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Sperm morphology and evidence for sperm competition among parrots

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Abstract

Sperm competition is an important component of post-copulatory sexual selection that has shaped the evolution of sperm morphology. Previous studies have reported that sperm competition has a concurrently directional and stabilizing effect on sperm size. For example, bird species that show higher levels of extrapair paternity and larger testes (proxies for the intensity of sperm competition) have longer sperm and lower coefficients of variation in sperm length, both within and between males. For this reason, these sperm traits have been proposed as indexes to estimate the level of sperm competition in species for which other measures are not available. The relationship between sperm competition and sperm morphology has been explored mostly for bird species that breed in temperate zones, with the main focus on passerine birds. We measured sperm morphology in 62 parrot species that breed mainly in the tropics and related variation in sperm length to life-history traits potentially indicative of the level of sperm competition. We showed that sperm length negatively correlated with the within-male coefficient of variation in sperm length and positively with testes mass. We also showed that sperm is longer in sexually dichromatic and in gregarious species. Our results support the general validity of the hypothesis that sperm competition drives variation in sperm morphology. Our analyses suggest that post-copulatory sexual selection is also important in tropical species, with more intense sperm competition among sexually dichromatic species and among species that breed at higher densities.

KEYWORDS
parrots, post-copulatory sexual selection, sperm competition, sperm morphology

1 | INTRODUCTION

When females copulate promiscuously, a competition arises among the sperm of different males to fertilize the same egg (Parker, 1970). This contest is a form of post-copulatory sexual selection referred to as sperm competition. Sperm competition plays an important role in the evolution of sperm morphology, having both a directional and a stabilizing effect on sperm length (Briskie, Montgomerie, & Birkhead, 1997; Calhim, Immler, & Birkhead, 2007; Immler, Calhim, & Birkhead, 2008; Kleven et al., 2009; Kleven, Laskemoen, Fossøy, Robertson, & Lifjeld, 2008; Lifjeld, Laskemoen, Kleven, Albrecht, & Robertson, 2010; Lüpold, Linz, Rivers, Westneat, & Birkhead, 2009). The directional effect has been shown in fish (Balshine, Leach, Neat, Werner, & Montgomerie, 2001), mammals (Gomendio & Roldan,

1991), insects (Morrow & Gage, 2000), anurans (Byrne, Simmons, & Roberts, 2003; Liao et al., 2018), reptiles (Tourmente, Gomendio, Roldan, Giojalas, & Chiaraviglio, 2009) and birds (Kleven et al., 2009; Lifjeld et al., 2010; Lüpold, Calhim, Immler, & Birkhead, 2009), whereby species exposed to higher sperm competition levels tend to have longer sperm. The stabilizing effect on sperm length, that is reduced variation in sperm length—both at the within- and between-male levels—with increasing levels of sperm competition, has been shown in passerine birds (Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010).

Different hypotheses have been proposed to explain selection for longer sperm in birds. First, the positive relationship between sperm length and speed (Briskie & Montgomerie, 1992; Lüpold, Calhim, et al., 2009) suggests that longer sperm might have evolved as a consequence of selection on speed, as longer sperm will then outcompete shorter sperm in the race to the ova. Second, the positive relationship between sperm length and the length of the sperm storage tubules (SSTs) in the females' utero-vaginal junction suggests that longer sperm might have evolved to fill the space within the SSTs (Briskie & Montgomerie, 1992; Briskie et al., 1997).

Regarding the stabilizing selection, species with higher levels of sperm competition show reduced variation in sperm length, both within and between males (Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010). This suggests that there is an "optimal" sperm morphology, probably achieved by selection that reduces errors during sperm production (Calhim et al., 2007; Hunter & Birkhead, 2002). It has been shown that sperm traits are highly heritable (Birkhead, Pellatt, Brekke, Yeates, & Castillo-Juarez, 2005) and less condition dependent (Birkhead & Fletcher, 1995; Birkhead, Fletcher, & Pellatt, 1999; but see Immler, Pryke, Birkhead, & Griffith, 2010; Lüpold, Birkhead, & Westneat, 2012). These sperm properties could explain the reduction of variation in sperm length under higher post-copulatory sexual selection (Immler et al., 2008).

Given the compelling evidence that sperm competition acts concurrently on sperm morphology in a directional and stabilizing manner, recent studies have used mean sperm length and the coefficients of variation of sperm length, both within and between males, as proxies of the intensity of sperm competition (Omotoriogun, Laskemoen, et al., 2016; Sardell & DuVal, 2014). These studies have been taxonomically restricted to passerine birds (Albrecht et al., 2012; Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010; Lüpold Linz & Birkhead, 2009; Lüpold, Linz, Rivers, et al., 2009; Omotoriogun, Albrecht, et al., 2016; Omotoriogun, Laskemoen, et al., 2016; Sardell & DuVal, 2014), with the exception of one study on shorebirds (Johnson & Briskie, 1999) and one on pheasants (Immler et al., 2007), though the latter found no effect of sperm competition on sperm morphology. Most studies focused on temperate zone species (Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010; but see Albrecht et al., 2012; Omotoriogun, Albrecht, et al., 2016; Omotoriogun, Laskemoen, et al., 2016). The temperate zone bias might be due to the general assumption of low levels of sperm competition in tropical birds (Stutchbury & Morton, 1995), possibly

associated with the different life-history traits between the species of these two regions (Ricklefs & Wikelski, 2002). For example, species with a shorter lifespan might tolerate higher levels of extrapair paternity (EPP, a proxy of the intensity of sperm competition) because they have fewer breeding opportunities throughout their lives (Arnold & Owens, 2002; Mauck, Marschall, & Parker, 1999). Accordingly, species that have high rates of adult mortality tend to show higher levels of EPP (Arnold & Owens, 2002). Tropical species are characterized by long lifespans (Ricklefs & Wikelski, 2002); hence, lower levels of sperm competition are predicted. However, empirical support for this theoretical prediction is limited (Macedo, Karubian, & Webst, 2008).

In a comparative analysis that included 99 passerine species from the temperate zone and 31 from the tropical zone, no difference was found in indicators of the level of sperm competition between these two groups of birds (Albrecht et al., 2012). For this reason and given that most birds live and breed in the tropics (Gaston, 2000; Hawkins, Porter, & Diniz-filho, 2003; Valcu, Dale, & Kempenaers, 2012), it is important to explore variation in the level of sperm competition, directly or through its proxies, in nonpasserine tropical species to be able to formulate general rules of how sexual selection operates among birds.

We explored variation in sperm morphology in 62 parrot species (~15% of all Psittaciformes; 30 genera, five families), breeding mainly in the tropics. The general aim of our study was to investigate whether findings from passerine birds can be generalized. Specifically, we tested whether mean sperm length and the within-male and between-male coefficients of variation in sperm length correlated with each other and with other known indicators of the intensity of sperm competition. Parrots are long-lived animals: the average lifespan is 26 years and ranges from 8.5 to 100 years (Wasser & Sherman, 2010). This group of birds is also characterized by social monogamy with lifelong pair bonds, even though there are exceptions (Toft & Wright, 2015). These traits suggest a low intensity of sexual selection, but few studies have used genetic markers to confirm genetic monogamy (Eastwood et al., 2018; Masello, Sramkova, Quillfeldt, Epplen, & Lubjuhn, 2002). On the other hand, Bublat et al. (2017) showed that socially monogamous macaws (species from the genera *Ara*, *Diopsittaca* and *Primolius*) had much lower sperm density compared with polygynandrous Eclectus parrots (*Eclectus roratus*), which might be an adaptation to intense sperm competition in the latter. Despite these life-history traits, parrots show striking coloration and up to 30% of the species are sexually dichromatic (estimated from del Hoyo, Elliott, Sargatal, Christie, & Kirwan, 2017). Parrots also exhibit high levels of cognitive capacities (Van Horik, Clayton, & Emery, 2012; Lambert, Jacobs, Osvath, & von Bayern, 2019) and problem-solving skills (Auersperg, von Bayern, Gajdon, Huber, & Kacelnik, 2011; Auersperg, Kacelnik, & von Bayern, 2013; O'Neill, Picaud, Maehner, Gahr, & von Bayern, 2019), and females may choose males based on these skills (Chen, Zou, Sun, & Cate, 2019). Hence, in parrots, ornamental colours and high cognitive abilities might be consequences of sexual selection. Studying indicators of

sperm competition intensity will allow us to explore variation in the genetic mating system of parrots and to understand the effect of post-copulatory sexual selection in this group.

Using a comparative approach, we tested whether sperm measures (CV and sperm length) were predicted by (a) relative testes mass, a proximate indicator of the intensity of sperm competition (Møller & Briskie, 1995; Pitcher, Dunn, & Whittingham, 2005), (b) sexual size dimorphism and dichromatism, traits considered to be sexually selected (Berry & Shine, 1980; Dale, Dey, Delhey, Kempenaers, & Valcu, 2015; Darwin, 1871; Owens & Hartley, 1998), and (c) gregariousness (proximity to other breeding pairs), a trait facilitating sexual selection (Shuster & Wade, 2003).

2 | MATERIAL AND METHODS

2.1 | Collection of sperm samples

We explored the variation in sperm morphology within and between males in 62 parrot species belonging to 30 genera and five families. In their natural habitat, these species primarily breed in the tropics, some of them extend their breeding range into the subtropical zone and a few into the temperate zone of the southern hemisphere (del Hoyo et al., 2017). We collected one sperm sample per male (total N = 138) from birds that were born in captivity and held in the breeding facility of the Loro Parque Fundación (LPF), Tenerife, Spain. Samples were collected between June 2012 and June 2013, and between April and May 2018. In February 2019, sperm samples from two Kākāpō (*Strigops habroptila*) were collected on Codfish Island, New Zealand, in collaboration with the Kākāpō Recovery Team. To collect the samples, we used the electro-stimulation technique (Lierz, Reinschmidt, Müller, Wink, & Neumann, 2013) with three probe sizes (length × diameter [mm]: 25 × 3, 35 × 4 and 50 × 5), depending on the size of the bird sampled. The electric current and the number of electric impulses were adapted to each species, as described by Bublat et al. (2017). Kākāpō samples were collected using this technique or by cloacal massage. Sperm samples were taken directly from the cloaca using scaled glass capillaries (Wiretrol II, 1–5 µl; Drummond Scientific Company). From the samples collected in 2012–2013 and the Kākāpō samples (the latter were previously diluted, one with NaCl and the other with the semen extender Blanco, Schneider et al., 2017), smears were made onto microscope slides, stained with Eosin B2% and covered with a mounting medium (Entellan New, 107961; Merck KGaA), whereas the ones from 2018 were fixed in 50–100 µl of 5% formalin solution. From the samples fixed in formalin, we pipetted a 10 µl aliquot onto a microscope slide, spread it with the side of the pipette tip and allowed it to air-dry. The different methods used did not have an effect on the sperm measurements. We inspected all samples at 200x magnification using a Zeiss Axio Imager.M2 microscope with bright field optics and took between 4 and 25 photographs per slide at 400× magnification with an Axiocam 506 colour camera.

2.2 | Sperm morphometry

We measured sperm morphometry (head and flagellum length) from the photographs using the software ZEN 2, blue edition (Carl Zeiss Microscopy GmbH), including only normal-looking spermatozoa (total N = 1,996). To minimize observer error, all measurements were taken by one person (K.T.). We calculated total sperm length as the sum of the two measurements. We did not measure mid-piece length separately, because in most samples it could not easily be distinguished. When the mid-piece was visible, we included it in the measure of flagellum length, assuming that the nonvisible mid-pieces would most likely blend into the tail. The average number of spermatozoa measured per male and species was 14.9 (range: 3–62). The repeatability of sperm measurements per male was 0.261 (95% CI: 0.17–0.36) and 0.419 (95% CI: 0.29–0.55) per species; these were obtained through 1,000 parametric bootstrap iterations (Stoffel, Nakagawa, & Schielzeth, 2017). We calculated coefficients of variation (CV = [SD/mean]) both within and between males, and adjusted them to correct for variation in sample size (CV_{adj} = [1 + 1/(4n)]*CV; Sokal & Rohlf, 1981). The adjusted coefficient of variation within males was denoted as CV_{wm} and for between males as CV_{bm}.

We collected sperm samples from one to twelve males of each species (median per species = 2). For six species (the Yellow-crowned amazon *Amazona ochrocephala*, the Yellow-headed amazon *Amazona oratrix*, the Sulphur-crested cockatoo *Cacatua galerita*, the Yellow-crested cockatoo *Cacatua sulphurea*, the Eclectus parrot and the Red-breasted parakeet *Psittacula alexandri*), we obtained samples from two or more subspecies, but these were averaged to obtain species-specific values for the analyses. We used the CV_{wm} of each individual to calculate a mean CV_{wm} for each species. To calculate CV_{bm}, we used the mean and standard deviation (SD) of sperm length for each of the males of a given species. Given the small number of males sampled per species (often only one, median 2), we only use CV_{wm} for further analyses. However, we note that even with the limited sampling, the total sperm CV_{wm} correlated positively with the CV_{bm} (Pearson's r = 0.496, N = 29 species).

Because our sperm samples came from males bred in captivity, we expect higher levels of inbreeding compared with males from wild populations. Studies exploring the effect of inbreeding on sperm characteristics of mammals and birds have shown that the proportion of abnormal sperm is higher, and sperm velocity lower in inbred compared with outbred males (Gomendio, Cassinello, & Roldan, 2000; Heber et al., 2012; Opatová et al., 2016). However, there is no evidence for inbreeding depression on the morphology (e.g., length, CV) of normal-looking sperm of fish, fruit flies and birds (Ala-Honkola et al., 2013; Mehlis, Frommen, Rahn, & Bakker, 2012; Opatová et al., 2016). Specifically, in zebra finches (*Taeniopygia guttata*) inbreeding depression seems to have no more than a modest effect on the length (Cohen's d = −0.55) and a small effect on the CV (d = 0.24) of normal-looking sperm (Opatová et al., 2016). We therefore assume that our results reflect the variation in sperm morphology observed in wild parrots.

2.3 | Explanatory variables: Data collection and analysis

We considered six explanatory variables potentially explaining variation in sperm morphology. These predictor variables were collected before the sperm morphology data were collected. At the time of performing the measurements, the person measuring the sperm was unaware of the predictor variables.

2.3.1 | Testes mass

Testes mass has been used as an indicator of the intensity of sperm competition, because species with higher levels of EPP show relatively larger testes (Møller & Briskie, 1995). Data on testes mass was obtained from the literature (Calhim & Birkhead, 2007; Krishnaprasadan, Kotak, Sharp, Schmedemann, & Haase, 1988; Wilkinson & Birkhead, 1995). We only found data for 10 of the 62 species studied here. For analysis, we log10-transformed this variable to improve normality. We added body mass in all the analyses that included testes mass to control for a possible allometric effect, as it has been reported that testes mass relates to body mass in birds and other taxa (Birkhead, 1998; Morrow & Gage, 2000).

2.3.2 | Body size

We measured wing, tarsus and tail length for an average of 4.8 (range: 1–22) females and 5.7 (range: 1–23) males of each species. Individuals could only be measured during a yearly veterinarian health check and due to time constraints some of the measurements could not be taken. In these cases, measurements were taken from the book *Parrots of the World* (Forshaw, 1978) (see online data repository). Measurements for the Kākāpō were also taken from this source, as this species is not present in the LPF collection. We estimated body size for males and females, using the first principal component (PC1) from a principal component analysis (PCA) that included the three measurements for both sexes. PC1 explained 65% of the variation in the data.

2.3.3 | Clutch size

We compiled data on clutch size from the records of the LPF from the 2012 to 2015 breeding seasons. Based on these data (1–105 clutches per species, mean: 16.4), we calculated average clutch size per species. Clutch size records were missing for 13 species (see online data repository). In those cases, we used data on clutch size from the *Handbook of the Birds of the World Alive* (HBW Alive, del Hoyo et al., 2017).

2.3.4 | Sexual size dimorphism

Sexual size dimorphism (SSD) is an indicator of the intensity of sexual selection in birds (Owens & Hartley, 1998; Szekely, Lislevand, & Figuerola, 2007). We calculated SSD as $PC1_{male\ body\ size} - PC1_{female\ body\ size}$. Hence, positive values reflected species where males are larger than females.

2.3.5 | Sexual dichromatism

Sexual selection is considered to be one of the most important factors causing sexual dichromatism in birds (Dale et al., 2015). A comparative study exploring the mechanisms behind sexual dimorphism in body size and plumage colouration among passerines has shown that sexual dichromatism is associated with the frequency of EPP (Owens & Hartley, 1998). Thus, we consider sexual dichromatism as an indicator of the intensity of sexual selection in birds (Badyaev & Hill, 2003; Dale et al., 2015). We scored dichromatism as present ("yes") or absent ("no") according to (a) visual inspection of the species' colour plates and (b) information from the section "descriptive notes" in the HBW Alive (del Hoyo et al., 2017). We defined a species as dichromatic if plumage colour of any body part differed between the sexes (e.g., male and female show different colours, or the same colour but different tones). We did not classify a species as dichromatic if the colour of a patch was the same, but the patch differed in size.

2.3.6 | Gregariousness

Opportunities for extrapair mating may be higher when species nest closer together. Hence, we scored gregariousness as "yes" or "no" based on information from the "breeding" section of the HBW Alive (del Hoyo et al., 2017). We scored a "yes" for gregariousness if the description suggests that breeding pairs nest in close proximity (i.e., several pairs occupying adjacent trees, two or three nests per tree, nests with multiple breeding pairs) or if the species is described as colonial. The Kākāpō was excluded from analyses that consider gregariousness as a predictor, because this is the only lekking species among parrots.

We reported all our measurements, the conditions in which they were collected, the sample size for each variable and the reason we excluded data from our analyses.

2.4 | Phylogeny

We used a recent phylogeny of 307 parrot species produced from a 30-gene supermatrix (Provost, Joseph, & Smith, 2017). Only one of the species we studied here, the Superb parrot (*Polytelis swainsonii*), was not included in this phylogeny. We added the Superb parrot to the phylogeny using the function pinTips in the package "TREEMAN" (Bennett, Sutton, & Turvey, 2017). This function finds the branch of the phylogenetic tree common for all *Polytelis* species and adds the missing taxon at a random position within this branch.

2.5 | Statistical analysis

All the statistical analyses were performed in R 3.5.2 (R Development Core Team, 2018). All data and code are available in the online repository https://osf.io/v23bw/.

We first tested whether mean sperm length was correlated with CV_{wm} using a linear model. As these variables were negatively correlated (Figure 1), we investigated simultaneous effects of the explanatory variables on both mean sperm length and CV_{wm}. To test our hypotheses, we ran a multivariate analysis of variance (MANOVA) using these two variables as response variables and including phylogeny, testes mass, male body size (PC1), clutch size, SSD, sexual dichromatism and gregariousness as predictors. To control for phylogeny, we used phylogenetic eigenvectors (Diniz-Filho, de Sant'Ana, & Bini, 1998) calculated using the package "ADEPHYLO" v. 1.1-11 (Jombart, Dray, & Bilgrau, 2016). These phylogenetic eigenvectors are equivalent to the PC axes obtained in a PCA (Swenson, 2014). Hence, the eigenvectors that describe the phylogenetic relationship between the species considered in this study were kept in subsequent analyses. We selected these eigenvectors based on the MANOVA analysis (Desdevises, Legendre, Azouzi, & Morand, 2003).

To identify the direction and magnitude of the relationships, we ran univariate phylogenetically informed linear models separately for mean sperm length and CV_{wm} and included each of the significant predictors identified by the MANOVA analysis.

To assess the combined effect of the significant predictors identified, we ran phylogenetically informed linear models, with multiple predictors, separately for mean sperm length and CV_{wm}. We included all significant predictors as explanatory variables, except testes mass, because the sample size was too small (N = 10 species).

Finally, to explore the difference in mean sperm length and CV_{wm} among different taxonomic groups (Psittaciformes, Passeriformes and Charadriiformes), we conducted post hoc comparisons using the package "MULTCOMP" (Hothorn, Bretz, & Westfall, 2008).
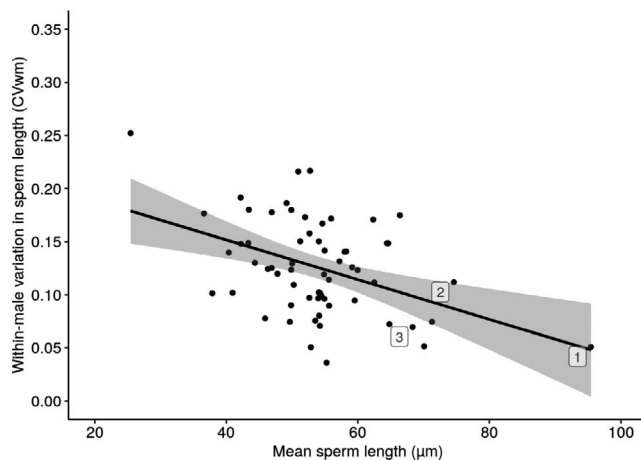
3 | RESULTS

Total sperm length varied from 25.45 μm in the Southern Festive amazon (*Amazona festiva*) to 95.43 μm in the Vasa parrot (*Coracopsis vasa*). Flagellum length ranged from 15.41 to 65.64 μm and head length from 7.9 to 29.79 μm (Figure S1). To explore the variation in the two components of sperm length, we calculated CV_{wm} for head and CV_{wm} for flagellum length separately. The CV_{wm} for head length (mean = 0.212 μm; range = 0.078–0.473 μm) was significantly larger than the CV_{wm} for flagellum length (mean = 0.152 μm; range = 0.053–0.522 μm; paired sample *t* test: estimate = 0.059 ± 0.012, t_{61} = 5.01, *p* < 0.001).

The MANOVA analysis showed a strong phylogenetic signal and a significant effect of sexual dichromatism, gregariousness and relative testes mass on both mean sperm length and CV_{wm} (Table 1). Given that only the first eigenvector explained the phylogenetic relationship between the species studied here, we included only this first eigenvector in all further models to control for phylogeny. We performed another MANOVA analysis based on those species for which we measured a minimum of 10 sperm (N = 50), as it has been shown that this sample size provides a representative estimate of the mean and variance of the sperm length (Kleven et al., 2008; Laskemoen, Kleven, Fossøy, & Lifjeld, 2007). The results of this analysis are qualitatively similar (Table S1). Therefore, we kept the complete data for the next analyses to include a larger sample size (N = 62).

Univariate, phylogenetically informed models (Table 2) showed that sperm were longer in sexually dichromatic and in gregarious species (Figure 2) and that sperm length increased with increasing relative testes mass (Figure 3a). There was a significant negative correlation between CV_{wm} and relative testes mass (Figure 3b).

FIGURE 1 Relationship between mean sperm length and the within-male coefficient of variation in sperm length (CV_{wm}) for 62 parrot species (Pearson's *r* = −0.43, *p* < 0.001; no control for phylogeny). Plotted points represent the mean values per species. The line and the 95% CI (grey) are based on a linear model. The only two parrot species described as polygynandrous (1: Vasa parrot; 2: Eclectus parrot) and the one species described as lekking (3: Kākāpō) are highlighted

TABLE 1 Results of a MANOVA analysing the effects of various predictors on both mean sperm length and the within-male coefficient of variation in sperm length (CV_{wm})

Predictors	V^b	Statistic	*p*
Phylogeny			
Eigenvector 1[a]	0.122	$F_{2,57}$ = 3.97	**0.024**
Eigenvector 2[a]	0.046	$F_{2,57}$ = 1.36	0.26
Eigenvector 3[a]	0.0006	$F_{2,57}$ = 0.017	0.98
Clutch size	0.003	$F_{2,59}$ = 0.095	0.91
Body size (PC1 male)	0.044	$F_{2,59}$ = 1.35	0.27
Sexual size dimorphism	0.017	$F_{2,59}$ = 0.495	0.61
Sexual dichromatism	0.139	$F_{2,59}$ = 4.75	**0.012**
Gregariousness	0.238	$F_{2,58}$ = 9.08	**<0.001**
Body mass (male)	0.285	$F_{2,6}$ = 1.2	0.37
log(testes mass)	0.794	$F_{2,6}$ = 11.6	**0.009**

[a]Phylogenetic eigenvectors (see Methods2 for details).
[b]Pillai's trace statistic; ranges from 0 to 1. Bold p values are statistically significant.

TABLE 2 Univariate linear models analysing the relationship between various predictors and mean sperm length and the within-male coefficient of variation in sperm length (CV$_{wm}$) separately

Response variable	Predictors	Estimate	SE	Statistic	p
Mean sperm length	(Intercept)	51.7	1.39		
	Phylogeny[a]	−2.7	1.19	$t_{59} = -2.27$	**0.027**
	Sexual dichromatism[b]	7.66	2.67	$t_{59} = 2.87$	**0.006**
Mean sperm length	(Intercept)	51.6	1.2		
	Phylogeny[a]	−2.7	1.1	$t_{58} = -2.45$	**0.017**
	Gregariousness[b]	13.0	3.13	$t_{58} = 4.17$	**<0.001**
Mean sperm length	(Intercept)	72.7	5.88		
	Phylogeny[a]	−3.86	19.4	$t_6 = -0.199$	0.85
	Body mass	−0.013	0.013	$t_6 = -1.04$	0.34
	log(Testes mass)	11.1	3.63	$t_6 = 3.06$	**0.022**
CV$_{wm}$	(Intercept)	0.129	0.007		
	Phylogeny[a]	0.011	0.006	$t_{59} = 1.95$	0.056
	Sexual dichromatism[b]	−0.012	0.013	$t_{59} = -0.96$	0.34
CV$_{wm}$	(Intercept)	0.129	0.006		
	Phylogeny[a]	0.011	0.006	$t_{58} = 1.99$	0.051
	Gregariousness[b]	−0.015	0.016	$t_{58} = -0.934$	0.35
CV$_{wm}$	(Intercept)	0.12	0.022		
	Phylogeny[a]	0.148	0.074	$t_6 = 2.0$	0.092
	Body mass	2.84×10^{-5}	4.78×10^{-5}	$t_6 = 0.594$	0.57
	log(Testes mass)	−0.035	0.014	$t_6 = -2.54$	**0.044**

[a]Eigenvector 1 (see Methods2 for details).
[b]1, "no"; 2, "yes. Bold p values are statistically significant.

A phylogenetically informed model that included all significant predictors showed that only gregariousness remained as a significant predictor of mean sperm length (estimate = 10.6 ± 3.48, t_{57} = 3.06, p = 0.003, see Table S2). Sexual dichromatism was no longer significant, probably because the two variables are related (Fisher's exact test: p = 0.001; seven out of nine gregarious species in our data set are also sexually dichromatic). Another model that included all the predictors measured in this study—not only the predictors identified in the MANOVA analysis—also showed gregariousness to be the only significant predictor of sperm length (Table S3).

A combined univariate analysis also showed a significant interaction between sexual dichromatism and gregariousness on mean sperm length (estimate = 16.0 ± 7.26, t_{56} = 2.21, p = 0.031; Table S4), with the longest sperm in those species that are both gregarious and sexually dichromatic. The sperm length of the species that are both sexual dichromatic and gregarious is ~33% longer than of the other species, and this difference was significant (estimate = 16.6 ± 3.35, t_{59} = 4.97, p < 0.001).

Parrots showed significantly smaller sperm compared to passerines (post hoc comparison; estimate = −90.46 ± 8.90, t_{58} = −10.17, p < 0.001), but not compared to shorebirds (post hoc comparison; estimate = −21.36 ± 13.47, t_{58} = −1.59, p = 0.21; Figure 4a). However, as the shorebirds study only included 16 species, the power of that

test is limited. Parrots also exhibited significantly larger variation in sperm length (within-male, CV$_{wm}$) compared to temperate zone passerines (Figure 4b, estimate = 0.108 ± 0.006, t_{59} = 17.57, p < 0.0001).

4 | DISCUSSION

We studied sperm morphology in 62 parrot species and found that mean sperm length and the within-male coefficient of variation in sperm length (CV$_{wm}$) were negatively correlated, as expected under the hypothesis that higher levels of sperm competition lead to both longer sperm and sperm that are less variable. Both measures were related to relative testes mass, another proxy of the intensity of sperm competition, though the sample size for this analysis was smaller (N = 10 species). We also found that on average, sperm were longer in sexually dichromatic and in gregarious species.

The significant relationship between relative testes mass and both mean sperm length and CV$_{wm}$ corresponds with previous findings in passerines (Immler et al., 2008). Within passerine species, it has been shown that testes mass is associated with the level of sperm competition, as species with higher levels of extrapair paternity have larger testes (Lüpold, Linz, Rivers, et al., 2009; Møller & Briskie, 1995). It has also been reported that CV$_{wm}$ is negatively related to the frequency of EPP (Kleven et al., 2008; Lifjeld et al.,
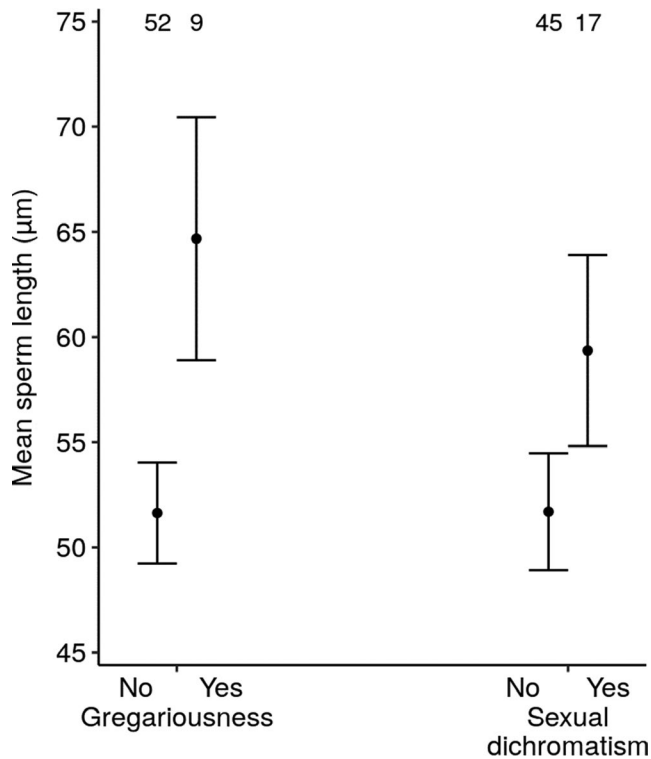
FIGURE 2 Mean total sperm length in relation to gregariousness and sexual dichromatism for 61 and 62 parrot species, respectively. Shown are estimates (dots) and their 95% CI (error bars) from the univariate models shown in Table 2. Numbers above the *X*-axis show sample sizes (number of species in each group)

FIGURE 3 Relationship between residual testes mass ($\log_{10}$-transformed) and (a) mean total sperm length and (b) the within-male coefficient of variation in sperm length (CV_{wm}) for the 10 parrot species for which testes mass data were available in the literature. The line and 95% CI (grey) are based on the model shown in Table 2. The only two parrot species described as polygynandrous (1: Vasa parrot; 2: Eclectus parrot) are highlighted

2010), which is clear evidence for the role of sperm competition in determining sperm morphology.

Our results also suggest that sexual dichromatism in parrots is associated with increased sperm competition, because dichromatic species had significantly longer sperm. Previous studies have suggested that sexual selection is the main driver of sexual dichromatism (Badyaev & Hill, 2003; Dale et al., 2015), and comparative analyses have shown that sexual dichromatism in birds is related to the level of extrapair paternity (Møller & Birkhead, 1994; Owens & Hartley, 1998). One possible scenario to explain this pattern is that sexual selection via sperm competition is the evolutionary force that has driven sexual dichromatism in parrots as well. This is supported by semen parameters, showing that the highly sexually dichromatic Eclectus parrot also has the highest semen density and total amount of sperm per ejaculate compared with other parrot species (Bublat et al., 2017).

Gregarious parrot species also had longer sperm, suggesting that species that breed in groups also experience higher levels of sperm competition. Breeding under higher local densities may increase opportunities to engage in mating outside the pair bond and reduce the costs of seeking extrapair copulations. Indeed, extrapair copulations seem to be more common among colonial breeders (Møller & Birkhead, 1993). Our finding also supports previous work showing that species that breed at high densities have larger testes (Pitcher et al., 2005). The significant correlation between sexual dichromatism
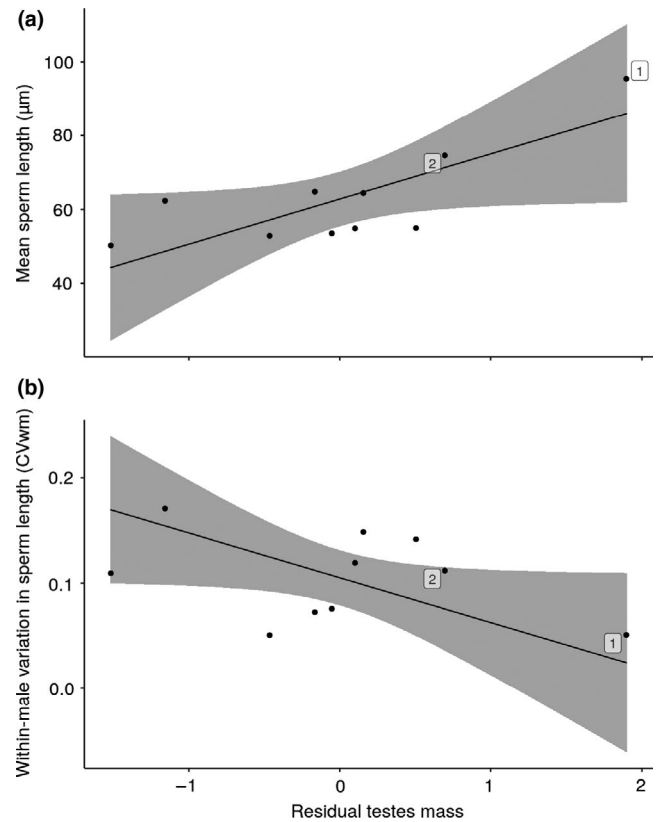
and gregariousness, at least in the parrot species under study, further suggests that sexual ornamentation in parrots might have evolved as a consequence of sexual selection which is stronger in gregarious species. However, this does not imply a direct causal link between sexual dichromatism and sperm length. We hypothesize an evolutionary scenario where gregariousness might have driven both sexual dichromatism and increased levels of EPP, and the latter might then have driven the evolution of ejaculate traits, such as longer sperm. Our findings simply suggest that sperm competition is higher among sexually dichromatic and gregarious species, and also that sexually dichromatic species are gregarious. Further work is needed to investigate potential causal links.

The two species from our data set with the longest sperm were the Vasa parrot and the Eclectus parrot (see online data repository). These species are polygynandrous (Ekstrom, Burke, Randrianaina, & Birkhead, 2007; Heinsohn, Legge, & Endler, 2005), a mating system that is typically associated with a high level of sperm competition (Pitcher et al., 2005). The sperm measurements reported here support this view. Both of these species are also sexually dichromatic—with the Vasa parrot showing sexual differences only during the breeding season—and the Vasa parrot exhibits a unique penis-like

FIGURE 4 (a) Mean sperm length for 62 parrot species (Psittaciformes), 16 shorebird species (Charadriiformes) and 55 passerine species (Passeriformes); scale on Y-axis is $\log_{10}$ transformed. (b) Within-male coefficient of variation in total sperm length (CV_{wm}) only for the parrot and passerine species; scale on Y-axis is $\log_{10}$ transformed. (c) Relationship between mean sperm length and the within-male coefficient of variation for the parrot and passerine species; the lines and 95% CI (grey) are based on a linear model without controlling for phylogeny; scale on Y-axis and X-axis is $\log_{10}$ transformed. The data for passerines are from Lifjeld et al., 2010 and those for shorebirds from Johnson & Briskie, 1999
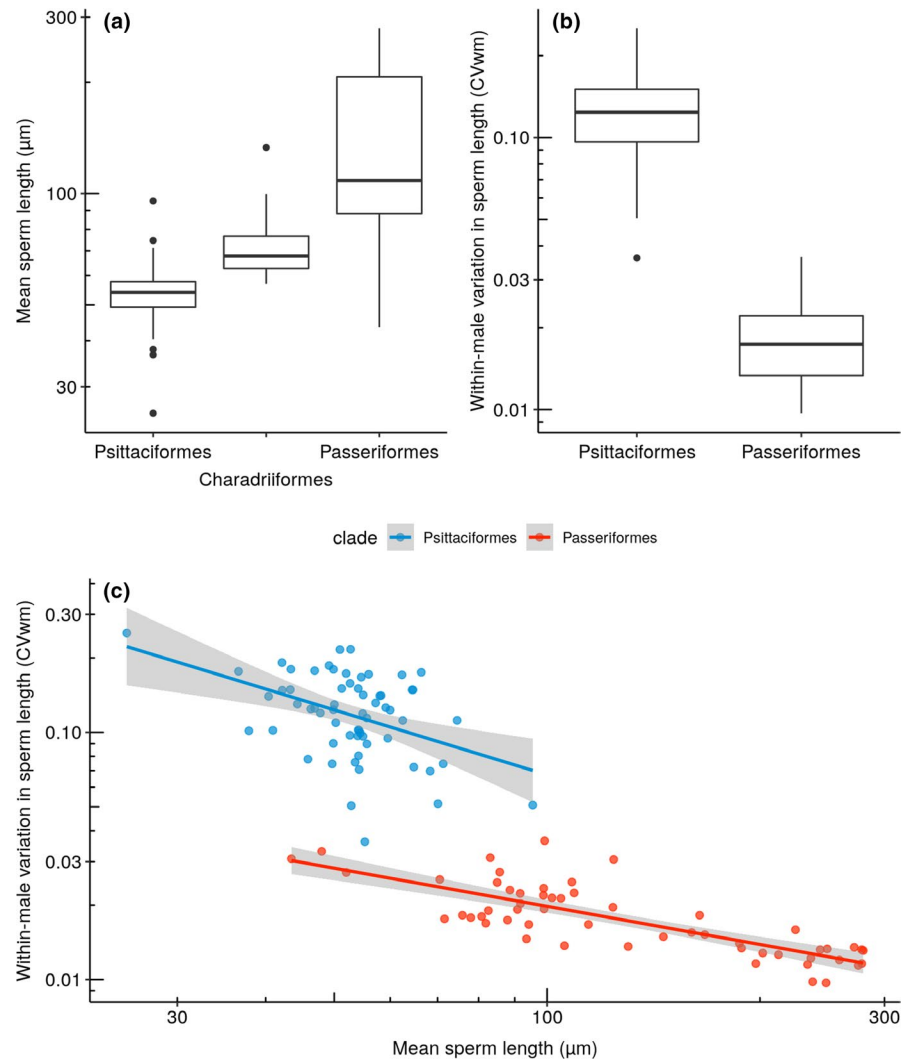
cloacal protrusion, which allows males to interlock their cloaca with the female's to prolong copulations (Wilkinson & Birkhead, 1995) and reflects the high level of sperm competition occurring in this species.

Besides the samples of the only two polygynandrous parrot species, we also obtained sperm measurements for the Kākāpō, the only lekking (and flightless) parrot species. The species is neither sexually dichromatic nor gregarious; hence, our general findings cannot explain their relatively long sperm (68.33 μm, the fifth longest, see online repository). However, it has been reported that Kākāpō females mate up to three times with the same or different males (Eason et al., 2006). Hence, sperm competition might still be high in this species and could thus be the evolutionary force that led to their relatively long sperm.

The effect of sperm competition on sperm morphology has been mainly explored for passerine species (Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Lifjeld et al., 2010). We now provide evidence suggesting that sperm competition has also shaped the morphology of parrot sperm. It is thus important to compare the variation in sperm morphology between these two taxonomic groups.

Mean sperm length in our data set of 62 parrot species ranged from 25.45 to 95.43 μm (a 3.8-fold difference between the shortest and longest). A study on variation in sperm size for 196 passerine species (Immler et al., 2011) reported that mean sperm length ranged from 41.8 to 284.8 μm (6.8-fold difference). Another study focusing on 12 Afrotropical sunbird species (Omotoriogun, Laskemoen, et al., 2016) reported mean sperm length ranging from 74.1 to 115.6 μm (1.6-fold difference), whereas a study on shorebird species (Johnson & Briskie, 1999) reported mean sperm length ranging from 57 to 133.2 μm (2.3-fold difference). Parrot mean sperm length thus overlaps with that of species from other taxonomic groups. Although our results show that parrots have significantly smaller sperm compared with passerines, the extent of the variation within parrots is similar to what has been found in other groups.

In agreement with the shorter sperm length, parrots also exhibited significantly larger variation in sperm length (within-male, CV_{wm}) compared with temperate zone passerines. Even though the relationship between sperm length and CV_{wm} is negative for passerines and for parrots (Figure 4c, Table S5), the magnitude of the effect is not the same for both groups (Table S6). Where sperm length

overlaps between passerines and parrots (~40–100 μm), the CV_{wm} is much lower for passerines (Figure 4c). The lower CV_{wm} for a given sperm length, together with the generally longer sperm in passerines, suggests that the level of sperm competition is lower in parrots compared with passerines. Nevertheless, the significant negative relationship between CV_{wm} and mean sperm length, together with the correlation of these two variables with testes mass, indicates that post-copulatory sexual selection is driving variation in parrot sperm morphology as well.

We note that the negative relationship between mean sperm length and CV_{wm} could be a simple consequence of sexual selection acting on sperm length only. Indeed, if the variance does not change along with the mean, then the coefficient of variation will decrease solely due to an increase in sperm length. However, if this negative relationship was simply a statistical artefact, we would expect a similar relationship (similar slope and intercept) in passerines and in parrots, but this was clearly not the case (Table S6). Thus, the most parsimonious explanation is that post-copulatory sexual selection has both a directional and a stabilizing effect on parrot sperm length, given that the relationship between sperm length and CV_{wm} is negative for passerines and parrots (Figure 4c, Table S5 and S6; Calhim et al., 2007; Kleven et al., 2008; Lifjeld et al., 2010; Lüpold, Linz, & Birkhead, 2009).

Our results also suggest that there is stronger post-copulatory sexual selection on sperm flagellum length than on sperm head length, as the CV_{wm} of flagellum length is lower than that of head length. However, it is important to consider that when visible, we included the mid-piece in the flagellum measurements as we assumed that when the mid-piece was not visible, it would most likely be blended into the tail. If this is not the case, and the mid-piece was actually included in the head measurements, then this result would be incorrect. However, as this finding goes in agreement with what has been found in passerine species (Briskie & Montgomerie, 1992; Lüpold, Linz, & Birkhead, 2009; Omotoriogun, Laskemoen, et al., 2016; Rowe et al., 2015), we consider that sexual selection might be acting mostly on flagellum length and less on head length within parrots. The sperm head contains the acrosome and nucleus (Jamieson, 2006), two components important for the sperm–egg interaction (Rowe et al., 2015). Any alteration in the sperm head could affect the sperm function during fertilization. Hence, head length may be under stabilizing selection, whereas the flagellum seems to be the target of directional, sexual selection. An increase in flagellum length would increase sperm swimming speed (Briskie & Montgomerie, 1992; Lüpold, Calhim, et al., 2009), making it a better competitor in the race to the ova.

In summary, our results support the view that tropical species experience varying levels of sperm competition (Albrecht et al., 2012). The greatest levels of sperm competition are probably found in the two species (the Vasa and Eclectus parrots) with the rarest mating system among parrots, in which females mate promiscuously and males provide food to and copulate with multiple females (Ekstrom et al., 2007; Heinsohn et al., 2005). Additionally, within parrots, the level of sperm competition seems generally higher for species that breed at higher densities, probably because of increased opportunities to mate outside the pair bond. Our results also indicate that sexual ornamentation in parrots is related to sperm competition, though the precise evolutionary mechanism has yet to be explored.

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REFERENCES

Ala-Honkola, O., Hosken, D. J., Manier, M. K., Lüpold, S., Droge-Young, E. M., Berben, K. S., ... Pitnick, S. (2013). Inbreeding reveals mode of past selection on male reproductive characters in *Drosophila melanogaster*. *Ecology and Evolution*, *3*(7), 2089–2102. https://doi.org/10.1002/ece3.625

Albrecht, T., Kleven, O., Kreisinger, J., Laskemoen, T., Omotoriogun, T. C., Ottosson, U., ... Lifjeld, J. T. (2012). Sperm competition in tropical versus temperate zone birds. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20122434. https://doi.org/10.1098/rspb.2012.2434

Arnold, K. E., & Owens, I. P. F. (2002). Extra-pair paternity and egg dumping in birds: Life history, parental care and the risk of retaliation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*, 1263–1269. https://doi.org/10.1098/rspb.2002.2013

Auersperg, A. M. I., Kacelnik, A., & von Bayern, A. M. P. (2013). Explorative learning and functional inferences on a five-step means-means-end problem in Goffin's cockatoos (*Cacatua goffini*). *PLoS ONE*, *8*(7), e68979. https://doi.org/10.1371/journal.pone.0068979

Auersperg, A. M. I., von Bayern, A. M. P., Gajdon, G. K., Huber, L., & Kacelnik, A. (2011). Flexibility in problem solving and tool use of kea

and new caledonian crows in a multi access box paradigm. *PLoS ONE*, *6*(6), e20231. https://doi.org/10.1371/journal.pone.0020231

Badyaev, A. V., & Hill, G. E. (2003). Avian sexual dichromatism in relation to phylogeny and ecology. *Annual Review of Ecology, Evolution, and Systematics*, *34*(34), 27–49. https://doi.org/10.1146/132441

Balshine, S., Leach, B. J., Neat, F., Werner, N. Y., & Montgomerie, R. (2001). Sperm size of African cichlids in relation to sperm competition. *Behavioral Ecology*, *12*(6), 726–731. https://doi.org/10.1093/beheco/12.6.726

Bennett, D. J., Sutton, M. D., & Turvey, S. T. (2017). treeman: an R package for efficient and intuitive manipulation of phylogenetic trees. *BMC Research Notes*, *10*(1), 30. https://doi.org/10.1186/s13104-016-2340-8

Berry, J. F., & Shine, R. (1980). Sexual size dimorphism and sexual selection in turtles (order testudines). *Oecologia*, *44*(2), 185–191. https://doi.org/10.1007/BF00572678

Birkhead, T. R. (1998). Sperm competition in birds. *Reviews of Reproduction*, *3*, 123–129. https://doi.org/10.1016/0169-5347(87)90033-4

Birkhead, T. R., & Fletcher, F. (1995). Male phenotype and ejaculate quality in the zebra finch *Taeniopygia guttata*. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *262*, 329–334.

Birkhead, T. R., Fletcher, F., & Pellatt, E. J. (1999). Nestling diet, secondary sexual traits and fitness in the zebra finch. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*, 385–390. https://doi.org/10.1098/rspb.1999.0649

Birkhead, T. R., Pellatt, E. J., Brekke, P., Yeates, R., & Castillo-Juarez, H. (2005). Genetic effects on sperm design in the zebra finch. *Nature*, *434*(7031), 383–387. https://doi.org/10.1038/nature03374

Briskie, J. V., & Montgomerie, R. (1992). Sperm size and sperm competition in birds. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *247*(1319), 89–95. https://doi.org/10.1098/rspb.1992.0013

Briskie, J. V., Montgomerie, R., & Birkhead, T. R. (1997). The evolution of sperm size in birds. *Evolution*, *51*(3), 937–945. https://doi.org/10.2307/2411167

Bublat, A., Fischer, D., Bruslund, S., Schneider, H., Meinecke-Tillmann, S., Wehrend, A., & Lierz, M. (2017). Seasonal and genera-specific variations in semen availability and semen characteristics in large parrots. *Theriogenology*, *91*, 82–89. https://doi.org/10.1016/j.theriogenology.2016.11.029

Byrne, P. G., Simmons, L. W., & Roberts, J. D. (2003). Sperm competition and the evolution of gamete morphology in frogs. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*, 2079–2086. https://doi.org/10.1098/rspb.2003.2433

Calhim, S., & Birkhead, T. R. (2007). Testes size in birds quality versus quantity—Assumptions, errors, and estimates. *Behavioral Ecology*, *18*, 271–275. https://doi.org/10.1093/beheco/arl076

Calhim, S., Immler, S., & Birkhead, T. R. (2007). Postcopulatory sexual selection is associated with reduced variation in sperm morphology. *PLoS ONE*, *2*(5), e413. https://doi.org/10.1371/journal.pone.0000413

Chen, J., Zou, Y., Sun, Y.-H., & ten Cate, C. (2019). Problem-solving males become more attractive to female budgerigars. *Science*, *363*(6423), 166–167. https://doi.org/10.1126/science.aau8181

Dale, J., Dey, C., Delhey, K., Kempenaers, B., & Valcu, M. (2015). The effects of life-history and social selection on male and female plumage coloration. *Nature*, *527*(7578), 1–17. https://doi.org/10.1038/nature15509

Darwin, C. (1871). *The descent of man and selection in relation to sex*.

del Hoyo, J., Elliott, A., Sargatal, J., Christie, D. A., & Kirwan, G. (2017). *Handbook of the birds of the world*. Barcelona, Spain: Lynx Edicions.

Desdevises, Y., Legendre, P., Azouzi, L., & Morand, S. (2003). Quantifying phylogenetically structured environmental variation. *Evolution*, *57*(11), 2647–2652. https://doi.org/10.1111/j.0014-3820.2003.tb01508.x

Diniz-Filho, J. A. F., de Sant'Ana, C. E. R., & Bini, L. M. (1998). An eigenvector method for estimating phylogenetic inertia. *Evolution*, *52*(5), 1247–1262. https://doi.org/10.1111/j.1558-5646.1998.tb02006.x

Eason, D. K., Elliott, G. P., Merton, D. V., Jansen, P. W., Harper, G. A., & Moorhouse, R. J. (2006). Breeding biology of kakapo (*Strigops habroptilus*) on offshore island sanctuaries, 1990–2002. *Notornis*, *53*(1), 27–36.

Eastwood, J. R., Berg, M. L., Ribot, R. F. H., Stokes, H. S., Martens, J. M., Buchanan, K. L., ... Bennett, A. T. D. (2018). Pair fidelity in long-lived parrots: Genetic and behavioural evidence from the Crimson Rosella (*Platycercus elegans*). *Emu - Austral Ornithology*, *118*(4), 369–374. https://doi.org/10.1080/01584197.2018.1453304

Ekstrom, J. M. M., Burke, T., Randrianaina, L., & Birkhead, T. R. (2007). Unusual sex roles in a highly promiscuous parrot: The greater vasa parrot *Caracopsis vasa*. *Ibis*, *149*(2), 313–320. https://doi.org/10.1111/j.1474-919X.2006.00632.x

Forshaw, J. M. (1978). *Parrots of the world (Second Edi)*. Melbourne, Australia: Lansdowne Editions.

Gaston, K. J. (2000). Global patterns in biodiversity. *Nature*, *405*, 220–227. https://doi.org/10.1038/35012228

Gomendio, M., Cassinello, J., & Roldan, E. R. S. (2000). A comparative study of ejaculate traits in three endangered ungulates with different levels of inbreeding: Fluctuating asymmetry as an indicator of reproductive and genetic stress. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1446), 875–882. https://doi.org/10.1098/rspb.2000.1084

Gomendio, M., & Roldan, E. R. (1991). Sperm competition influences sperm size in mammals. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *243*(1308), 181–185. https://doi.org/10.1098/rspb.1991.0029

Hawkins, B. A., Porter, E. E., & Diniz-filho, J. A. F. (2003). Productivity and history as predictors of the latitudinal diversity gradient of terrestrial birds. *Ecology*, *84*(6), 1608–1623. https://doi.org/10.1890/0012-9658(2003)084[1608:PAHAPO]2.0.CO;2

Heber, S., Varsani, A., Kuhn, S., Girg, A., Kempenaers, B., & Briskie, J. (2012). The genetic rescue of two bottlenecked South Island robin populations using translocations of inbred donors. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20122228. https://doi.org/10.1098/rspb.2012.2228

Heinsohn, R., Legge, S., & Endler, J. A. (2005). Extreme reversed sexual dichromatism in a bird without sex role reversal. *Science*, *309*(5734), 617–619. https://doi.org/10.1126/science.1112774

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous interference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Hunter, F. M., & Birkhead, T. R. (2002). Sperm viability and sperm competition in insects. *Current Biology*, *12*(2), 121–123. https://doi.org/10.1016/S0960-9822(01)00647-9

Immler, S., Calhim, S., & Birkhead, T. R. (2008). Increased postcopulatory sexual selection reduces the intramale variation in sperm design. *Evolution*, *62*(6), 1538–1543. https://doi.org/10.1111/j.1558-5646.2008.00393.x

Immler, S., Pitnick, S., Parker, G. A., Durrant, K. L., Lupold, S., Calhim, S., & Birkhead, T. R. (2011). Resolving variation in the reproductive tradeoff between sperm size and number. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(13), 5325–5330. https://doi.org/10.1073/pnas.1009059108

Immler, S., Pryke, S. R., Birkhead, T. R., & Griffith, S. C. (2010). Pronounced within-individual plasticity in sperm morphometry across social environments. *Evolution*, *64*(6), 1634–1643. https://doi.org/10.1111/j.1558-5646.2009.00924.x

Immler, S., Saint-Jalme, M., Lesobre, L., Sorci, G., Roman, Y., & Birkhead, T. R. (2007). The evolution of sperm morphometry in pheasants. *Journal of Evolutionary Biology*, *20*, 1008–1014. https://doi.org/10.1111/j.1420-9101.2007.01302.x

Jamieson, B. G. M. (2006). Avian spermatozoa: Structure and phylogeny. In B. G. M. Jamieson (Ed.), *Reproductive biology and phylogeny of aves* (pp. 249–511). Enfield, NH: Science Publishers Inc.

Johnson, D. D. P., & Briskie, J. V. (1999). Sperm competition and sperm length in shorebirds. *The Condor*, 101(4), 848–854. https://doi.org/10.2307/1370074

Jombart, T., Dray, S., & Bilgrau, A. E. (2016). adephylo: Adephylo: Exploratory analyses for the phylogenetic comparative method. *Bioinformatics*, 26(15), 1907–1909.

Kleven, O., Fossøy, F., Laskemoen, T., Robertson, R. J., Rudolfsen, G., & Lifjeld, J. T. (2009). Comparative evidence for the evolution of sperm swimming speed by sperm competition and female sperm storage duration in passerine birds. *Evolution*, 63(9), 2466–2473. https://doi.org/10.1111/j.1558-5646.2009.00725.x

Kleven, O., Laskemoen, T., Fossøy, F., Robertson, R. J., & Lifjeld, J. T. (2008). Intraspecific variation in sperm length is negatively related to sperm competition in passerine birds. *Evolution*, 62(2), 494–499. https://doi.org/10.1111/j.1558-5646.2007.00287.x

Krishnaprasadan, T. N., Kotak, V. C., Sharp, P. J., Schmedemann, R., & Haase, E. (1988). Environmental and hormonal factors in seasonal breeding in free-living male Indian rose-ringed parakeets (*Psittacula krameri*). *Hormones and Behavior*, 22(4), 488–496. https://doi.org/10.1016/0018-506X(88)90053-0

Lambert, M. L., Jacobs, I., Osvath, M., & von Bayern, A. M. P. (2019). Birds of a feather? Parrot and corvid cognition compared. *Behaviour*, 156(5–8), 391–407. https://doi.org/10.1163/1568539x-00003527

Laskemoen, T., Kleven, O., Fossøy, F., & Lifjeld, J. T. (2007). Intraspecific variation in sperm length in two passerine species, the bluethroat *Luscinia svecica* and the willow warbler *Phylloscopus trochilus*. *Ornis Fennica*, 84, 131–139.

Liao, W. B., Huang, Y., Zeng, Y., Zhong, M. J., Luo, Y., & Lüpold, S. (2018). Ejaculate evolution in external fertilizers: Influenced by sperm competition or sperm limitation? *Evolution*, 72(1), 4–17. https://doi.org/10.1111/evo.13372

Lierz, M., Reinschmidt, M., Müller, H., Wink, M., & Neumann, D. (2013). A novel method for semen collection and artificial insemination in large parrots (Psittaciformes). *Scientific Reports*, 3, 2066. https://doi.org/10.1038/srep02066

Lifjeld, J. T., Laskemoen, T., Kleven, O., Albrecht, T., & Robertson, R. J. (2010). Sperm length variation as a predictor of extrapair paternity in passerine birds. *PLoS ONE*, 5(10), e13456. https://doi.org/10.1371/journal.pone.0013456

Lüpold, S., Birkhead, T. R., & Westneat, D. F. (2012). Seasonal variation in ejaculate traits of male red-winged blackbirds (*Agelaius phoeniceus*). *Behavioral Ecology and Sociobiology*, 66(12), 1607–1617. https://doi.org/10.1007/s00265-012-1415-3

Lüpold, S., Calhim, S., Immler, S., & Birkhead, T. R. (2009). Sperm morphology and sperm velocity in passerine birds. *Proceedings of the Royal Society B: Biological Sciences*, 276, 1175–1181. https://doi.org/10.1098/rspb.2008.1645

Lüpold, S., Linz, G. M., & Birkhead, T. R. (2009). Sperm design and variation in the New World blackbirds (Icteridae). *Behavioral Ecology and Sociobiology*, 63(6), 899–909. https://doi.org/10.1007/s00265-009-0733-6

Lüpold, S., Linz, G. M., Rivers, J. W., Westneat, D. F., & Birkhead, T. R. (2009). Sperm competition selects beyond relative testes size in birds. *Evolution*, 63(2), 391–402. https://doi.org/10.1111/j.1558-5646.2008.00571.x

Macedo, R. H., Karubian, J., & Webst, M. S. (2008). Extrapair paternity and sexual selection in socially monogamous birds: Are tropical birds different? *The Auk*, 125(4), 769–777. https://doi.org/10.1525/auk.2008.11008

Masello, J. F., Sramkova, A., Quillfeldt, P., Epplen, J. T., & Lubjuhn, T. (2002). Genetic monogamy in burrowing parrots *Cyanoliseus*

patagonus? *Journal of Avian Biology*, 33, 99–103. https://doi.org/10.1034/j.1600-048X.2002.330116.x

Mauck, R. A., Marschall, E. A., & Parker, P. G. (1999). Adult survival and imperfect assessment of parentage: Effects on male parenting decisions. *The American Naturalist*, 154(1), 99–109. https://doi.org/10.1086/303216

Mehlis, M., Frommen, J. G., Rahn, A. K., & Bakker, T. C. M. (2012). Inbreeding in three-spined sticklebacks (*Gasterosteus aculeatus* L.): Effects on testis and sperm traits. *Biological Journal of the Linnean Society*, 107, 510–520. https://doi.org/10.1111/j.1095-8312.2012.01950.x

Møller, A. P., & Birkhead, T. R. (1993). Cuckoldry and sociality: A comparative study of birds. *The American Naturalist*, 142(1), 118–140. https://doi.org/10.1086/285531

Møller, A. P., & Birkhead, T. R. (1994). The evolution of plumage brightness in birds is related to extrapair paternity. *Evolution*, 48(4), 1089–1100. https://doi.org/10.1111/j.1558-5646.1994.tb05296.x

Møller, A. P., & Briskie, J. V. (1995). Extra-pair paternity, sperm competition and the evolution of testis size in birds. *Behavioral Ecology and Sociobiology*, 36(5), 357–365. https://doi.org/10.1007/BF00167797

Morrow, E. H., & Gage, M. J. (2000). The evolution of sperm length in moths. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267, 307–313. https://doi.org/10.1098/rspb.2000.1001

O'Neill, L., Picaud, A., Maehner, J., Gahr, M., & von Bayern, A. M. P. (2019). Two macaw species can learn to solve an optimised two-trap problem, but without functional causal understanding. *Behaviour*, 156(5–8), 691–720. https://doi.org/10.1163/1568539x-00003521

Omotoriogun, T. C., Albrecht, T., Hořák, D., Laskemoen, T., Ottosson, U., Rowe, M., ... Lifjeld, J. T. (2016). Sperm size evolution in African greenbuls (Passeriformes: Pycnonotidae). *Biological Journal of the Linnean Society*, 117(2), 337–349. https://doi.org/10.1111/bij.12663

Omotoriogun, T. C., Laskemoen, T., Rowe, M., Albrecht, T., Bowie, R. C. K., Sedláček, O., ... Lifjeld, J. T. (2016). Variation in sperm morphology among Afrotropical sunbirds. *Ibis*, 158(1), 155–166. https://doi.org/10.1111/ibi.12334

Opatová, P., Ihle, M., Albrechtová, J., Tomášek, O., Kempenaers, B., Forstmeier, W., & Albrecht, T. (2016). Inbreeding depression of sperm traits in the zebra finch *Taeniopygia guttata*. *Ecology and Evolution*, 6(1), 295–304. https://doi.org/10.1002/ece3.1868

Owens, I. P. F., & Hartley, I. R. (1998). Sexual dimorphism in birds: Why are there so many different forms of dimorphism? *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1394), 397–407. https://doi.org/10.1098/rspb.1998.0308

Parker, G. (1970). Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, 45(4), 525–567. https://doi.org/10.1111/j.1469-185X.1970.tb01176.x

Pitcher, T. E., Dunn, P. O., & Whittingham, L. A. (2005). Sperm competition and the evolution of testes size in birds. *Journal of Evolutionary Biology*, 18(3), 557–567. https://doi.org/10.1111/j.1420-9101.2004.00874.x

Provost, K. L., Joseph, L., & Smith, B. T. (2017). Resolving a phylogenetic hypothesis for parrots: Implications from systematics to conservation. *Emu - Austral Ornithology*, 118(1), 7–21. https://doi.org/10.1080/01584197.2017.1387030

R Development Core Team (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ricklefs, R. E., & Wikelski, M. (2002). The physiology/life history nexus. *Trends in Ecology and Evolution*, 17(10), 462–469. https://doi.org/10.1016/S0169-5347(02)02578-8

Rowe, M., Albrecht, T., Cramer, E. R. A., Johnsen, A., Laskemoen, T., Weir, J. T., & Lifjeld, J. T. (2015). Postcopulatory sexual selection is associated with accelerated evolution of sperm morphology. *Evolution*, 69(4), 1044–1052. https://doi.org/10.1111/evo.12620

Sardell, R. J., & DuVal, E. H. (2014). Small and variable sperm sizes suggest low sperm competition despite multiple paternity in a lekking

suboscine bird. *The Auk*, *131*(4), 660–671. https://doi.org/10.1642/AUK-14-38.1

Schneider, H., Fischer, D., Failing, K., Ehling, C., Meinecke-Tillmann, S., Wehrend, A., & Lierz, M. (2017). Investigations on different semen extenders for cockatiel semen. *Journal of Zoo Biology*, *1*(1), 1–12.

Shuster, S. M., & Wade, M. J. (2003). *Mating systems and strategies*. Princeton, NJ: Princeton University Press.

Sokal, R., & Rohlf, F. (1981). *Biometry: The principles and practice of statistics in biological research* (2nd ed.). New York, NY: W. H. Freeman and Co.

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *8*(11), 1639–1644. https://doi.org/10.1111/2041-210X.12797

Stutchbury, B. J., & Morton, E. S. (1995). The effect of breeding synchrony on extra-pair mating systems in songbirds. *Behaviour*, *132*(9–10), 675–690. https://doi.org/10.1163/156853995X00081

Swenson, N. G. (2014). *Functional and phylogenetic ecology in R*. New York, NY: Springer.

Szekely, T., Lislevand, T., & Figuerola, J. (2007). Sexual size dimorphism in birds. In D. J. Fairbairn, W. U. Blanckehorn & T. Szekely (Eds.), *Sex, size and gender roles: Evolutionary studies of sexual size dimorphism* (pp. 27–37). Oxford, UK: Oxford University Press.

Toft, C. A., & Wright, T. F. (2015). *Parrots of the wild: A natural history of the world's most captivating birds*. Oakland, CA: University of California Press.

Tourmente, M., Gomendio, M., Roldan, E. R. S., Giojalas, L. C., & Chiaraviglio, M. (2009). Sperm competition and reproductive mode influence sperm dimensions and structure among snakes. *Evolution*, *63*, 2513–2524. https://doi.org/10.1111/j.1558-5646.2009.00739.x

Valcu, M., Dale, J., & Kempenaers, B. (2012). RangeMapper: A platform for the study of macroecology of life-history traits. *Global Ecology and Biogeography*, *21*(9), 945–951. https://doi.org/10.1111/j.1466-8238.2011.00739.x

Van Horik, J. O., Clayton, N. S., & Emery, N. J. (2012). Convergent evolution of cognition in corvids, apes and other animals: In J. Vonk & T. K. Shackelford (Eds.), *The Oxford handbook of comparative evolutionary psychology* (pp. 80–101). New York, NY: Oxford University Press.

Wasser, D. E., & Sherman, P. W. (2010). Avian longevities and their interpretation under evolutionary theories of senescence. *Journal of Zoology*, *280*(2), 103–155. https://doi.org/10.1111/j.1469-7998.2009.00671.x

Wilkinson, R., & Birkhead, T. R. (1995). Copulation behaviour in the Vasa parrots *Coracopsis vasa* and *C. nigra*. *Ibis*, *137*, 117–119. https://doi.org/10.1111/j.1474-919X.1995.tb03228.x

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Additional supporting information may be found online in the Supporting Information section at the end of the article.