Pre- and postcopulatory sexual selection favor aggressive, young males in polyandrous groups of red junglefowl

Running head: Sexual selection favors aggressive, young males

Keywords
Aggression; *Gallus*; Polyandry; Reproductive Senescence; Sexual Networks; Sperm Competition

Data archival location
Authors intend to deposit supporting data on Dryad digital repository on acceptance.

Abstract

A challenge in evolutionary biology is to understand the operation of sexual selection on males in polyandrous groups, where sexual selection occurs before and after mating. Here, we combine fine-grained behavioural information (>41,000 interactions) with molecular parentage data to study sexual selection in replicated, age-structured groups of polyandrous red junglefowl, *Gallus gallus*. Male reproductive success was determined by the number of females mated (precopulatory sexual selection) and his paternity share, which was driven by the polyandry of his female partners (postcopulatory sexual selection). Pre- and postcopulatory components of male reproductive success covaried positively; males with high mating success also had high paternity share. Two male phenotypes affected male pre- and postcopulatory performance: average aggressiveness towards rival males and age. Aggressive males mated with more females and more often with individual females, resulting in higher sexual exclusivity. Younger males mated with more females and more often with individual females, suffering less intense sperm competition than older males. Older males had a lower paternity share even allowing for their limited sexual exclusivity, indicating they may produce less competitive ejaculates. These results indicate that - in these populations - postcopulatory sexual selection reinforces precopulatory sexual selection, consistently promoting younger and more aggressive males.

Introduction

Arising from competition between members of one sex for access to reproductive opportunities with members of the opposite sex, sexual selection is a powerful agent of evolutionary exaggeration and diversification (Darwin 1859, 1871; Andersson 1994). In polyandrous populations, where females mate with multiple males, males compete both before mating (i.e. competition for mates; precopulatory competition) and after mating (i.e. competition over paternity share; postcopulatory competition) (Parker 1970; Parker and Birkhead 2013; Firman et al. 2017). Under these conditions, males should therefore invest in both precopulatory competition, e.g. by establishing territories and/or social dominance to attract mates and exclude competitors (Andersson 1994; Simmons et al. 1999; Montrose et al. 2008; Collet et al. 2012; Procter et al. 2012), and postcopulatory competition, through sperm numbers (e.g. large ejaculates and/or high remating rates; Wedell et al. 2002; Parker and Pizzari 2010) or traits associated with the fertilising efficiency of an ejaculate (Snook 2005).

While some degree of polyandry is prevalent in natural populations (Taylor et al. 2014), we are only beginning to unravel the complex interplay between precopulatory and postcopulatory episodes of sexual selection (Pizzari and Wedell 2013; Evans and Garcia-Gonzalez 2016). Untangling this complexity is an outstanding challenge in sexual selection studies (Andersson and Simmons 2006; Jones and Ratterman 2009; Parker and Birkhead 2013; Evans and Garcia-Gonzalez 2016), with implications for our understanding of alternative mating tactics, patterns of sexual conflict and the maintenance of genetic variation within populations (Holman and Kokko 2013; Kvarnemo and Simmons 2013; Evans and Garcia-Gonzalez 2016). For example, a negative covariance between male mating success and paternity share indicates that males who are successful in precopulatory competition are disadvantaged in postcopulatory competition and *vice versa*. This suggests potential trade-offs between pre- and poctopulatory male investment. Negative covariances may therefore promote the evolution of alternative mating tactics and the maintenance of polymorphism (Moore and Moore 1999; Taborsky et al. 2008). Positive covariances on the other hand, create the opportunity for pre- and postcopulatory sexual selection to consistently favour the same male phenotype, suggesting a lack of trade-offs between pre- and poctopulatory male investment.

Addressing this challenge hinges on resolving the way in which the overall level of polyandry of a group influences the operation of sexual selection on males (Devigili et al. 2015; Evans and Garcia-Gonzalez 2016). A number of empirical studies have begun to exlpore this through detailed information of sexual interactions, mating behaviour and reproductive success in promiscuous groups under realistic conditions (Collet et al. 2012; Pélissié et al. 2014; Devigili et al. 2015; Turnell and Shaw 2015). In addition to group-level polyandry, variation in polyandry among females within a group may also play a key role in modulating sexual selection on males (Sih et al. 2009; McDonald et al. 2013; McDonald and Pizzari 2016). For example, a recent study of a natural population of field crickets, *Gryllus campestris*, found that males employing the most successful precopulatory strategies were unable to prevent postcopulatory competition because they failed to curtail polyandry. This meant that the males that mated with more females necessarily included more polyandrous females among their sexual partners (Fisher et al. 2016). Lack of paternity data however, prevented this study from estimating the consequences of these patterns for male reproductive success and postcopulatory sexual selection. To do this, we must establish the way in which the distribution of mating within a group determines patterns of postcopulatory competition, and how this in turn shapes the relationship between pre- and postcopulatory selection (Muniz et al. 2015; Wey et al. 2015; Fisher et al. 2016; McDonald and Pizzari 2016).

Here, we combine detailed behavioural data and molecular parentage to study the operation of pre- and postcopulatory sexual selection in replicate freely-mating, age-structured social groups of red junglefowl, *Gallus gallus*. This system allows the experimental engineering of replicate social units, while enabling fine-grained recording of the complex behavioural interactions occurring naturally in these groups. This system thus represents a helpful compromise between studying sexual selection in the laboratory *versus* in nature. Fowl groups are highly polyandrous and both pre- and postcopulatory processes contribute to variation in male reproductive success (Pizzari and Birkhead 2000; Collet et al. 2012, 2014). Previous work has shown that male social status is a key determinant of male reproductive success in small social groups of red junglefowl or feral populations of the related domestic chicken, *G. domesticus* (Pizzari and Birkhead 2000; Johnsen et al. 2001; Pizzari et al. 2002; Dean et al. 2010; see Pizzari 2016 for a recent review). Social dominance provides males with privileged mating access to females (Collet et al. 2012; Pizzari 2016). Furthermore, socially dominant males may be able to defend paternity in different non-mutually exclusive ways. First, they might reduce the level of sperm competition by preventing subordinates from mating with the same females (Dean et al. 2010). Second, they may ‘top up’ their sperm representation within the sperm storage organs of a female by mating repeatedly with the same females (Collet et al. 2012). One mechanism through which dominant males can achieve this monopoly across animal societies is through aggressive interactions with their rivals (Packer 1979; Moore and Moore 1999; Muller and Wrangham 2004). For example, more aggressive males might be able to both mate with more females and simultaneously prevent other males from mating with the same females. This would result in a negative relationship between their mating success and the intensity of sperm competition faced by their ejaculates (McDonald and Pizzari 2016). Little is known however about the mechanisms through which social status is favoured by pre- and postcopulatory sexual selection in polyandrous groups.

An additional complication is that previous studies of social status have often neglected the potentially confounding effects of male age. For example, in coalitions of Barbary macaques, *Macaca sylvanus*, older (i.e. post-prime) males are socially subordinate to younger adult males in their prime (Berghänel et al. 2011). Similarly, in lekking fallow deer, *Dama dama*, male status peaks at intermediate ages (Farrell et al. 2011). Male age has been shown to shape male reproductive success in fowl populations through male reproductive senescence, which penalizes older competitors (Dean et al. 2010; Noguera et al. 2012; Cornwallis et al. 2014). In small groups of feral domestic fowl, old males were just as likely as younger males to dominate hierarchies (Dean et al. 2010). However, old dominant males were unable to fertilise all the eggs produced by females but their status enabled them to prevent younger subordinates from mating with females. This resulted in a considerable proportion of eggs remaining unfertilised in groups dominated by old males (Dean et al. 2010). It is unclear however, whether dominant males may be similarly able to avoid sharing partners with subordinates in larger groups, where it is harder for a male to enforce sexual monopoly, and whether in such groups old males might be able to display sufficient levels of aggressiveness to retain top-ranking status and monopolise access to females (Dean et al. 2010). The way in which male status interacts with male age to determine reproductive success, in polyandrous groups therefore remains unresolved. In this study we address three objectives.

First, we study the architecture of male reproductive success by dissecting the key sources of variation such as mating success and paternity share. Previous work on smaller social units of this population indicated that paternity share due to postcopulatory competition represents the major source of variation in male reproductive success, followed by precopulatory mating success and positive covariance between these sources (Collet et al. 2012). We wanted to confirm these patterns in larger, more complex social groups.

Second, we investigated the independent roles that male social status and age play in pre- and postcopulatory processes. Based on previous work, we predicted male status to confer a competitive advantage in both pre- and postcopulatory episodes (Pizzari 2016). We further predicted that the role of male status in pre- and postcopulatory sexual selection is explained by male propensity to attack other males (male average aggressiveness). Also based on previous work, we predicted old males (i.e. past their prime) to be disfavoured in both pre- and postcopulatory competition (Dean et al. 2010). It is also possible that younger males may be less competitive than males in their prime as has been found in other species (e.g. Farrell et al. 2011), but the evidence for this effect is less clear in male fowl (Dean et al. 2010; Cornwallis et al. 2014). We show that male average aggressiveness and age play important and independent roles in sexual selection.

Third and finally, we examined the specific mechanisms through which male aggressiveness and age independently influence postcopulatory sexual selection. We predicted that male aggression towards other males enables a male to both prevent other rivals from mating with his partners, and remate with the same females more frequently. Similarly, we predicted older males to be less successful in both such strategies than younger males.

Methods

Study population and replicate groups

We studied replicate groups of red junglefowl (*Gallus gallus*), a species that naturally lives in social groups ranging from 2 to 28 individuals (Collias and Collias 1996), with variable levels of polyandry (Collet et al. 2012; Pizzari 2016). All individuals used in this study originate from a population kept at the University of Oxford field station in Wytham, UK (see Gillingham et al. 2009; Worley et al. 2010; Collet et al. 2012; Løvlie et al. 2013; Collet et al. 2014 for more information). All experimental work was conducted over three breeding seasons (April-October, 2011-2013) under semi-natural conditions (free-ranging in outdoor pens). We studied 20 experimental mixed-sex groups in total, each consisting of a unit of 10 adult males and a unit of 12 adult females. Each experimental group had a unique 10-male unit that was created from a pool of 127 unique males. In total 61 males were re-used across experimental groups due to limitations on available males (figure S1). Females on the other hand, were assembled into only 10 unique female units that were combined with different male units to form each mixed-sex group (figure S1). Each female unit had 12 females, created from a pool of 78 unique females, with 48 females used across multiple 10 female units due to limitations on available females (figure S1). On six occasions a female was removed from a trial due to death or illness. In these cases the female was replaced immediately with a new female to maintain consistent sex ratios, and the female present for the shortest length of time was excluded from analysis. Throughout our analyses (see Data analysis), we include random effects for male identity and the identity of female units to account for these sources of non-independence within our data.

We housed females in single-sex groups for a minimum of 14 days before each trial to ensure the depletion of sperm potentially stored from previous mating (Etches 1996). Male units were housed in the experimental enclosure for 3 days prior to the release of females to allow male dominance hierarchies to form and stabilize and ensure sperm reserves were not depleted from prior copulations (Etches 1996). On the morning of the first day of each trial all females joined the males in the experimental enclosure.

Behavioural observations and male traits

Behavioural observations commenced on the first day, when females joined males in the experimental enclosure, and lasted for a total of 10 days. Observations were made twice every day for 3 hours at 0500-0800 GMT and again at 1800-2100 GMT, totalling 1,200 hours of behavioural observations across the 20 mixed-sex groups. At the end of trials, females were isolated from males and males were returned to single-sex enclosures with other males from the stock population. Throughout trials we used *ad libitum* sampling techniques as all individuals could be observed at once (Altmann 1974). We recorded all male-male interactions including aggressive interactions (i.e. pecks, chases, fights, waltzes) and avoidances (Johnsen et al. 2001). A male was considered the loser in any aggressive interaction if he retreated one body length or more from the aggressing male (Johnsen et al. 2001; Froman et al. 2002; Wilson et al. 2009). Male avoidances occur when a focal male retreats more than a body length away from an approaching male. We recorded all copulation attempts, defined as any occasion when a male attempts to grab and/or mount a female (Løvlie et al. 2005), where separate attempts must be isolated by at least five seconds. Copulations were considered successful when cloacal contact was observed or when the male tail was lowered over the female cloaca and cloacal contact was assumed to occur (Pizzari and Birkhead 2000; Collet et al. 2012). We recorded 17,562 attempted copulations, 3,510 successful copulations and 23,448 male-male interactions.

To explore how male competitive behaviours shape male reproductive success through pre- and postcopulatory competition we characterised interactions between males in two ways: male social status and male average aggressiveness. Because we aimed to test how these intrasexual behaviours predict male mating success and the frequency at which a male remates with the same female, we excluded competitive interactions directly related to mating opportunities (i.e. copulation interruptions) from our measure of male average aggressiveness and status. This is because interruptions are largely manifested as copulation attempts themselves and as such do not represent a measure independent of mating success or remating rates (e.g. Pizzari 2001)

Male social status was calculated based on all male-male aggressive interactions and male-male avoidances (see above) using David's score (David 1987; de Vries et al. 2006). David's score is a ranking method used to calculate male social status based on the proportion of wins and losses between interacting individuals and is appropriate when data sets have high asymmetry in interaction strength between pairs (Gammell et al. 2003; de Vries et al. 2006; full details provided in supporting information A1). Male average aggressiveness was calculated as the mean number of aggressive interactions initiated by the focal male across all males in the group.

We measured male age as the number of breeding seasons (late spring to autumn) that the male has experienced. All birds hatch between summer and winter in a given year then mature over winter and spring before their first breeding season. Males in their first breeding season are thus deemed yearlings (1 year old), males in their second breeding season 2 years old and so on. Across the whole population males ranged from 1-7 yrs. old with a mean (±SE) of 2.815yrs ± 0.142. Each replicate group included males in their first reproductive season (1yr old) and a number of older males aged up to at least 4 yrs. old (table S1).

Molecular methods

We collected eggs laid by the females of a group during the 10 days of the trial and the 11th day after a trial was complete. Eggs were incubated artificially for 5-9 days before taking tissue samples. Embryo tissue samples were subsequently stored in absolute ethanol at 4°C until later parentage assignment. We ignored eggs laid on day one of a trial because these are unlikely to be fertilized (Etches 1996), and instead only considered parentage data for eggs laid from days 2-10 of each trial, plus the 11th day after the trial was complete. DNA was extracted following a standard ammonium acetate precipitation protocol (Nicholls et al. 2000). All samples were genotyped at 10 variable microsatellite loci in a single multiplex reaction using primers designed to amplify in domestic chicken (table S2; Cheng and Crittenden 1994; Crooijmans et al. 1996, 1997; Gibbs et al. 1997; Hanotte et al. 1997; Dawson et al. 1998; Groenen et al. 2000) or across a wide range of bird species (table S2; Dawson et al. 2010). PCRs were carried out in 2 µl volumes, using the reagents and methods described in Kenta et al. (2008). Cycling conditions for both multiplexes were as follows: an initial heating step of 95˚C for 15 minutes was followed by 35 cycles of 94˚C for 30 seconds, 56˚C for 90 seconds and 72˚C for 60 seconds. A final extension step of 60˚C for 30 minutes completed the reaction. PCR products were diluted by 1 in 150, and fragments were separated on an ABI 3730 capillary sequencer. Allele sizes were assessed against ROX 500 size standard using the software GeneMapper version 3.1 (Applied Biosystems).

Null allele frequencies for each locus were estimated using CERVUS version 3.0.1 (Kalinowski et al. 2007). Molecular parentage analyses were carried out in CERVUS, using the approaches outlined in Dean et al. (2010). Because of the larger number of closely related potential parents in our experimental design, we could not assign parentage to all offspring with 95% confidence (135 eggs). Subsequent analyses using parentage data were therefore carried out including only the individual eggs that could be assigned parentage with 95% confidence, totalling 847 eggs. None of the 10 microsatellite loci had high null allele frequencies (highest = 0.03), so all were used for parentage analyses. In some cases a male sired offspring with a female despite this pair never being observed copulating. We added this female to the male's mating success and assumed that this pair copulated once to better inform the structure of our intersexual and male competitive networks (see below). In total, we observed 1224 unique pairs copulating, with an additional 96 pairs that were inferred to have copulated from molecular parentage analysis alone.

Data analysis

(i) Mating success, paternity share and reproductive success

We first assessed the potential for sexual selection on males. We calculated male total reproductive success (T) as the sum of all zygotes he sired. Male T can be broken down into three constituent components: his mating success (M, i.e. the number of unique females with which he copulated successfully), the average fecundity of his sexual partners (N), and the proportion of all his partner's zygotes that he fertilized (P), such that: $T = M \times N \times P$. We calculated the standardized variance in T and each reproductive component as in Webster et al. (1995). Because of the low level of variation in *N* over the 10 days eggs were collected, we focus on *M* as the main source of precopulatory reproductive success here but continue to explore *N* in further sections below.

We then calculated precopulatory sexual selection on male mating success across all groups using mixed-effects models with a Poisson error distribution, with *T* as response variable and *M* as an explanatory variable. This approach measured the male Bateman gradient across replicate groups (Bateman 1948; Arnold and Duvall 1994; Jones 2009).

To study the impact of the polyandry of a male's sexual partners on his reproductive success, we calculated the "sperm competition intensity" (*SCI*) experienced by each male. Assuming a simple null model of sperm competition where the fertilising efficiency of the ejaculate of each male that mated a given female is equal, male paternity share is inversely proportional to the number of males that mate with the same partner (i.e. $1/k$, where k = number of males mating with the same female) (Shuster and Wade 2003). Using this null model, male *SCI* can be estimated as the harmonic mean mating success of his sexual partners, (i.e. the average mating success of his female partners; McDonald and Pizzari 2016). This is given as $SCI_i = 1/\frac{1}{M}(\sum_j^M \frac{1}{k_j})$, where *M* is the number of mates for male *i* and k_j is the total number of mates for the j_{th} female that mated male *i*. A male's *SCI* is thus the average polyandry of his female partners and will equal 1 when no other male mates with his partners. *SCI* is thus an indicator of the intensity of sperm competition faced by males. This can then be used to understand the extent to which mating patterns alone shape variation in male paternity share and male reproductive success. Importantly, males can increase their paternity share in face of sperm competition by copulating multiple times with the same female over a given reproductive period (i.e. remating). We therefore also calculated *SCI* weighted by the number of copulations between individual male and female pairs (“weighted sperm competition intensity”, SCI_w). This is calculated as $SCI_{w_i} = 1/\frac{1}{M}(\sum_j^M \frac{c_{ij}}{C_j})$, where c_{ij} is the number of times the i_{th} male copulated with female *j* and C_j is the total number of times female *j* copulated with all her sexual partners. We used mixed-effects models with male *T* as response variable and *M* together with either male *SCI* or male SCI_w as explanatory variables. Males that never mated are not exposed to sperm competition and so were not included. This approach enabled us to quantify precopulatory sexual selection on *M*, controlling for sperm competition intensity (bivariate Bateman gradients).

To demonstrate the importance of *SCI* and SCI_w in postcopulatory competition, we then used binomial mixed effect models with male paternity share (*P*) as response variable with *SCI* or SCI_w as an explanatory variable. All models included male identity, the identity of the mating group and identity of the female unit as random effects. The inclusion of a random effect for male identity is important to account for non-independence within our data due the re-use of individual males across replicate experimental groups. Similarly, the random effect for female unit allows us to statistically control for the use of entire female units across replicate experimental groups, while the our random effect for group identity controls for differences in intercepts driven by group level variation.

(ii) Pre- and postcopulatory sexual selection on male status, aggressiveness and age

We investigated the strength of sexual selection on male status, controlling for male age using mixed-effects models. We measured selection through each component (*M*, *N* and *P*) of male reproductive success separately, and on total male reproductive success (*T*). We included both male status and male age as explanatory variables, as there was limited evidence for collinearity between these two variables (overall Pearson's $r = 0.248$). We used a Poisson error distribution for models for *T* and *M,* a Gaussian error structure for *N* and a binomial error structure for models where *P* was the response variable. All models included male identity, the identity of the mating group and identity of the female unit as random effects. Due to the small number of unique female units (10), we repeated analyses with female unit as a fixed effect; results were qualitatively similar.

We then explored the idea that the role of male status in sexual selection might be mediated by male average aggressiveness. We first investigated the relationship between male average aggressiveness and status. As expected, male average aggressiveness and social status were highly positively correlated with each other across all groups (overall Pearson's $r = 0.693$, figure S2; table S3), although an appreciable proportion of the variation in male average aggressiveness remained unexplained by status (figure S2 & S3A). This variation is intuitive given that male social status and average aggressiveness capture different aspects of male competitive behaviour. First, males can assert dominance without overt aggression and the average aggressiveness of a male can diverge from that expected based solely on his social status. Second, male social status takes into account third-party relationships, e.g. the most dominant male may not aggress males at the bottom of the hierarchy, but will achieve a high status if he dominates other individuals that subsequently dominate many other males. Finally, not all aggressions result in avoidances and so do not necessarily contribute to male social status. To further investigate the role of male average aggressiveness, we explored the possibility that the most aggressive males may only aggress a small subset of male competitors. We found that although some males were highly aggressive to a small subset of competitors, the males that were most aggressive on average also aggressed more individual males (figure S3B-C). This result indicates that male average aggressiveness captures a generalized tendency of a male to attack other males. We therefore conducted complementary analyses with male average aggressiveness, replacing male social status (because these variables were strongly correlated) with random effects and error structures as described above. We again assessed the potential for collinearity between male age and male average aggressiveness prior to analysis and found little evidence of collinearity between these traits (overall $r = 0.065$; see figure S4).

We further investigated the role of male age through longitudinal analyses of male reproductive success over successive years for the 48 males that were used more than once across multiple years. These longitudinal analyses enabled us to explore the extent to which population-level patterns are determined by age-related declines in male sexual behaviours within males and the extent to which they are driven by cohort effects such as selective mortality.

To provide an overall description of the causal structure through which average aggressiveness and male age affect male reproductive success through different reproductive components (i.e. M, N and P), we constructed an *a priori* path analysis scheme (Sih et al. 2002; figure 3). The main aim of this analysis is to better visualize the complexity of pre- and postcopulatory competition. This analysis thus serves as hypothesis as to how male average aggressiveness and male age influence different components of male reproductive success both directly and indirectly, via the frequency at which males remate with the same females and male SCI_w.

Finally, we investigated whether variation in the relationship between male age and average aggressiveness across groups modulates total sexual selection on average male aggressiveness. For each group we calculated the Pearson correlation coefficient between male average aggressiveness and male age. We then calculated standardized sexual selection gradients on male average aggressiveness, where male reproductive success (T) was divided by its mean and male average aggressiveness was standardized to have a mean of zero and a standard deviation of 1, within each group. We used a linear model, with female unit identity as a covariate, to test whether the correlation between male age and average aggressiveness modifies total selection on male average aggressiveness.

(iii) Mechanisms modulating sexual selection on male average aggressiveness and age

We followed up our selection analyses on male average aggressiveness and male age in section (ii), by exploring the mechanisms through which male average aggressiveness and male age affect male reproductive success.

To do this, we first explored the role of both male traits in predicting male sperm competition intensity and weighted sperm competition intensity (SCI and SCI_w respectively), using mixed-effects models. A male's SCI_w was log-transformed, and all models included male identity, the identity of the mating group and identity of the female unit as random effects. Importantly, the SCI (and SCI_w) of different males are not independent because males represent reciprocal members of each other's competitive environments and correlations between male traits and sperm competition values may driven by male mating success alone. We therefore used randomisations of our mating data to test whether the relationship between male SCI (and SCI_w) with male average aggressiveness and male age is more extreme than can be expected by chance due to the variation in male and female mating success alone (i.e. randomisation tests; Croft et al. 2008; Farine and Whitehead 2015). For both *SCI* and SCI_w we generated 1,000 simulated data sets, each including all 20 mating groups (see supporting information A2 for details). We repeated the same models used for our empirical data above for each of the 1,000 simulated data sets for *SCI* and SCI_w respectively, generating a null distribution of regression slopes. To test whether the observed empirical slope is greater than would be expected by chance, we compared our observed parameter estimates to their respective simulated distribution of parameter estimates (Farine and Whitehead 2015).

Male phenotype may shape a male's postcopulatory competitive success (*P*) in multiple ways. For example, male aggressiveness may modulate a male's sperm competitive intensity: (i) by providing increased access to females, allowing a male to defend his paternity by mating repeatedly with the same female, and/or (ii) by limiting the access of other males to females. We explored these possibilities using two complementary approaches. First, to test whether a male's average aggressiveness and/or age impact his access to females, we used mixed-effects models with male average number of mating attempts (i.e. number of copulations attempts divided by number of females with whom he attempted to copulate) as a response variable. One male that was never observed attempting to copulate was excluded. Male average aggressiveness and male age were added as fixed effects. We then used mixed-effects models to assess the relationship between male average remating rate (i.e. his mean number of copulations per mating partner) as a response variable, male average aggressiveness and male age as fixed effects. Both male average number of mating attempts and average remating rate were log transformed and random effects were included as described above.

Second, we asked whether aggression between males shapes their *SCI* and SCI_w directly by effectively excluding competitor males from copulating with their partners e.g. preventing other males from mating with their female partners. To test this, we constructed male-male aggressive and sperm competition networks for each group, where links between males represent the number of aggressive interactions or the number of copulations a male delivered to a rival's female partners, respectively (see supporting information A3 for details). We then constructed a mixed effect model with a Poisson error structure, the number of copulations a focal male "received" from each competitor male as the response variable and the number of aggressive interactions the focal male initiated with each competitor male as a fixed effect. Random effects included mating group and female unit identity, and the identity of focal and competitor males. This analysis asks whether aggression by a focal male towards his rivals covaries with the number of times his rivals copulate with the females mated by the focal male (i.e. the focal male's sexual partners). To test for significance we used randomisations of our mating data as above (see supporting information A3) and compared the observed statistic to the simulated distribution. Specifically, this simulation approach asks whether the number of copulations competitors deliver to a focal male's female partners varies with the outgoing aggression by focal males, controlling for the observed distribution mating success and total remating rate of all males.

All statistical analyses were carried out using R statistical software (R Core Team 2014) and mixed effect models using lme4 (Bates et al. 2014).

Results

(i) Mating success, paternity share and reproductive success

Groups varied in the degree of polyandry, with females mating with an average of 2.83 to 7.75 different males each across different groups (Table 1). Groups were characterized by substantial variation in male reproductive success (Table 1). Consistent with previous work (Collet et al. 2012), postcopulatory paternity share was the most important source of variation in male reproductive success, followed by precopulatory mating success, and by a positive covariance between male mating success and paternity share (Table 1; Table S4). Average fecundity of males' partners on the other hand, showed little variation (Table 1), and standardized covariances including partner fecundity were close to zero (i.e. <|0.02|). There was strong precopulatory selection on male mating success (i.e. strong positive Bateman gradients; Table 2; Fig. 1). We confirmed the role of postcopulatory processes in shaping male reproductive success, showing that while controlling for male mating success (*M*) and partner fecundity (*N*), male paternity share (*P*) had a strong positive effect on male reproductive success (*T*) (mean standardized $\beta_{TP \cdot MN} = 0.927, \chi_1^2 = 248.57, p < 0.001$).

Bivariate Bateman gradients identified similarly strong sexual selection for male mating success and against sperm competition intensity measures (*SCI* or SCI_w, Table 2). The negative effect of *SCI* and SCI_w on total male reproductive success was driven by their influence on male paternity share, such that males facing higher intensity had lower paternity share (*SCI*: $\chi_1^2 = 35.512, p < 0.001$, SCI_w: $\chi_1^2 = 44.854$, $p < 0.001$; Fig. 1; see table S4 for a summary of unstandardised and standardized values per group). Therefore, male mating success is under strong positive precopulatory sexual selection, whereas mating with highly polyandrous females (i.e. high *SCI* and SCI_w) was under strong negative postcopulatory sexual selection.

(ii) Pre- and postcopulatory sexual selection on male average aggressiveness and age

Controlling for male age, male status was significantly positively related with male mating success but had no effect on average partner fecundity or male paternity share (Fig. S5). This resulted in an overall non-significant positive effect of male average social status on male reproductive success (Fig. S5). Male age on the other hand, had a strong negative impact on male reproductive success (Fig. 2).

Replacing male status with male average aggressiveness yielded a qualitatively similar pattern. Controlling for male age, male average aggressiveness significantly and positively predicted male mating success (*M*: $\chi_1^2 = 15.483$, $p <$ 0.001; Fig. 2E) but had no effect on average partner fecundity (*N*: $\chi_1^2 = 0.071$, $p =$ 0.790; Fig. S6) or male paternity share (*P*: $\chi_1^2 = 0.000$, $p = 0.992$; Fig. 2F). This resulted in an overall weak significant positive effect of male average aggressiveness on male reproductive success (*T*: $\chi_1^2 = 4.878$, $p = 0.027$; Fig. 2D). Again, male age had a strong negative relationship with both M ($\chi_1^2 = 10.697, p = 0.001$; Fig. 2B) and P ($\chi_1^2 = 33.553, p < 0.001$; Fig. 2C), but no effect on partner fecundity ($\chi_1^2 = 0.526$, $p = 0.468$; Fig. S6). This resulted in an overall reduction in the reproductive success of older males (*T:* $\chi_1^2 = 23.585, p < 0.001$; Fig. 2A).

We further explored the role of male age by investigating the extent to which age-dependent effects are caused by changes within males through longitudinal analyses of males replicated across multiple breeding seasons. The results of the longitudinal analyses suggest variable patterns within males rather than a consistent age-dependent decline in competitive behaviours (Fig. S7). However, these results should be treated with caution due to the limited number of males and low replication within males (average of 2.25 times each).

Our path analysis confirmed the above results and also suggested that male average aggressiveness has a positive effect on mating success and on the rate at which a male remates with the same females (remating rate), which in turn conveys an advantage in postcopulatory paternity share. Male age, on the other hand, has a direct effect on male P independent from SCI_w, suggesting that the ejaculates of older males may be disfavoured in competition with the sperm of younger males (Fig. 3). To explore this result further, we conducted a post-hoc mixed effect model using a binomial error structure with male paternity share as a response variable and male SCI_w, male age and male average aggressiveness as explanatory variables. We included male identity, the identity of the mating group and identity of the female unit as random effects. Model results confirmed those of the path analysis, demonstrating firstly that male SCI_w was associated with a significant decline in paternity share ($\chi_1^2 = 40.97$, $p < 0.001$). Secondly this analysis revealed that male age explained reductions in male paternity share beyond that explained by male SCI_w ($\chi_1^2 = 26.62, p < 0.001$), whereas male average aggressiveness had no effect ($\chi_1^2 = 1.195, p = 0.274$).

To further clarify the role of male traits in postcopulatory sexual selection, we calculated the proportion of the standardized variation in male paternity share (I_P) explained by male age, male average aggression and male SCI_w for all replicate groups (Moorad and Wade 2013). On average these three traits combined explained 54.61% ± 4.94 (±SE) of the variation in I_P. Male age contributed substantially to the variation in P (median = 26.50%, IQR = 7.02-43.91), followed by male SCI_w (median = 16.51%, IQR = 1.06-33.17). Male average aggressiveness consistently explained less variation in I_P (median = 3.85%, IQR 0.225-12.91). These results confirm the patterns presented above, suggesting that male aggression largely affects variation in male paternity through remating rates whereas male age explains variation above and beyond male SCI_w.

Finally, we investigated how the relationship between age and male average aggressiveness modulates sexual selection on male average aggressiveness, and found a non-significant tendency for weaker selection on aggressiveness in groups with strong positive relationship between age and aggressiveness, which was driven by the reduced performance of old males (t = -2.103, *d.f.* = 9, p = 0.065; Fig. 4).

(iii) Mechanisms modulating sexual selection on male aggressiveness and age

We tested whether the effects of male average aggressiveness and male age reported above were determined by the structure of the sexual network within groups. We found that male age positively predicted *SCI,* so that older males faced higher sperm competition intensities, whereas more aggressive males had reduced *SCI* values (Table 3). However, randomisation tests revealed that this pattern was not more than expected by chance, when controlling the distribution of mating across males and females within groups (Table 3; Fig. S8). Thus, there was no evidence that more aggressive or younger males have higher exclusivity than can be expected solely as a result of the distribution of male and female mating success. In other words, more aggressive and younger males secure the least polyandrous females of a group simply as a result of the fact that they mate with more females, and not because they preferentially target the least polyandrous females. Similarly, our randomization tests of the relationship between male age and male average aggressiveness with SCI_w

show that younger and more aggressive males experience lower SCI_w values but not lower than one would expect if males distributed copulations across their sexual partners randomly (Table 3; Fig. S8).

The result that aggressive males do not have lower sperm competition intensities compared to less aggressive males than can be predicted by the distribution of male and female mating success, was further supported by our aggressiveness randomisations. These randomisations showed that the number of aggressive acts performed by a male towards other males did little to prevent those competitors from mating with his sexual partners ($p_{rand} = 0.832$, Fig. S9). Instead, the reduced SCI_w faced by younger, more aggressive males is driven by a positive relationship between male average aggressiveness and remating rate ($\chi^2_1 = 11.222$, $p < 0.001$; Fig. 5; result for male social status were qualitatively similar, Table S5), and by a negative relationship between male age and remating rate ($\chi^2_1 = 8.258$, $p = 0.004$; Fig. 5, all sexual networks are presented in Fig. S10). These results were corroborated by the relationship between the average number of attempted copulations with male age and average aggressiveness, showing that younger males and more aggressive males attempted to copulate with given females more frequently (male average aggressiveness: $\chi^2_1 = 16.144$, $p < 0.001$; male age: $\chi^2_1 = 16.931$, $p < 0.001$, Fig. 5; Fig. S11).

Discussion

We are only beginning to unravel the complex architecture of sexual selection in polyandrous populations. In this study, we used detailed behavioural observations in combination with molecular parentage data to investigate pre- and postcopulatory sexual selection in replicate social groups of red junglefowl. We found that male reproductive success is highly variable within replicate groups and largely explained by: paternity share, the postcopulatory component of sexual selection, male mating success, a precopulatory component of sexual selection, and their positive covariance (i.e. positive correlation between mating success and paternity share; COV_{MP}). Variation in the fecundity of a male's sexual partners (N) on the other hand, contributed relatively little to the total variation in male reproductive success. The limited role of N in this population is largely due to: (a) the promiscuity of the mating system, which prevents males from monopolizing access to more fecund females, and (b) the relatively small variation in female fecundity (Collet et al. 2012; 2014). It is also possible that the limited period of time of a trial may have influenced the role N in our study. The duration of a trial (10 days) was chosen to capture sexual interactions over a period of time broadly consistent with the production of a clutch of eggs in this species. Prolonging the duration of a trial will likely reduce variation in female fecundity, by enabling more females to lay their entire clutch within a trial. This should further limit the role of N in male sexual selection. It is however possible that in more natural populations, harsher environmental conditions might increase individual variation in female fecundity, promoting opportunity of sexual selection on males through N.

The patterns of variance and covariance in male reproductive success observed in our study are strikingly consistent with previous results reported for smaller social units (3 males and 4 females) of the same study population (Collet et al. 2012; 2014), suggesting that the architecture of male reproductive success is largely reproducible and robust to moderate variation in group size or sex ratios. The predominant role of paternity share is also consistent with studies of other similarly polyandrous taxa (e.g. Pélissié et al. 2014; Morimoto et al. 2016). In addition, the positive covariance observed between male pre- and postcopulatory success demonstrates that these selection episodes reinforce each other, promoting the same male phenotypes (i.e. aggressive and young, see below). Such positive covariances have been found in other species under more restricted experimental conditions (e.g. Evans et al. 2003; Sbilordo and Martin 2014) but only more recently in replicate naturalistic social groups (Devigili et al. 2015). Positive covariances are expected when traits that contribute both to success in pre- and postcopulatory competition are condition-dependent (Helfenstein et al. 2010; Rahman et al. 2013; Turnell and Shaw 2015; Sasson et al. 2016). This is because males in better condition will be able to invest more in both pre- and postcopulatory traits, overall outperforming males in poorer condition. This may limit the scope for alternative mating tactics, which instead may occur when trade-offs between strategies (e.g. between defending current paternity and mating with more partners) result in disruptive or balancing selection on differing strategies (Taborsky et al. 2008). Our results indicate that pre- and postcopulatory sexual selection act synergistically on the same phenotypes.

We identify two independent male phenotypic traits that strongly influenced male performance both in pre- and postcopulatory competition: average aggressiveness and age. Male average aggressiveness positively predicted male mating success and was associated with a relative reduction in postcopulatory competition, whereas older males suffered relatively more intense postcopulatory competition and were less successful in precopulatory competition. Thus, this study shows that pre- and postcopulatory processes act in concert favouring young, aggressive males at the expense of older, less aggressive rivals. By applying a network approach with randomisations of our data, we show that the reduced sperm competition intensity (SCI_w) experienced by aggressive males is not caused by their ability to exclude individual rival males from copulating with their female partners, but by the higher remating rates that aggressive males achieve with their sexual partners. This result was also highlighted by the path analysis, suggesting that male aggressiveness (or traits associated with aggressiveness) enables a male to remate more often with individual females, rather than prevent his competitors from mating with these females. This is consistent with previous findings in smaller groups of this population demonstrating that postcopulatory sexual selection promotes males that remate frequently with the same females (Collet et al. 2012). Furthermore, postcopulatory sexual selection on male remating rates was stronger in groups with stronger postcopulatory sexual selection on male social status, suggesting that a male’s ability to remate with the same female may be determined by traits associated with his social competitive ability (Collet et al. 2012). The results of the present study make sense of these earlier observations, by showing that the average aggressiveness of a male simultaneously predicts his social dominance and his ability to remate repeatedly with his sexual partners. In contrast, a study of small groups of feral domestic fowl (2 males, 4 females), suggested that male social status is favored by sexual selection because it enables males to exclude competitors from mating (Dean et al. 2010). Together, these results suggest that while male status and aggressiveness are consistently favored by sexual selection, the role of these phenotypes and the specific way in which they convey an advantage in intrasexual competition changes with the number of competitors, through variation in group size or sex ratio.

In small groups with only two males, the effect of social status may be sufficiently strong to enable the dominant male to prevent the subordinate from mating, while this may be more difficult in larger groups, and groups with more males. As groups become larger, the scope for competitive exclusion between males may be reduced because male aggression becomes less effective at excluding males. In line with this, previous work in red junglefowl has suggested that while socially dominant males may reduce the sexual behavior of subordinates, there are limits to the number of females, that a dominant male can effectively guard (Johnsen et al. 2001). Moreover, in very large flocks of domestic fowl, ordered/delineated social hierarchies may break down (Hughes et al. 1997; Pagel and Dawkins 1997; Estevez et al. 2007). While the group sizes used in this study are within the range of naturally forming group sizes in red junglefowl (Collias and Collias 1996), it is likely that the high population density accentuated patterns of pre- and postcopulatory competition observed. While in our study females always had the possibility to avoid males (e.g. via perches and artificial cover), females may have more opportunities to avoid male harassment in natural, unconfined groups with lower densities, potentially reducing female mating rates. In such natural groups, behaviours such as male courtship feeding and predator vigilance may also play a role in shaping patterns of sexual interactions. Work in both domestic and red junglefowl suggests both these traits are associated with male social status (Stokes 1971; Pizzari 2003). Dominant male fowl spend more time being vigilant and are more likely to courtship feed females than subordinate males (Stokes 1971; Pizzari 2003). In the present study variation in food quality and availability was minimal as all birds had *ad libitum* access to commercial feed. This is likely different from a natural population, where only some males will be able to secure nutritious food items and present them to females (e.g. McBride et al. 1969). In these more natural settings, such status-related behaviours may play a more important role in shaping female responses to males.

Similarly, the present study and several previous studies of smaller social units of fowl populations (Dean et al. 2010; Collet et al. 2012; 2014) used moderately female-biased adult sex ratios (0.83, 0.5 and 0.75, respectively). However, in nature adult sex ratios in red junglefowl groups are variable (Collias and Collias 1967; 1996). This variation may change patterns of selection. For example, previous work has shown that at highly female-biased sex ratios, females face lower sexual harassment by males and actively solicit more copulations compared to strongly male-biased sex ratios (Løvlie and Pizzari 2007). Therefore, we may predict that the relative importance and intensity of postcopulatory sexual selection on males to be reduced as groups become more female-biased.

Variation in group size and sex ratio may also contribute to explain differences in the role of male aggression detected in studies of other taxa. For example, a recent study of pre- and postcopulatory competitive networks in natural populations of crickets (*Gryllus campestris*) showed that more aggressive males suffered more –rather than less- sperm competition (Fisher et al. 2016). In contrast, several studies have shown that male aggressiveness can reduce the intensity of sperm competition through competitive exclusion. For example, larger, socially dominant male lizards are able to exclude smaller males from mating with females (Keogh et al. 2013). Similarly, male *Drosophila melanogaster* use aggression towards rivals as a form of mate guarding, reducing the rate at which rivals are able to copulate with their female partners (Baxter et al. 2015). Sex ratio variation may also affect the ability of males to avoid sperm competition with rivals. For example, in Soay Sheep (*Ovis aries*), bigger males with larger horns are able to exclude smaller males from mating with females via aggressive contests, when receptive females are in short supply (Preston et al. 2003). However, when more females are available, the relative siring success of larger males is reduced as they are unable to monopolise many females (Preston et al. 2003).

A number of mechanisms may explain the double advantage experienced by aggressive males. First, proximate mechanisms such as higher plasma steroid levels, may simultaneously control male aggressiveness towards other males and libido, leading to more aggressive males mating with more females and more often (i.e. male aggressiveness and mating performance are not causally inter-related but controlled by a third variable). Second, females may prefer to associate and mate with these males. Previous work has suggested that female fowl may prefer to remain close to (McBride et al. 1969; Johnsen et al. 2001) and mate with socially dominant males (i.e. male aggressiveness favours male mating performance through female preference). Finally, it is also possible that males that are more aggressive to rivals may also be more aggressive to females. Male fowl can aggressively coerce females into copulation and male harassment of females is often intense (Pizzari and Birkhead 2000; Løvlie and Pizzari 2007). Such increased male aggressiveness and harassment towards females may reduce or overcome female resistance to remating (i.e. male aggressiveness favours male mating performance through male harassment of females). A positive relationships between male aggressiveness and female behavior is evident in some species of water striders, where highly aggressive males often have high mating success in closed groups, and harassment of females results in reduced female resistance and convenience polyandry (Rowe 1992; Eldakar et al. 2009; Wey et al. 2015; Devost and Turgeon 2016). Moreover, a recent study of the water strider, *Aquarius remigis*, demonstrated that in groups with male biased sex ratios, aggressive males with higher mating success also had the lowest intensity of sperm competition (Wey et al. 2015), although this study was unable to quantify paternity success. The results of our study provide some support for the idea that male harassment of females leads to higher remating rates as more aggressive males also attempt to copulate with females more often than less aggressive males. However, these results may also be explained by males with higher steroid plasma levels simultaneously being more aggressive and having higher libido. Unpacking the relative roles of these mechanisms will likely require experimental manipulations.

Our results also reveal a strong impact of male age on both pre- and postcopulatory male performance. A large source of the effect of male age on paternity share appears to be the reduced ability of older males to mate with multiple females and defend their paternity by remating frequently with these females. However, our results suggest substantial variation in male paternity share was also determined by direct effects of male age. Importantly, we also show that controlling for this reduction in remating rate (and increased SCI_w), male age explains substantial variation in male paternity share (P) and has a direct negative relationship with P. This result suggests that older males not only suffer from a reduction in remating rates but also experience a decline in fertilising performance. This may be the result of reduced ejaculate quality and/or sperm numbers, cryptic female choice against older males, or a combination of the above. Consistent with the first mechanism, reproductive senescence in male fowl can result in marked declines in mulitple male reproductive traits including libido, the ability to successfully transfer sperm, the number of sperm inseminated and sperm swimming velocity (Dean et al. 2010; Noguera et al. 2012; Cornwallis et al. 2014).

Intense competition for access to females can accentuate the decline in age-specific male reproductive success, especially in polyandrous species, where age-related declines in semen traits further impact variation in male reproductive success, and in age-structured populations where old males may compete with younger males. Our results suggest variable patterns in within-male trajectories rather than a consistent deterioration in remating rates within males. This is similar to previous studies in fowl that showed within male changes in copulation propensity with age were highly variable (Dean et al. 2010). In our study, males measured across multiple years always experienced different male and female social groups. This suggests that within-male variation across years may be largely dominated by differences in the social environment experienced by a male during a trial. In principle, it is also possible that the social environment experienced by a male prior to a trial may have carry-over effects that influence his behaviour during the trial. Between trials, males were housed in larger groups of males as part of general flock husbandry, and variation in the social groups during this period may also contribute to variability of males used across multiple trials. Therefore, the results of our study indicate that age-related differences in male reproductive success are more consistent with variation across cohorts. Cohort effects are potentially driven by selective mortality and life history trade-offs, rather than by longitudinal age-dependent declines within males. However, the limited sample size and variable social environments in our study limit our power test for longitudinal effects.

One important outcome of the reduced fertility of older males who are still able to monopolize female partners, is a reduced contribution of viable sperm that may result in unfertilized female ova (Dean et al. 2010). Male reproductive ageing therefore represents a potential important contributor for sexual conflict (Dean et al. 2010; Carazo et al. 2011). In this study we were unable to document the number of unfertilized eggs, however our results suggest that in large promiscuous groups the potential for strong impacts of male age on female fertility may be reduced because older males tend to share their female partners with many males.

Finally, our results show that the relationship between male age and average aggressiveness was variable across groups. An important outcome of this is that in some groups, more aggressive males may be the youngest and most fertile, whereas in other groups less fertile, older males may be more aggressive. In natural populations, such between-group variation could be generated by differences in the way competitive traits (e.g. aggression) change throughout male lifetimes, or differences in local environmental conditions and mortality regimes. These differences may represent an important axis of variation in cross-sectional studies of male competition, e.g. by generating variation in patterns of selection on male traits, such as aggression, and the potential for sexual conflict driven by male senescence. Group-specific relationships between age and aggressiveness may represent an important mechanism preserving additive genetic variance in sexually competitive traits in the face of consistent directional pre- and postcopulatory sexual selection.

Conclusions

Our study demonstrates that pre- and postcopulatory sexual selection operate in the same direction on multiple male traits in replicate age-structured polyandrous groups of red junglefowl. Our results shed light on the mechanisms through which male social status, mediated by male aggression, determine male pre- and postcopulatory competitive success. While aggressive interactions between pairs of males had no effect on how frequently rivals copulated with each other's sexual partners, more aggressive males were able to both mate with more females and to defend paternity by remating frequently with the same females. Older males were instead poor competitors in both pre- and postcopulatory competition, mating with fewer females and potentially delivering less competitive ejaculates. These results throw light on the complexity of sexual selection acting on males in polyandrous groups, with implications for the evolution of alternative reproductive tactics and conflict between the sexes.

Literature cited

Altmann, J. 1974. Observational study of behavior: sampling methods. Behaviour 49:227–267.

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ.

Andersson, M., and L. W. Simmons. 2006. Sexual selection and mate choice. Trends Ecol. Evol. 21:296–302.

Arnold, S. J., and D. Duvall. 1994. Animal mating systems: a synthesis based on selection theory. Am. Nat. 143:317–348.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2:349–368.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7.

Baxter, C. M., R. Barnett, and R. Dukas. 2015. Aggression, mate guarding and fitness in male fruit flies. Anim. Behav. 109:235–241.

Berghänel, A., J. Ostner, and O. Schülke. 2011. Coalitions destabilize dyadic dominance relationships in male Barbary macaques (Macaca sylvanus). Behaviour 148:1256–1274.

Carazo, P., P. Molina-Vila, and E. Font. 2011. Male reproductive senescence as a potential source of sexual conflict in a beetle. Behav. Ecol. 22:192–198.

Cheng, H. H., and L. B. Crittenden. 1994. Microsatellite Markers for Genetic Mapping in the Chicken. Poult. Sci. 73:539–546.

Collet, J. M., R. F. Dean, K. Worley, D. S. Richardson, and T. Pizzari. 2014. The measure and significance of Bateman's principles. Proc. R. Soc. B Biol. Sci. 281:20132973.

Collet, J., D. S. Richardson, K. Worley, and T. Pizzari. 2012. Sexual selection and the differential effect of polyandry. Proc. Natl. Acad. Sci. U. S. A. 109:8641–5.

Collias, N. E., and E. C. Collias. 1967. A field study of the Red junglefowl in north-central India. The Condor 69:360–386.

Collias, N. E., and E. C. Collias. 1996. Social organization of a red junglefowl, *Gallus gallus*, population related to evolution theory. Anim. Behav. 51:1337–1354.

Cornwallis, C. K., Dean Rebecca, and T. Pizzari. 2014. Sex-specific patterns of aging in sexual ornaments and gametes. Am. Nat. 184:E66–E78.

Croft, D., R. James, and J. Krause. 2008. Exploring animal social networks. Princeton University Press, Princeton, NJ.

Crooijmans, R. P., R. J. Dijkhof, J. J. van der Poel, and M. A. Groenen. 1997. New microsatellite markers in chicken optimized for automated fluorescent genotyping. Anim. Genet. 28:427–437.

Crooijmans, R. P., P. A. van Oers, J. A. Strijk, J. J. van der Poel, and M. A. Groenen. 1996. Preliminary linkage map of the chicken (*Gallus gallus domesticus*) genome based on microsatellite markers: 77 new markers mapped. Poult. Sci. 75:746–754.

Darwin, C. 1859. On the Origin of Species. John Murray, London, UK.

Darwin, C. 1871. The descent of man and selection in relation to sex. John Murray, London, UK.

David, H. A. 1987. Ranking from unbalanced paired-comparison data. Biometrika 74:432–436.

Dawson, D. A., G. J. Horsburgh, C. Küpper, I. R. K. Stewart, A. D. Ball, K. L. Durrant, B. Hansson, I. Bacon, S. Bird, A. Klein, A. P. Krupa, J.-W. Lee, D. Martín-Gálvez, M. Simeoni, G. Smith, L. G. Spurgin, and T. Burke. 2010. New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility - as demonstrated for birds. Mol. Ecol. Resour. 10:475–494.

Dawson, D., S. McConnell, A. Wardle, M. Gibbs, and T. Burke. 1998. Characterization and mapping of 15 novel chicken microsatellite loci. Anim. Genet. 29:159–160.

de Vries, H., J. M. G. Stevens, and H. Vervaecke. 2006. Measuring and testing the steepness of dominance hierarchies. Anim. Behav. 71:585–592.

Dean, R., C. K. Cornwallis, H. Løvlie, K. Worley, D. S. Richardson, and T. Pizzari. 2010. Male reproductive senescence causes potential for sexual conflict over mating. Curr. Biol. 20:1192–1196.

Devigili, A., J. P. Evans, A. Di Nisio, and A. Pilastro. 2015. Multivariate selection drives concordant patterns of pre- and postcopulatory sexual selection in a livebearing fish. Nat. Commun. 6:8291.

Devost, E., and J. Turgeon. 2016. The combined effects of pre- and post-copulatory processes are masking sexual conflict over mating rate in Gerris buenoi. J. Evol. Biol. 29:167–177.

Eberhard, W. G. 2009. Postcopulatory sexual selection: Darwin's omission and its consequences. Proc. Natl. Acad. Sci. U. S. A. 106:10025–10032.

Eldakar, O. T., M. J. Dlugos, J. W. Pepper, and D. S. Wilson. 2009. Population structure mediates sexual conflict in Water striders. Science 326:816–816.

Estevez, I., I.-L. Andersen, and E. Nævdal. 2007. Group size, density and social dynamics in farm animals. Appl. Anim. Behav. Sci. 103:185–204.

Etches, R. J. 1996. Reproduction in poultry. First edition. CABI, Wallingford, Oxon.

Evans, J. P., and F. Garcia-Gonzalez. 2016. The total opportunity for sexual selection and the integration of pre- and post-mating episodes of sexual selection in a complex world. J. Evol. Biol., doi: 10.1111/jeb.12960.

Evans, J. P., L. Zane, S. Francescato, and A. Pilastro. 2003. Directional postcopulatory sexual selection revealed by artificial insemination. Nature 421:360–363.

Farine, D. R., and H. Whitehead. 2015. Constructing, conducting and interpreting animal social network analysis. J. Anim. Ecol. 84:1144–1163.

Farrell, M. E., E. Briefer, and A. G. McElligott. 2011. Assortative Mating in Fallow Deer Reduces the Strength of Sexual Selection. PLOS ONE 6:e18533.

Firman, R. C., Gasparini, C., Manier, M. K., and Pizzari, T. 2017. Postmating female control: 20 years of cryptic female choice. Trends Ecol. Evol., in press.

Fisher, D. N., R. Rodríguez-Muñoz, and T. Tregenza. 2016. Comparing pre- and post-copulatory mate competition using social network analysis in wild crickets. Behav. Ecol. arv236.

Froman, D. P., T. Pizzari, A. J. Feltmann, H. Castillo-Juarez, and T. R. Birkhead. 2002. Sperm mobility: mechanisms of fertilizing efficiency, genetic variation and phenotypic relationship with male status in the domestic fowl, *Gallus gallus domesticus*. Proc. R. Soc. B Biol. Sci. 269:607–612.

Gammell, M. P., H. de Vries, D. J. Jennings, C. M. Carlin, and T. J. Hayden. 2003. David's score: a more appropriate dominance ranking method than Clutton-Brock et al.'s index. Anim. Behav. 66:601–605.

Gibbs, M., D. A. Dawson, C. McCamley, A. F. Wardle, J. A. Armour, and T. Burke. 1997. Chicken microsatellite markers isolated from libraries enriched for simple tandem repeats. Anim. Genet. 28:401–417.

Gillingham, M. A. F., D. S. Richardson, H. Løvlie, A. Moynihan, K. Worley, and T. Pizzari. 2009. Cryptic preference for MHC-dissimilar females in male red junglefowl, Gallus gallus. Proc. Biol. Sci. 276:1083–1092.

Groenen, M. A. M., H. H. Cheng, N. Bumstead, B. F. Benkel, W. E. Briles, T. Burke, D. W. Burt, L. B. Crittenden, J. Dodgson, J. Hillel, S. Lamont, A. P. de Leon, M. Soller, H.

Takahashi, and A. Vignal. 2000. A consensus linkage map of the chicken genome. Genome Res. 10:137–147.

Hanotte, O., A. Pugh, C. Maücher, D. Dawson, and T. Burke. 1997. Nine novel chicken microsatellite loci and their utility in other Galliformes. Anim. Genet. 28:311–313.

Helfenstein, F., S. Losdat, A. P. Møller, J. D. Blount, and H. Richner. 2010. Sperm of colourful males are better protected against oxidative stress. Ecol. Lett. 13:213–222.

Holman, L., and H. Kokko. 2013. The consequences of polyandry for population viability, extinction risk and conservation. Philos. Trans. R. Soc. B Biol. Sci. 368:20120053.

Hughes, B. O., N. L. Carmichael, A. W. Walker, and P. N. Grigor. 1997. Low incidence of aggression in large flocks of laying hens. Appl. Anim. Behav. Sci. 54:215–234.

Johnsen, T. S., M. Zuk, and E. A. Fessler. 2001. Social dominance, male behaviour and mating in mixed-sex flocks of red Junglefowl. Behaviour 138:1–18.

Jones, A. G. 2009. On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. Evolution 63:1673–84.

Jones, A. G., and N. L. Ratterman. 2009. Mate choice and sexual selection: what have we learned since Darwin? Proc. Natl. Acad. Sci. U. S. A. 106 Suppl:10001–8.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16:1099–1106.

Kenta, T., J. Gratten, N. S. Haigh, G. N. Hinten, J. Slate, R. K. Butlin, and T. Burke. 2008. Multiplex SNP-SCALE: a cost-effective medium-throughput single nucleotide polymorphism genotyping method. Mol. Ecol. Resour. 8:1230–1238.

Keogh, J. S., K. D. L. Umbers, E. Wilson, J. Stapley, and M. J. Whiting. 2013. Influence of alternate reproductive tactics and pre- and postcopulatory sexual selection on paternity and offspring performance in a lizard. Behav. Ecol. Sociobiol. 67:629–638.

Kvarnemo, C., and L. W. Simmons. 2013. Polyandry as a mediator of sexual selection before and after mating Polyandry as a mediator of sexual selection before and after mating. Philos. Trans. R. Soc. B Biol. Sci. 368:1–16.

Løvlie, H., C. K. Cornwallis, and T. Pizzari. 2005. Male mounting alone reduces female promiscuity in the fowl. Curr. Biol. 15:1222–1227.

Løvlie, H., M. A. F. Gillingham, K. Worley, T. Pizzari, and D. S. Richardson. 2013. Cryptic female choice favours sperm from major histocompatibility complex-dissimilar males. Proc. R. Soc. B Biol. Sci. 280:20131296.

Løvlie, H., and T. Pizzari. 2007. Sex in the morning or in the evening? Females adjust daily mating patterns to the intensity of sexual harassment. Am. Nat. 170:E1–E13.

McBride, G., I. P. Parer, and F. Foenander. 1969. The social organization and behaviour of the feral domestic fowl. Anim. Behav. Monogr. 1:125–181.

McDonald, G. C., R. James, J. Krause, and T. Pizzari. 2013. Sexual networks: measuring sexual selection in structured, polyandrous populations. Philos. Trans. R. Soc. B Biol. Sci. 368:1–10.

McDonald, G. C., and T. Pizzari. 2016. Why patterns of assortative mating are key to study sexual selection and how to measure them. Behav. Ecol. Sociobiol. 70:209–220.

Montrose, V. ., W. E. Harris, A. J. Moore, and P. J. Moore. 2008. Sperm competition within a dominance hierarchy: investment in social status vs. investment in ejaculates. J. Evol. Biol. 21:1290–1296.

Moorad, J., and M. J. Wade. 2013. Selection gradients, the opportunity for selection, and the coefficient of determination. Am. Nat. 181:291–300.

Moore, A. J., and P. J. Moore. 1999. Balancing sexual selection through opposing mate choice and male competition. Proc. R. Soc. Lond. B Biol. Sci. 266:711–716.

Morimoto, J., T. Pizzari, and S. Wigby. 2016. Developmental Environment Effects on Sexual Selection in Male and Female Drosophila melanogaster. PLOS ONE 11:e0154468.

Muller, M. N., and R. W. Wrangham. 2004. Dominance, aggression and testosterone in wild chimpanzees: a test of the "challenge hypothesis." Anim. Behav. 67:113–123.

Muniz, D. G., P. R. Guimarães, B. A. Buzatto, and G. Machado. 2015. A sexual network approach to sperm competition in a species with alternative mating tactics. Behav. Ecol. 26:121–129.

Nicholls, J. A., M. C. Double, D. M. Rowell, and R. D. Magrath. 2000. The evolution of cooperative and pair breeding in Thornbills acanthiza (Pardalotidae). J. Avian Biol. 31:165–176.

Noguera, J. C., R. Dean, C. Isaksson, A. Velando, and T. Pizzari. 2012. Age-specific oxidative status and the expression of pre- and postcopulatory sexually selected traits in male red junglefowl, Gallus gallus. Ecol. Evol. 2:2155–2167.

Packer, C. 1979. Male dominance and reproductive activity in Papio anubis. Anim. Behav. 27 Pt 1:37–45.

Pagel, M., and M. S. Dawkins. 1997. Peck orders and group size in laying hens: `futures contracts' for non-aggression. Behav. Processes 40:13–25.

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

Parker, G. A., and T. R. Birkhead. 2013. Polyandry: the history of a revolution. Philos. Trans. R. Soc. B Biol. Sci. 368:1–13.

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

Pélissié, B., P. Jarne, V. Sarda, and P. David. 2014. Disentangling precopulatory and postcopulatory sexual selection in polyandrous species. Evolution 68:1320–1331.

Pizzari, T. 2003. Food, vigilance, and sperm: the role of male direct benefits in the evolution of female preference in a polygamous bird. Behav. Ecol. 14:593–601.

Pizzari, T. 2001. Indirect partner choice through manipulation of male behaviour by female fowl, *Gallus gallus domesticus*. Proc. R. Soc. B Biol. Sci. 268:181–186.

Pizzari, T. 2016. The Wood-Gush legacy: A sociobiology perspective to fertility and welfare in chickens. Appl. Anim. Behav. Sci. 181:12–18.

Pizzari, T., and T. R. Birkhead. 2000. Female feral fowl eject sperm of subdominant males. Nature 405:787–789.

Pizzari, T., D. P. Froman, and T. R. Birkhead. 2002. Pre- and post-insemination episodes of sexual selection in the fowl, *Gallus g. domesticus*. Heredity 88:112–116.

Pizzari, T., and N. Wedell. 2013. The polyandry revolution. Philos. Trans. R. Soc. B Biol. Sci. 368:20120041.

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

Procter, D. S., A. J. Moore, and C. W. Miller. 2012. The form of sexual selection arising from male-male competition depends on the presence of females in the social environment. J. Evol. Biol. 25:803–812.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rahman, M. M., J. L. Kelley, and J. P. Evans. 2013. Condition-dependent expression of pre- and postcopulatory sexual traits in guppies. Ecol. Evol. 3:2197–2213.

Rowe, L. 1992. Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. Anim. Behav. 44:189–202.

Saavedra, S., and D. B. Stouffer. 2013. “Disentangling nestedness” disentangled. Nature 500:E1–E2.

Sasson, D. A., P. R. Munoz, S. A. Gezan, and C. W. Miller. 2016. Resource quality affects weapon and testis size and the ability of these traits to respond to selection in the leaf ‐ footed cactus bug, Narnia femorata. Ecol. Evol. 6:2098–2108.

Sbilordo, S. H., and O. Y. Martin. 2014. Pre- and postcopulatory sexual selection act in concert to determine male reproductive success in Tribolium castaneum. Biol. J. Linn. Soc. 112:67–75.

Shuster, S., and M. J. Wade. 2003. Mating systems and strategies. Princeton University Press, Princeton, NJ.

Sih, A., S. F. Hanser, and K. a. McHugh. 2009. Social network theory: new insights and issues for behavioral ecologists. Behav. Ecol. Sociobiol. 63:975–988.

Sih, A., M. Lauer, and J. J. Krupa. 2002. Path analysis and the relative importance of male–female conflict, female choice and male–male competition in water striders. Anim. Behav. 63:1079–1089.

Simmons, L. W., J. L. Tomkins, and J. Hunt. 1999. Sperm competition games played by dimorphic male beetles. Proc. R. Soc. Lond. B Biol. Sci. 266:145–150.

Snook, R. R. 2005. Sperm in competition: not playing by the numbers. Trends Ecol. Evol. 20:46–53.

Stokes, A. W. 1971. Parental and courtship feeding in Red junglefowl. The Auk 88:21–29.

Taborsky, T., R. F. Oliveira, and H. J. Brockmann. 2008. The evolution of alternative reproductive tactics: concepts and questions. P. *in* Alternative Reproductive Tactics: An Integrative Approach. Cambridge University Press, Cambridge, UK ; New York.

Taylor, M. L., T. A. R. Price, and N. Wedell. 2014. Polyandry in nature: a global analysis. Trends Ecol. Evol. 29:376–383.

Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly. Am. Nat. 122:765–788.

Turnell, B. R., and K. L. Shaw. 2015. High opportunity for postcopulatory sexual selection under field conditions. Evol. Int. J. Org. Evol. 69:2094–2104.

Webster, M. S., S. Pruett-Jones, D. F. Westneat, and S. J. Arnold. 1995. Measuring the effects of pairing success, extra-pair copulations and mate quality on the opportunity for sexual selection. Evolution 49:1147–1157.

Wedell, N., M. J. G. Gage, and G. A. Parker. 2002. Sperm competition, male prudence and sperm-limited females. Trends Ecol. Evol. 17:313–320.

Wey, T. W., A. T. Chang, S. Fogarty, and A. Sih. 2015. Personalities and presence of hyperaggressive males influence male mating exclusivity and effective mating in stream water striders. Behav. Ecol. Sociobiol. 69:27–37.

Wilson, D. R., X. J. Nelson, and C. S. Evans. 2009. Seizing the opportunity: subordinate male fowl respond rapidly to variation in social context. Ethology 115:996–1004.

Worley, K., J. Collet, L. G. Spurgin, C. Cornwallis, T. Pizzari, and D. S. Richardson. 2010. MHC heterozygosity and survival in red junglefowl. Mol. Ecol. 19:3064–3075.

Supporting information for:

Pre- and postcopulatory sexual selection favor aggressive, young males in polyandrous groups of red junglefowl

(A1) Calculating male social status using David's Score

We calculated male social status using David's score (DS). David's score is calculated based on the proportion wins and losses between dyads. The proportion of wins between individual i and an interactant j (P_{ij}) is calculated the number of dominance interactions in which individual i dominated individual j, divided by the total number of dominance interactions between individuals i and j. Similarly, the proportion of losses for individual i with individual j (P_{ji}) is calculated as the number of times j dominated i divided by the total number of dominance interactions between both individuals. DS for each individual is calculated using the following formula:

$$DS = w + w_2 - l - l_2$$

where w is the sum of P_{ij} values for individual i. w_2 is the sum of P_{ij} values for individual i weighted by the w value of its interactants. Parameter l is the sum of the proportion losses (P_{ji}) for individual i, and l_2 is the sum of the proportion losses of individual i weighted by the l value of its interactants.

Importantly, the above calculation based on P_{ij} does not take the number of interactions between dyads into account: i.e. if A beats B in one out of one interactions, its P_{AB} is 1, and when A beats B in five out of five interactions, its P_{AB} is also 1. Because interaction frequencies differed greatly between interacting dyads in our data we used a modified version of the above formula as proposed by de Vries at al. (2006) that replaces P_{ij} with the proportion of contests won corrected for chance (D_{ij}). This approach takes into account the number of interactions between dyads and lends more weight to those relationships with more interactions. For example, taking the example from de Vries at al. (2006) above, if individual A beats individual B in one out of one contests, the D_{AB} for individual A would be 0.75, whereas if individual A beats individual B in five out of five contests, its D_{AB} would be 0.917. For full details see de Vries at al (2006) cited in main text.

(A2) SCI and SCI_w randomisation tests

We used randomisations of our mating data to test the significance of the relationship between male *SCI* and SCI_w with male average aggressiveness and male age This approach asks whether the relationship between male average aggressiveness and both measures of sperm competition intensity is greater than we would expect by chance given the observed distribution of male mating success in a group. For male *SCI* we generated 1,000 networks for each mating group using randomisations of our observed sexual networks that randomly shuffles copulating pairs of males and females but holds male mating success and male traits (average aggressiveness and age) constant (i.e. controlling for average polyandry and the variance in male and female mating success) (Saavedra and Stouffer 2013). We then recalculated male *SCI* for every randomised network. For male SCI_w we again generated 1,000 networks for each mating group where randomisations hold male and female mating success, and male traits constant as above, but randomly allocate a male's total number of copulations across his female mating partners. We then recalculated male SCI_w for every randomised network.

(A3) Male-male aggression and sperm competition networks

Here, we ask whether aggression between males shapes their *SCI* and SCI_w directly by effectively excluding competitor males from copulating with their partners e.g. more aggressive males prevent other males from mating with their female partners. To test this, we constructed male-male aggressive networks and male-male sperm competition networks for each group. Male-male aggressive networks contained males as nodes and edges between males were both weighted by the number of aggressive interactions and directed, i.e. if male A aggressed male B four times and male B aggressed male A seven times this pair would share two edges where the edge from A to B would be weighted as 4 and the edge from B to A would be weighted as 7. If males did not initiate an aggressive interaction with another male then edge values were zero. Sperm competition networks were similarly directed and weighted, but here weights instead represent the number of copulations delivered by males (i.e. if male A mated a total of five times with all the females with which male B mated, the edge from male A to B would carry a weight of 5). If males never shared females then edge values were zero.

We then constructed a mixed effect model with a Poisson error structure and the number of copulations a focal male "received" from each competitor male, from the sperm competition network, as the response variable. As a fixed effect we included the number of aggressive interactions the focal male initiated with that competitor male, from the aggression network. These analyses therefore ask; "does the number of aggressive interaction initiated from male A towards male B, predict the number of copulations male B has with male A's females?" Males that never mated or never initiated an aggressive interaction could not differentially deliver copulations or aggressive interactions across competitors and so were not included in the analysis. Random effects included mating group and female unit identity, and the identity of focal and competitor males.

It is important to note that, as the mating success of a focal male increases, so will the total number of copulations with which he competes with any other male, because males will on average share increasingly more sexual partners. In addition, some males may be able to achieve a high average remating rate across all females mated, even if aggression by competitors may reduce their remating rates with certain females. To control for this, we again used a randomisations of our mating data as above. These randomisations hold constant the observed sexual network but allow males to allocate their total number of copulations randomly across their females. For each randomised male-female sexual network, we re-calculated the male-male sperm competition network. In total this generated 1,000 randomised sperm competition networks for each mating group independently; producing 1,000 simulated data sets each containing all 20 groups. We then repeated the above mixed-effects model for each simulated data set and compared the observed statistic to the distribution of statistics across all simulations. Specifically, this simulation approach asks whether outgoing aggression by focal males varies with the number of copulations competitors deliver to his females, controlling for the mating success and remating rate of all males as a whole.

Table S1 Number of males of given ages in all replicate groups of red junglefowl.

Group Identity	Male age (years)						
	1	2	3	4	5	6	7
G1	5	-	-	-	-	5	-
G2	5	-	-	-	2	3	-
G3	5	-	-	-	-	5	-
G4	3	2	3	-	-	1	1
G5	3	2	3	-	1	-	1
G6	3	2	3	-	1	-	1
G7	3	2	3	1	-	-	1
G8	3	2	3	1	1	-	-
G9	5	-	1	-	4	-	-
G10	5	-	1	-	1	3	-
G11	5	3	1	1	-	-	-
G12	5	3	2	-	-	-	-
G13	5	-	-	-	5	-	-
G14	5	-	-	1	4	-	-
G15	5	-	1	1	3	-	-
G16	5	1	1	-	3	-	-
G17	5	-	-	1	4	-	-
G18	6	-	1	-	3	-	-
G19	5	-	-	1	-	4	-
G20	5	-	1	-	-	4	-

Table S2 Primer details of microsatellite loci used for parentage analyses

Locus	**Label**	**Size**	**No. of alleles**	**Reference**
MCW0123	FAM	77-85	4	Crooijmans et al. 1996
LEI0028	FAM	153-173	5	Hanotte et al. 1997
LEI0109	FAM	204-208	3	Gibbs et al. 1997
LEI0127	FAM	222-256	4	Gibbs et al. 1997
ROS0081	FAM	307-317	5	Groenen et al. 2000
MCW0295	HEX	85-97	3	Crooijmans et al. 1997
ALD0188	HEX	140-154	4	Cheng et al. 1994
LEI0196	HEX	170-192	9	Dawson et al. 1998
LEI0068	HEX	221-235	5	Gibbs et al. 1997
MCW0183	HEX	292-316	4	Crooijmans et al. 1997

Table S3 Model results for the relationship between male social status and male average aggressiveness across replicate groups of red junglefowl. Estimates with their standard errors (SE) and *P*-values obtained from likelihood ratio tests (LRT) excluding only the parameter of interest.

Response	Parameter	Estimate	SE	Δ AIC	LRT
Social status	Male average aggressiveness	1.329	0.097	127.7	$\chi^2 = 129.7$, $p < 0.001$

Table S4 Summary information for individual groups of red junglefowl. Mean male reproductive success ($\bar{T}$), Mean number of female partners per males ($\bar{M}$), mean male remating rate, mean female polyandry, opportunity for sexual selection ($I_s = \sigma_M^2/\bar{M}^2$). unstandardized Bateman gradient (β_M), mean standardized Bateman gradient (β_M^*), Opportunity for selection ($I_T = \sigma_T^2/\bar{T}^2$), standardized variance components for mating success (I_M), partner fecundity (I_N), paternity share (I_P) and the covariance between M and P (COV_{MP}), the percentage contribution of standardized variance components to the Opportunity for selection (%M, %N, %P, %COV_{MP}). Results across groups are broadly consistent with Collet et al (2012) demonstrating a trend for reduced I_T and I_M with increasing polyandry, whereas %P tended to increase with increasing polyandry.

$\bar{\bar{T}}$	$\bar{\bar{M}}$	Mean remating rate	Mean polyandry	I_s	β_M	β_M^*	I_T	I_M	I_N	I_P	COV_{MP}	%M	%N	%P	% COV_{MP}
4.9	7.4	19.4	6.17	0.24	0.84	1.27	0.87	0.19	0.01	0.41	0.24	21.47	1.62	46.63	27.84
4.6	6.8	18	5.67	0.27	1.09	1.62	1.45	0.19	0.14	0.48	0.08	13.07	9.70	33.32	5.64
4.7	6.7	19.5	5.58	0.32	1.08	1.54	1.53	0.20	0.06	0.80	0.57	13.35	3.92	52.12	37.43
4.8	6.4	19.6	5.33	0.41	0.43	0.58	0.58	0.42	0.01	0.44	-0.03	72.74	1.96	75.67	-5.42
5	9.3	29.3	7.75	0.10	0.93	1.74	0.59	0.08	0.01	0.29	0.17	13.99	0.91	49.84	28.42
4.8	8.3	18.3	6.92	0.16	0.43	0.75	1.07	0.13	0.02	0.84	0.16	12.43	1.47	78.92	15.34
5.3	5	12.4	4.17	0.76	1.23	1.16	1.88	0.69	0.01	1.22	0.19	36.79	0.54	64.89	9.86
3.8	5.7	11.5	4.75	0.29	0.84	1.26	1.03	0.25	0.05	0.40	0.24	24.26	4.79	38.96	23.62
4.3	8.1	25.5	6.75	0.12	0.55	1.03	0.81	0.12	0.02	0.72	-0.05	14.56	2.05	88.95	-6.23
5.6	4.8	17.9	4.00	0.53	1.72	1.47	1.94	0.36	0.04	0.45	0.16	18.78	2.17	23.07	8.46
6	9.3	33.4	7.75	0.11	0.87	1.35	0.82	0.09	0.00	0.63	0.14	11.00	0.20	76.31	17.52
5.6	7.8	21.8	6.50	0.33	1.13	1.57	1.43	0.22	0.01	0.64	0.45	15.19	1.05	44.90	31.29
3.5	6.4	13.9	5.33	0.26	0.76	1.38	0.91	0.19	0.03	0.57	0.45	20.40	2.98	62.29	49.48
3	6.5	20.1	5.42	0.30	0.49	1.06	1.75	0.28	0.02	1.63	0.18	15.97	1.25	92.85	10.50
1.4	6.2	13.4	5.17	0.15	0.08	0.35	1.16	0.21	0.09	1.13	-0.28	17.94	7.61	97.68	-24.17
3.2	5	13	4.17	0.40	0.96	1.49	1.43	0.37	0.06	0.78	-0.03	25.75	3.88	54.83	-2.29
2.6	4.9	9.9	4.08	0.32	0.47	0.89	1.26	0.30	0.03	0.77	0.03	23.58	2.44	61.37	2.01
2.4	3.4	7.2	2.83	0.64	1.08	1.52	3.13	0.25	0.05	0.62	0.49	7.91	1.48	19.80	15.61
4.5	7.1	19.4	5.92	0.19	0.96	1.52	0.83	0.15	0.01	0.35	0.15	18.65	1.29	42.93	18.67
4.7	6.9	17.1	5.75	0.11	1.44	2.12	0.58	0.09	0.01	0.32	0.26	15.09	2.02	55.44	45.27

Table S5 Model results for the relationship between male social status, male age and male average remating rate across replicate groups of red junglefowl. Estimates with standard errors (SE) and *P*-values obtained from likelihood ratio tests (LRT) excluding only the parameter of interest. Male traits are scaled to have a mean of zero and standard deviation of one

Response	Parameter	Estimate	SE	Δ AIC	LRT
Average remating rate	Male social status	0.114	0.035	8.31	$\chi^2 = 10.989$, *d.f. = 1,* p < 0.001
	Male age	-0.143	0.042	8.99	$\chi^2 = 10.31$, *d.f. = 1,* p = 0.001

Figure S1. Diagrammatic representation of the experimental design showing how 20 replicate experimental mating groups of red junglefowl (*Gallus gallus*) group were formed from 10 unique units of 12 female (female units = squares) and 20 unique units of 10 males (male units = circles). Numbers within squares and circles indicate unique male and female units. The diagram shows how female units were used across male units (i.e. female unit 1 was used with two unique male units, female unit 2 was used with one unique male unit and so on). In total we used 20 units of 10 males. Each unit of males was a unique combination of 10 males taken from a pool of 127 unique males. In total we used 10 unique units of females. Each unit of females contained 12 females taken from a pool of 78 unique females. We were able to control for the use of the same individual males in multiple trials for individual level male analyses using mixed-models that contained random effects for male identity. To control for the use of the same female units across multiple units of males, all models either controlled for female unit as a random effect or fixed effect.

Figure S2. The relationship between male status and male average aggressiveness for each individual mating group of red junglefowl

Figure S3. (A) Relationship between the unique number of males aggressed and male social status across all males in the replicate groups of red junglefowl. (B) Relationship between the unique number of males aggressed and male average aggressiveness. (C) Relationship between male average aggressiveness across all males in the group and the average aggressiveness of males over only the subset of males they aggressed.

Figure S4. The relationship between male age and male average aggressiveness in replicate groups of red junglefowl. The overall correlation between these traits was low (*r* = 0.065). To further explore this relationship we also tested for a quadratic relationship between male average aggressiveness and age using mixed effect models, with male average aggressiveness as a response variable and male age as an explanatory variable. Random effects included male identity, group identity and female unit identity. Male average aggressiveness was log+1 transformed. Model results suggested an initial increase in male age may be associated with an increased aggressiveness before an eventual decline, although because several groups consisted largely of two age groups this result should be interpreted with caution (male age^2: $\chi_1^2 = 6.754$, $p = 0.009$). (B) Boxplot of the Pearson correlations coefficient between male age and male average aggressiveness calculated independently for each group.

Figure S5. The relationship between male reproductive success (*T*) and male social status across replicate groups red junglefowl. Results are also shown for each individual component of male reproductive success i.e. mating success (*M*), partner fecundity (*N*) and paternity share (*P*) with social status all groups. Traits are standardized to have a mean of zero and standard deviation of 1 within groups, and fitness components are mean standardized within groups.

Figure S6. Panels show the relationship between partner fecundity (*N*) and both male age and male average aggressiveness across replicate groups red junglefowl. Traits are standardized to have a mean of zero and standard deviation of 1 within groups, and partner fecundity is mean standardized within groups.

Figure S7. Longitudinal changes in male traits for (A) average aggressiveness (B) number of female mating partners (C) average number of copulations per female mating partner (remating rate) across replicate groups of red junglefowl. Lines connect repeat measurements of the same male. Large points represent a mean of two values.

Figure S8. Panels show the distribution of 1000 effect sizes calculated from mixed effect models on simulated sperm competition intensity (*SCI*) and weighted sperm competition intensity (SCI_w) values generated from randomisations of empirical data across replicate groups od red junglefowl. Bars show frequency distribution of simulated effect sizes, red dotted lines represent observed effect sizes and solid lines represent 95% range of simulated effect sizes. Clockwise from top left (A) effect of male age on *SCI*, (B) effect male average aggressiveness on *SCI*, (C) effect of male age on SCI_w and (D) the effect of male average aggressiveness on SCI_w.

Figure S9. The distribution of 1000 effect sizes calculated from mixed effect models examining the how number of aggressive acts a focal male delivers towards other males affects the number of copulations that competitor males delivered to the focal male's female partners females generated from randomisations of empirical data across replicate groups od red junglefowl. Bars show frequency distribution of simulated effect sizes, red dotted lines represent observed effect sizes and solid lines represent 95% range of simulated effect sizes.

Figure S10. All sexual networks for all 20 replicate groups of red junglefowl in this study. Blue nodes are males red nodes are females, edges between nodes represent sexual interactions. The thickness of edges represents the number of interactions and is comparable within behaviours within groups. The same males and females are represented twice in each network. Male and female nodes are ordered such that nodes closer to the center have the highest mating success. The size of male nodes represents male age standardized within groups. Networks can be read anti-clockwise starting from the central top male axis; yellow edges represent mating attempts, green edges represent successful copulations and orange edges represent fertilized ova. Older males tend to have lower mating success and more aggressive males tend to remate with females more often.

Figure S11. Panels show the relationship between male age and male average aggressiveness with the number of unique females that males attempted to copulate (A & B), the total number of attempted copulations per male (C & D), the average number of attempted copulations across all females with which he attempted to copulate (E & F) across replicate groups red junglefowl.

Tables (1-3)

Table 1. Summary of male reproductive success, mating success and female polyandry across replicate groups of red junglefowl. Grand means presented ± SE. Opportunity for Selection (I_T) is calculated as the mean standardised variation in T (i.e. $\sigma_T^2/\bar{T}^2$). Standardised variation in mating success (I_M), partner fecundity (I_N) and paternity share (I_P) and covariances between mating success and paternity share (COV_{MP}) were calculated as Webster et al. (1995).

Average male reproductive success (T)	Average male mating success (M)	Average male remating rate	I_T	I_M	I_N	I_P	COV_{MP}	Average polyandry
4.235 (0.277)	6.6 (0.352)	18.03 (1.459)	1.252 (0.14)	0.239 (0.033)	0.034 (0.008)	0.675 (0.077)	0.179 (0.047)	5.5 (0.293)

Table 2. Bateman gradient and bivariate Bateman gradient models results across replicate groups of red junglefowl. Gradients presented are mean standardized by dividing reproductive success (T), mating success (M) and sperm competition intensities and weighted sperm competition intensities (SCI and SCI_w) by their respective means within replicate groups. Estimates provided with their standard errors (SE) and P-values obtained from likelihood ratio tests (LRT) excluding only the parameter of interest.

Full model	Parameter	Estimate	SE	Δ AIC	LRT
$T \sim M$	M	1.261	0.115	110.513	$\chi^2 = 112.513$, *d.f. = 1,* $p < 0.001$
$T \sim M + SCI$	M	1.177	0.162	91.658	$\chi^2 = 93.658$, *d.f. = 1,* $p < 0.001$
	SCI	-0.688	0.477	15.160	$\chi^2 = 17.160$, *d.f. = 1,* $p < 0.001$
$T \sim M + SCI_w$	M	1.133	0.177	49.215	$\chi^2 = 51.215$, *d.f. = 1,* $p < 0.001$
	$SCIw$	-0.238	0.152	23.757	$\chi^2 = 25.757$, *d.f. = 1,* $p < 0.001$

Table 3. Results from mixed-effects models describing the relationship between male age and male average aggressiveness with male sperm competition intensity (*SCI*) and weighted sperm competition intensity (SCI_w) across replicate groups of red junglefowl. Results for SCI_w are presented for log-transformed data. Two tailed *P*-values are calculated by comparing observed model estimates to model estimates from 1000 models generated from randomized versions of the empirical data. Male traits are scaled to have a mean of zero and standard deviation of one, so effect sizes are comparable within models.

Response	Parameter	Estimate	p_{rand}
SCI	Male age	0.149	0.792
	Male average aggressiveness	-0.158	0.820
SCI_w	Male age	0.144	0.482
	Male average aggressiveness	-0.099	0.114

Figure Legends (1-5)

Figure 1. (A) The effect of male mating success on male reproductive success in replicate groups of red junglefowl. Colours represent the magnitude of weighted male sperm competition intensities (SCI_w). Values are mean standardized within groups. (B) the effect of male SCI_w on male paternity share. Red line shows the null expectation when paternities are shared out among males based only on the relative representation of their ejaculates, assuming relatively constant fertility across a male's female partners (i.e. based on their weighted sperm competition intensities; SCI_w). Deviations from the red line are due: to male traits affecting paternity share above and beyond his share of copulations, variation in female fecundity, and random variation.

Figure 2. The relationship between male reproductive success (*T*) with male average aggressiveness and male age across replicate groups of red junglefowl. Results are also shown for individual components of male reproductive success i.e. mating success (*M*) and paternity share (*P*) with male average aggressiveness and male age across all groups. Traits are standardized to have a mean of zero and standard deviation of 1 within groups, and fitness components are mean standardized within groups.

Figure 3. A path diagram showing the results of a path analysis for male age and male average aggressiveness and male reproductive success across replicate groups of red junglefowl. Arrows show the direction of effect, solid lines represent significant results, dotted lines represent non-significant results. Signs represent whether effects are positive or negative. Components of male reproductive success (mating success (*M*), average partner fecundity (*N*), paternity share (*P*)) and reproductive success (T) are in circles. Male traits including male age and male average aggressiveness, weighted sperm competition intensities; SCI_w) and male average remating rate are in boxes. All potential directions of influence that were included in our *a priori* path analysis design are included.

Figure 4. The relationship between standardised selection gradients on male average aggressiveness and the correlation between male average aggressiveness and male age across replicate groups of red junglefowl. Shaded area represents 95% confidence intervals.

Figure 5. Relationships between (A) male remating rate and male age, (B) between male remating rate and male average aggressiveness, (C) the relationship between male paternity share (*P*) rate and male remating rate across replicate groups of red junglefowl. Male remating rate is mean standardized within groups and male traits are values standardized to have a mean of zero and a standard deviation of 1 within groups. (D) Example sexual networks for eight of the twenty groups in this study. Blue nodes are males red nodes are females, edges between nodes represent sexual interactions. The thickness of edges represents the number of interactions and is comparable within behaviours within groups. The same males and females are represented twice in each network. Male and female nodes are ordered such that nodes closer to the center have the highest mating success. The size of male nodes represents male age standardized within groups. Networks can be read anti-clockwise starting from the central top male axis; yellow edges represent mating attempts, green edges represent successful copulations and orange edges represent fertilized ova. Older males tend to have lower mating success and more aggressive males tend to remate with females more often.