Changes in Sperm Quality and Numbers in Response to Experimental Manipulation of Male Social Status and Female Attractiveness

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ABSTRACT: In promiscuous species, male reproductive success is determined by the interaction between the ability to access and choose females of the highest reproductive quality and, after copulation, the ability to outcompete the ejaculates of rival males. Disentangling the factors regulating the interplay between traits conferring a reproductive advantage before and after copulation is therefore crucial to understanding how sexual strategies evolve. Here we show in the fowl *Gallus gallus*, where social status determines copulation success, that dominant males produce more sperm than subordinates but that the quality of dominant males' sperm decreases over successive copulations, whereas that of subordinates remains constant. Experimentally manipulating male social status confirmed that ejaculate quality (the number and quality of sperm produced) was a response to the social environment rather than the result of intrinsic differences between dominant and subordinate males. We further show that dominant males responded to variation in female sexual ornamentation, which signals reproductive quality, by adjusting the number and quality of sperm they transferred, whereas subordinate males did not: they transferred ejaculates of similar quality to females with different ornament sizes. These results indicate that trade-offs between traits influencing reproductive success before and after copulation, combined with variation in social dynamics and female quality, may favor the evolution of phenotypically plastic alternative reproductive strategies.

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Throughout the animal kingdom, females commonly copulate with multiple males within a single reproductive event (Parker 1970; Birkhead and Møller 1998; Simmons 2001). Such promiscuity plays a fundamental role in the evolution of sexual strategies because it generates sexual selection both before insemination, on the ability to access and choose partners conveying the highest reproductive benefits, and after insemination, on the ability of males and females to bias the paternity of offspring (Andersson 1994; Eberhard 1996; Wigby and Chapman 2004). Therefore, to understand the causes of variation in reproductive success and the processes underpinning sexual evolution, it is important to establish the mechanisms regulating the expression and interactions between traits under pre- and postcopulatory sexual selection (Andersson and Simmons 2006).

It is well established that competition between individuals for access to sexual partners promotes the evolution of a wide array of traits (Darwin 1871; Andersson 1994). Sexual competition is often more intense between males because of their potentially higher rate of reproduction and lower investment per offspring compared to females (Bateman 1948; Parker 1979). This, in turn, is thought to explain why females are usually more discriminating when selecting sexual partners than males (Trivers 1972; Bateson 1983; Andersson 1994). However, male mate choice is predicted to evolve when the probability of attaining future copulations is high, when females vary in reproductive quality, and when males suffer mating/parental costs (Burley 1977; Johnstone et al. 1996; Kokko and Monaghan 2001; Chenoweth et al. 2006). Although male mate choice has received less attention than female choice, there is increasing empirical evidence that males discriminate between females on the basis of body size and mass (Bonduriansky 2001), fecundity (Marconato and Shapiro 1996; Byrne and Rice 2006), and ornamentation (Amundsen

2000; Domb and Pagel 2001; Chenoweth and Blows 2003; Lebas et al. 2003). The environmental conditions that males experience and the relative costs and benefits of mate choice can, however, change, generating variation both within and between males in the degree of discrimination they exert over females (Johnstone et al. 1996). For example, males in favored mating roles, such as socially dominant positions, are predicted to exercise more stringent mate choice, all else being equal, because their probability of acquiring future copulations is higher than that of males in disfavored mating roles, such as subordinate positions (Parker 1983). There is some empirical support for this idea, with the strength of mate choice being dependent on the ability of males to access females (Ptasek and Travis 1997; Amundsen and Forsgren 2003; Preston et al. 2003; Cornwallis and Birkhead 2006).

Male mate choice can continue after copulation through the strategic adjustment of ejaculate quality (cryptic male choice; Parker 1998; Reinhold et al. 2002). Ejaculates can be costly to produce (Dewsbury 1982; Nakatsuru and Kramer 1982; Birkhead and Fletcher 1995; Pitnick 1996), and in numerous species the sperm reserves of males can become depleted, limiting their reproductive success (Preston et al. 2001; Sæther et al. 2001). Fertilization success is highly dependent on the number and quality (measured as sperm swimming velocity) of sperm that males inseminate (Martin et al. 1974; Wishart and Palmer 1986; Birkhead et al. 1995*b*; Birkhead and Møller 1998; Donoghue et al. 1998; Vladić and Järvi 2001; Froman et al. 2002; Gage et al. 2004; Snook 2005; Pattarini et al. 2006), a relationship that becomes even more pronounced when males compete for fertilizations (Allen and Champion 1955; Dziuk 1996). The fertilization advantage resulting from inseminating more and/or higher quality sperm, in combination with limited sperm resources, is expected to lead to the evolution of strategic sperm investment (Parker 1998; Wedell et al. 2002). Males have been shown to allocate more sperm to ejaculates when copulating with higher-quality females and when there is a greater risk of sperm competition (Birkhead and Møller 1998; Wedell et al. 2002; Pizzari et al. 2003; Pound and Gage 2004; Rubolini et al. 2006).

Evidence that males adjust the quality of their sperm in response to the risk of sperm competition and female quality is more limited. In a variety of species with alternative mating strategies, it has been shown that males in favored roles, which face a reduced risk of sperm competition, produce lower-quality sperm than males in disfavored roles, which continually face sperm competition (Vladić and Järvi 2001; Froman et al. 2002; Neff et al. 2003; Gage et al. 2004). It has been unclear whether differences in sperm quality between males in favored and disfavored mating roles were intrinsic or a facultative response to varying risks of sperm competition. Nevertheless, it is now emerging that males may respond to variation in the risk of sperm competition by adjusting their sperm quality (Kilgallon and Simmons 2005; Rudolfsen et al. 2006; Pizzari et al. 2007), but this research has limitations because sperm were not collected during copulation, making it difficult to assess how sperm quality changes across different mating contexts and over series of copulations. The number of copulations that males gain usually varies with factors such as mating role, so the way ejaculate quality changes with copulation order and rate has important implications for reproductive success but remains to be quantified. The number of sperm that males ejaculate often decreases with successive copulations (Birkhead and Møller 1998), which can influence the fertilization success of males in favored and disfavored mating roles (Preston et al. 2001), and there is also some evidence that sperm quality may decrease the more frequently males copulate, although whether this varies across males is unknown (Birkhead et al. 1995*a*). It is also uncertain whether males adjust their sperm quality in response to variation in female quality, despite evidence that they adjust sperm numbers (Wedell et al. 2002), which may be dependent on copulation order and frequency because of processes such as male mate choice.

The sexual strategies that males adopt may arise via different mechanisms (Gross 1996). Where trade-offs exist between sexual traits, disruptive selection may ensue, leading to the evolution of alternative reproductive strategies. Alternative reproductive strategies may be due to genetic polymorphism, with the expression of genotypes being fixed across different environmental conditions (alternative strategies), or phenotypically plastic, where genotypic expression is environmentally dependent and individuals change strategies according to the conditions that they experience (conditional strategy; Gross 1996). Where phenotypic plasticity occurs, variance in the strength of gene × environment interactions, in combination with the direction and strength of sexual selection, will determine the evolutionary trajectory of phenotypic plasticity (Roff 1997). Quantifying whether sexual strategies are phenotypically plastic and, if so, whether individuals vary in their level of plasticity has crucial implications for understanding the diversity of reproductive strategies.

The aims of this study were threefold: first, to assess whether males in favored and disfavored mating roles differ in the number and quality, measured as velocity, of sperm they produce over successive copulations; second, to determine whether the number and velocity of sperm that males produce is fixed or phenotypically plastic with respect to mating role; and finally, to establish whether males in different mating roles adjust the number and velocity of sperm they invest in females of differing re-

productive quality and whether this is dependent on copulation order.

The study was carried out using an old Swedish breed of fowl, *Gallus gallus*, which are behaviorally and morphologically similar to the ancestor of all chicken breeds, the red jungle fowl *G. gallus* spp. (Harrison 1987; Schütz and Jensen 2001). The fowl live in social groups that range from male-female pairs up to 12 males and 16 females, but they are usually found in groups of one or two males and three to five females (Collias and Collias 1967; Ali and Ripley 1981; Nishida et al. 2000). Consequently, males and females consistently encounter multiple copulation partners. Males form dominance hierarchies, and social status mediates access to females, placing dominant and subordinate males in favored and disfavored mating roles, respectively (Pizzari et al. 2002). Dominant males have higher copulation success and face a lower risk of sperm competition than subordinate males, but females frequently copulate with multiple males, generating intense postcopulatory sexual selection (Collias and Collias 1967, 1996; McBride et al. 1969; Pizzari et al. 2002). When females are promiscuous, the number and velocity of sperm that males inseminate relative to rivals determines fertilization success (Martin et al. 1974; Wishart and Palmer 1986; Froman et al. 2002). The promiscuous nature of the breeding system and the high frequency with which males can copulate (more than 40 copulations within a few hours have been observed; Pizzari et al. 2003) can lead to sperm depletion, and we have previously shown that males, especially dominant individuals, are economical with their gametes, adjusting the number of sperm they ejaculate according to the number of competing males, female novelty, and female reproductive quality (Pizzari et al. 2003; Cornwallis and Birkhead 2006, 2007). Females are adorned with fleshy head ornaments, called combs, that signal reproductive quality; females with larger combs are in better condition, are more likely to be sexually receptive, lay heavier eggs and eggs with more yolk, and have a tendency to be socially dominant (Cloutier and Newberry 2000; Joseph et al. 2003; Pizzari et al. 2003; Cornwallis and Birkhead 2007). Females' comb size varies, and males choose and allocate sperm to females with large combs relative to other available females (Pizzari et al. 2003; Cornwallis and Birkhead 2006, 2007).

Methods

Study Population

We studied a population of fowl at Tovetorp Zoological Research Station, University of Stöckholm, Sweden, from April to July 2002 and from May to August 2003. Males and females were randomly assigned to groups at the start of each breeding season. Males were kept in pairs in outdoor aviaries (6 m × 6 m) adjacent to aviaries (8 m × 6 m) containing groups of four females. Males could observe females in the adjacent aviary but were prevented from mixing with females to ensure that they were sexually rested. Social status was monitored throughout the breeding season by recording aggressive pairwise interactions (Clutton-Brock et al. 1979), and body mass was measured to the nearest 10 g every 2 weeks. The body size of individuals was measured at the beginning and the end of each breeding season and was calculated using PC1 of a principal-component analysis of tarsus length, wing length, and head length, which explained 79% of variation in morphological traits (Gosler and Harper 2000).

Measuring Sperm Numbers and Velocity

All individuals were fully habituated to human presence, which allowed natural ejaculates to be collected. Females were fitted with small plastic harnesses and gently held on the ground in front of the male in a soliciting position (Pizzari et al. 2003; Cornwallis and Birkhead 2006). Males readily mounted females presented in this way, and after copulation, ejaculates were collected, their volume was measured using a Gilson pipette, and ejaculates were stored in 5% formalin for sperm counting, which was performed using standardized methods (Bakst and Cecil 1997).

Sperm velocity was recorded by diluting 1 μL of semen in 50 μL Dulbecco's modified Eagle's medium (DMEM). Subsequent dilutions using DMEM were made to obtain a concentration of approximately 2×10^6 sperm mL^{-1}. Thirty microliters of this solution was placed on a microscope slide on a heated microscope stage at 41°C and videorecorded with a CCD KP-M1E/K Hitachi Denshi camera (Tokyo) connected to a BH-2 Olympus microscope (Tokyo) with dark-field optics at a magnification of × 20. The velocity of individual sperm was measured using a Hobson Sperm Tracker (Froman and McLean 1996; Pizzari et al. 2004). Average path velocity was used as the measure of sperm velocity because it positively correlates with fertilization success (Wishart and Palmer 1986) and was calculated by dividing the smoothed distance that each sperm traveled by the time taken to cover that distance. For each sample, 100 sperm were individually tracked within 3 min of being placed on the heated stage, and the mean value of the 100 tracks was used in analyses. Sperm velocity was measured over successive ejaculates. Because of the time required to videorecord each sperm sample (travel to and from the laboratory and recording time), only every other ejaculate that a male produced was measured. All samples were videorecorded within 20 min of ejaculation.

The Relationship between Social Status and Sperm Number and Velocity

To measure the number and velocity of sperm that males produced, they were subjected to controlled mating trials. Males, chosen at random, were visually isolated from the male they were paired with 30 min before trials began. All trials took place between 1630 and 2030 hours local time, the peak time of sexual activity in the fowl (Parker et al. 1940), and males were sexually rested (no copulations in the previous 48 h; Etches 1996). Single females (not in the groups adjacent to males) were randomly chosen from the population, fitted with a harness for collecting sperm, and gently held in front of males in a soliciting position. Males were allowed to copulate to satiation with the female. After 10 min had passed where the male had made no further attempt to copulate, a new female was introduced, and the male was again allowed to copulate to satiation. This procedure was repeated until males failed to copulate with two females in succession, which was done to reduce the influence of differential sperm allocation on measurements of the total number of sperm that males could produce. After copulations, ejaculates were collected and sperm numbers and velocity were measured. Males ($n = 26$) were exposed to up to two trials, at least 48 h apart, with each trial following the same protocol but using different females.

Generalized linear mixed models (GLMMs) were used to analyze variation in (1) the number of sperm ejaculated over successive copulations, (2) the total number of sperm produced over all copulations, (3) the total number of copulations, and (4) the velocity of sperm ejaculated over successive copulations. The terms entered into each analysis are detailed in the results tables (appendix in the online edition of the *American Naturalist*) and were classified in the following way: fixed factors = social status; covariates = copulation order, number of copulations, ejaculate volume, number of sperm in ejaculate, male body mass, and body size; random factors = year and male identity nested within group. The distribution of the residuals from the analyses of sperm number and velocity ejaculated over successive copulations (analyses 1 and 4) differed from normality (sperm numbers, Kolmogorov-Smirnov: $P < .001$, deviance = 2,284.71; sperm velocity, Kolmogorov-Smirnov: $P < .05$, deviance = 1,434.2). Data were normalized using ln transformations (sperm numbers, Kolmogorov-Smirnov: $P > .05$, deviance = 1,133.36; sperm velocity, Kolmogorov-Smirnov: $P > .20$, deviance = 73.95). Data on the number of copulations that males performed are counts and were therefore analyzed using a generalized linear mixed model with a Poisson error distribution. With data on successive copulations, there is a possibility that residuals from ejaculates produced closer together are more similar than values from ejaculates produced further apart, leading to spatially/temporally correlated errors within the data, which should be taken into account (Littell et al. 2006). Therefore, in the analyses of sperm number and sperm velocity over successive copulations, different spatial variance-covariance structures (spatial Gaussian, spatial power, and spatial exponential) were investigated that estimate the degree of correlation between observations and take into account unbalanced data (Littell et al. 2006). These spatial variance-covariance structures did not explain a significant amount of variation in either analysis and therefore were not used in the analyses (log-likelihood ratio tests [LRTs] of spatially structured model vs. null model, sperm number: spatial Gaussian $P = 1.0$, spatial power $P = .30$, spatial exponential $P = .30$; sperm velocity: spatial Gaussian $P = 1.0$, spatial power $P = 1.0$, spatial exponential $P = .95$). In all analyses, only data on males before any changes in social status occurred were used.

Fixed or Plastic Sperm Production: Manipulation of Social Status

The social status of males ($n_{\text{males}} = 11$) was experimentally manipulated halfway through the breeding season to ascertain whether the number and velocity of sperm that males produced were fixed or phenotypically plastic with respect to social status. Social status was manipulated by placing two males of the same dominance rank together, forcing one male to change status. After the manipulation, groups were left for 2 weeks to acclimatize before sperm number and sperm velocity were measured again. The velocity and number of sperm males produced were measured up to two times in each dominance position with the protocol outlined in "The Relationship between Social Status and Sperm Number and Velocity."

Variation in the total number of sperm produced and the velocity of sperm ejaculated over successive copulations when males were occupying different social positions was analyzed using GLMMs. The terms entered into each analysis are given in the results tables (appendix) and were classified in the following way: fixed factors = social status and initial status (1 = males started in a dominant position, 2 = males started in a subordinate position); covariates = copulation order and body mass; random factors = male identity and year. The residuals from the model of sperm velocity over successive copulations once again differed from normality (Kolmogorov-Smirnov: $P < .01$, residual deviance = 1,589.01) and were corrected using an ln transformation (Kolmogorov-Smirnov: $P > .05$, residual deviance = 12.50). Spatial variance-covariance structures were again investigated for data on sperm velocity over successive copulations, but they did not significantly reduce residual deviance and were therefore not

used (LRTs: spatial Gaussian $P = .40$, spatial power $P = .79$, spatial exponential $P = .82$).

Female Ornamentation

To assess whether males adjusted the number and velocity of sperm they transferred to females according to their reproductive value, males were exposed to a different type of controlled mating trial. Trials again took place between 1630 and 2030 hours, and males, chosen at random, were isolated from the male they were paired with 30 min before each trial. Two females that the male had not seen for at least 17 days (length of time females store sperm; Etches 1996) were randomly chosen from the population and fitted with harnesses. The two females were each randomly assigned to a person and held 1 m apart in front of the male with their heads oriented toward the male for 1 min, allowing him to observe the females. After the observation minute, females were placed in a soliciting position (head away from the males), and the male was allowed to copulate freely until he made no attempt to copulate for 10 min. The number and velocity of sperm males allocated to each female was measured. Males were exposed to up to two trials at least 48 h apart, and each trial followed exactly the same protocol, but different pairs of females were used each time. Female comb size was measured by taking a digital picture against a standard background under standard lighting conditions. After the breeding season, female comb size was calculated from pictures using Photoshop (Pizzari et al. 2003; Cornwallis and Birkhead 2007).

We analyzed the effect of female comb size on (1) the order in which males copulated with females over successive copulations, (2) the number of copulations females received, (3) the total number of sperm males allocated to females, and (4) the velocity of sperm females obtained over successive ejaculates. The terms included in each analysis are specified in the results tables (appendix) and were classified in the following way: fixed factors = social status; covariates = female comb size, number of copulations, and copulation order; random factors = year and male identity nested within group. In all analyses, female comb size was standardized in order to capture the differences between the two females that a male had an opportunity to copulate with (standardization = ln [comb size female A/comb size female B]). The order in which males copulated with females was analyzed using a generalized linear mixed model with binomial error distribution and the probability that a male copulated with a female (1 or 0) over successive copulations as the response variable. Because the probability that a male copulated with a female is not independent of the probability that he copulated with the other female (1 − probability of copulating with other female), only data on the female with the larger comb were analyzed. Variation in the number of copulations females received was analyzed using a generalized linear mixed model with Poisson error distribution. The total number of sperm that females received and the velocity of sperm that males ejaculated with different females over successive copulations were analyzed using GLMMs. The residuals from the GLMM of sperm velocity over successive copulations were normalized using an ln transformation (before transformation, Kolmogorov-Smirnov: $P < .05$, residual deviance = 507.99; after transformation, Kolmogorov-Smirnov: $P > .05$, residual deviance = −13.44). Spatial variance-covariance structures were again investigated in the analyses of copulation order and sperm velocity over successive copulations, but in neither case did any of the structures significantly reduce residual deviance (LRTs; copulation order: spatial Gaussian $P = 1.00$, spatial power $P = .51$, spatial exponential $P = .27$; sperm velocity: spatial Gaussian $P = 1.0$, spatial power $P = .66$, spatial exponential $P = 1.0$).

All analyses were performed in SAS, version 9.1, using "Proc Glimmix." Restricted maximum likelihood estimation (REML) was used in GLMMs, and restricted pseudolikelihood estimation (REPL) was used in generalized linear mixed models (Wolfinger and O'Connell 1993). The significance of fixed effects was examined using Wald-type tests, and the significance of random effects was assessed using LRTs: 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 between the two models. The fixed effect with the highest P value was sequentially dropped until only significant terms ($P < .05$) remained in the model (Grafen and Hails 2002).

Results

The Relationship between Social Status and Sperm Number and Velocity

The number of sperm that dominant and subordinate males ejaculated decreased over successive copulations ($P < .0001$; table A1; all tables are in the online edition of the *American Naturalist*), and there was no significant difference between dominant and subordinate males in the number of sperm they invested in individual ejaculates (table A1; fig. 1*A*). However, the sum total of sperm produced by dominant males was significantly greater than that produced by subordinates ($P = .007$; table A2; fig. 1*B*). After the effects of social status were accounted for, body mass was negatively related to the total number of sperm that males produced ($P = .005$; table A2), but body size and the number of time males copulated had no influence on the number

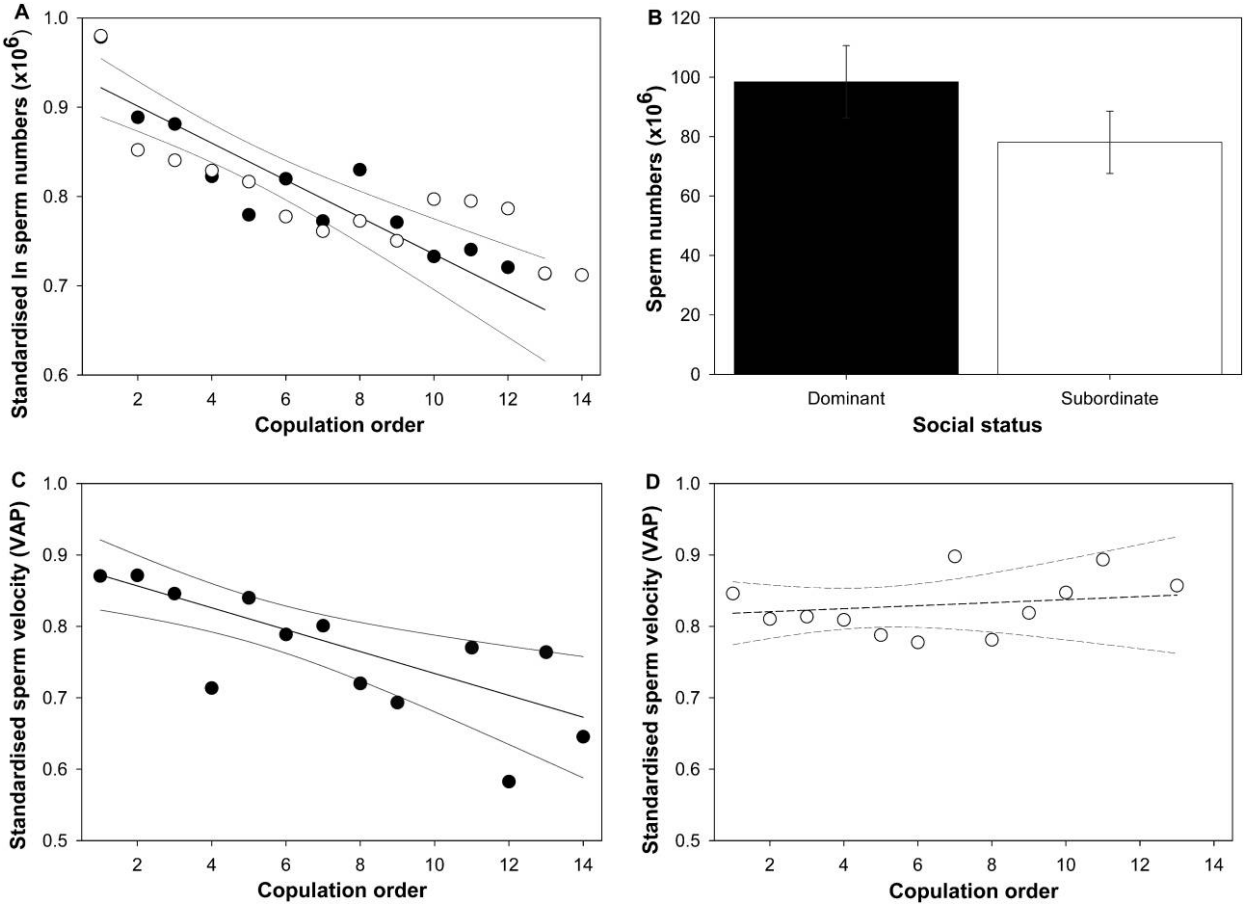

Figure 1: Number (*A*, *B*) and velocity (*C*, *D*) of sperm dominant and subordinate males ejaculated over successive copulations. Thick lines are regression lines, thin lines are 95% confidence intervals, and circles represent means. In *A*, *C*, and *D*, data are standardized for graphical purposes to show how sperm numbers and velocity changed within males with repeated copulation. Standardization = value for ejaculate/highest value for any ejaculate from a male in that group on that day. Sperm numbers were ln transformed before being standardized to generate a linear relationship. *A*, Rate at which males ejaculated sperm over successive copulations. Dominant (*filled circles*) and subordinate (*open circles*) males did not differ in the rate at which they ejaculated sperm, but the number of sperm they produced significantly declined with copulation order (regression line represents overall relationship). *B*, Total number of sperm dominant males (*filled bar*) produced over successive copulations was significantly greater than that produced by subordinate males (*open bar*). *C*, Velocity of dominant males' sperm over successive copulations declined significantly, whereas that of subordinate males (*D*) remained constant.

of sperm produced (table A2). There were also differences in the velocity of sperm that dominant and subordinate males ejaculated. Although average sperm velocity did not differ between males of different social status (table A3), over successive copulations the velocity of sperm produced by dominant males declined significantly (table A3; fig. 1*C*), whereas that of subordinate males remained constant (social status × copulation order, $P = 0.01$; table A3; fig. 1*D*). The difference in the velocity of sperm that dominant and subordinate males produced did not appear to be influenced by body mass or body size and was not dependent on the number of sperm contained within ejaculates or the ejaculate volume (table A3). Furthermore, dominant and subordinate males did not differ in the number of times they copulated with females (social status: $F = 0.19$, df = 1, 37, $P = .67$; body mass: $F = 2.78$, df = 1, 39, $P = .10$; body size: $F = 0.36$, df = 1, 38, $P = .55$; male (group) LRT $P = 1.0$; year LRT $P = .89$).

Fixed or Plastic Sperm Production: Manipulation of Social Status

When social status was experimentally manipulated, the number and velocity of sperm that males produced over successive copulations changed, indicating that investment in gametes was dependent on social environment (tables

A4, A5; fig. 2). When males were dominant, they produced a significantly greater total number of sperm than when they were subordinate ($P = .02$; table A4; fig. 2*A*). Furthermore, male identity explained a significant amount of variance in total sperm numbers ($P = .02$; table A4), indicating that there were inherent differences between males in the number of sperm they produced, although the interaction between male identity and social status was not significant, suggesting that plasticity in sperm production when social status changed was similar across males (table A4). Sperm velocity also changed in relation to social status: when males were dominant, they underwent a greater decline in the velocity of their sperm over successive copulations in comparison to when they were subordinate ($P = .02$; table A5; fig. 2*B*). A significant amount of variation in sperm velocity was also attributable to male identity ($P < .0001$; table A5), and the interaction between male identity and social status, indicating that males differed in their average sperm velocity and in the amount their average sperm velocity, changed with social status ($P = .005$; table A5). However, the interaction between male identity, social status, and copulation order was not significant, suggesting that males were similar in the way their sperm velocity changed over successive copulations when occupying different status positions (table A5). Changes in the number and velocity of sperm that males produced when their social status changed were independent of whether a male started in a dominant or subordinate position (initial status; tables A4, A5), revealing that phenotypic plasticity in ejaculate traits was independent of prior social experience.

Female Ornamentation

When presented with two females, males were more likely to copulate with the female with the larger comb ($P = .01$; table A6). This, however, changed over successive copulations, with the likelihood of the female with the larger comb receiving a copulation decreasing as males continued to copulate ($P = .006$; table A6). Females with relatively larger combs also received more copulations from both dominant and subordinate males ($P = .006$; table A7).

Consistent with previous research (Pizzari et al. 2003; Cornwallis and Birkhead 2006, 2007), dominant males transferred significantly more sperm to females with relatively larger combs (table A8; fig. 3*A*), whereas subordinate males did not (social status × comb size $P = .027$; table A8; fig. 3*B*). The number of times males copulated with females had a significant positive effect on the number of sperm they transferred ($P < .0001$; table A8), although once this effect had been taken into account, female comb size still explained a significant amount of variance ($P = .004$; table A8) in sperm numbers, demonstrating that males adjusted not only the number of copulations they allocated to females but also the number of sperm within their ejaculates (table A8). Interestingly, dominant males also allocated sperm of higher velocity to females with larger combs (table A9; fig. 3*C*), whereas the velocity of sperm that subordinate males ejaculated did not change significantly in relation to female comb size (social status × comb size $P = .02$; table A9; fig. 3*D*). The differences in the velocity of sperm that females with different comb sizes received from dominant males were not

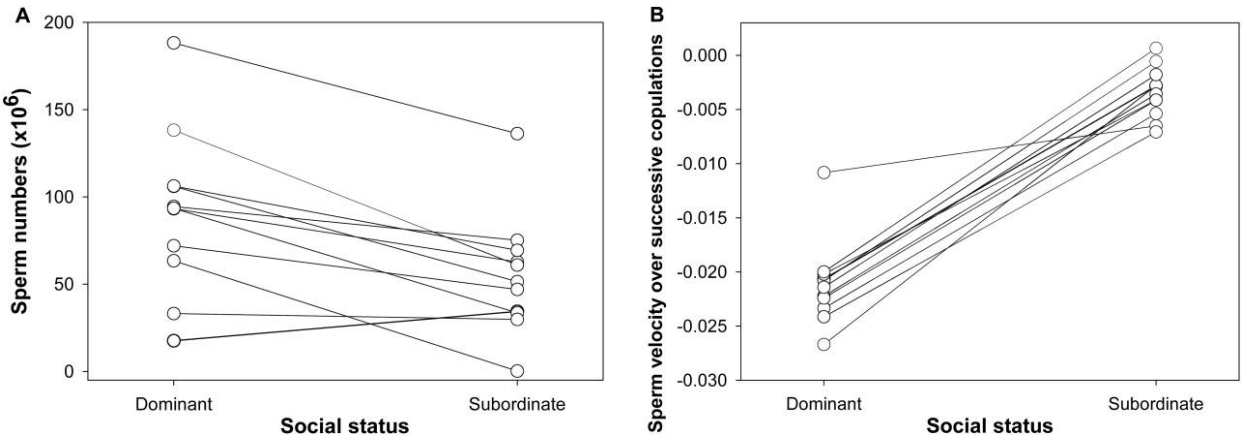

Figure 2: Changes in the total number of sperm produced (*A*) and the velocity of sperm ejaculated (*B*) over successive copulations when social status was manipulated. Circles represent means for individual males. Data presented in *B* are the individual slopes of sperm velocity for each male over successive copulations, acquired using the random term (best linear unbiased predictors, or BLUPs) from the general linear mixed model analysis. When males were dominant, they produced more sperm (*A*), but the velocity of their sperm over successive copulations (*B*) declined to a greater extent than when they were subordinate.

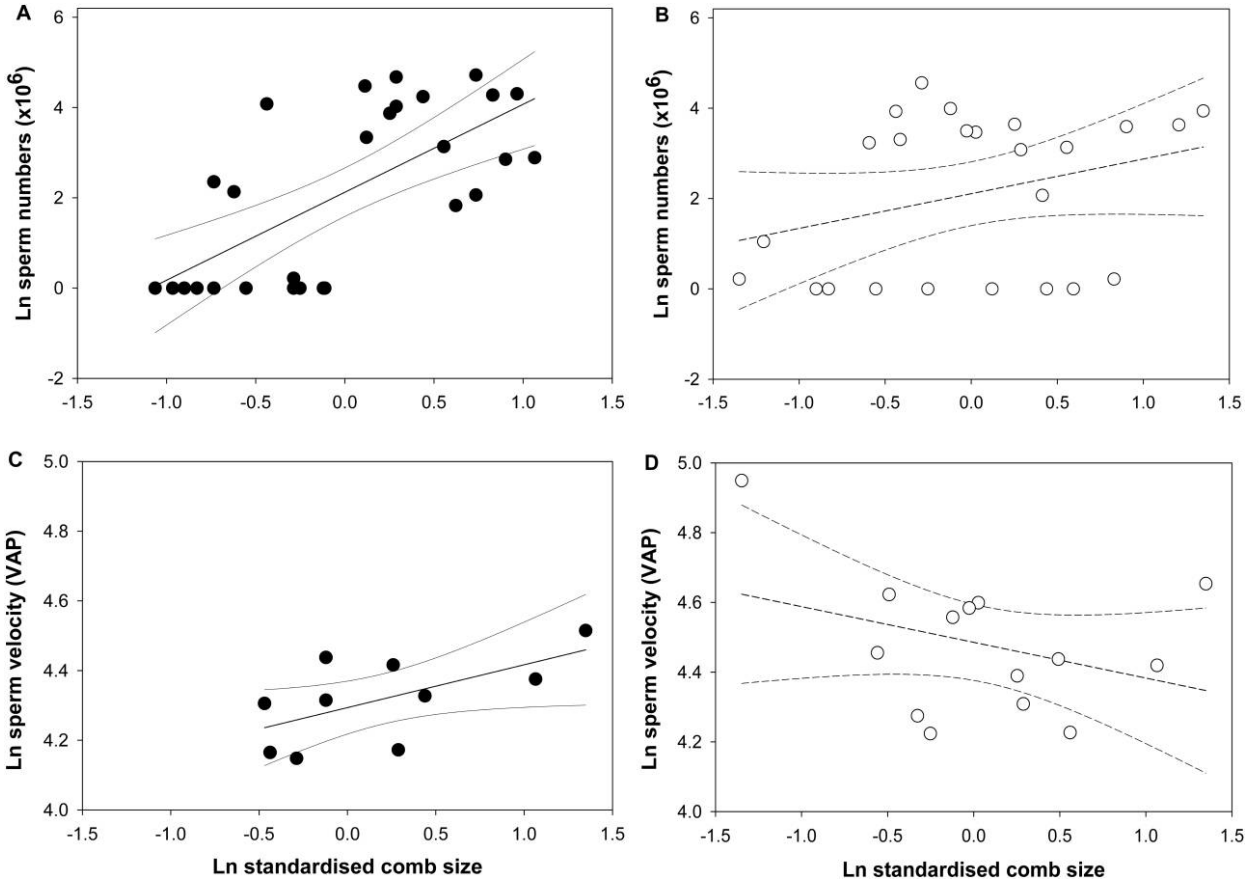

Figure 3: Number (*A*, *B*) and velocity (*C*, *D*) of sperm dominant and subordinate males invested in females with different comb sizes. Males were presented with pairs of females. The difference between the comb sizes of the two females was standardized as ln (comb size of female/comb size of other female). Dominant males invested more sperm (*A*) and sperm of higher velocity (*C*) in females with larger combs, whereas the number (*B*) and velocity (*D*) of sperm subordinate males ejaculated were unrelated to female comb size.

due to the order in which males copulated with females (table A9). In fact, the effects of copulation order on sperm velocity disappeared when males had the opportunity to copulate with two females (table A9), suggesting that the observed patterns of sperm velocity in this study may be due to males differentially adjusting the velocity of sperm within their ejaculates.

Discussion

This study illustrates that variation in female quality and social status, which determines access to females and the risk of sperm competition, have pronounced effects on ejaculate quality, which is likely to have an important effect on patterns of paternity. The influences of social status on the sperm that males produced were detectable only when copulation history was taken into account and were not the result of intrinsic differences between dominant and subordinate males but rather were phenotypic responses to the social environment. Social status also influenced the degree to which males adjusted their sperm investment with respect to female ornamentation, which signals reproductive quality. In accordance with theory (Johnstone et al. 1996; Reinhold et al. 2002), dominant males showed greater mate discrimination, investing both more and higher-velocity sperm in females with relatively larger ornaments than did subordinate males, which may influence the evolution of female ornamentation (Amundsen 2000). These results begin to reveal how changes in social dynamics, which in turn generate variation in male mating roles and the quality of females that males gain access to, interact to shape the evolution and expression of phenotypically plastic alternative reproductive strategies.

Previous studies have revealed relationships between sexual traits influencing pre- and postcopulatory success (Koyama and Kamimura 1999; Evans et al. 2003; Gage et

al. 2004; Fitzpatrick et al. 2006), some of which have been negative, suggesting that trade-offs exist between sexual traits (Zamudio and Sinervo 2000; Vladić and Järvi 2001; Froman et al. 2002). Few studies, however, have experimentally manipulated individuals to determine how investment in one trait affects others and instead have relied mainly on observations across males. Examining differences across males has limitations because it (1) does not reveal whether there is a causal link between the traits of interest, (2) may fail to detect relationships between traits because individuals differ in quality, and (3) does not provide insight into the selective mechanisms maintaining variation between individuals (phenotypic plasticity vs. genetic polymorphism). More recently, experimental approaches have been adopted that expose individuals to different environmental conditions and suggest that males may combat higher risks of sperm competition by adjusting the quality and/or the number of sperm they allocate to copulations (Kilgallon and Simmons 2005; Cornwallis and Birkhead 2006). Rudolfsen et al. (2006) and Pizzari et al. (2007) found, in arctic charr *Salvelinus alpinus* and domestic fowl *Gallus gallus*, respectively, that when males increase in social status, their sperm quality declines, whereas males that remain at the same status have consistent sperm quality. These studies, however, did not collect sperm during copulation, removing the possibility of estimating the effects of copulation history on ejaculate quality and making it difficult to interpret how these effects feed in to different mating scenarios.

In this study, the relationships between ejaculate traits and social status were apparent only when ejaculates were examined over a series of copulations. This highlights the importance of taking into account mating history and indicates that ejaculate quality, and in turn fertilization success, is influenced by the copulation rates of competing males. Attaining higher social status was associated with increased sperm production, which may enable males to capitalize on the mating advantage that social dominance provides (McBride et al. 1969; Pizzari et al. 2002), but it was also associated with a decline in sperm velocity over successive copulations, suggesting that males may trade off investment in sperm velocity against social competitive ability and/or number of sperm produced. In contrast, an increase in sperm production is unlikely to translate into more fertilizations for subordinate males because they gain few copulations. Therefore, the reproductive success of subordinate males may not be constrained by the total number of sperm produced, alleviating any trade-off that may exist between sperm production and sperm velocity, whereas dominant males' fertilization success may be dependent on such trade-offs. Trade-offs between traits may generate disruptive selection, facilitating the maintenance of variation within the sexes through the evolution of alternative reproductive strategies.

Whether alternative reproductive strategies evolve to be phenotypically plastic will depend on the frequency at which individuals occupy different mating roles, which is often in the fowl (Collias and Collias 1996), and on the amount of additive genetic variance attributable to gene × environment interactions (Roff 1997). This study examined only phenotypic responses to different social conditions, and therefore the underlying genetic variation in plasticity remains to be established. Nevertheless, at the phenotypic level, variation in plasticity in sperm numbers and velocity between males with respect to changes in social status were limited. This suggests that the potential for plasticity in these traits to evolve further is restricted. Males did, however, vary in the amount by which their average sperm velocity changed when they switched social status (male × social status), indicating that if these changes are genetically determined, then plasticity in average sperm velocity may respond to selection. A significant amount of variance in the number of sperm produced and sperm velocity was also explained by male identity, suggesting that there are intrinsic differences between males in these traits across different mating contexts.

The mechanisms regulating phenotypic plasticity in ejaculate traits remain to identified. However, spermatogenesis in the fowl takes approximately 16 days (Etches 1996), and the changes that occurred in sperm velocity in this study took place over a series of copulations (hours), suggesting that the adjustment of sperm velocity may be mediated by other factors, such as seminal fluid composition, rather than by changes in the sperm per se. Seminal fluid composition can influence sperm velocity in the fowl, and the volume and composition of seminal fluid in ejaculates systematically changes over successive ejaculates (Nishiyama 1955; Fujihara 1992; Etches 1996). Male fowl may therefore adjust the velocity of their sperm by differentially allocating seminal fluid to copulations. The composition of seminal fluid is also influenced by testosterone (McDowell et al. 1996), and in the fowl, dominant males have elevated levels of androgens, especially testosterone (Johnsen and Zuk 1995). Testosterone influences the activity of Leydig and Sertoli cells in the testes and, in turn, a male's daily sperm production (de Reviers and Williams 1984). It is therefore plausible that increases in testosterone levels when males become dominant may influence the rate of sperm production and change sperm velocity by influencing the seminal fluid contained within ejaculates.

The allocation of different volumes and/or compositions of seminal fluid may explain the way males adjusted the velocity of their sperm in relation to female ornament size. Males are expected to differentially invest in females when

the probability of gaining future reproductive opportunities is high, females vary in quality, and males suffer mating/parental costs (Parker 1983; Johnstone et al. 1996). These conditions are often met in the fowl (Collias and Collias 1996; Cornwallis and Birkhead 2007), especially for dominant males, which in comparison to subordinates have a higher probability of gaining future copulations and suffer higher costs to mating that arise from sperm depletion and reductions in sperm velocity. In a number of species, including the fowl (Amundsen and Forsgren 2003; Preston et al. 2003; Cornwallis and Birkhead 2007), dominant males respond to variation in female quality by adjusting the number of sperm they ejaculate, making it plausible that they also adjust the seminal fluid in their ejaculates, generating changes in their sperm velocity. If dominant males' sperm velocity is constrained by the availability of seminal fluid, then the observed patterns of sperm velocity may result from the differential allocation of limited resources of seminal fluid. This may explain why dominant males' sperm velocity decreased with copulation order when females were presented singly but not when they were in pairs. Dominant males have been shown to allocate more sperm to initial copulations when females are on their own and the probability of gaining future copulations is low, in comparison to when females are in pairs, which causes males to allocate sperm according to female ornamentation (Cornwallis and Birkhead 2006). Allocation patterns of seminal fluid may be similar, giving rise to the observed changes in sperm velocity.

In contrast to dominant males, the velocity of the sperm that subordinate males invested in females remained constant, which is perhaps not surprising because they were able to continually produce high-velocity sperm, enabling maximum investment in each female. However, the number of sperm that they ejaculated declined steeply over successive copulations, indicating limited sperm reserves. It is possible that these limits are not reached under natural mating conditions, because subordinate males gain only a few copulations, and so there may be little need for males to adjust their sperm investment. Alternatively, the failure of subordinate males to invest more sperm in females with larger ornaments may be due to sperm competition in these females being too intense, which theoretically will lead to a reduction in investment, enabling sperm to be conserved for situations with less sperm competition (Parker 1998; Engqvist and Reinhold 2006). The sperm allocation patterns of subordinate males may therefore be under balancing selection, shaped by the opposing forces of female quality and the avoidance of intense sperm competition (Reinhold et al. 2002; Engqvist and Reinhold 2006).

Investing in females with large combs is likely to be beneficial because they lay larger eggs with more yolk, are in better condition, and are more likely to be socially dominant (Cornwallis 2004; Cornwallis and Birkhead 2007). Collias et al. (1994) also found that dominant female red jungle fowl have higher lifetime reproductive success than subordinate females. In turn, reproducing with dominant males may drive the evolution of female ornamentation. Dominant males provide females with greater direct benefits (Pizzari 2003) and may also offer superior genes (Collias and Collias 1996; Parker and Garant 2004). Despite dominance changing, there is a heritable component to social status (Craig et al. 1965), and dominant males have higher lifetime reproductive success (Collias et al. 1994; Collias and Collias 1996). Sexual selection is thus predicted to favor female sexual traits that promote the probability of reproducing with dominant males, and by possessing large combs, females are able to secure more sperm of higher velocity from dominant males.

Together, these results suggest that variation in social dynamics can favor the evolution of phenotypically plastic alternative reproductive strategies. It appears that social competitive ability has important influences on the ejaculate traits of males, and although it is well established that males can vary the number of sperm in ejaculates, we show that they can also adjust the velocity of their sperm. It now remains for the implication of these results to be assessed under natural mating conditions and for the mechanisms underlying the adjustment of sperm velocity to be examined.

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Left, male fowl. *Right*, a group of female fowl (photographs by Charlie K. Cornwallis).