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Social competitiveness associated with rapid fluctuations in sperm quality in male fowl

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When females copulate with multiple males, paternity is determined by the competitive ability of a male to access females and by the ability of its ejaculates to out-compete those of other males over fertilization. The relationship between the social competitiveness of a male and the fertilizing quality of its sperm has therefore crucial implications for the evolution of male reproductive strategies in response to sexual selection. Here, we present a longitudinal experimental study of the relationship between social status and sperm quality. We monitored sperm quality in socially naive male domestic fowl, *Gallus gallus domesticus*, before and after exposure to a social challenge which comprised two stages. In the first stage, social dominance was established in male pairs divergent in sperm quality, and in the second, social status was experimentally manipulated by re-shuffling males across pairs. We show that sperm quality fluctuates within males both before and after a social challenge. Importantly, such fluctuations followed consistently different patterns in males that displayed different levels of social competitiveness in the social challenge. In particular, following the social challenge, sperm quality dropped in males that won both contests while the sperm quality of males that lost both contests remained constant. Together, these results indicate that males of different social competitiveness are predisposed to specific patterns of fluctuations in sperm quality. These rapid within-male fluctuations may help explain the recent findings of trade-offs between male social and gametic competitive abilities and may help maintain phenotypic variability in these traits.

Keywords: alternative mating strategies; *Gallus*; phenotypic plasticity; social status; sperm competition; sperm mobility

1. INTRODUCTION

Variation in paternity depends on multiple, functionally integrated traits that work in concert to determine different components of male reproductive success. In promiscuous species, sexual selection arises before insemination from variation in male copulation success, which in many social species is determined by the ability of a male to monopolize females (Darwin 1871; Andersson 1994), and after insemination, from variation in the ability of its ejaculates to fertilize ova under sperm competition (Parker 1998; Pizzari & Birkhead 2002; Snook 2005; Andersson & Simmons 2006). After controlling for the number of sperm inseminated, an important source of post-insemination variation in paternity is often explained by the relative fertilization efficiency of an ejaculate (e.g. Birkhead *et al.* 1999; Gage *et al.* 2004; Garcia-Gonzalez & Simmons 2005), which is often referred to as 'sperm quality', and which comprises a diverse range of traits, including sperm size, morphology, swimming velocity, metabolic performance, longevity and seminal fluid effects (Birkhead & Pizzari 2002; Pizzari & Birkhead 2002; Snook 2005). The relationship between pre-insemination components of male reproductive success, such as male monopolization of females as reflected by social status, and post-insemination components, such as sperm quality, has crucial implications for the evolution of male reproductive strategies and is the focus of intense interest (Schwagmeyer *et al.* 1987; Koyama & Kamimura 2000; Preston *et al.* 2001; Vladic & Jarvi 2001; Froman *et al.* 2002; Evans *et al.* 2003; Neff *et al.* 2003; Shuster & Wade 2003; Cornwallis 2004; Pizzari *et al.* 2004; Hermes *et al.* 2005; Andersson & Simmons 2006; Fitzpatrick *et al.* 2006; Rudolfsen *et al.* 2006). A negative relationship between social status and ejaculate quality may be determined by a trade-off in investment in different pre- and post-insemination reproductive traits (e.g. Parker 1998). Combined with high variance in male reproductive success (Shuster & Wade 2003), this trade-off may generate disruptive sexual selection promoting the evolution of genetically fixed or phenotypically plastic alternative male reproductive strategies, in which socially subdominant phenotypes compensate for low social competitiveness by investing in more competitive ejaculates. On the other hand, the trade-off between social status and ejaculate quality may be condition dependent, with males in good condition being able to invest in both status and ejaculate quality. This scenario may result in a positive phenotypic relationship between social status and ejaculate quality across males, triggering directional sexual selection for genotypes that are less constrained by such trade-off and eroding additive genetic variation. Finally, social status and ejaculate quality may operate and thus evolve independently from each other.

Across-male correlations between social status and sperm quality have produced ambiguous results. In some species, this correlation is positive, possibly driven by

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dominant males inhibiting the reproductive potential of subordinates (Koyama & Kamimura 2000; Hermes *et al.* 2005; Fitzpatrick *et al.* 2006), while in others, a negative relationship between social status and fertilizing efficiency suggests alternative male reproductive strategies (Preston *et al.* 2001; Vladic & Jarvi 2001; Froman *et al.* 2002; Neff *et al.* 2003; Cornwallis 2004; Rudolfsen *et al.* 2006). Part of this ambiguity may lie in the correlational approach of most of these studies. Phenotypic correlations across males fail to: (i) distinguish between constraints (e.g. dominants inhibiting subordinates) and adaptations (e.g. subordinates investing more in sperm quality), (ii) demonstrate the causality of a relationship, and (iii) determine whether a relationship is mediated by phenotypic plasticity or genetic polymorphism. Two additional issues must be considered when studying the relationship between social status and ejaculate quality. First, due to status-specific copulation rates, differential rates of sperm depletion may result in males of different status producing ejaculates that contain not only different numbers of sperm (Preston *et al.* 2001), but also sperm of different qualities (e.g. Cornwallis 2004). Second, the baseline level of fluctuations in the sperm quality of a male must be monitored to demonstrate the extent to which changes in sperm quality are induced by changes in social status. Although some studies are based on the assumption that sperm quality is constant until a male is challenged socially (e.g. Rudolfsen *et al.* 2006), this assumption is seldom verified. In this study, we overcome these constraints by experimentally investigating the relationship between social status and ejaculate quality within male domestic fowl, *Gallus gallus domesticus*, producing divergent sperm quality. In the fowl, male access to females is strongly mediated by male social hierarchy (Kratzer & Craig 1980; Cheng & Burns 1988; Parker & Ligon 2002; Pizzari *et al.* 2002). Male social status also mediates the number of sperm that a male inseminates into a female, a major determinant of the fertilizing efficiency of an insemination under sperm competition (Martin *et al.* 1974; Parker 1998; Pizzari & Birkhead 2002), through status-dependent sperm allocation strategies (Pizzari *et al.* 2003; Cornwallis & Birkhead 2006), and preferential ejection of inseminations by subdominant males by females (Pizzari & Birkhead 2000). Consistent with these mechanisms, socially dominant males have been found to father more offspring in small populations (Guhl & Warren 1946; Johnes & Mench 1991). In addition, sperm mobility, which is a measure of the metabolic performance of an ejaculate, reflects the fertilizing efficiency of an ejaculate in both competitive and non-competitive contexts (Froman & Feltmann 1998; Birkhead *et al.* 1999; Froman *et al.* 1999) and provides an objective measure of sperm quality in this species. We are interested in determining the relationship between two traits, male social status and sperm mobility, that mediate male reproductive success in the fowl and the phenotypic plasticity of this relationship.

We exposed males of high and low sperm mobility (Froman & Kirby 2005) to a two-stage social challenge, in which we monitored social dominance in pairs of males of divergent sperm mobility in the first stage, and then experimentally manipulated male social status by re-shuffling males across pairs in the second stage. We measured the sperm mobility of each male, when males were sexually rested (thus controlling for sperm depletion) twice before and twice following the social challenge. This experimental design identified four social male phenotypes of varying social competitiveness and tested: (i) whether social phenotype was related to sperm mobility across males and (ii) whether sperm mobility fluctuated to different degrees and/or in different patterns in different social phenotypes, i.e. the phenotypic plasticity of the relationship between social phenotype and sperm mobility. By comparing changes in sperm mobility occurring after the social challenge with baseline fluctuations (i.e. fluctuations occurring before the social challenge), we also investigated the causal relationship between social status and sperm mobility. In addition, by monitoring the sperm mobility of individual males on different timescales, we investigated both rapid fluctuations in sperm mobility that occur through changes in sperm, which were already developed at the time of the social challenge, and longer-term fluctuations that may have occurred through changes during spermatogenesis.

2. MATERIAL AND METHODS

(a) *Study population*

We studied a population of New Hampshire domestic fowl, characterized by males of highly repeatable sperm mobility (Froman & Feltmann 1998), at the University Farm of Oregon State University, Corvallis (US) in 2004. Sperm mobility is an *in vitro* assay which measures the ability of a population of sperm to penetrate a solution of an inert medium (Accudenz: Accurate Chemicals and Scientific Corporation, Westbury, NY, USA) in light absorbance units with a spectrophotometer (Froman & Feltmann 1998). The light absorbance of a sperm population is proportional to the number of sperm within the sample that have a straight-line velocity greater than 30 $\mu m\, s^{-1}$ (Froman & Feltmann 2000; Froman & Kirby 2005).

In other words, mobile sperm are necessarily motile (Froman & Kirby 2005). We used 30 males producing sperm of high mobility (mean($\pm$s.e.) 0.454 ± 0.0243 absolute units) and 30 males of low sperm mobility (0.059 ± 0.007 absolute units), originating from a breeding programme that has maintained sperm mobility high (0.425–0.525 absolute units) in one line and low (0.050–0.150 absolute units) in another since 2000 (Froman & Kirby 2005).

(b) *The social challenge experiment*

The aim of this challenge was to create four social phenotypes of varying social competitiveness to test whether: (i) social phenotype was related to sperm mobility across males and (ii) the phenotypic plasticity of this relationship, by investigating whether sperm mobility fluctuated to different degrees and/or in different patterns in males of different social competitiveness. Males were individually housed in battery cages ($30 \times 46 \times 63$ cm) and maintained on a 14L : 10D photoperiod (Froman *et al.* 2002). On day 1, we set up 30 pairs of males, matching one male from the high- and one from the low-sperm mobility line that were not cage neighbours and thus had no social experience of each other. We simultaneously released the two males in an indoor pen containing four hens (Froman *et al.* 2002). We allowed the males to familiarize with the new environment and with each other for 1 day and determined their social hierarchy based on the outcome of competitive interactions and the frequency of dominance-related behaviours displayed in the following

2 days (days 2–3) through focal watches during the daily evening peak in copulation and competitive activity, following a standardized protocol (Froman *et al.* 2002). In all the cases, male hierarchy was clear, with one male consistently avoiding the other which also performed dominance-related behaviours at higher frequency (Froman *et al.* 2002). On day 4, we reconstituted male pairs, by swapping males assortatively across pens, matching together two dominant or two subdominant males (e.g. dominant from pair A with dominant from pair B). Males from two pens were swapped (i.e. male A from pen 1 to pen 2, male B from pen 2 to pen 1) simultaneously and rapidly (i.e. within few minutes), preventing the resident male from copulating with the hens while the other male was being replaced. We determined male social hierarchy in these new pairs on days 5 and 6 as above and placed the males back in their cages on day 7. This manipulation imposed a change of status on one of the two males (e.g. dominant from pair A becomes subdominant, dominant from pair B remains dominant), creating four equally represented social phenotypes: (DD) males that remained dominant across the manipulation; (Ds) males that were dominant and became subdominant; (sD) males that were subdominant and became dominant; and (ss) males that remained subdominant. We therefore tested whether sperm mobility fluctuated to different degrees and/or in different patterns in males of different social phenotypes. By comparing changes in sperm mobility occurring after the social challenge with baseline fluctuations, we also investigated whether the outcome of the social challenge caused changes in sperm mobility and thus inferred the causal relationship between social status and sperm mobility.

The sperm mobility of each male was measured four times. Four semen samples were obtained from each male through cloacal massage, two before the social challenge and two afterwards. Basal sperm mobility was measured once for each male between 23 and 31 August 2004, 21–27 days before males were exposed to the social challenge, and a second time on day 1, before males were introduced in the indoor pens for the social challenge (i.e. 3 days before the completion of first stage of the social challenge). In addition, sperm mobility was measured again 2–3 days after males were returned to their individual cages at the end of the social challenge (i.e. longer than 48 h from their last exposure to hens), and 14 days following the social challenge. Males were always sampled in their individual cages, thus controlling for potential direct interference by other males. In addition, all males were sampled when they were sexually rested and their sperm reserves fully replenished. It takes a male fowl 48 h to completely restore its sperm reserves once these are completely depleted by manual massage (Parker *et al.* 1942) or by a series of consecutive natural copulations (Pizzari *et al.* 2003). We specifically sampled males more than 48 h following their last copulation opportunity with the hens present during the social challenge to eliminate any risk that differential copulation rates would have resulted in differential depletion rates and different sperm mobilities. Fowl spermatids take on average 14 days to fully develop into spermatozoa and reach the distal end of the vas deferens, in proximity of the cloaca (Lake 1984; Kirby & Froman 2000; see also Clulow & Jones 1988). Here, these extra-gonadal sperm reserves undergo a process of maturation, possibly mediated by ion exchange with seminal plasma of the vas deferens (Froman *et al.* 2006). Abdominal massage delivers most of the male extra-gonadal sperm reserves and results in sperm depletion (Parker *et al.* 1942; C. K. Cornwallis & T. Pizzari 2006, unpublished data). Therefore, the first two sperm mobility measurements provided information on the baseline (i.e. independent of status changes) level of within-male fluctuations in sperm mobility. The third sperm mobility measurement analysed sperm that developed prior to the social challenge and were maturing in the vas deferens when the male was socially challenged. The fourth measurement, 14 days following the social challenge, analysed sperm that begun their development at the time of the social challenge. Therefore, any change in sperm mobility within 14 days will be mediated by changes to developed sperm, maturing in the vas deferens, while changes occurring after 14 days or longer are likely due to new sperm developing in the testis. This design enabled us to monitor the mobility of sperm that were mature and the mobility of sperm that were developing at the time of the social challenge. In other words, by measuring sperm mobility shortly after the social challenge, we tested for rapid exogenous changes associated with the social challenge occurring in fully developed extra-gonadal sperm, maturing in the vas deferens. By measuring sperm mobility 14 days after the social challenge, we tested for long-term endogenous changes occurring in sperm that were developing in the testes at the time of the social challenge.

Experimental animal care was in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.

(c) *Data analysis*

(i) *Inter-male relationship between sperm mobility and social status*

We investigated the inter-male relationship between sperm mobility and social status in two ways. First, we examined the probability (0, 1) of males from the high sperm mobility line becoming socially dominant using a generalized linear mixed model (GLMM) with a binomial error distribution and restricted pseudo-likelihood estimation (Wolfinger & O'Connell 1993). Only data from males in the high line were included because they were always paired with a male from the low line and the status of one male in a pair is dependent upon the other male. The order of social challenge (first and second) was entered as a fixed factor and male identity (defined as the experimental subject) was specified as a random factor. The back-transformed least squares (LS) means for the first and second social challenges were tested against 0.5 using a one sample *t*-test to assess whether males from the high line were more or less likely to become dominant in the first and second social challenges. Second, we analysed variation in sperm mobility between dominant and subordinate males during the first two measurements, before any experimental manipulation was imposed, using a GLMM with lognormal error distribution and restricted maximum-likelihood estimation (REML). Sperm mobility was positively skewed and therefore the model was defined with a lognormal error distribution, which normalized the data (residuals followed a normal distribution, Kolmogorov–Smirnov: $p > 0.05$). Social status during the first social challenge and sperm mobility line were included as fixed effects and male identity nested within pair (defined as the subject) was entered as a random factor.

(ii) *Intra-male relationship between sperm mobility and social status*

Intra-male relationship between sperm mobility and social status was analysed in two ways. First, variation in the sperm mobility of males of different status across all the four measurements were analysed using a GLMM with lognormal error distribution and REML estimation. Social status (DD, Ds, sD and ss), sperm mobility line (high and low) and measurement (1–4) were entered as fixed effects. We partitioned variance in sperm mobility attributable to male identity and changes (i.e. plasticity) in sperm mobility over the four measurements by fitting male identity (defined as the subject) and a male identity×measurement interaction as random effects (referred to as a multilevel mixed model or random coefficients model; Hruschka *et al.* 2005). To test whether the variance in sperm mobility attributable to male identity and male identity×measurement differed between status classes (DD, Ds, sD and ss), we ran separate GLMMs for each status group to generate variance components for the male and male×measurement terms for DD, Ds, sD and ss, which were then compared using one-way ANOVAs.

Second, we further explored changes in sperm mobility within males across the four status categories by examining three comparisons in sperm mobility: (i) sperm mobility in measurement 1−sperm mobility in measurement 2, (ii) sperm mobility in measurement 2−sperm mobility in measurement 3, and (iii) sperm mobility in measurement 3−sperm mobility in measurement 4. Variation in changes in sperm mobility was analysed using a GLMM with a normal error distribution and REML estimation as changes in sperm mobility between measurements followed a normal distribution (residuals were normally distributed, Kolmogorov–Smirnov: $p>0.05$). Social status (DD, Ds, sD and ss), sperm mobility line and sperm measurement comparison (1–2, 2–3 and 3–4) were entered as fixed effects and male identity (defined as the subject) was entered as a random effect.

The residuals from models were used to assess homogeneity of variance and outliers. The significance of random effects was assessed using likelihood ratio tests (LRT): the change in residual log-likelihood values when random factors were sequentially added was calculated and tested against a χ^2-distribution with degrees of freedom equal to the difference in the number of parameters added (Self & Liang 1987). The significance of fixed effects was examined using Wald-type tests (type III for main effects and type I for interactions; Grafen & Hails 2002). The fixed effect with the highest *P*-value was sequentially dropped until only significant terms ($p<0.05$) remained in the model. All analyses were performed using SAS v. 9.1.

3. RESULTS

(a) *Inter-male relationship between sperm mobility and social status*

Male status was randomly distributed with respect to high and low sperm mobility lines across male pairs and across social challenges (stage: $F_{1,29}=0.06$, $p=0.80$. LS means: first stage 0.50 ± 0.09 (mean±s.e.) tested against 0.5, $t=0.0$, d.f.$=29$, $p=1.0$; second stage 0.47 ± 0.09 (mean±s.e.) tested against 0.5, $t=0.36$, d.f.$=29$, $p=0.72$). After controlling for line differences, dominant and subdominant males did not differ significantly in sperm mobility (line: $F_{1,59}=263.84$, $p<0.0001$; status: $F_{1,59}=0.04$, $p=0.84$; line×status: $F_{1,59}=0.34$, $p=0.56$; figure 1).

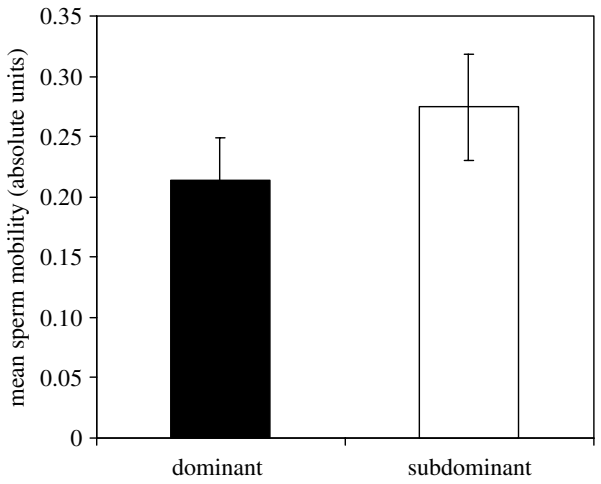

Figure 1. Mean sperm mobility of the males that became dominant and subdominant in the first social challenge. Subdominant males tended to have higher sperm mobility but this difference was not significant. Vertical bars represent s.e.

(b) *Intra-male relationship between sperm mobility and social status*

The sperm mobility of a male changed significantly across measurements. Importantly, consistent with the idea of a dynamic relationship between social status and sperm mobility, we found that the sperm mobility of males changed across measurements according to the social status they gained in the social challenge, resulting in a status×sperm mobility measurement interaction (line: $F_{1,164}=217.03$, $p<0.0001$; status: $F_{3,164}=0.14$, $p=0.94$; sperm measurement: $F_{3,164}=4.11$, $p=0.008$; status×measurement: $F_{9,164}=2.12$, $p=0.03$). Within each status phenotype, there was a significant amount of variation explained by male identity (table 1), but there was no difference in the variance attributable to male identity between status phenotypes (ANOVA, $F_{3,58}=2.16$, $p=0.10$; table 1). Furthermore, the degree to which sperm mobility changed over measurements was similar both within and between status groups (within groups: male×sperm measurement did not explain a significant amount of variation in mobility in any status group (LRTs $p>0.05$); between groups: ANOVA, $F_{3,58}=0.32$, $p=0.81$). We further explored the status×measurement interaction by analysing changes in sperm mobility within males across the four different status categories. We analysed relative changes in sperm mobility through three mobility comparisons: (i) sperm mobility measurement 2−measurement 1 (baseline changes before the social challenge), (ii) measurement 3−measurement 2 (rapid changes following the challenge), and (iii) measurement 4−measurement 3 (long-term changes following the challenge). Different status categories experienced distinct patterns of sperm mobility fluctuations, suggesting that social competitiveness predisposed males to specific fluctuations of sperm mobility. Importantly, the social competitiveness of a male was associated with both changes in baseline sperm mobility and changes in sperm mobility following the social challenge (line: $F_{1,110}=0.58$, $p=0.45$; status: $F_{3,110}=0.13$, $p=0.95$; sperm mobility comparison: $F_{2,110}=0.51$, $p=0.6034$; status×sperm mobility comparison: $F_{6,110}=2.56$, $p=0.024$). Consistent with the idea of a trade-off between status and sperm quality, in males that were consistently

Table 1. Status-specific phenotypic plasticity in sperm mobility. (Fluctuations in sperm mobility of individual males in the four social phenotypes. Likelihood ratio tests (LRT) were used to assess the significance of each random effect.)

social phenotype	term	*N*	variance component	s.e.	LRT (G statistic)	*p*
DD	male	15	0.63	0.26	45.36	<0.0001
DD	male×measurement		0.00	0.04	0.00	1.0
Ds	male	15	0.19	0.10	12.20	0.0004
Ds	male×measurement		0.00	0.05	0.00	1.0
sD	male	14	0.06	0.05	4.10	0.04
sD	male×measurement		0.08	0.05	0.00	1.0
ss	male	15	0.32	0.16	17.70	<0.0001
ss	male×measurement		0.01	0.06	0.00	1.0

dominant across the social challenge (DD), sperm mobility peaked immediately before the challenge (i.e. measurement 2), remained approximately constant shortly following the challenge (i.e. measurement 3) and dropped 14 days following the challenge (i.e. measurement 4). In males that remained subdominant (ss), on the other hand, sperm mobility remained constant across the social challenge (figure 2*a*). The sperm mobility of males of intermediate social phenotypes (Ds and sD) diverged shortly following the social challenge (measurement 3), increasing in males that were initially subdominant (sD) and decreasing in males that were initially dominant (Ds), both social phenotypes showing little change in sperm mobility in the 14 days following the social challenge (measurement 4; figure 2*b*).

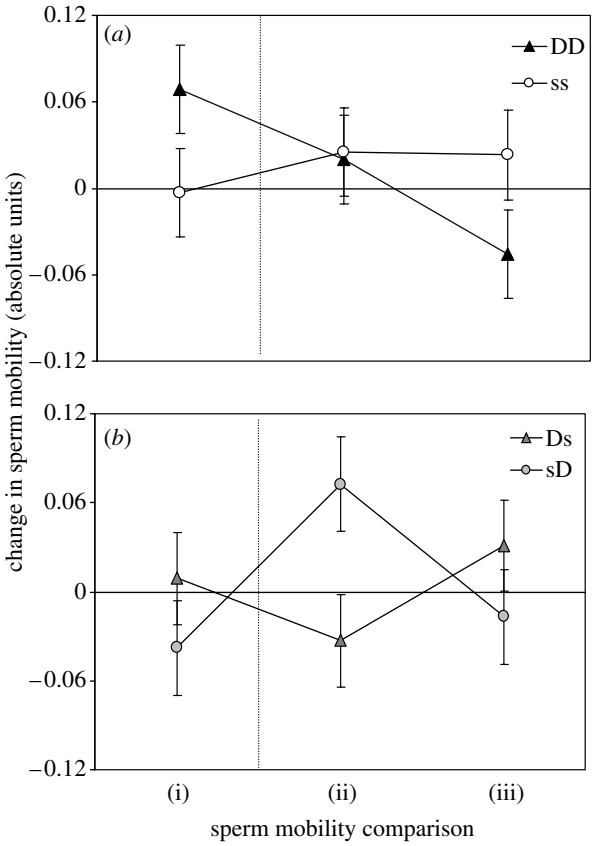

Figure 2. Mean(±s.e.) change in sperm mobility between measurements of (*a*) males that remained dominant (DD) and subdominant (ss) and (*b*) males that switched from dominant to subdominant (Ds) and from subdominant to dominant (sD). Relative changes in sperm mobility of across measurements were monitored through three mobility comparisons: (i) sperm mobility measurement 2−measurement 1, (ii) measurement 3−measurement 2, and (iii) measurement 4−measurement 3. (status×comparison *post hoc* LS means significance tests, DD 1 versus DD 2, $p=0.0101$; DD 1 versus Ds 1, $p=0.0215$; DD 1 versus sD 1, $p=0.0183$; DD 1 versus sD 3, $p=0.0575$; DD 3 versus sD 2, $p=0.0091$; Ds 2 versus sD 2, $p=0.0192$; sD 1 versus sD 2, $p=0.0163$; sD 2 versus sD 3, $p=0.0511$; all other tests non-significant). The vertical dotted line indicates when the social challenge occurred in relation to the three sperm mobility comparisons.

4. DISCUSSION

The present study is one of the first to analyse the degree of phenotypic plasticity in sperm quality, particularly in relation to social status. While the significant male effect is consistent with the previous results that sperm mobility is repeatable within males (Froman & Feltmann 1998), the study also revealed rapid and longer-term intra-male fluctuations in sperm mobility. Together, these behavioural and physiological data indicate that males undergo rapid and longer-term fluctuations in sperm mobility and that different social phenotypes are characterized by distinct patterns of sperm mobility fluctuations. Following the social challenge, sperm mobility dropped in males that won both social contests (DD) and remained constant in males that lost both (ss). Similarly, shortly after the social challenge, sperm mobility increased in males that lost the first and won the second contest (sD) and tended to drop in males that won the first but lost the second contest (Ds). These results are partly consistent with within-male trade-offs between sperm quality and male status suggested by the previous studies. First, recent evidence indicates that in humans the quality of ejaculated sperm may change rapidly in response to perceived risk of sperm competition (Kilgallon & Simmons 2005). Second, in the Arctic charr, *Salvelinus alpinus*, sperm velocity dropped in males that won a social contest, while it remained constant in males that lost a social contest within 4 days of the social challenge (Rudolfsen *et al.* 2006). However, little is known about the mating history of male Arctic charr before the experiment and the way the sperm velocity fluctuates independent of social challenges. Third, in sexually rested male feral fowl, a measure of sperm quality correlated with sperm mobility (average path swimming velocity, VAP) declined over successive natural ejaculates in socially dominant males but remained constant in subdominant males (Cornwallis 2004). This pattern was reversed within individual males when their status was experimentally changed (Cornwallis 2004). It remains unclear whether changes in relative sperm quality within males associated with social

challenges translate into phenotypic relationships between social status and sperm quality at a population level. Froman *et al.* (2002) found a weak, but significant, negative relationship between status and the mobility of the extra-gonadal sperm reserves (obtained through abdominal massage as in the present study) in males of the random-bred stock population from which the lines of the present study originated. However, in feral fowl, differences in the sperm quality of dominant and subdominant males only emerged towards the end of a succession of natural ejaculates (Cornwallis 2004). Average sperm quality across the succession of natural ejaculates did not differ significantly between dominant and subdominant males (Cornwallis 2004). The present study was based on the manual collection of semen samples which is likely to contain most of the extra-gonadal sperm reserves of a male (Parker *et al.* 1942), thereby potentially masking more marked variation that may occur across natural ejaculates. Interestingly, the status-specific baseline fluctuations in sperm mobility that occurred before the social challenge (i.e. when focal males were still socially naive) were of a similar magnitude to the changes that were recorded following the social challenge. This makes the causality of the changes in sperm mobility unclear because it is difficult to determine whether changes in sperm mobility are the direct consequences of the outcome of social challenges and/or whether changes in sperm mobility are due to males of different social competitiveness being predisposed to certain patterns of change in sperm mobility. An important cautionary note emerging from this study is therefore that baseline fluctuations must be considered when testing the response of sperm quality in relation to specific social or environmental challenges. Together, these results suggest that negative correlations between status and sperm quality across males in a population may arise from these intra-male fluctuations as transient properties of natural populations. The possibility that phenotypic relationships between social status and sperm mobility are transient would explain why the present study failed to detect a significant relationship between absolute sperm mobility and male social competitiveness. It is also possible that the sample size (i.e. number of male pairs) may have been too small to detect an effect of line on social status. However, previous studies have used similar sample sizes to detect significant negative phenotypic relationships between status and sperm quality in the fowl (Froman *et al.* 2002; Cornwallis 2004). Although individual males differed significantly in their absolute sperm mobility, the degree to which sperm mobility fluctuated within individual males was consistent across males of the same social phenotype and the degree of plasticity in sperm mobility did not vary across social phenotypes. This indicates that while there appears to be potential for sperm mobility to respond to selection at least at the phenotypic level, the strength of this response is unlikely to differ between males of different social competitiveness.

The results of the present study suggest the exciting possibility that the competitive quality of developed sperm can be re-programmed over few days, possibly in patterns dictated by the social phenotype of a male. Some changes in sperm mobility were observed over a period longer than 14 days and thus may have been explained by both physiological changes in developing sperm and changes in developed sperm, maturing in the ductus deferens. However, other changes occurred within 3 days of the social challenge and were too rapid to be explained by the production of new spermatozoa of different qualities, suggesting instead that they were determined by exogenous changes in seminal fluid composition and extra-gonadal milieu (Froman *et al.* 2006). Sperm mobility is determined by the ability of sperm mitochondria to consume oxygen for the conversion of fatty acids into ATP, through oxidative phosphorylation and thus reflects the metabolic performance of sperm mitochondrial function (Froman & Feltmann 1998; Froman *et al.* 1999). Froman & Kirby (2005) demonstrated that sperm from males of high and low sperm mobility show a twofold difference in sperm oxygen consumption, a twofold difference in motile sperm concentration between lines and a 10-fold difference in frequency of sperm with aberrant mitochondrial ultrastructure in the low line. This divergence was associated with multiple single nucleotide polymorphisms within the coding region of the mitochondrial DNA, including one within the arginine tRNA gene, which may play a crucial role in sperm ATPase and maturation across vertebrates and invertebrates (Radany 1979; Osanai & Chen 1993; Strong & Ellington 1993; Froman & Kirby 2005). In the fowl, maturation of fully developed sperm occurs in the ductus deferens (Kirby & Froman 2000), possibly through mitochondrial uptake of Ca^{++} and loss of K^{+} (Kirby & Froman 2000). Variation in sperm mobility may then be determined by mitochondrial ability to exchange Ca^{++} and K^{+} during maturation. It is therefore possible that rapid changes in sperm mobility following the social challenge may occur in maturing spermatozoa by sudden changes in the exogenous chemical milieu of the seminal plasma in the ductus deferens (Lake 1966; Fujihara 1992), possibly mediated by rapid changes in plasma steroid levels associated with changes in status (Johnsen & Zuk 1995). A possible, albeit untested, mechanism for some of the observed changes in sperm mobility is driven by changes in the rate of spermatogenesis associated with changes in status. All else being equal, an increased rate of sperm production in dominant males may reduce the access of individual sperm to seminal fluid, possibly resulting in decreased mobility. Sperm mobility influences the rate at which sperm are lost from the sperm storage tubules of a female, and thus for a given number of sperm inseminated, sperm mobility determines how long an ejaculate will be competitive in a female (Froman *et al.* 2002). Males investing in social status will have privileged access to females; therefore, they will have the opportunity of replenishing the sperm reserves stored by individual females and the risk of sperm competition faced by their ejaculates will be relatively low. Subdominant males, on the other hand, have limited opportunities to copulate with the same female and their ejaculates are likely to face sperm competition. Therefore, all else being equal, inseminating ejaculates of high sperm mobility may be more important for subdominant than dominant males. In other words, subdominant males will anticipate infrequent mating opportunity and increased probability of sperm ejection. Dominant males will enjoy female monopolization and frequent mating. The differences in mobility might therefore reflect an optimum ejaculate for the immediate social status of the male. This process may promote variance in paternity and thus may

help explain the paradox of the maintenance of genetic variation in spite of strong sexual selection in many promiscuous species where male reproductive success is mediated by both social status and ejaculate quality.

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