Dunnock social status correlates with sperm speed, but fast sperm does not always equal high fitness

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ABSTRACT

Sperm competition theory predicts that males should modulate sperm investment according to their social status. Sperm speed (one proxy of sperm quality) also influences the outcome of sperm competition because fast sperm cells may fertilize eggs before slow sperm cells. We evaluated whether the social status of males predicted their sperm speed in a wild population of dunnocks (*Prunella modularis*). In addition to the traditional analysis of the average speed of sperm cells per sample, we systematically evaluated ranked groups of sperm, ranging from the 5-fastest sperm cells to the 100-fastest sperm cells in a sample. We further evaluated whether fitness, defined here as the number of chicks sired per male per breeding season, relates to the sperm speed in the same population. We found that males in monogamous pairings (i.e. low levels of sperm competition), produced the slowest sperm cells whereas subordinate males in polyandrous male-male coalitions, (i.e. high levels of sperm competition), produced the fastest sperm cells. This result was consistent across all the ranked groups of sperm, but statistical support was conditional on the number of sperm cells included in the analysis. Surprisingly, we found no significant relationship between fitness and sperm speed, contrary to theory – it is possible that the differential mating opportunities across social status leveled out any possible difference. Our study also suggests that it is important to identify biologically meaningful rankings of fastest sperm and cutoffs for inclusions for assessing sperm competition via sperm speed.

INTRODUCTION

Competition between the sperm cells of two or more males to fertilize the same ovum is common across the animal kingdom, and this phenomenon is widely known as sperm competition (Parker, 1970, 1990). Sperm competition theory predicts that when males compete for the same female, males should either adjust their behavior (e.g., mate guarding) or modulate sperm allocation to maximize their fitness (Parker, 1970; Birkhead & Hunter, 1990). In species with social dominance hierarchies, males with a high social status (i.e., dominant individuals) are predicted to have relatively more mating access to females, thereby decreasing their risk of sperm competition and favoring their fertilization chances (Birkhead & Hunter, 1990; Birkhead et al., 1991). In contrast, subordinate males with limited mating access to females are subject to intense sperm competition, and have fewer fertilization chances (Birkhead & Hunter, 1990; Birkhead et al., 1991). Empirical evidence demonstrates that dominant males can decrease sperm allocation given their social advantage, while subordinate males can increase sperm allocation to counteract their social disadvantage (Cornwallis & Birkhead, 2007; Montrose et al., 2008; Wedell et al., 2002).

Such differential sperm allocation has traditionally been measured in terms of sperm count (Del Barco-Trillo, 2011; Kelly & Jennions, 2011). Yet, theory predicts that the outcome of sperm competition is not only dependent on the number of sperm cells, but also on sperm quality traits such as mobility, seminal fluid concentration, and sperm swimming speed (Birkhead et al., 1999; Snook, 2005; Kelly & Jennions, 2011). Laboratory-based studies in insects, fish, mammals, and birds find support for the prediction that males also actively modulate sperm quality in response to perceived sperm competition (e.g., Bartlett, Steeves, Gemmell, & Rosengrave, 2017; Cornwallis & Birkhead, 2007; Montrose, Harris, Moore, & Moore, 2008; Ramm et al., 2015). However, whether such a prediction holds true in wild populations remains less explored. For example, in birds, studies of social status vs.

sperm swimming speed have been mainly conducted in laboratory settings using captive individuals of model species such as *Gallus gallus* (Birkhead et al., 1999; Froman et al., 2002; Pizzari et al., 2007). Although Kleven et al. (2009) conducted a comprehensive study of sperm swimming speed using 42 passerine bird species in wild conditions, they did not directly assess sperm swimming speed in relation to social status.

Sperm swimming speed (hereafter referred to as sperm speed) is a sperm quality trait that may influence the outcome of sperm competition (Gage et al., 2004; Kleven et al., 2009; Lupold et al., 2009). In birds, the specific relevance of the sperm speed in fertilization seems to occur after the female release the sperm cells from the sperm storage tubules (SSTs) (Froman et al., 2002; Hemmings & Birkhead, 2017). While most studies of sperm speed primarily focus on the average sperm per sample, recent studies have stressed the importance of using sub-samples, ranked groups of fastest sperm cells, within a sample. Such sub-samples usually consist of 5%, 10%, or 20% of the fastest sperm cells of the sperm cells in a sample (Bennison et al., 2016; Haugland et al., 2009; Mossman et al., 2009; Rudolfsen et al., 2006; Vaz Serrano et al., 2006, 2006). The rationale for using a ranked group of fastest sperm cells is that only a small portion of the fastest sperm cells represent the most viable cells of an individual's sample that will not only traverse the lower female reproductive tract quickly, but that will also approximate the maximum obtainable sperm speed of an individual sperm phenotype (Bennison et al., 2016; Birkhead et al., 1999; Mossman et al., 2009; Snook, 2005). Consequently, some sperm speed studies deem that faster sperm cells are more important and, thus, remove slow sperm cells from analyses (also see: Sasson, Johnson, & Brockmann, 2015).

Evaluating whether male social status correlates with sperm speed in the wild requires a species with an observable social dominance hierarchy. The dunnock (*Prunella modularis*), provides such a study system. The breeding system of *P. modularis* varies by population, but

it often includes monogamous pairs and polyandrous trios. Although polygyny and polygynandry also occur, these two types of breeding groups are less common in our studied population (Santos & Nakagawa, 2013). In *P. modularis*, the males can be categorized as monogamous males, polyandrous dominant alpha males, or polyandrous subordinate beta males according to their social behavior (e.g., male–male interactions, mate guarding; Davies, 1992). A monogamous male has almost exclusive social access to his female partner, but socially monogamous males are not completely free from sperm competition, as females engage in extra-pair/group copulations with other males (Burke, Davies, Bruford, & Hatchwell, 1989; Santos et al., 2015). Nevertheless, among these three types of males, socially monogamous males are likely to experience the least sperm competition. Among co-breeding males in polyandrous groups, dominant alpha males and subordinate beta males directly compete for mating access to their social females. Dominant alpha males usually gain more access to females (compared to beta males) and, consequently, they may experience lower sperm competition than subordinate beta polyandrous males, which have fewer opportunities to copulate (Davies, 1983). Thus, the variation in the risk of sperm competition among these three social status makes *P. modularis* suitable for sperm competition studies. In addition, dunnocks are an ideal species to study sperm competition because they are adapted for high levels of sperm production. In other words, the dunnock seminal glomera contains an enormous reserve of sperm at any point in time during their breeding season, as well as they are also adapted to avoid sperm cell depletion (Birkhead et al., 1991).

Here, our primary goal is to evaluate whether the social status of males predicts sperm speed in a wild population of *P. modularis*. To thoroughly explore this prediction, we systematically evaluate the effect of social status on sperm speed in monogamous males, alpha polyandrous males, and beta polyandrous males. We predict, according to sperm competition theory, that beta polyandrous males will produce the fastest sperm cells and

monogamous males the slowest sperm cells, as these males experience the most and least sperm competition, respectively. Accordingly, we expect the sperm speed for the alpha polyandrous males to fall somewhere between the two. Our secondary goal is to quantify the changes of between- and within-individual variance (and intra-class correlation, ICC) of sperm speed when the number of sperm cells included in the analysis is changed. This additional analysis aims to quantify whether the variation across different ranked groups of fastest sperm cells is consistent. We predict that sperm speed consistency decreases (as variance increases) when the number of sperm cells included in analysis increases. Our third goal is to explore whether the reproductive fitness of males, expressed here as the number of chicks sired per adult male within a season, relates to the sperm speed in the same studied population. We expect that, given the differential mating opportunities that occur among the different male social status, there would not be any strong correlation between the reproductive fitness of males and their sperm speed. Theory predicts such a correlation to be positive if all the males of a given population have equal or similar access to copulations but, as described above, in *P. modularis* access to females depends on the male's status. In other words, the benefits of differential female access and differential sperm speed could cancel each other when these two variables are negatively correlated, which is likely in dunnocks.

METHODS

Study population and social status monitoring

We studied a wild population of *P. modularis* in the Dunedin Botanic Garden, New Zealand (45.856° S and 170.518° E, area 7.2 ha, ca. 80 breeding dunnocks per year: Santos & Nakagawa, 2013; Santos et al., 2015; Holtmann, Santos, Lara, & Nakagawa, 2017) All adults and nestlings in this population were monitored and banded over seven breeding seasons (September to January 2009–2016). Using daily visual observations of the social breeding

groups in the field, and following Davies (1992), we classified males as monogamous males, which were exclusively associated to a single female with no additional or permanent males observed in the same territory (hereafter, $\alpha_{[monogamous]}$), or cooperatively breeding polyandrous males, which were divided into dominant polyandrous alpha and subordinate polyandrous beta (hereafter $\alpha_{[polyandrous]}$ and $\beta_{[polyandrous]}$, respectively). We determined the status of the polyandrous males based on visual observations of social/antagonistic interactions between the males within the breeding groups.

Sperm collection and sperm speed measurements

We analyzed 44 semen samples from different male dunnocks captured with mist nests over two breeding seasons (Season A: September to January 2014–2015, $N_{[individuals]} = 20$, $N_{[\alpha_{monogamous}]} = 4$, $N_{[\alpha_{polyandrous}]} = 7$, $N_{[\beta_{polyandrous}]} = 9$. Season B: September to January 2015–2016, $N_{[individuals]} = 24$, $N_{[\alpha_{monogamous}]} = 2$, $N_{[\alpha_{polyandrous}]} = 12$, $N_{[\beta_{polyandrous}]} = 10$. Average age ± SD = 2.72 ± 1.87 years, minimum age = 1-year, maximum age = 7-years). We collected semen samples (ca. 5–10 µl) using cloacal massage (Wolfson 1952). The samples were rapidly (within a minute) diluted in 10 µl of Dulbecco's Modified Eagle Medium, DMEM, (ThermoFisher Scientific, USA). After gentle mixing, we pipetted 1 µl of the semen mixture onto a 20 µM slide (Leja, Netherlands) and then added 3 µl of DMEM. We placed the slide with the semen mixture onto a slide warmer heated to 37°C under a microscope (Eclipse E200, Nikon Instruments Inc, USA). We set the microscope to a negative phase and 100× magnification, and we connected it to a Gigabit Ethernet camera (Basler Scout ACA780-75GC, Germany). We used the camera in tandem with the Sperm Class Analyzer software (SCA, Microptic, Spain) to capture and measure sperm speed in multiple 1-second videos for each semen sample. Slides, pipette tips, Eppendorf tubes, DMEM, and anything else that would come into contact with the semen sample were always preheated to 37°C. If the

concentration of the sperm cells in the sample was too high to produce a good recording, we further diluted the sample in the tube with DMEM and then made a new slide.

We recorded on average 5.72 (SD = 1.42, minimum = 3, maximum = 10) video clips per sample (1 second each). Using the SCA software, we removed debris, immotile spermatozoa, and spermatozoa with overlapping trajectories that could not be distinguished by the software from our dataset. After processing the videos, we obtained, on average, 121 ± 63 sperm cells (minimum = 22 sperm cells, maximum = 322 sperm cells) per sample. The SCA software provides measures of average path velocity (VAP $\mu m \times s^{-1}$), curvilinear velocity (VCL $\mu m \times s^{-1}$), and straight-line velocity (VSL $\mu m \times s^{-1}$) per sperm cell. These three speed proxies were highly correlated (VCL *vs*. VAP $r = 0.942$, 95% CIs = 0.939 to 0.945; VCL *vs*. VSL $r = 0.926$, 95% CIs = 0.922 to 0.930; and VAP *vs*. VSL $r = 0.993$, 95% CIs = 0.993 to 0.994). We opted to analyze the raw VCL data instead of obtaining an eigenvector from a principal component analysis (PCA) between these three proxies for two main reasons. First, eigenvector units are not in the original scale of the variable, thus, biologically meaningful interpretations are limited. Second, VCL tracks the point-to-point trajectory of each sperm cell, and this measure is ideal when ovarian fluids are not present to facilitate sperm cell orientation. Hence, sperm cell trajectories are not expected to be linear (i.e., VSL) (Rudolfsen et al., 2006; Kleven et al., 2009). We exclusively analyzed one sample per individual, and we assumed that this sample would appropriately represent each individual's sperm speed as suggested by Laskemoen et al. (2013).

Quantifying reproductive fitness

We defined reproductive fitness as the number of chicks sired per breeding male within a field season. To assess paternity and quantify this individual proxy of fitness, we blood sampled all adults and chicks during each breeding season and stored the blood in ethanol at

4°C. Additionally, we collected tissue from the embryos of all unhatched eggs that contained embryos. DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Auckland, New Zealand). For paternity assignment, we genotyped adults and chicks at 16 microsatellite markers (for a detailed description of the genotyping procedure see: Holtmann et al., 2017; Santos et al., 2015). We assigned paternity using a Bayesian approach implemented in the R package MasterBayes 2.5.5 (Hadfield, Richardson, & Burke, 2006) in R 3.5.1 (R Core Team, 2019). MasterBayes allowed us to incorporate genotypic as well as non-genetic data to estimate the likelihood of parentage (Walling et al., 2010). Along with the genetic data, we included the distance (longitude and latitude) between the nest of a social pair/group and the nests of all other potential sires. In addition, we included males as potential fathers that did not have a nest but were known to be alive. For each breeding season, we ran a separate model and estimated the number of unsampled males. In all models, we specified Wang's genotyping error (Wang, 2004), and the MCMC chains were run with 1,300,000 iterations, a thinning interval of 1,000, and a burn-in of 30,000. For the analyses, we used the data of 156 offspring for which paternity was assigned with an average confidence of 0.96 (standard deviation = 0.11).

Statistical analyses

Sperm speed and social status – We conducted all statistical analyses in R 3.5.2 (R Core Team, 2019). To assess differences in sperm speed among male *P. modularis* social status, we fitted Bayesian generalized linear mixed models (BLMMs) using Markov chain Monte Carlo (MCMC) techniques implemented in the *MCMCglmm* package (Hadfield, 2010) with the Gaussian error distribution. The speed (VCL $\mu \times s^{-1}$) of individual sperm cells was the response variable. We included social status as a categorical variable ($\alpha_{[monogamous]}$, $\alpha_{[polyandrous]}$, and $\beta_{[polyandrous]}$), and the individual's age (in years) as fixed effects. We fitted

male age as a fixed factor because sperm speed might decrease with increasing male age (Johnson et al., 2015; Johnson & Gemmell, 2012; Møller et al., 2009). Age was z-transformed to be on a comparable scale (Schielzeth, 2010). We included individual identity as a random effect. In total, we fitted six BLMMs, each, as mentioned, with sperm speed (VCL $\mu \times s^{-1}$) as the response variable. We fitted one model using the average sperm speed per sample, and for the other five models, we used five different groups of fastest sperm cells, as follows: from the 1st to the 5th fastest sperm cell, from the 1st to the 10th fastest sperm cells, from the 1st to the 20th fastest sperm cells, from the 1st to the 50th fastest sperm cells, and from the 1st to the 100th fastest sperm cells. For all the BLMMs, we report estimates of regression coefficients as the mean of 1,000 posterior samples with 95% credible intervals (CIs) and considered the effects to be statistically significant if the CIs did not overlap zero. To obtain 1,000 posterior samples, we ran each BLMM for 2,600,000 MCMC iterations with a burn-in period of 600,000 iterations and a thinning interval of 2,000. We used and inverse-gamma prior for the residuals (*V = 0.002, nu = 1*), a parameter expanded prior for the random effects (*V = 1, nu = 1, alpha.mu = 0, alpha.V = 1,000*), and default priors for the fixed effects.

Analysis of variance components and ICC for sperm speed – To quantify the between- and within-individual variance of sperm speed (hereafter $\sigma^2_{[between]}$ and $\sigma^2_{[within]}$, respectively), we obtained variance components and their associated 95% CIs from null BLMMs (i.e. the model with only intercept as a fixed factor). We fitted the BLMMs using *MCMCglmm* and settings described above. In total, we fitted five BLMMs per breeding season, each with a different number of fastest sperm cells as described above. Sperm speed (VCL $\mu \times s^{-1}$) was z-transformed and included as a response variable, and individual identity was included as a random effect. We calculated the ICC as the proportion of the between-individual variance in relation to the total phenotypic variance (Nakagawa & Schielzeth, 2010).

Sperm speed and male fitness – To assess the relationship between sperm speed and the reproductive fitness of *P. modularis* males, we fitted six BLMMs using the techniques described above. Similarly, we used the speed (VCL $\mu \times s^{-1}$) of individual sperm cells as the response variable (using the average sperm speed and the same five groups of fastest sperm cells, as described above). We included fitness (measured as the number of chicks sired per individual) and the individual's age (in years) as fixed effects, both as continuous variables. We included individual identity as a random effect. In addition, we fitted six additional BLMM models, very similar to the ones described above, but including: (1) social status, and (2) the interaction between social status and the total number of chicks, as fixed effects. These additional models were designed to further explore whether the slope of the correlation between VCL and fitness for a specific social status was significant. This is important given that the previous models only evaluated this correlation (slope) for the individual fitness without considering the social status per se. Therefore, this final set of models can be seen as a type of sensitivity analysis.

RESULTS

Sperm speed in the social status

We found that $\beta_{[polyandrous]}$ males had the fastest average sperm speed (VCL $\mu \times s^{-1}$) followed by $\alpha_{[polyandrous]}$ males, whereas $\alpha_{[monogamous]}$ males had the slowest sperm (Table S1, Figure 1). This pattern was consistent across the five sperm groups evaluated (1^{st} to 5^{th}, 1^{st} to 10^{th}, 1^{st} to 20^{th}, 1^{st} to 50^{th}, and 1^{st} to 100^{th}), and also in the average sperm speed model (Figure 1, Table S1). We found that the significant differences in sperm speed only occurred between $\beta_{[polyandrous]}$ and $\alpha_{[monogamous]}$ and only in the first three sperm groups (1^{st} to 5^{th}, 1^{st} to 10^{th}, 1^{st} to 20^{th}, Figure 1, Table S1). More precisely, we found that the difference in sperm speed

between $\beta_{[polyandrous]}$ and $\alpha_{[monogamous]}$ was significantly different up to the first 22 fastest sperm cells (Figure S1). This is mainly because the uncertainty around the mean speed estimates increased as more sperm cells were included. The differences in speed between the other two social status ($\alpha_{[monogamous]}$ *vs*. $\alpha_{[polyandrous]}$ and $\alpha_{[polyandrous]}$ vs. $\beta_{[polyandrous]}$) were relatively small and non-significant (Figure 1, Table S1).

Analysis of variance components and ICC in the sperm speed and social status models

The estimates of the variance components ($\sigma^2_{[between]}$ and $\sigma^2_{[within]}$) and ICC were similar in both field seasons (Figure 2). We found that the highest between-individual variance ($\sigma^2_{[between\ 2014\text{-}2015]} = 0.881$ and $\sigma^2_{[between\ 2015\text{-}2016]} = 0.867$, Figure 2) and lowest within-individual variance ($\sigma^2_{[within\ 2014\text{-}2015]} = 0.158$ and $\sigma^2_{[within\ 2015\text{-}2016]} = 0.161$, Figure 2) occurred when we evaluated the first five (1^{st} to 5^{th}) fastest sperm cells. We also observed that when the number of sperm cells analyzed increased (i.e., from the 1^{st} to 100^{th} fastest sperm cells), the $\sigma^2_{[between]}$ variance steadily decreased, whereas the $\sigma^2_{[within]}$ steadily increased (Figure 2). Both variance components reached similar values when including the 1^{st} to 100^{th} fastest sperm cells per individual ($\sigma^2_{[between\ 2014\text{-}2015]} = 0.456$ and $\sigma^2_{[within\ 2014\text{-}2015]} = 0.490$; and $\sigma^2_{[between\ 2014\text{-}2015]} = 0.572$ and $\sigma^2_{[within\ 2015\text{-}2016]} = 0.529$). The ICC closely mirrored the between-individual variance (Figure 2). The maximum ICC values occurred when we included the first five (1^{st} to 5^{th}) fastest sperm cells only ($ICC_{[2014\text{-}2015]} = 0.848$, 95% CIs = 0.708. to 0.912; $ICC_{[2015\text{-}2016]} = 0.844$, 95% CIs = 0.702 to 0.909; Figure 2). The ICC gradually decreased, reaching the minimum value when including the first 100 (1^{st} to 100^{th}) fastest sperm cells ($ICC_{[2014\text{-}2015]} = 0.485$, 95% CIs = 0.295 to 0.628; $ICC_{[2015\text{-}2016]} = 0.519$, 95% CIs = 0.351 to 0.640, Figure 2).

Sperm speed and male fitness

We found a weak, non-significant negative relationship between sperm speed (VCL $\mu \times s^{-1}$) and male reproductive fitness in *P. modularis* (Figure 3 and Table S2). Notably, the slight reduction in sperm speed was very consistent across the five sperm groups evaluated (1^{st} to 5^{th}, 1^{st} to 10^{th}, 1^{st} to 20^{th}, 1^{st} to 50^{th}, and 1^{st} to 100^{th}), and also in the average sperm model (Figure 3, Table S2). In addition, we found that none of the slopes of the BLMMs that included social status (Table S3) were significant. In other words, there were no significant correlations between VCL and reproductive fitness for any of the three social status evaluated (Table S3).

DISCUSSION

This study primarily assessed whether male social status predicts sperm speed in a wild population of dunnocks. It also systematically evaluated changes in between- and within-individual variance (and ICC) for five different groups of fastest sperm cells. Finally, we evaluated whether sperm speed correlates with fitness in the same population. We found that polyandrous beta males, which experience the highest sperm competition in dunnocks, produced the fastest average sperm speed, whereas monogamous alpha males, which have the lowest sperm competition, produced the slowest swimming sperm (Figure 1, Table S1). This result is in line with previous, laboratory-based studies in birds, which predicted that the social status of an individual should dictate their sperm speed (Froman et al., 2002; Pizzari et al., 2007).

The trends regarding social status and sperm speed persisted regardless of which group of fastest sperm cells (or even including the average sperm speed) was considered in the analyses, but significant differences between polyandrous beta and monogamous alpha males were only present when up to the 22 fastest swimming sperm cells were used (Figure

S1). When the number of fastest sperm cells included in analyses was increased, the between-individual variance (and ICC) of sperm speed declined while the within-individual variance increased (Figure 2). This explains why statistically significant differences between the social status (i.e., between beta polyandrous and alpha monogamous) vanished when more sperm cells per individual were included in the analysis.

Importantly, we found no evidence for an association between sperm speed and male reproductive fitness in the studied population. This result may indicate that the differential mating opportunities between the three different social status evaluated negated differences in sperm speed between these same groups. In other words, the advantage of having fast sperm speed is offset by male status and associated differences in mating opportunities.

Sperm speed and social status

We did not find significant differences in sperm speed within polyandrous males (i.e., between alpha polyandrous males and beta polyandrous males, Figure 1, Table S1), either in the average sperm speed or in any of the five groups of fastest sperm cells evaluated, although the sperm speed values of the polyandrous alpha males were consistently lower than those of polyandrous beta males (Figure 1, Table S1). It is possible that a larger sample size (ours was limited to 44 individuals) would have detected significant statistical differences between the polyandrous males. We predicted significant differences in sperm speed between the alpha and beta males in polyandry because they exhibit a clear social hierarchy (Davies, 1986; Santos & Nakagawa, 2013), and because the risk of competition is expected to be the highest when only two rival males (instead of multiple males) compete for a female (Kelly & Jennions, 2011). However, polyandrous female dunnocks copulate with both alpha and beta males and engage in extra-group mating. Paternity in polyandrous groups in our dunnock population is known to be shared evenly, on average, between polyandrous alpha and beta

males with 46% and 45%, respectively (Santos et al., 2015). Further, extra-group paternity is relatively common (ca. 9%) in these polyandrous groups (Burke et al., 1989; Santos et al., 2015). It is likely that both co-breeding males watch each other when one of them is engaging the female. Moreover, these co-breeding males might even see their females copulating with extra-group males in addition to copulations with their co-breeding 'associate'. Consequently, co-breeding males could perceive intense sperm competition, which in turn could lead to an increase in sperm investment by both males in the group.

Analysis of variance components and ICC in the sperm speed and social status models

A secondary finding from this study is that the number of fastest sperm cells included in the analyses has the potential to alter conclusions drawn from the same data. This highlights the importance of understanding how many of the fastest sperm cells play a role in fertilization success. For instance, if we accept that fewer than the 22 fastest sperm cells play a critical role in fertilization, we would conclude that sperm speed between alpha monogamous and beta polyandrous males is significantly different in a way that is biologically meaningful (Figure S1). Conversely, if we assume that more than the 22 fastest sperm cells are needed to outcompete rivals, we would conclude that sperm speed between alpha monogamous and beta polyandrous males is not necessarily different (Figure S1). Our detailed analyses allowed us to pinpoint that the between-individual variance and ICC in sperm speed decreases as more fastest sperm cells per individual are included in the analyses (Figure 2). Therefore, the statistical significance between the social status was dependent on the number of sperm cells analyzed in our work. It is important to note that statistical significance also heavily depends on the number of individuals included, which was fixed in our study. While this issue could apply to most of the studies that have focused on sperm quality traits such as swimming speed (e.g., Vaz Serrano et al., 2006; Rudolfsen, Figenschou, Folstad, & Kleven,

2008; Haugland et al., 2009; Mossman et al., 2009; Bennison et al., 2016), it has rarely been acknowledged. We suggest that the 'ideal' number of sperm cells to include in analyses of sperm traits varies across species. This variation is created by the number of fast sperm cells required to guarantee fertilization and/or outcompete rivals, yet to our knowledge, this information is still unavailable. Therefore, we still need to opt for a more comprehensive and transparent type of analysis (e.g., evaluating how varying the number of fastest sperm cells included in analyses might affect the results). We suggest the procedure followed here as a method that allows for more robust and comparable results and conclusions. This may be particularly important in birds where more than one sperm to reach the egg for successful fertilization to occur and for subsequent embryo survival (Hemmings & Birkhead, 2015; Mizushima et al., 2014)

Sperm speed and male reproductive fitness

Our findings indicate that there was not a significant relationship between sperm speed and the individual reproductive fitness of *P. modularis* males in our study system (Figure 3, Table S2). This finding initially appears contradictory to theoretical expectations, as theory posits that individuals possessing faster sperm cells would be able to gain more reproductive fitness (Birkhead & Hunter, 1990; Birkhead et al., 1991). However, we have to consider that the frequency of mating for individual males varies according to their social status. For instance, dominant *P. modularis* males can have more frequent copulations while subordinate males have few (Davies, 1992; Davies, 1983). Such differential mating rates have the potential to counteract the effect of sperm speed on fitness. Although beta polyandrous males face the highest level of sperm competition in *P. modularis* and produce the faster swimming sperm cells, they mate very infrequently. We therefore argue that the number of sired chicks of a polyandrous beta male is mainly influenced by its mating opportunities rather than its sperm

speed. Hence, we suggest that the behavioral component of male–male competition (e.g., male dominance, male guarding) could still be a strong driver of male reproductive fitness and that extra-pair paternity may play only a minor role in our studied population (Santos et al., 2015).

An additional question, which is difficult to answer with the evidence gathered in this study, is why monogamous males do not produce faster sperm to achieve higher reproductive fitness. A possible answer to this includes, but is not limited to, a potential trade-off for alpha males. For instance, alpha males may need more energy to defend their mates and their territories. Alternatively, it could be possible that monogamous males might suffer some costs for increasing their sperm speed (e.g., dying younger). Such an effect may not be reflected in the short-term (within a breeding season), but probably could be traceable across multiple breeding seasons. Such possibilities remain open to future research.

Final remarks

Although conditional, we found a relationship between male social status and sperm speed, which is in line with the predictions from sperm competition theory. Further, we employed a new statistical approach for sperm competition studies that assay sperm speed and recommend that researchers conduct comprehensive profiling of sperm speed on a case-by-case basis rather than by using an arbitrarily fixed fraction of the fastest sperm cells. A single fraction may not capture the between-individual variance in sperm speed in a population and the appropriateness of the fraction could be dependent on the number of individuals used in the analysis. As a final note, we have shown that behavioral aspects such as male-male coalitions resulted in differential sperm quality and this, coupled with differential mating opportunities, may have profound implications for the fitness of individuals.

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ETHICS

The New Zealand Department of Conservation (Permit 36716-FAU) and the University of Otago Animal Ethics Committee (Permits 12/49 and 89/14) approved this research.

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FIGURES

Figure 1. Outputs of the BLMM models that compared sperm speed (VCL $\mu \times s^{-1}$) and dunnock social status ($\alpha_{[mon]}$ = alpha monogamous, red; $\alpha_{[pol]}$ = alpha polyandrous, blue; $\beta_{[pol]}$ = beta polyandrous, black). Each plot displays different cumulated groups of fastest sperm cells, ranging from 1st to 5th $N_{[sperm]}$ = 220, 1st to 10th $N_{[sperm]}$ = 440, 1st to 20th $N_{[sperm]}$ = 880, 1st to 50th $N_{[sperm]}$ = 2,135, and 1st to 100th $N_{[sperm]}$ = 3,683, and average sperm speed $N_{[sperm]}$ = 5,338. $N_{[individuals]}$ = 44 in all the GLMMs. The colored dots represent the posterior mean in each social status and vertical lines represent 95% credible intervals. Raw data (horizontally jittered) are presented in the background with black dots. See BLMM regression coefficients and confidence intervals in Table S1.

Figure 2. Between- and within-individual variance in sperm speed (VCL $\mu \times s^{-1}$) and the intra-class correlation coefficient (ICC) from different groups of fastest sperm cells. Sperm groups are as follows: 1st to 5th, 1st to 10th, 1st to 20th, 1st to 50th, and 1st to 100th. Data are separated into two breeding seasons (2014–2015 and 2015–2016).

Figure 3. Comparison of sperm speed (VCL $\mu \times s^{-1}$) and individual fitness across five groups of fastest sperm cells (1st to 5th, 1st to 10th, 1st to 20th, 1st to 50th, 1st to 100th, and average sperm speed). Raw data (horizontally jittered) are presented in the background with grey dots. See BLMM regression coefficients and confidence intervals in Table S2.

Figure 1.

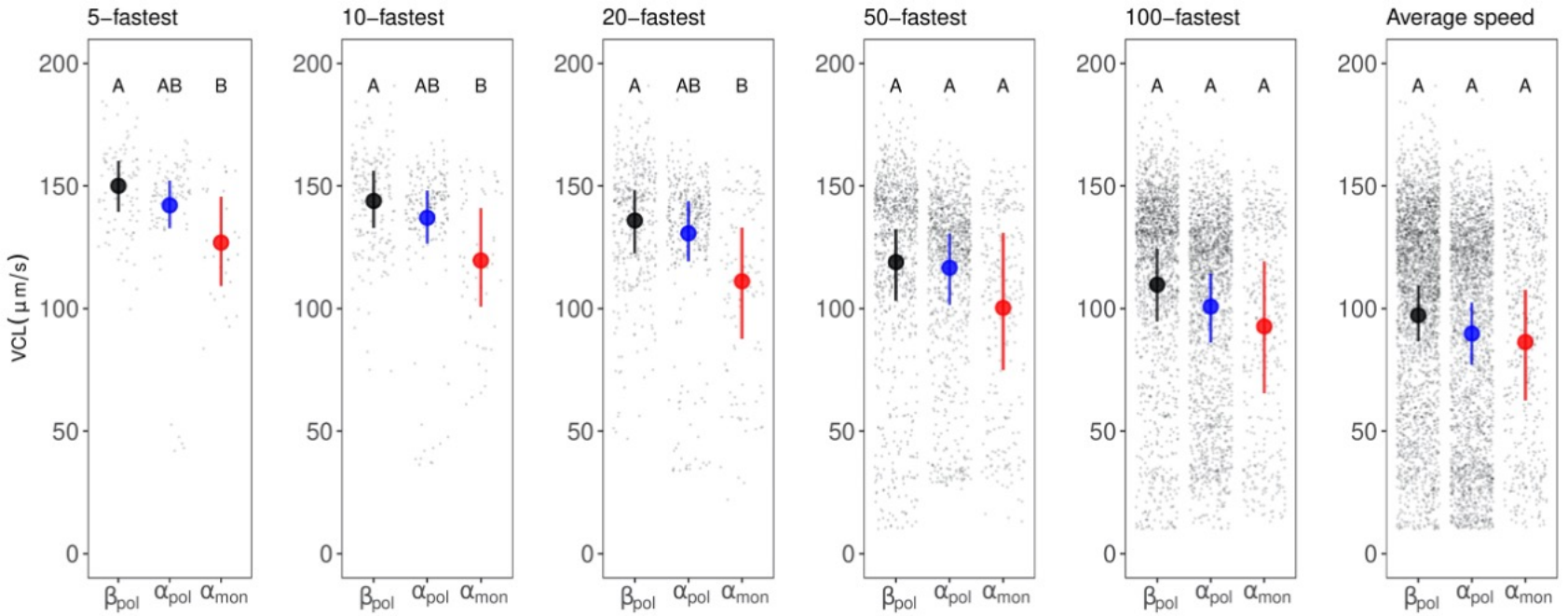

5−fastest
10−fastest
20−fastest
50−fastest
100−fastest
Average speed
VCL(μm/s)
200
150
100
50
0
A
AB
B
A
A
A
βpol
αpol
αmon

Figure 2.

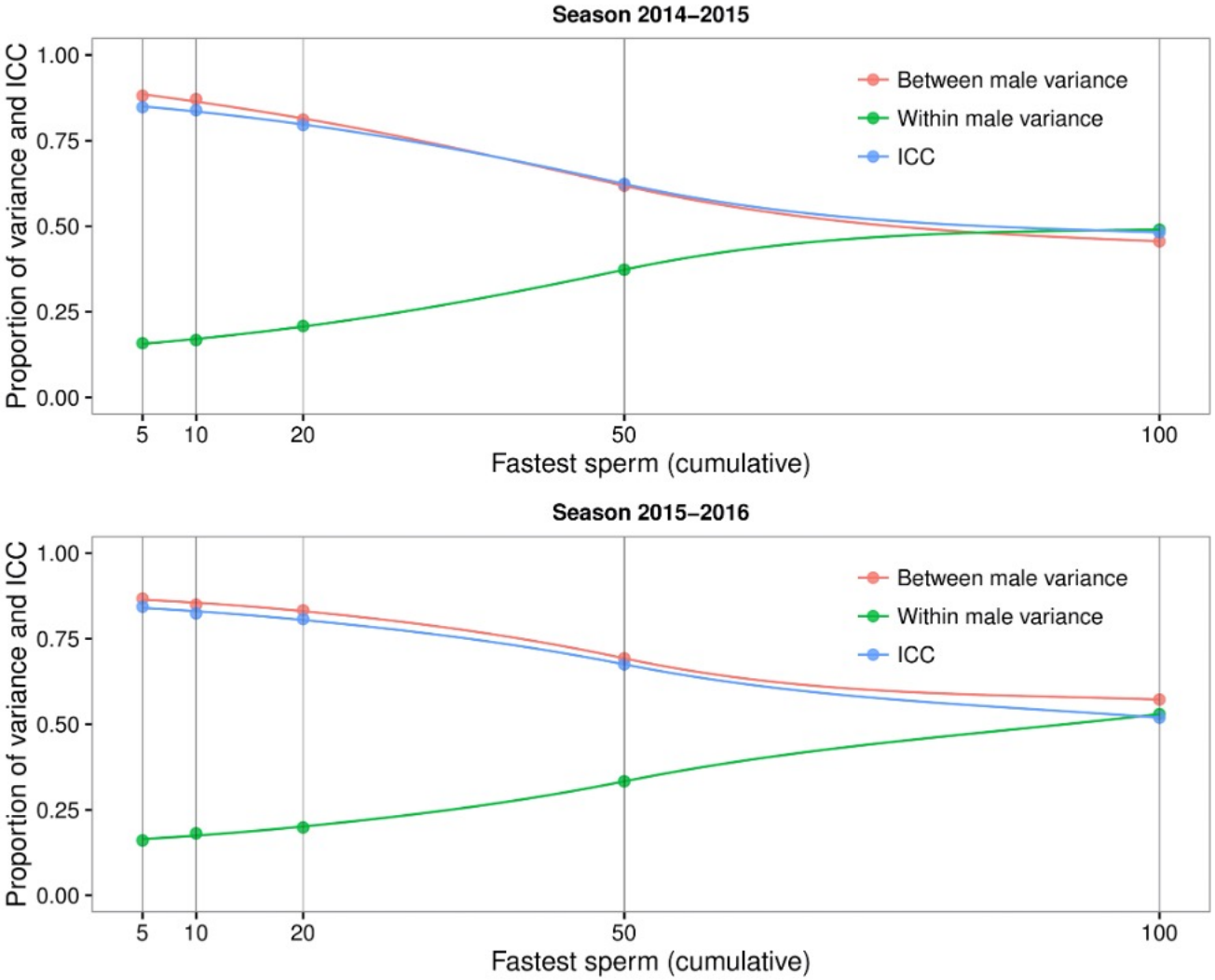
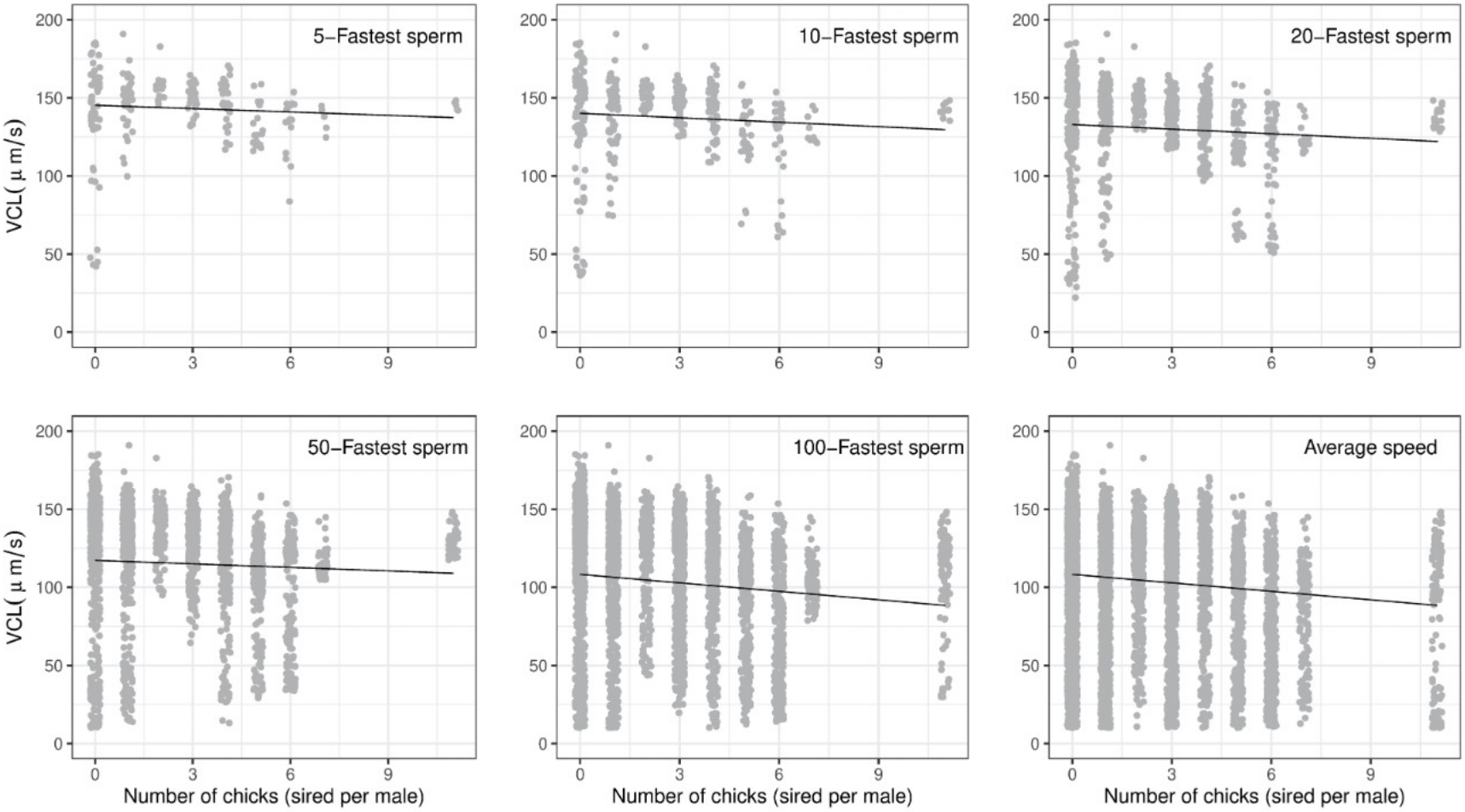

Season 2014–2015
Proportion of variance and ICC
1.00
0.75
0.50
0.25
0.00
Between male variance
Within male variance
ICC
5
10
20
50
100
Fastest sperm (cumulative)
Season 2015–2016
Proportion of variance and ICC
1.00
0.75
0.50
0.25
0.00
Between male variance
Within male variance
ICC
5
10
20
50
100
Fastest sperm (cumulative)

Figure 3.


5−Fastest sperm
10−Fastest sperm
20−Fastest sperm
50−Fastest sperm
100−Fastest sperm
Average speed
VCL(μ m/s)
Number of chicks (sired per male)
0
50
100
150
200
0
3
6
9

SUPPLEMENTARY MATERIAL

Dunnock social status correlates with sperm speed, but fast sperm does not always equal high fitness

Table S1. Outputs from the six BLMMs assessing differences in sperm speed (VCL $\mu \times s^{-1}$) among three social statuses ($\alpha_{[mon]}$ = alpha monogamous, $\alpha_{[pol]}$ = alpha polyandrous, and $\beta_{[pol]}$ = beta polyandrous) in dunnocks. We present the differences between the social status pairs along with their 95% credible intervals. Significant differences are in bold.

1st - 5th fastest	***Fixed effects***	**Posterior mean**	**95% Credible intervals**
	Intercept ($\alpha_{[mon]}$)	126.86	107.47 to 146.04
	Social status (difference $\alpha_{[pol]}$ vs. $\alpha_{[mon]}$)	15.20	-7.64 to 34.04
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[mon]}$)	**23.26**	**0.90 to 47.55**
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[pol]}$)	8.06	-5.54 to 23.77
	Age	0.81	-6.65 to 7.90
	Random effects	σ^2	
	Sample identity	491	291 to 720
1st - 10th fastest	***Fixed effects***	***Posterior mean***	***95% Credible intervals***
	Intercept ($\alpha_{[mon]}$)	119.93	100.62 to 140.31
	Social status (difference $\alpha_{[pol]}$ vs. $\alpha_{[mon]}$)	17.43	-6.90 to 38.79
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[mon]}$)	**23.92**	**2.88 to 48.29**
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[pol]}$)	6.49	-10.39 to 22.77
	Age	0.37	-8.64 to 6.80
	Random effects	σ^2	
	Sample identity	579	346 to 864
1st - 20th fastest	***Fixed effects***	**Posterior mean**	**95% Credible intervals**
	Intercept ($\alpha_{[mon]}$)	111.69	88.37 to 132.79
	Social status (difference $\alpha_{[pol]}$ vs. $\alpha_{[mon]}$)	18.81	-4.83 to 44.54
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[mon]}$)	**23.93**	**0.32 to 52.89**
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[pol]}$)	5.11	-12.07 to 22.10
	Age	-1.29	-10.28 to 7.19
	Random effects	σ^2	
	Sample identity	727	448 to 1,095
1st - 50th fastest	***Fixed effects***	**Posterior mean**	**95% Credible intervals**
	Intercept ($\alpha_{[mon]}$)	99.64	73.09 to 125.80
	Social status (difference $\alpha_{[pol]}$ vs. $\alpha_{[mon]}$)	17.54	-12.18 to 47.26
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[mon]}$)	19.04	-10.30 to 51.45
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[pol]}$)	1.49	-20.80 to 21.98
	Age	-4.53	-14.70 to 6.06
	Random effects	σ^2	

	Sample identity	1016	606 to 1,512
1st - 100th fastest	***Fixed effects***	**Posterior mean**	**95% Credible intervals**
	Intercept ($\alpha_{[mon]}$)	93.17	67.45 to 119.75
	Social status (difference $\alpha_{[pol]}$ vs. $\alpha_{[mon]}$)	7.72	-20.55 to 36.03
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[mon]}$)	15.95	-16.19 to 46.07
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[pol]}$)	8.23	-12.35 to 27.27
	Age	-3.90	-13.45 to 6.37
	Random effects	σ^2	
	Sample identity	951	548 to 1,368
Average speed	***Fixed effects***	**Posterior mean**	**95% Credible intervals**
	Intercept ($\alpha_{[mon]}$)	85.25	63.13 to 106.37
	Social status (difference $\alpha_{[pol]}$ vs. $\alpha_{[mon]}$)	4.45	-21.26 to 29.37
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[mon]}$)	12.17	-14.63 to 35.46
	Social status (difference $\beta_{[pol]}$ vs. $\alpha_{[pol]}$)	7.72	-12.27 to 22.70
	Age	-1.84	-8.96 to 7.55
	Random effects	σ^2	
	Sample identity	689	429 to 1,035

Table S2. Outputs from the six BLMMs assessing differences between sperm speed (VCL $\mu \times s^{-1}$) and individual reproductive fitness in dunnocks (number of chicks sired per male). We present regression coefficients with their 95% credible intervals. Significant differences are in bold.

1st - 5th fastest	***Fixed effects***	**Posterior mean**	**95% Credible intervals**
	Intercept	145.29	135.42 to 154.74
	No. total chicks	-0.72	-3.43 to 2.14
	Age (centred)	-1.77	-8.38 to 5.60
	Random effects	**σ^2**	
	Sample identity	526	304 to 754
1st - 10th fastest	***Fixed effects***	***Posterior mean***	***95% Credible intervals***
	Intercept	140.14	128.79 to 150.05
	No. total chicks	-0.95	-3.81 to 2.08
	Age (centred)	-2.53	-8.94 to 5.35
	Random effects	**σ^2**	
	Sample identity	614	347 to 909
1st - 20th fastest	***Fixed effects***	**Posterior mean**	**95% Credible intervals**
	Intercept	132.94	121.39 to 144.76
	No. total chicks	-0.98	-4.02 to 2.84
	Age (centred)	-3.98	-11.31 to 4.20
	Random effects	**σ^2**	
	Sample identity	758	473 to 1,144
1st - 50th fastest	***Fixed effects***	**Posterior mean**	**95% Credible intervals**
	Intercept	117.30	104.73 to 130.76
	No. total chicks	-0.75	-4.44 to 3.36
	Age (centred)	-6.115	-14.71 to 3.12
	Random effects	**σ^2**	
	Sample identity	1,031	617 to 1,465
1st - 100th fastest	***Fixed effects***	**Posterior mean**	**95% Credible intervals**
	Intercept	108.31	95.88 to 121.29
	No. total chicks	-1.81	-5.31 to 2.09
	Age (centred)	-5.69	-14.80 to 2.59
	Random effects	**σ^2**	
	Sample identity	948	598 to 1,393
Average speed	***Fixed effects***	**Posterior mean**	**95% Credible intervals**
	Intercept	96.75	86.40 to 107.85
	No. total chicks	-1.50	-4.75 to 1.53
	Age (centred)	-3.37	-11.12 to 4.64
	Random effects	**σ^2**	
	Sample identity	677	407 to 996

Table S3. Outputs from the six BLMMs assessing differences between sperm speed (VCL $\mu \times s^{-1}$) and individual reproductive fitness (number of chicks sired per male) but including the slopes of the three social statuses ($\alpha_{[mon]}$ = alpha monogamous, $\alpha_{[pol]}$ = alpha polyandrous, and $\beta_{[pol]}$ = beta polyandrous). We present regression coefficients with their 95% credible intervals.

	Fixed effects	**Posterior mean**	**95% Credible intervals**
1st - 5th fastest	Intercept	133.40	103.52 to 168.40
	No. total chicks	-1.98	-11.05 to 5.74
	Social status $\alpha_{[pol]}$	3.53	-34.67 to 37.42
	Social status $\beta_{[pol]}$	20.32	-12.90 to 54.62
	Age (centred)	0.02	-7-80 to 7.76
	Total chicks x Social status $\alpha_{[pol]}$	3.57	-6.19 to 12.32
	Total chicks x Social status $\beta_{[pol]}$	-0.54	-9.50 to 10.00
	Random effects	σ^2	
	Sample identity	503	286 to 754
	Fixed effects	**Posterior mean**	**95% Credible intervals**
1st - 10th fastest	Intercept	131.59	99.28 to 168.12
	No. total chicks	-3.57	-12.87 to 4.75
	Social status $\alpha_{[pol]}$	1.60	-36.39 to 40.61
	Social status $\beta_{[pol]}$	16.61	-19.27 to 55.54
	Age (centred)	-0.66	-8.18 to 6.93
	Total chicks x Social status $\alpha_{[pol]}$	4.87	-4.78 to 14.70
	Total chicks x Social status $\beta_{[pol]}$	0.95	-9.48 to 11.13
	Random effects	σ^2	
	Sample identity	592	327 to 869
	Fixed effects	**Posterior mean**	**95% Credible intervals**
1st - 20th fastest	Intercept	121.46	82.66 to 155.07
	No. total chicks	-3.07	-12-90 to 7.66
	Social status $\alpha_{[pol]}$	5.64	-34.43 to 49.09
	Social status $\beta_{[pol]}$	18.16	-22.39 to 57.63
	Age (centred)	-2.04	-10.99 to 8.39
	Total chicks x Social status $\alpha_{[pol]}$	4.20	-6.09 to 16.25
	Total chicks x Social status $\beta_{[pol]}$	0.50	-10.21 to 13.57
	Random effects	σ^2	
	Sample identity	758	456 to 1,141
	Fixed effects	**Posterior mean**	**95% Credible intervals**
1st - 50th fastest	Intercept	116.69	66.85 to 161.43
	No. total chicks	-5.28	-17.44 to 7.20
	Social status $\alpha_{[pol]}$	-2.13	-54.78 to 50.41
	Social status $\beta_{[pol]}$	5.29	-46.08 to 55.88
	Age (centred)	-5.02	-16.04 to 5.88
	Total chicks x Social status $\alpha_{[pol]}$	6.07	-6.47 to 19.28
	Total chicks x Social status $\beta_{[pol]}$	3.31	-13.27 to 16.61
	Random effects	σ^2	
	Sample identity	1084	610 to 1660

	Fixed effects	**Posterior mean**	**95% Credible intervals**
1st - 100th fastest	Intercept	110.41	66.19 to 153.29
	No. total chicks	-5.51	-17.21 to 5.16
	Social status $\alpha_{[pol]}$	-12.53	-62.08 to 35.83
	Social status $\beta_{[pol]}$	5.12	-38.88 to 55.19
	Age (centred)	-5.04	-15.51 to 4.35
	Total chicks x Social status $\alpha_{[pol]}$	6.62	-5.10 to 19.30
	Total chicks x Social status $\beta_{[pol]}$	1.69	-12.63 to 13.69
	Random effects	σ^2	
	Sample identity	985	585 to 1451
	Fixed effects	**Posterior mean**	**95% Credible intervals**
Average speed	Intercept	99.00	62.33 to 137.59
	No. total chicks	-4.16	-13.79 to 6.23
	Social status $\alpha_{[pol]}$	-8.84	-53.37 to 31.96
	Social status $\beta_{[pol]}$	1.96	-37.61 to 40.11
	Age (centred)	-2.60	-11.67 to 4.91
	Total chicks x Social status $\alpha_{[pol]}$	4.23	-6.70 to 15.69
	Total chicks x Social status $\beta_{[pol]}$	1.94	-10.09 to 13.78
	Random effects	σ^2	
	Sample identity	720	416 to 1075

Figure S1. The average difference in sperm speed VCL $\mu \times s^{-1}$ (red line) between $\alpha_{[monogamous]}$ and $\beta_{[polyandrous]}$ males and 95% credible intervals (green and blue lines). The grey area represents statistical significance (i.e., credible intervals do not overlap with zero).