North Kent Plastic Cages Ltd).

Experiment 2

A second experiment was designed to quantify and compare sperm number per ejaculate and the size of copulatory plugs produced by dominant ($N = 10$) and subordinate ($N = 11$) males. As in experiment 1, each subject male was allowed to copulate with a previously sexually naive female using the protocol described above, except that in this case the copulatory series was interrupted after the first ejaculation to recover the ejaculate. Three males that did not transfer any sperm during their first copulation were allowed to mate a second time, at least 1 week later. Copulations with no sperm transfer were excluded from the analysis.

Immediately after the first ejaculation, females were killed humanely using an overdose of halothane. Sperm number was recorded following Ramm & Stockley (2007) to collect and count sperm from the female reproductive tract. After abdominal incision of the female, oviducts were clamped to prevent sperm migration. The tract was opened via a longitudinal incision down each uterine horn and placed in a Perspex Sterilin tube containing 1 mL 1% citrate solution (Perspex, Surrey, UK). The tube was agitated for 5 min to let sperm disperse from the oviduct. Sperm counts were performed on an improved Neubauer haemocytometer (Hawksley, Medical and Laboratory Equipment, Lancing, UK) using standard protocols (European Society of Human Reproduction and Embryology 2002). Female reproductive tracts were frozen for several weeks, then defrosted and gently dissected to remove and weigh the copulatory plug using an electronic balance.

Measurement of male reproductive organs and sperm velocity

At the end of experiments 1 and 2, all male subjects ($N = 46$) were rehoused in paired cages for at least 1 week to allow for replenishment of sperm reserves before they were killed humanely using an overdose of halothane. Body mass and the paired masses of preputial glands, seminal vesicles and testes were recorded using an electronic balance.

For males from experiment 2 ($N = 21$), we also measured sperm velocity from the left epididymis following the method fully described by Lemaître *et al.* (2011). Swimming sperm were recorded at ×20 magnification using a Flea®2 camera (FL2-03S2M-C; Point Grey Research, Inc., Richmond, BC, Canada) attached to a Leica DM1000 microscope (Leica Microsystems, Wetzlar, Germany), approximately 30 min after the start of the dissection. The duration of each recording was 2 s (75 frames s^{-1}, 150 frames in total for each recording). Recordings were analysed using the Computer-Assisted Sperm Analysis (CASA) plugin (Wilson-Leedy & Ingermann, 2007) for IMAGEJ software (version 1.38x, http://rsbweb.nih.gov/ij/) using parameters optimized for bank vole sperm (Lemaître *et al.*, 2011). We measured blindly with respect to male social status: (i) curvilinear velocity (VCL, μm s^{-1}), which estimates the velocity point to point along the trajectory, (ii) average path velocity (VAP, μm s^{-1}), which estimates the point-to-point velocity over a constructed smooth path, and (iii) straight line velocity (VSL, μm s^{-1}), which estimates the velocity point to point along a straight line. To compare sperm velocity between treatment groups, we used two recordings of swimming sperm for each individual. First, we analysed each recording twice to test for repeatability of the measures taken by the CASA plugin on the same recording. These measures were highly repeatable for each variable (e.g. for VAP: intraclass coefficient of correlation, ICC = 0.88; $F_{1,41} = 15.75$; $P < 0.001$) and were therefore averaged. Next, we tested repeatability of the measures between the two different recordings for each subject, using the average value of each recording previously calculated. These measures were also highly repeatable (e.g. for VAP: intraclass coefficient of correlation: ICC = 0.95; $F_{1,20} = 34.46$; $P < 0.001$) and were therefore averaged to obtain a mean value of each sperm velocity trait for each male.

Statistical analysis

Raw data were log-transformed or square-root-transformed as appropriate to improve normality (assessed by Kolmogorov–Smirnov tests). To compare investment in testes or seminal vesicles between males of different social status, we used a general linear model with the trait of interest (log-transformed) as the dependent variable, social status (dominant vs. subordinate) as a fixed factor, log body mass as a covariate and the

interaction between social status and the covariate log body mass (Tomkins & Simmons, 2002). This interaction term takes into account the homoscedasticity of the variances between the two groups of males and is removed from the model only where $P > 0.2$ to avoid type II errors because the ability to detect significant interactions is weak (Hendrix *et al.*, 1982). Similarly, we used this method to compare male investment in sperm number per ejaculate and copulatory plug size according to male social status with log testes mass or log seminal vesicles mass as covariates instead of body mass, because the size of these organs may reflect differences of investment in the production of sperm and copulatory plugs, respectively. These analyses were intended to explore whether subordinate males might be investing a significantly higher proportion of their total sperm reserves or plug-producing capacity in any given copulation compared to dominant males. To test whether males adjust sperm numbers or copulatory plug size according to female body condition, we also ran a GLM with female body mass as the independent variable and sperm number per ejaculate or copulatory plug mass as the dependent variable.

As the three descriptors of sperm velocity (VCL, VAP and VSL) were highly correlated, a principal component analysis of the variance–covariance matrix of these three log-transformed variables was used to reduce the number of parameters in subsequent analyses. The first principal component summarizing multivariate velocity variation explained 94.35% of the variance and had an eigenvalue of 2.83. The loadings of the three velocity measurements on this first factor were: 0.95 (VCL), 0.99 (VAP) and 0.97 (VSL). The factor score is thus the single variable used in the subsequent analyses to represent sperm velocity (hereafter called sperm velocity). Independent *t*-tests compared behavioural traits and sperm velocity of dominant and subordinate males. In this case, homogeneity of variance was assessed by Levene's test for equality of variances, and where not respected, we used the unequal variance *t*-test advocated by Ruxton (2006). Data are presented as means ± standard error of the mean (SEM), and differences are regarded as statistically significant at $P < 0.05$. All tests are two-tailed and performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Relative investment in sperm components of the ejaculate

Our analyses reveal that dominant males transferred significantly more sperm per ejaculate than subordinate males (Table 1). Dominant males also appear to invest relatively more sperm per ejaculate than subordinates, after controlling for both body and testes size (Table 2A). Moreover, we found no evidence that subordinate males compensate for ejaculating fewer sperm per ejaculate by performing more ejaculations than dominant males, because there was no significant difference between dominant and subordinate males in the number of ejaculations achieved within a given copulatory series (Table 1). Dominant males also performed significantly more intromissions than did subordinates during the first copulation but not during the whole copulatory series (Table 1).

Our findings for relative testes size are more ambiguous than those for sperm numbers, in part because the interaction between social status and the covariate log body mass was retained in the model (see Methods – this interaction term is removed from the model only where $P > 0.2$, to avoid type II errors (Tomkins & Simmons, 2002). Hence, although the testes of dominant males tended to be relatively larger than those of subordinate males after controlling for body mass, this difference is not statistically significant (Table 2A). However, the absolute testes mass of dominant males was significantly greater than that of subordinates (Table 1), as in previous studies (Kruczek & Styrna, 2009), and correlated with body mass ($n = 46$; $r = 0.68$; $P < 0.001$). Finally, sperm velocity did not differ significantly in relation to male social status (Table 1), and sperm number per ejaculate was not significantly related to female body mass ($\beta = 0.22 \pm 0.33$; $t_{18} = 0.66$; $P = 0.52$).

Relative investment in non-sperm components of the ejaculate

Seminal vesicles mass was significantly correlated with body mass ($n = 46$; $r = 0.54$; $P < 0.001$) and testes mass ($n = 46$; $r = 0.76$; $P < 0.001$), and the seminal vesicles of dominant males were relatively larger than those of subordinate males when controlling for body mass (Table 2B). We also compared the size of copulatory plugs produced by dominant and subordinate males, using log body mass or log seminal vesicles mass as a covariate, respectively. We found no significant difference in the size of copulatory plugs produced by dominant and subordinate males relative to body mass or seminal vesicles mass (Table 2B). Similarly, the absolute size of the copulatory plug did not differ significantly according to male social status (Table 1) although absolute seminal vesicles mass was significantly greater for dominant males (Table 1), as reported previously (Kruczek & Styrna, 2009). The size of copulatory plugs was not significantly related to female body mass ($\beta = 0.03 \pm 0.33$; $t_{17} = 0.09$; $P = 0.93$).

Discussion

Our study offers a direct test of current ejaculate investment theory where male mating roles (dominant vs. subordinate) are consistent and clearly identifiable. Contrary to theoretical expectations, we find that dominant male bank voles invest more sperm per ejaculate

Table 1 Mean value (± SEM) of body mass, reproductive and copulatory traits investigated for dominant and subordinate males. Differences in these traits between dominant and subordinate males are tested using independent *t*-tests.

	Dominant	Subordinate	d.f.	*t*	*P*
Body mass (g)	26.66 ± 0.95	25.08 ± 0.81	44	1.25	0.22
Testes mass (mg)	724.50 ± 17.58	643.35 ± 22.45	44	2.81	0.007
Sperm number per ejaculate	$28.36 \pm 2.07 \times 10^6$	$21.00 \pm 2.00 \times 10^6$	17	2.65	0.02
Sperm motility	−0.01 ± 0.27	0.01 ± 0.35	19	−0.48	0.96
Seminal vesicles mass (mg)	278.10 ± 13.69	223.99 ± 12.85	44	2.87	0.006
Copulatory plug mass (mg)	27.44 ± 2.88	32.06 ± 2.73	17	−1.32	0.21
Time to first ejaculation (min)	18.18 ± 1.76	18.47 ± 2.91	23	0.42	0.67
Number of ejaculations	3.4 ± 0.3	3.1 ± 0.3	23	0.63	0.53
Number of intromissions (first series)	21.1 ± 1.8	16.4 ± 1.9	42	2.01	0.05
Total number of intromissions	48.43 ± 4.27	51.27 ± 3.52	23	−0.62	0.54

than subordinate males. Sperm competition games predict the opposite result for defined male roles under sperm competition – that is, assuming no differential constraint on investment, subordinate males should invest relatively more sperm per ejaculate than dominant males because they are typically mating in a disfavoured role (Parker, 1990; Parker & Pizzari, 2010; Tazzyman *et al.*, 2009). Previous empirical studies do provide some indirect evidence in support of this theory. For example, the stripped ejaculates of subordinate male Arctic charr (*Salvelinus alpinus*) have a higher percentage of milt volume occupied by sperm compared to dominant males (Rudolfsen *et al.*, 2006), and subordinate male Iberian ibex (*Capra pyrenaica*) have larger testes relative to body size compared to dominants (Sarasa *et al.*, 2010; see also Stockley & Purvis, 1993). Studies of male fowl have also revealed differences in sperm allocation strategies according to social status in a variety of contexts, different to those explored here. For example, subordinate males were shown to ejaculate more sperm than dominants in the presence of a single competitor, but this pattern was reversed in the presence of three rival males (Pizzari *et al.*, 2003). Discrete male investment strategies in sperm competition have also been studied in other theoretical contexts such as alternative mating tactics or male phenotypes (e.g. Gage *et al.*, 1995; Hettyey & Roberts, 2007; Simmons *et al.*, 2007) or phenotypic plasticity (Cornwallis & Birkhead, 2007, 2008).

Why then, might our findings for male bank voles fail to support predictions of current sperm competition theory? The theoretical predictions of non-random roles models tested here are relatively robust across a range of scenarios (reviewed in Parker & Pizzari, 2010), which appear generally applicable to our study system. For example, Ball & Parker (2000) explored situations where roles depend on male phenotype and are non-random but not strictly constant, which may be a more realistic scenario in relation to mating opportunities of male bank voles under natural conditions. Predicted sperm allocation under these conditions is again dependent on which role a male typically occupies, with more sperm allocated in the disfavoured role (Ball & Parker, 2000; Parker & Pizzari, 2010) – the opposite to our findings here. The main exception to these predictions is whether sperm limitation is strong, in which case a greater investment can be predicted for the favoured or dominant male (Ball & Parker, 2000; see also Mesterton-Gibbons, 1999), consistent with our results. There is growing evidence that sperm limitation may be widespread in mammals under certain conditions (Preston *et al.*, 2001; Stockley & Bro-Jørgensen, 2011). Nonetheless, it seems unlikely that this can explain our results, as Ball & Parker (2000) found little evidence in the literature to suggest that internal fertilizers suffer sufficient sperm limitation to reverse predictions of the non-random roles models.

As the pattern of sperm investment that we report for male bank voles is not consistent with theoretical expectations based on sperm competition roles, it is possible that our findings might instead be explained by differential constraints or costs of investment according to male social status. Constraints on sperm investment could be behavioural and/or physiological in origin. For example, if subordinate male bank voles are more likely to experience aggressive interference or take-overs by dominant males during a copulatory series (e.g. Ratkiewicz & Borkowska, 2000), they may be constrained to ejaculate with fewer intromissions and thus to transfer fewer sperm per ejaculate (see also Ramm & Stockley, 2007). In support of this idea, we found significant differences in the copulatory behaviour of dominant and subordinate males; dominant males performed more intromissions prior to their first ejaculation (see also Horne & Ylönen, 1996), which might explain why they delivered more sperm per ejaculate than subordinates if a high level of stimulation during copulation increases the quantity of sperm ejaculated (Toner & Adler, 1986; Stockley & Preston, 2004). Under this scenario, subordinate males could then compensate for a lower sperm investment per ejaculate by increasing their ejaculation frequency (Stockley & Preston, 2004) and could thus still achieve greater relative sperm investment per female than dominant males in accordance with predictions of sperm competition theory (Parker, 1990; Parker & Pizzari, 2010). However, we found no evidence that

Table 2. Analysis of testes mass (controlled for body mass) and sperm number per ejaculate (controlled for body mass and testes mass) in relation to male social status (A) and analysis of seminal vesicles mass (controlled for body mass) and copulatory plug mass (controlled for body mass and seminal vesicles mass) in relation to male social status (B). Social status is included as a covariate in these models.

	d.f.	Estimate ± SE	Mean square	*F*	*P*
(A) Testes mass and sperm number per ejaculate models					
Testes mass					
Model	3		0.04	18.77	< 0.001
Social status	1	0.53 ± 0.15	0.008	3.57	0.07
Body mass	1	0.81 ± 0.15	0.09	38.34	< 0.001
Social status*body mass	1	−0.35 ± 0.20	0.007	3.06	0.09
Error	42				
Sperm number per ejaculate					
Model	2		1.65	4.66	0.03
Social status	1	1.02 ± 0.33	3.30	9.31	0.009
Body mass	1	−3.29 ± 2.36	0.69	1.95	0.18
Error	14		0.35		
Sperm number per ejaculate					
Model	2		1.49	3.95	0.04
Social status	1	0.99 ± 0.37	2.78	7.36	0.02
Testes mass	1	1.94 ± 1.96	0.37	0.97	0.34
Error	14		0.38		
(B) Seminal vesicles and copulatory plug mass models					
Seminal vesicles mass					
Model	2		0.14	13.61	< 0.001
Social status	1	0.46 ± 0.59	0.07	6.77	0.01
Body mass	1	0.98 ± 0.32	0.16	16.18	< 0.001
Error	43		0.01		
Copulatory plug mass					
Model	3		0.02	1.62	0.24
Social status	1	−2.26 ± 1.44	0.04	2.46	0.14
Body mass	1	−0.19 ± 0.62	0.009	0.65	0.44
Social status*Body mass	1	1.49 ± 1.00	0.032	2.23	0.16
Error	12		0.01		
Copulatory plug mass					
Model	2		0.02	1.40	0.28
Social status	1	−0.14 ± 0.09	0.04	2.74	0.12
Seminal vesicles mass	1	0.32 ± 0.33	0.01	0.94	0.35
Error	13		0.02		

All traits are log-transformed except sperm number per ejaculate, which is square-root-transformed. An interaction between social status and the control trait (body mass or testes mass) was removed from the model only if $P > 0.2$ (see Methods).

subordinate male bank voles compensate for lower sperm numbers by performing more ejaculations than dominant males or for other forms of compensation such as increased sperm velocity. Our findings thus strongly imply that the total sperm investment per female of dominant males is significantly greater than that of subordinate males. Importantly, this may at least partly explain why dominant males have higher reproductive success than subordinate males in a competitive context (Klemme *et al.*, 2006). It is also noteworthy that some males in our study failed to transfer any sperm, even though they appeared to copulate normally, suggesting that multiple mating by females may function in part to ensure fertilization (Jennions & Petrie, 2000; Zeh & Zeh, 2001).

More broadly, our findings are consistent with contrasting hormonal profiles of dominant and subordinate males that might reflect constraints on sperm investment decisions. For example, it is known that male bank voles with naturally high testosterone levels are more likely to be dominant than those with low levels (Mills *et al.*, 2009) and that aggressiveness of dominant male bank voles is positively correlated with androgen levels (Kruczek, 1997). Kruczek & Styrna (2009) therefore suggested that low testosterone levels of subordinate males may explain the (absolutely) smaller size of their testes and seminal vesicles compared to those of dominant males, as also reported here, as well as their lower sperm quality. In the context of interpreting findings of the present study, low testosterone levels of subordinate males could also lead to higher costs of obtaining copulations compared to dominant males, due to lower competitive and mate searching ability (see also Mills *et al.*, 2009). This is relevant to the interpretation of our findings because the sperm competition models that we are aiming to test assume roughly equivalent costs for males in different sperm competition roles (Parker, 1990; Ball & Parker, 2000; but see Tazzyman *et al.*, 2009), and hence, the predictions may not apply if costs differ widely according to social status. As noted by Kruczek & Styrna (2009), subordinate males may also experience significant social stress, with potential consequences for their reproductive physiology and competitive ability (e.g. Arnold & Dittami, 1997; Faulkes *et al.*, 2001). In some cases, the reproductive capabilities of subordinate males may even be suppressed in the presence of dominant males (Koyama, 2004). Social suppression of reproductive function under competitive conditions could thus create significant constraints on sperm investment decisions made by subordinate male mammals, such that assumptions of current sperm competition models may not always apply in cases where social status is linked to sperm competition roles.

Our findings also provide relatively little support for predictions of current sperm competition theory incorporating non-sperm components of the ejaculate. As predicted by Cameron *et al.* (2007), we find evidence that males mating in a favoured role are investing more overall in seminal products and ejaculate production, because dominant males in our study had larger seminal vesicles for their body size than subordinates, suggestive of greater overall investment in the production of seminal fluid proteins. However, as discussed above, subordinate males ejaculated relatively fewer sperm compared to dominant males (rather than more as

predicted), and there was no difference in either the absolute mass of plugs produced by dominant and subordinate males or plug mass relative to body mass or the mass of the seminal vesicles. In general, there is good reason to expect that plugs have important functions in mammalian sperm competition such that adaptive variation in both plug size and composition could be selectively favoured (Ramm *et al.*, 2005, 2008, 2009). More specifically, male bank voles have been shown to develop larger seminal vesicles in response to high levels of sexual competition experienced during sexual maturation, suggesting an important role for the products of these accessory reproductive glands in sperm competition (Lemaître *et al.*, 2011). It may therefore be possible that seminal fluid proteins other than those in the copulatory plug are produced or allocated strategically according to male roles in sperm competition. There is increasing evidence of strategic allocation of non-sperm components of the ejaculate, although this is currently restricted to invertebrate taxa. For example, in *Drosophila melanogaster*, males adjust their investment in two specific seminal proteins in relation to the level of sperm competition (Wigby *et al.*, 2009), and in a ladybird beetle (*Adalia bipunctata*) high-quality males invest more in non-sperm ejaculate components than low-quality males (Perry & Rowe, 2010).

Why then, in the present study, did we find no evidence for variation in plug size according to male roles in sperm competition or indeed in relation to the number of sperm ejaculated? First, it could be that there is no advantage to producing a relatively large plug under increased sperm competition risk, for example if it is more important that the plug is appropriately positioned within the female reproductive tract to achieve maximum functional efficiency. Unfortunately, there are currently little empirical data available to test this idea, although in other rodent species it is known that an unusually small plug can be disadvantageous to fertility under non-competitive conditions (Carballada & Esponda, 1992). A second possibility is that differences in anticipated mating rate influence plug size, such that dominant males (with higher mating rates) partition their seminal expenditure into more ejaculates (Rogers *et al.*, 2005). If so, the plugs of dominant and subordinate males may be of similar size even though dominant males have larger seminal vesicles. A further possibility is that adaptive variation in plug size between males exists but is difficult to quantify accurately based on a single measurement, as suggested by a more detailed investigation into intra- and inter-individual variation in the size of copulatory plugs produced by ring-tailed lemurs (*Lemur catta*, Parga *et al.*, 2006). Hence, we suggest that in future studies, it will be important to test for within-male variation in plug size and more importantly also to explore variation in the composition of plugs and other non-sperm components of the ejaculate, as well as plug size *per se*.

Finally, as dominant males in our study appear to be investing more in ejaculates than subordinate males, we note that our findings are not consistent with a trade-off between effort spent obtaining matings and investment in ejaculate expenditure, as typically assumed in sperm competition games (e.g. Parker, 1990, 1998). Rather, dominant males appear capable of investing more both in achieving greater mating success than subordinates *and* in greater ejaculate expenditure. Interpreted more broadly in the light of life-history theory, this suggests that dominant males may pay a cost of higher investment in reproduction through a decrease in survival (Stearns, 1992). Recent studies of bank voles support this idea because high social status and associated reproductive success was found to be closely associated with high levels of circulating testosterone and with a decrease in survival, probably due to the immunosuppressive role of this hormone (Mills *et al.*, 2009, 2010).

In summary, our findings for male bank voles do not support predictions of sperm competition games in relation to male roles, perhaps because such predictions do not always apply in cases where males differ in social status. Further investigation is now required to explore the consequences of different mating costs and hormonal constraints on ejaculate production.

Acknowledgments

We thank all members of the Mammalian Behaviour and Evolution Group for useful feedback and discussion, L. Burgess, A. Davidson, J. Fick, R. Humphries, S. Jopson and J. Waters for help conducting the experiment, and J. Wilson-Leedy and S. Koyama for advice on sperm velocity measurements. JFL and SAR were supported by a grant from the Leverhulme Trust to PS (F/00 025/W). We thank Prof. David Coltman and two anonymous referees for their thoughtful comments. This research adhered to the Association for the Study of Animal Behaviour/Animal Behaviour Society guidelines for the Use of Animals in Research, the legal requirements of the country in which the work was carried out and all institutional guidelines. No specific licences were required for this work.

References

Alonzo, S.H. & Pizzari, T. 2010. Male fecundity stimulation: conflict and cooperation within and between the sexes: model analyses and coevolutionary dynamics. *Am. Nat.* **175**: 174–185.

Arnold, W. & Dittami, J. 1997. Reproductive suppression in male alpine marmots. *Anim. Behav.* **53**: 53–66.

Ball, M.A. & Parker, G.A. 2000. Sperm competition games: a comparison of loaded raffle models and their biological implications. *J. Theor. Biol.* **206**: 487–506.

delBarco-Trillo, J. & Ferkin, M.H. 2004. Male mammals respond to a risk of sperm competition conveyed by odours of conspecific males. *Nature* **431**: 446–449.

Birkhead, T.R. & Møller, A.P. 1998. *Sperm Competition and Sexual Selection*. Academic Press, London.

Borkowska, A. 2010. Copulatory behaviour of the bank vole *Myodes glareolus*: matings with one and two males do not make a difference. *Acta Theriol.* **55**: 343–349.

Cameron, E., Day, T. & Rowe, L. 2007. Sperm competition and the evolution of ejaculate composition. *Am. Nat.* **169**: E158–E172.

Carballada, R. & Esponda, P. 1992. Role of fluid from seminal vesicles and coagulating glands in sperm transport into the uterus and fertility in rats. *J. Reprod. Fertil.* **95**: 639–648.

deCatanzaro, D. & Ngan, E.T. 1983. Dominance in intermale encounters and subsequent sexual success in mice. *J. Comp. Psychol.* **97**: 269–278.

Clarke, J.R., Clulow, F.V. & Greig, F. 1970. Ovulation in the bank vole, *Clethrionomys glareolus*. *J. Reprod. Fertil.* **23**: 531.

Cornwallis, C.K. & Birkhead, T.R. 2007. Changes in sperm quality and numbers in response to experimental manipulation of male social status and female attractiveness. *Am. Nat.* **170**: 758–770.

Cornwallis, C.K. & Birkhead, T.R. 2008. Plasticity in reproductive phenotypes reveals status-specific correlations between behavioral, morphological, and physiological sexual traits. *Evolution* **62**: 1149–1161.

Dewsbury, D.A. 1975. Diversity and adaptation in rodent copulatory behavior. *Science* **190**: 947–954.

Drews, C. 1993. The concept and definition of dominance in animal behaviour. *Behaviour* **125**: 283–313.

Engqvist, L. & Reinhold, K. 2005. Pitfalls in experiments testing predictions from sperm competition theory. *J. Evol. Biol.* **18**: 116–123.

Engqvist, L. & Reinhold, K. 2006. Theoretical influence of female mating status and remating propensity on male sperm allocation patterns. *J. Evol. Biol.* **19**: 1448–1458.

European Society of Human Reproduction and Embryology. 2002. *ESHRE Monographs: Manual on Basic Semen Analysis*. Oxford University Press, Oxford.

Faulkes, C.G., Bennett, N.C. & Faulkes, C. 2001. Family values: group dynamics and social control of reproduction in African mole-rats. *Trends Ecol. Evol.* **16**: 184–190.

Fricke, C., Wigby, S., Hobbs, R. & Chapman, T. 2009. The benefits of male ejaculate sex peptide transfer in *Drosophila melanogaster*. *J. Evol. Biol.* **22**: 275–286.

Gage, M.J.G., Stockley, P. & Parker, G.A. 1995. Effects of alternative mating strategies on characteristics of sperm production in the Atlantic salmon *(Salmo salar)*: theoretical and empirical investigations. *Philos. Trans. R. Soc. Lond. B* **350**: 391–399.

Hendrix, L.J., Carter, M.W. & Scott, D.T. 1982. Covariance analyses with heterogeneity of slopes in fixed models. *Biometrics* **38**: 641–650.

Hettyey, A. & Roberts, J.D. 2007. Sperm traits in the quacking frog (*Crinia georgiana*), a species with plastic alternative mating tactics. *Behav. Ecol. Sociobiol.* **61**: 1303–1310.

Horne, T.J. & Ylönen, H. 1996. Female bank voles (*Clethrionomys glareolus*) prefer dominant males; but what if there is no choice? *Behav. Ecol. Sociobiol.* **38**: 401–405.

Jennions, M.D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits *Biol. Rev.* **75**: 21–64.

Klemme, I., Eccard, J.A. & Ylönen, H. 2006. Do female bank voles (*Clethrionomys glareolus*) mate multiply to improve on previous mates? *Behav. Ecol. Sociobiol.* **60**: 415–421.

Klemme, I., Ylönen, H. & Eccard, J.A. 2008. Long-term fitness benefits of polyandry in a small mammal, the bank vole *Clethrionomys glareolus*. *Proc. R. Soc. Lond. B* **275**: 1095–1100.

Koyama, S. 2004. Primer effects by conspecific odors in house mice: a new perspective in the study of primer effects on reproductive activities. *Horm. Behav.* **46**: 303–310.

Kruczek, M. 1997. Male rank and female choice in the bank vole, *Clethrionomys glareolus*. *Behav. Proc.* **40**: 171–176.

Kruczek, M. & Styrna, J. 2009. Sperm quantity and quality correlate with bank voles males' social status. *Behav. Proc.* **82**: 279–285.

Kruczek, M. & Zatorska, M. 2008. Male rank affects reproductive success and offspring performance in bank voles. *Physiol. Behav.* **94**: 611–615.

Lemaître, J.F., Ramm, S.A., Hurst, J.L. & Stockley, P. 2011. Social cues of sperm competition influence accessory reproductive gland size in a promiscuous mammal. *Proc. R. Soc. Lond. B* **278**: 1171–1176.

Lemaître, J.F., Ramm, S.A., Jennings, N. & Stockley, P. 2012. Genital morphology linked to social status in the bank vole (*Myodes glareolus*). *Behav. Ecol. Sociobiol.*, **66**: 97–105.

Mesterton-Gibbons, M. 1999. On sperm competition games: raffles and roles revisited. *J. Math. Biol.* **39**: 91–108.

Mills, S.C., Grapputo, A., Jokinen, I., Koskela, E., Mappes, T., Oksanen, T.A. *et al.* 2009. Testosterone-mediated effects on fitness-related phenotypic traits and fitness, *Am. Nat.* **173**: 475–487.

Mills, S.C., Grapputo, A., Jokinen, I., Koskela, E., Mappes, T. & Poikonen, T. 2010. Fitness trade-offs mediated by immunosuppression costs in a small mammal. *Evolution* **64**: 166–179.

Parga, J.A., Maga, M. & Overdorff, D.J. 2006. High-resolution X-ray computed tomography scanning of primate. *Am. J. Phy. Anthropol.* **129**: 567–576.

Parker, G.A. 1970. Sperm competition and its evolutionary consequences in insects. *Biol. Rev.* **45**: 525–567.

Parker, G.A. 1990. Sperm competition games: raffles and roles. *Proc. R. Soc. Lond. B* **242**: 120–126.

Parker, G.A. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In: *Sperm Competition and Sexual Selection* (T.R. Birkhead & A.P. Møller, eds), pp. 3–49: Academic Press, London.

Parker, G.A. & Ball, M. 2005. Sperm competition, mating rate and the evolution of testis and ejaculate sizes: a population model. *Biol. Lett.* **1**: 235–238.

Parker, G.A. & Pizzari, T. 2010. Sperm competition and ejaculate economics. *Biol. Rev.* **85**: 897–934.

Perry, J.C. & Rowe, L. 2010. Condition-dependent ejaculate size and composition in a ladybird beetle. *Proc. R. Soc. Lond. B* **1700**: 3639–3647.

Pizzari, T., Cornwallis, C.K., Lovlie, H., Jakobsson, S. & Birkhead, T.R. 2003. Sophisticated sperm allocation in male fowl. *Nature* **426**: 70–74.

Pohorecky, L.A., Blakley, G.G., Ma, E.W., Soini, H.A., Wiesler, D., Bruce, K.E. *et al.* 2008. Social housing influences the composition of volatile compounds in the preputial glands of male rats. *Horm. Behav.* **53**: 536–545.

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

Polak, M., Wolf, L.L., Starmer, W.T. & Barker, J.S.F. 2001. Function of the mating plug in *Drosophila hibisci* Bock. *Behav. Ecol. Sociobiol.* **49**: 196–205.

Preston, B. & Stockley, P. 2006. Risk of sexual competition stimulates premature and repeated ejaculation in a mammal. *Curr. Biol.* **16**: 239–241.

Preston, B.T., Stevenson, I.R., Pemberton, J.M. & Wilson, K. 2001. Dominant rams lose out by sperm depletion: a waning success in siring counters a ram's high score in competition for ewes. *Nature* **409**: 681–682.

Preston, B., Stevenson, I., Pemberton, J.M., Coltman, D. & Wilson, K. 2003. Overt and covert competition in a promiscuous mammal: the importance of weaponry and testes size to male reproductive success. *Proc. R. Soc. Lond. B* **270**: 633–640.

Ramm, S.A. & Stockley, P. 2007. Ejaculate allocation under varying sperm competition risk in the house mouse, *Mus musculus domesticus*. *Behav. Ecol.* **18**: 491–495.

Ramm, S.A., Parker, G.A. & Stockley, P. 2005. Sperm competition and the evolution of male reproductive anatomy in rodents. *Proc. R. Soc. Lond. B* **272**: 949–955.

Ramm, S.A., Oliver, P.L., Ponting, C.P., Stockley, P. & Emes, R.D. 2008. Sexual selection and the adaptive evolution of mammalian ejaculate proteins. *Mol. Biol. Evol.* **25**: 207–219.

Ramm, S.A., McDonald, L., Hurst, J.L., Beynon, R.J. & 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.

Ratkiewicz, M. & Borkowska, A. 2000. Multiple paternity in the bank vole (*Clethrionomys glareolus*): field and experimental data. *J. Mamm. Biol.* **65**: 6–14.

Raveh, S., Heg, D., Dobson, F.S., Coltman, D.W., Gorrell, J.C., Balmer, A. *et al.* 2010. Mating order and reproductive success in male Columbian ground squirrels (*Urocitellus columbianus*). *Behav. Ecol.* **21**: 537–547.

Rogers, D.W., Chapman, T., Fowler, K. & Pomiankowski, A. 2005. Mating-induced reduction in accessory reproductive organ size in the stalk-eyed fly *Cyrtodiopsis dalmanni*. *BMC Evol. Biol.* **5**: 37.

Rozenfeld, F.M. & Rasmont, R. 1991. Odor cue recognition by dominant male bank voles, *Clethrionomys glareolus*. *Anim. Behav.* **41**: 839–850.

Rozenfeld, F.M., Leboulangé, E. & Rasmont, R. 1987. Urine marking by male bank voles (*Clethrionomys glareous* Schreber 1780 – Microtidae, Rodentia) in relation to their social rank. *Can. J. Zool.* **65**: 2594–2601.

Rudolfsen, G., Figenschou, L., Folstad, I., Tveiten, H. & Figenschou, M. 2006. Rapid adjustments of sperm characteristics in relation to social status. *Proc. R. Soc. Lond. B* **273**: 325–332.

Ruxton, G.D. 2006. The unequal variance t-test is an underused alternative to Student's t-test and the Mann–Whitney *U* test. *Behav. Ecol.* **17**: 688–690.

Sarasa, M., Serrano, E., Pérez, J.M., Soriguer, R.C., Gonzalez, G., Joachim, J. *et al.* 2010. Effects of season, age and body condition on allocation to testes mass in Iberian ibex. *J. Zool.* **281**: 125–131.

Schwagmeyer, P.L. & Foltz, D.W. 1990. Factors affecting the outcome of sperm competition in 13-lined ground-squirrels. *Anim. Behav.* **39**: 156–162.

Shapiro, L.E. & Dewsbury, D.A. 1986. Male dominance, female choice and male copulatory behavior in two species of voles (*Microtus ochrogaster* and *Microtus montanus*). *Behav. Ecol. Sociobiol.* **18**: 267–274.

Simmons, L.W., Emlen, D.J. & Tomkins, J.L. 2007. Sperm competition games between sneaks and guards: a comparative analysis using dimorphic male beetles. *Evolution* **61**: 2684–2692.

Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.

Stockley, P. & Bro-Jørgensen, J. 2011. Female competition and its evolutionary consequences in mammals. *Biol. Rev.* **86**: 341–366.

Stockley, P. & Preston, B.T. 2004. Sperm competition and diversity in rodent copulatory behaviour. *J. Evol. Biol.* **17**: 1048–1057.

Stockley, P. & Purvis, A. 1993. Sperm competition in mammals: a comparative study of male roles and relative investment in sperm production. *Funct. Ecol.* **7**: 560–570.

Tazzyman, S.J., Pizzari, T., Seymour, R.M. & Pomiankowski, A. 2009. The evolution of continuous variation in ejaculate expenditure strategy. *Am. Nat.* **174**: E71–E82.

Tomkins, J.L. & Simmons, L.W. 2002. Measuring relative investment: a case study of testes investment in species with alternative male reproductive tactics. *Anim. Behav.* **63**: 1009–1016.

Toner, J.P. & Adler, N.T. 1986. The pre-ejaculatory behavior of male and female rats affects the number of sperm in the vagina and uterus. *Physiol. Behav.* **36**: 363–367.

Wigby, S., Sirot, L.K., Linklater, J.R., Buehner, N., Calboli, F.C.F., Bretman, A. *et al.* 2009. Seminal fluid protein allocation and male reproductive success. *Curr. Biol.* **19**: 1–7.

Williams, P.D., Day, T. & Cameron, E. 2005. The evolution of sperm allocation strategies and the degree of sperm competition. *Evolution* **59**: 492–499.

Wilson-Leedy, J.G. & 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.

Zeh, J.A. & Zeh, D.W. 2001. Reproductive mode and the genetic benefits of polyandry. *Anim. Behav.* **62**: 427–433.

Data deposited at Dryad: doi: 10.5061/dryad.jj72n4t1

Received 5 August 2011; revised 14 March 2012; accepted 15 March 2012