Received: 18 December 2018 | Revised: 16 June 2019 | Accepted: 22 June 2019

DOI: 10.1111/jeb.13501

RESEARCH PAPER

Journal of Evolutionary Biology eseb WILEY

Sexual selection and the evolution of sperm morphology in sharks

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Funding information
Fundação para a Ciência e Tecnologia, Grant/Award Number: UID/MAR/04292/2013; Erasmus+; Australian Research Council; University of Western Australia; Veneto Region (Italy) Law 15/2007, Grant/Award Number: 4069; Western Australia Government; Spanish Ministry of Economy/European Development Fund, Grant/Award Number: CGL2012-34685, CGL2016-76173-P; Spanish Ministry of Economy, Grant/Award Number: SEV-2012-0262; Biotechnology and Biological Sciences Research Council

Abstract

Post-copulatory sexual selection, and sperm competition in particular, is a powerful selective force shaping the evolution of sperm morphology. Although mounting evidence suggests that post-copulatory sexual selection influences the evolution of sperm morphology among species, recent evidence also suggests that sperm competition influences variation in sperm morphology at the intraspecific level. However, contradictory empirical results and limited taxonomic scope have led to difficulty in assessing the generality of sperm morphological responses to variation in the strength of sperm competition. Here, we use phylogenetically controlled analyses to explore the effects of sperm competition on sperm morphology and variance in sharks, a basal vertebrate group characterized by wide variation in rates of multiple mating by females, and consequently sperm competition risk. Our analyses reveal that shark species experiencing greater levels of sperm competition produce sperm with longer flagella and that sperm flagellum length is less variable in species under higher sperm competition risk. In contrast, neither the length

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of the sperm head and midpiece nor variation in sperm head and midpiece length was associated with sperm competition risk. Our findings demonstrate that selection influences both the inter- and intraspecific variation in sperm morphology and suggest that the flagellum is an important target of sexual selection in sharks. These findings provide important insight into patterns of selection on the ejaculate in a basal vertebrate lineage.

KEYWORDS
elasmobranch, Sperm competition, sperm morphology

1 | INTRODUCTION

Sperm exhibit extraordinary morphological diversity and are among the most variable of all known cell types (Pitnick, Hosken, & Birkhead, 2009). However, the evolutionary processes that promote sperm diversification remain hotly debated (Lüpold & Pitnick, 2018). Although fertilization environments and phylogenetic effects undoubtedly influence sperm evolution (Lüpold & Pitnick, 2018; Pitnick et al., 2009; Simpson, Humphries, Evans, Simmons, & Fitzpatrick, 2014), sexual selection is now recognized as a particularly powerful selective force driving the evolution of sperm morphological diversity (Fitzpatrick & Lüpold, 2014; Pizzari & Parker, 2009; Simmons & Fitzpatrick, 2012). When females mate with multiple males, the temporal and spatial overlap of sperm from rival males within the fertilization environment can result in sperm competition, where sperm from different males compete to fertilize the available ova (Parker, 1970), and cryptic female choice, where females bias the outcome of sperm competition in favour of preferred males (Eberhard, 1996). These episodes of post-copulatory sexual selection therefore impose strong selective pressures on males to produce more effective ejaculates. When male fertility is influenced by the number of sperm present at the site of fertilization, sperm competition is expected to favour increases in the number of sperm that males produce (Parker, 1998; Pizzari & Parker, 2009). Indeed, evolutionary increases in sperm number in response to sperm competition are commonly observed across species (Rowley, Daly-Engel, & Fitzpatrick, 2019; Simmons & Fitzpatrick, 2012). However, sperm number is not the only target of post-copulatory sexual selection, as sperm competition and cryptic female choice can also influence the evolution of sperm morphology and size (Pitnick et al., 2009; Simmons & Fitzpatrick, 2012). If trade-offs exist between sperm number and size (Parker, 1982), then post-copulatory sexual selection for increased sperm production may result in evolutionary reductions in sperm size (Immler et al., 2011), making it challenging to predict how selection will shape sperm morphology.

Each component of the sperm cell (i.e. the head, midpiece and flagellum) influences sperm function, and therefore, post-copulatory sexual selection may act on each or all of these components provided they influence male fertilization success (Gage et al., 2004; Simmons & Fitzpatrick, 2012). For example, the size of the sperm midpiece is expected to increase in response to sperm competition to provide the cell with more energy (Anderson, Nyholt, & Dixson, 2005), and can influence the beat frequency of the flagellum (Cardullo & Baltz, 1991), which may in turn be targeted by selection to increase thrust (Fitzpatrick et al., 2009; Gomendio & Roldan, 1991). Sperm competition is also hypothesized to select for smaller relative sperm head size to reduce drag that opposes the thrusting force of the flagellum (Humphries, Evans, & Simmons, 2008). However, comparative studies evaluating how sperm morphology respond to variation in the strength of sperm competition show mixed, and often taxon-specific, results (Immler & Birkhead, 2007; Simmons & Fitzpatrick, 2012). Moreover, post-copulatory sexual selection may also influence sperm production efficiency (Birkhead, Pellat, Brekke, Yeates, & Castillo-Juarez, 2005), filtering out sperm with suboptimal morphologies during spermatogenesis (Lüpold, Wistuba, Damm, Rivers, & Birkhead, 2011). When sperm competition risk and/or intensity are high, selection should favour consistent production of an optimal sperm phenotype and correspondingly act to erode variation in sperm morphology within the ejaculate (Birkhead et al., 2005; Parker, 1993). Indeed, the evidence to date from phylogenetically controlled studies has revealed consistent negative relationships between intraspecific variation in sperm morphology and the level of sperm competition, although such relationships have been evaluated in only a handful of studies of passerine birds, social insects and rodents (Calhim, Immler, & Birkhead, 2007; Fitzpatrick & Baer, 2011; Immler, Calhim, & Birkhead, 2008; Kleven, Laskemoen, Fossøy, Robertson, & Lifjeld, 2008; Varea-Sánchez, Montoto, Tourmente, & Roldan, 2014). However, the way in which selection acts on sperm morphology and sperm variation across a broader taxonomic scale remains unclear.

Here, we examine how sperm competition shapes sperm morphology in sharks, an internally fertilizing, ancestral vertebrate group. Observations of matings in the wild suggest that female sharks commonly mate with more than one male within a reproductive cycle (e.g. Carrier, Pratt, & Martin, 1994; Whitney, Pratt, & Carrier, 2004). However, multiply-sired litters occur at vastly different frequencies across a wide variety of species, ranging from infrequent (e.g. 11% multiple paternity in the shortspine spurdog, *Squalus* cf. *mitsukurii)* to frequent (e.g. 92% multiple paternity in the small-spotted catshark, *Scyliorhinus canicula)* (reviewed in Byrne & Avise, 2012; Fitzpatrick, Kempster, Daly-Engel, Collin, & Evans, 2012; Rowley et al., 2019). Moreover, sharks are one of the few taxonomic groups in which relative testes mass has been validated against genetic estimates of sperm competition risk (the percentage of litters sired by more than

one male) and intensity (the mean number of sires per litter) (Rowley et al., 2019), thereby allowing broad comparative studies to be conducted using validated proxy measures for the level of sperm competition. In addition, female sharks retain sperm in specialized storage organs (i.e. oviducal glands). Although the length of sperm storage may vary (Pratt, 1993), in some species offspring can be produced using sperm stored for up to four years after mating (Bernal et al., 2015). The potential for long-term sperm storage uncouples mating from fertilization, increasing competition between rival ejaculates, and imposes selection on sperm morphology to enter and remain viable in sperm storage organs (Fitzpatrick et al., 2012; Orr & Brennan, 2015; Orr & Zuk, 2014). We take advantage of the considerable variation in sperm competition risk and intensity observed among sharks to consider how post-copulatory sexual selection influences sperm morphology and variance. Specifically, we examine the relationship between the size of each sperm component (head, midpiece and flagellum) and the level of sperm competition and consider how sperm competition acts on within-male variation in sperm component size.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Sperm was collected opportunistically from 122 individuals representing 25 shark species (mean ± SE number of males per species = 4.88 ± 1.10, range = 1–19, Table S1). Only individuals caught previously as part of commercial and artisanal fisheries, during scientific surveys, for research purposes, or as bycatch were sampled. Samples were collected at 10 field sites spanning 7 countries across five continents over a 12-year period (Table S1). We assessed males for maturity by examining the claspers, with fully calcified claspers indicating a sexually mature individual (Hamlett, 2005). Immature males were not sampled. Semen (sperm and seminal fluid) was extracted from mature males in breeding condition (defined as those males currently producing sperm) by manually applying pressure to the sperm sac or claspers (Figure 1a). Whenever possible, we measured the total length (mm) and body mass (g) of each individual prior to dissection, and the testes of mature males were excised and weighed to the nearest 0.01 g. However, we were unable to collect body and testes mass data directly from all individuals sampled in the field due to logistical constraints (e.g. when sharks were too large to be weighed or could not be dissected because they were being sold at market). In cases where we lacked body or testes mass data, we searched the literature using the species name in combination with the words 'testes mass', 'body mass' or 'gonadosomatic index' (GSI). If raw data were not reported in the studies examined, we contacted the authors directly to request the data, or calculated body and testes mass from GSI, using the program GraphClick v3.0.3 (Boyle, Samaha, Rodewald, & Hoffmann, 2012) to extract data from figures (summarized in Table S1). In this way, we supplemented the data we collected in the field with data on male body mass and testes mass from an additional two and four species, respectively (Table S1).

2.2 | Sperm analysis

Semen samples were either examined fresh or preserved in 1 ml of 10% neutral buffered formalin for subsequent examination. Fresh ejaculates were processed at field sites within a few hours of extraction, whereas preserved samples were taken back to the laboratory where they were examined. Sperm component length did not differ between fresh and preserved samples (Figure S1, paired t test performed on six species where sperm was preserved using both methods: head + midpiece: $t = 0.43$, $p = .68$; head + midpiece CV: $t = 0.66$, $p = .53$; flagellum: $t = 0.74$, $p = .49$; flagellum CV: $t = 0.24$, $p = .82$; note that we were not able to apply mixed-effects models to compare fresh and preserved samples as four of the six species had only one male represented in either of the sampling methods). Microscope slides were loaded with 10 μl of sea water-diluted (for fresh samples) or formalin-diluted (for preserved samples) semen samples and covered with a coverslip. Slides were left for up to two hours after loading to allow sperm to settle onto a single plane of focus prior to viewing under the microscope. For each male sample, we haphazardly selected and photographed between 20–30 individual morphologically normal sperm cells. Within-ejaculate variation in sperm length stabilizes after ~20 sperm cells are measured (Figure S2). All sperm images were captured at 400× magnification.

The number of field sites, number of years of data collection and the logistical constraints of sampling sharks introduced some differences in how images were captured. Images of sperm taken for later use in standardized downstream analyses (see below) were collected using different microscope and camera systems depending on field sites and sampling conditions. Specifically, when collecting semen samples at field stations, sperm images were captured using three different microscope and camera systems, which differed based on sampling locations (Adriatic Sea: a Leica DMLB30 light microscope fitted with a Leica DFC 420 camera (n = 3 species); Sardinian Sea: a Zeiss Axioskop light microscope fitted with a Canon EOS 1100D camera (n = 4 species); Azores: a Leica DM 6000B light microscope fitted with a Leica DFC340 camera (n = 6 species)). When microscopes and cameras were not available during sampling (e.g. in remote field locations or on boats), we preserved sperm in the field, then performed subsequent analyses in the laboratory using a Leica DM750 light microscope fitted with a Canon 600D camera (n = 18 species; note that six species were sampled in multiple locations and thus our total sample of field-collected samples remains at 25 species). Importantly, although differences in microscope and camera systems may add noise to the overall dataset, there is no *a priori* reason to assume that the variation in sampling will systematically bias the results in favour of the hypotheses being tested, but rather will attenuate regression coefficients towards zero (Hansen, 2016; Hansen & Bartoszek, 2012). Thus, any error in our final dataset on sperm length introduced by our sampling protocol is likely comparable with error introduced by collecting data from the literature, which is a common practice in comparative analyses in general, and in comparative analyses of sperm evolution in particular (e.g. Gage

& Freckleton, 2003; Gomendio, Tourmente, & Roldan, 2011; Lüpold & Fitzpatrick, 2015).

Sperm components were measured from digital images using the segmented line tool in ImageJ (Rasband, 1997). Mean sperm component lengths (μm) were calculated for each species. The division between the sperm head and midpiece was difficult to distinguish in seven species, which affected sample sizes of our analyses of head and midpiece length (see below).

2.3 | Phylogenetic linear models

We used phylogenetically controlled general least squares (PGLS) multiple regressions to examine associations between sperm morphology and relative testes mass, a proxy measure of sperm competition risk and/or intensity. All analyses were performed in R version 3.4.1 (R Core Team 2017). Phylogenetic relationships were derived from a recent elasmobranch phylogeny constructed using genetic data from 610 species (Stein et al., 2018). Using the original set of 500 phylogenetic trees, we generated a consensus tree using the function *consensus.tree* with the function *consensus.edges* to set branch lengths in the package phytools (Revell, 2012). To assess phylogenetic dependence of the data, likelihood ratio tests were used to calculate the phylogenetic scaling parameter λ (Freckleton, Harvey, & Pagel, 2002; Pagel, 1999), where a value of 0 indicates no phylogenetic signal, and 1 indicates total phylogenetic dependence. All data were $\log_{10}$ transformed prior to analysis, which improved the distribution of model residuals (see Mundry, 2014). Of the 25 species for which sperm components were measured, 19 had testes and body mass data available, allowing for tests of the effect of sperm competition on component length. One of these species (*Squalus blainville*) was not present in phylogeny, reducing the sample size of phylogenetically controlled analyses of flagellum length to 18. In two of these 18 species, the head and midpiece could not be distinguished, reducing the sample size to 16 for analyses examining the head and midpiece length.

To investigate evolutionary responses in sperm morphology to sperm competition in sharks, we examined the relationship between each sperm component (head, midpiece and the length of the flagellum) and relative testes mass (to control for the allometric relationship between testes mass and body size, body mass was included as a covariate in all models). Relative testes mass was used as a proxy for sperm competition risk/intensity, due to the close association between relative testes mass and the level of sperm competition across a wide range of taxa (Simmons & Fitzpatrick, 2012). Specifically, relative testes mass is correlated positively with multiple paternity rates (i.e. sperm competition risk) and the number of males siring offspring in a brood (i.e. sperm competition intensity) among shark species (Rowley et al., 2019), supporting the assertion that relative testes mass represents a valid estimate of the sperm competition risk/intensity in our analyses.

We calculated the mean within-male coefficient of variation (CV) for head and midpiece length and flagellum length using the formula CV = (standard deviation/mean)*100. We did not evaluate between-male CV, as 16 of the 25 species we examined had ≤ 2 individuals sampled per species and only six species had ≥ 10 individuals sampled, above which point between-male CV begins to stabilize (see Figure S3). We used PGLS regressions to test the relationship between the within-male CV of each sperm component and relative testes mass. Although CV is commonly used to assess standardized variation in sperm morphology (e.g. Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; Malo et al., 2006), the use of CV as an estimate of variation has been criticized for potentially yielding biased results in the absence of an isometric relationship between the mean and variance (Fitzpatrick & Baer, 2011; Tomkins & Simmons, 2002). Therefore, we performed additional analyses to examine the association between the standard deviation of sperm component length and relative testes mass while accounting for mean-variance relationships by adding the mean sperm component length as a covariate in the model (Fitzpatrick & Baer, 2011). All results remained consistent when we assessed variance by inspecting the response of the standard deviation of sperm component length to relative testes mass while controlling for mean sperm component length (Table S2).

A clear limitation of our dataset is the low number of within-species sampling. To address this issue, we performed an additional set of analyses using the *gls* function in the *nlme* package, where model effects were weighted by the sample size of the number of males assessed per species. We assessed various phylogenetic correlation structures in these weighted regressions (i.e. corPagel, corBrownian, corMartins) and used AIC model comparisons to identify the best fitting correlation structure for the models. In all models, corPagel and corBrownian best fit to our data (see Table S3).

For all models, the strength of the effects of the predictor variables on the dependent variables was generated by calculating the effect sizes, r and noncentral 95% confidence from model *t* values following Nakagawa and Cuthill (2007).

3 | RESULTS

Sperm morphology was variable across sharks, with head length ranging from 26.81 to 63.90 μm, midpiece length from 5.40 to 16.77 μm and flagellum length from 67.88 to 146.13 μm. In sharks, flagellum length was significantly positively associated with relative testes mass (Table 1, Figure 1). However, flagellum length was negatively associated with body mass in sharks, suggesting that larger-bodied shark species produce sperm with smaller flagella (Table 1). Similarly, sperm total length was positively associated with testes mass and negatively associated with body mass. Neither sperm head nor midpiece length was associated with relative testes mass (Table 1). The phylogenetic signal (λ) in the residual covariance in models assessing sperm head and midpiece exhibited strong phylogenetic dependence, whereas the phylogenetic signal in models assessing flagellum length was low, suggesting more labile evolutionary responses in the flagellum compared with the sperm head and midpiece.

There was a significant negative association between within-male CV of sperm flagellum length and relative testes mass (Table 1,

TABLE 1 Phylogenetically controlled generalized least squares (PGLS) regressions between sperm traits and testes mass. Models assess the relationship between sperm length and variance and testes mass in sharks. Body mass was included as a covariate in all models to control for the allometric relationship between body and testes size. The phylogenetic scaling parameter λ indicates the level of phylogenetic dependence of the data, ranging from 0 (low phylogenetic signal) to 1 (high phylogenetic signal). The slope of the regression with standard error (*SE*), t-statistic (*t*), degrees of freedom (*df*) and *p*-value are presented for each model. The effect size (*r*) and noncentral 95% confidence intervals (95% CI) are also presented for each model. Significant effects (i.e. cases where $p < .05$ and the 95% CI do not overlap zero) are highlighted in bold

Sperm trait	Predictors	λ	Slope	*SE*	*t*	*df*	*p*	*r*	95% CI
Head length	Testes mass	1.00	0.04	0.07	0.53	13	.60	.14	−0.37 to 0.57
	Body mass		0.002	0.06	0.03	13	.98	.26	−0.47 to 0.48
Midpiece length	Testes mass	1.00	0.12	0.11	1.10	13	.29	.08	−0.25 to 0.65
	Body mass		−0.08	0.09	−0.86	13	.40	.11	−0.62 to 0.30
Flagellum length	Testes mass	0.00	0.18	0.07	2.49	15	**.02**	**.54**	**0.08 to 0.77**
	Body mass		−0.17	0.06	−2.86	15	**.01**	**−.59**	**−0.79 to −0.16**
Total length	Testes mass	1.00	0.15	0.05	2.75	15	**.01**	**.57**	**0.13 to 0.78**
	Body mass		−0.12	0.04	−2.84	15	**.01**	**−.59**	**−0.79 to −0.15**
Within-male head length CV	Testes mass	0.73	0.02	0.12	0.17	13	.86	.23	−0.45 to 0.51
	Body mass		−0.03	0.10	−0.25	13	.81	−.22	−0.52 to 0.43
Within-male midpiece length CV	Testes mass	0.03	−0.27	0.19	−1.44	13	.17	−.37	−0.69 to 0.17
	Body mass		0.15	0.16	0.91	13	.38	.24	−0.29 to 0.62
Within-male flagellum length CV	Testes mass	0.00	−0.50	0.17	−2.92	15	**.01**	**−.60**	**−0.80 to −0.17**
	Body mass		0.29	0.14	2.06	15	.06	.47	−0.02 to 0.73
Within-male total length CV	Testes mass	0.00	−0.20	0.20	−0.97	13	.35	−.26	−0.63 to 0.28
	Body mass		0.02	0.17	0.11	13	.91	.03	−0.46 to 0.50

Figure 1). However, within-male CV of sperm head, midpiece length or total sperm length was not related with our proxy measure of sperm competition risk. These results did not qualitatively change when we assessed the effect of sperm competition on variance in sperm morphology by using within-male standard deviation of sperm component length as a response variable, while controlling for mean component length (Table S2).

Finally, to account for the variation in the number of males assess for each species, we assessed the relationship between sperm length and variance and relative testes mass in models that where weighted by intraspecific sampling effort (Table S3). These additional analyses revealed broadly similar results to those presented in our main set of analyses (see Table 1). Specifically, when weighting the regression models by sampling effort, flagellum length was

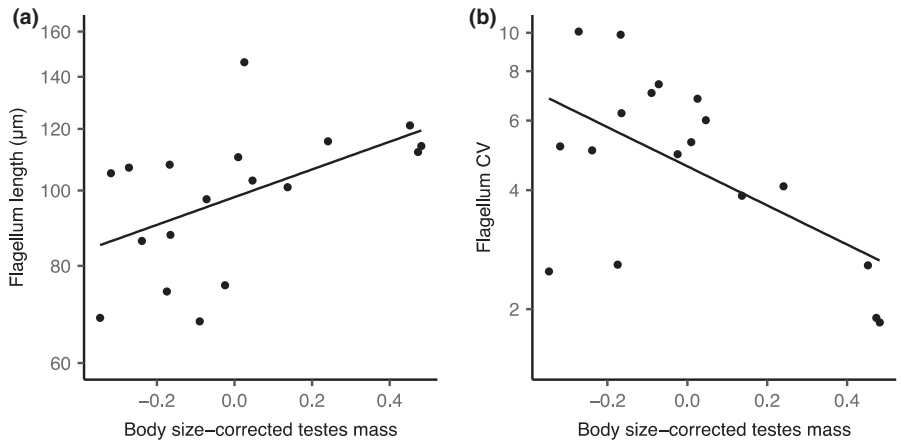

FIGURE 1 The associations between sperm morphological traits and body size-corrected testes mass, a proxy measure for sperm competition risk/intensity in sharks. Data are from the association between (a) sperm flagellum length, (b) the within-male coefficient of variation (CV) of flagellum length and body size-corrected testes mass. Sperm morphological traits are plotted on a log-scale, and body size-corrected testes mass values are residual values obtained from linear regression of log-transformed testes mass on log-transformed body mass for the shark species present in the analysis

positively associated and within-male CV of flagellum length was negatively associated with relative testes mass (Table S3). Sperm head, midpiece and total length were not associated with relative testes mass in weighted models (Table S3). However, in contrast to our main findings, within-male CV of sperm head, midpiece and total length were all negatively associated with relative testes mass in models that accounted for variation in intraspecific sampling effort (Table S3).

4 | DISCUSSION

Our results demonstrate that sperm competition acts to shape the evolution of the sperm morphology in sharks. We found that species experiencing higher levels of sperm competition produce ejaculates with longer sperm flagella and less variation in flagellum length. In contrast, sperm head and midpiece length and variance were not associated with sperm competition level in our main analyses. However, in models that accounted for the variation in the number of males sampled from each species, we detected reductions in the variance of every sperm trait assessed as sperm competition risk increased. Taken in combination, these results provide evidence for distinct patterns of selection on the length of different sperm components in sharks and suggest that the flagellum is an important target of sexual selection, with longer flagella likely offering an advantage during sperm competition.

This positive association between flagellum length and our proxy measure of sperm competition risk/intensity supports the theoretical prediction that longer sperm should be favoured in sperm competition (Gomendio & Roldan, 1991). Longer flagella can be advantageous during competitive fertilizations under three possible mechanistic scenarios, none of which is mutually exclusive. First, longer flagella may provide greater thrusting force to propel sperm more quickly as they swim towards the egg (Fitzpatrick et al., 2009; Lüpold, Calhim, Immler, & Birkhead, 2009), particularly as sperm swimming speed is an important predictor of competitive fertilization success in a wide range of taxa (Simmons & Fitzpatrick, 2012). Second, sperm with longer flagella may be better able to displace rival sperm from advantageous positions within the female's reproductive tract, for example by being better positioned to fertilize the egg or enter sperm storage organs (Lüpold et al., 2012). In sharks, sperm are commonly retained in specialized tubules in the oviducal gland after mating, which may impose specific selective pressures on sperm morphology. For example, sperm with longer flagella may be able to reach—and fill—the oviducal gland more quickly or be better able to displace rival sperm from the storage tubules, although these alternatives have yet to be investigated in sharks. Finally, longer sperm may be selected for if cryptic female choice favours the use and storage of sperm with longer flagella (Baer, Schmid-Hempel, Høeg, & Boomsma, 2003; Miller & Pitnick, 2002). Regardless of the mechanistic explanation, our results suggest that post-copulatory sexual selection selects for longer flagella in sharks.

Variation in sperm component length also showed divergent responses to sperm competition risk in sharks. Within-male variance in sperm flagellum length—but not sperm head and midpiece length—is reduced in species that experience higher levels of sperm competition. Such variance reduction in response to increases in sperm competition suggests that polyandrous mating selects for increased 'quality control' in male sperm production (Birkhead et al., 2005; Hunter & Birkhead, 2002) and supports the pattern of decreasing variation in sperm morphology in response to sexual selection previously documented in social insects (Fitzpatrick & Baer, 2011), passerine birds (Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008) and rodents (Varea-Sánchez et al., 2014). Thus, post-copulatory sexual selection appears to exert directional (resulting in longer flagellum lengths) and either directional or stabilizing selection (resulting in reduced variance in flagellum length) on the sperm flagellum in sharks. Cryptic female choice at the site of sperm storage may exert stabilizing selection for optimal sperm length, as was recently demonstrated in a passerine bird (Hemmings, Bennison, & Birkhead, 2016). Such sperm selection may also be possible in sharks, particularly given the long-term sperm storage observed in some species (Bernal et al., 2015; Hamlett, 2005). If female sperm storage organs preferentially retain specific sperm morphologies, competition among sperm for access to the oviducal gland will likely drive the evolution of less-variable sperm (sensu Fitzpatrick & Baer, 2011). It is well known that female storage organs impose selective pressures on sperm morphology (Briskie, Montgomerie, & Birkhead, 1997; García-González & Simmons, 2007; Pattarini, Starmer, Bjork, & Pitnick, 2006; Pitnick, Markow, & Spicer, 1999), and variation in total sperm length is negatively related to the duration of sperm storage in passerine birds (Kleven et al., 2009). With the exception of rodents (Varea-Sánchez et al., 2014), a commonality among the most taxa where sperm variance is negatively related with sperm competition risk (i.e. passerine birds, Calhim et al., 2007; Immler et al., 2008; Kleven et al., 2008; social insects, Fitzpatrick & Baer, 2011; and sharks, this study) is that females retain sperm for prolonged periods (i.e. several days, weeks or years) in specialized storage organs after copulation. Thus, our findings suggest that sperm–female interactions, specifically mediated by sperm storage organs, may represent a convergent mechanism underpinning reductions in sperm variation across phylogenetically distinct taxa. Future studies comparing the relationships between the level of sperm competition and sperm variance across species both with and without female sperm storage organs would represent an important test of this hypothesis.

In conclusion, our findings suggest that sperm competition arising from female polyandry influences the evolution of sperm flagellum length and variance in sharks. Sharks represent a useful model for studying the evolution of reproductive traits due to their wide range of reproductive systems and behaviours and unique position as one of the first vertebrates to develop internal fertilization. Further work examining evolutionary relationships between the

female sperm storage organ and patterns of sperm morphology in sharks would be a logical first step towards disentangling the roles of sperm competition and cryptic female choice in shaping selection on sperm in this group. This would aid in moving towards a more comprehensive understanding of how post-copulatory sexual selection operates in sharks.

ACKNOWLEDGMENTS

We thank Clinton Duffy and Malcolm Francis for contributing unpublished data, the Lonja Pesquera de Algeciras (and in particular Trudy, Vicente, Rosa and Curro) for facilitating access to fish caught as bycatch, Emilio Riginella, Vittoria Correale and Licia Finotto for helping in data collection, and Niclas Kolm for helpful comments on the manuscript. Thanks to staff at the NOAA Southeast Fisheries Science Center's Pelagic Longline Observer Program for samples from the northern Gulf of Mexico, and the University of West Florida's Jim Hammond for logistical support. Research was supported by a Biotechnology and Biological Sciences Research Council (BBSRC) Doctoral Training Program studentship to AR, Stockholm University (AR and JLF) and a Knut and Alice Wallenberg Academy Fellowship to JLF. CM was financially supported by Clodia project funded by the Veneto Region (Italy) Law 15/2007 (DGR no 4069). LL was supported by Erasmus + mobility program 2017, University of Padova (Italy). EG acknowledges Fundação para a Ciência e Tecnologia (FCT), through the strategic project UID/MAR/04292/2013 granted to MARE. FGG was supported by grants (CGL2012-34685 and CGL2016-76173-P, co-funded by the European Regional Development Fund) from the Spanish Ministry of Economy. Funding for the project was also provided by the Western Australia Government, the University of Western Australia, the Australian Research Council and a Doñana Biological Station project financed by the Spanish Ministry of Economy, through the Severo Ochoa Program for Centres of Excellence (SEV-2012-0262).

AUTHOR CONTRIBUTIONS

AR, LL, JPE, FGG, CM and JLF involved in conceptualization; AR, LL, MR, AB, EGG, RMK, SPC, EG, MCF, CP, FH, FGG, TDE and JLF collected the data; AR, LL, AK and JLF involved in data analysis; AR and JLF wrote the original draft; All authors involved in writing, reviewing and editing; LL, FGG, CM and JLF involved in funding acquisition.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Anderson, M. J., Nyholt, J., & Dixson, A. F. (2005). Sperm competition and the evolution of sperm midpiece volume in mammals. *Journal of Zoology*, *267*, 135–142. https://doi.org/10.1017/S0952836905007284

Baer, B., Schmid-Hempel, P., Høeg, J. T., & Boomsma, J. J. (2003). Sperm length, sperm storage and mating system characteristics in bumblebees. *Insectes Sociaux*, *50*, 101–108. https://doi.org/10.1007/s00040-003-0641-0

Bernal, M. A., Sinai, N. L., Rocha, C., Gaither, M. R., Dunker, F., & Rocha, L. A. (2015). Long-term sperm storage in the brownbanded bamboo shark Chiloscyllium punctatum. *Journal of Fish Biology*, *86*, 1171–1176. https://doi.org/10.1111/jfb.12606

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

Boyle, M. A., Samaha, A. L., Rodewald, A. M., & Hoffmann, A. N. (2012). Evaluation of the reliability and validity of GraphClick as a data extraction program. *Computers in Human Behavior*, *29*, 1023–1027.

Briskie, J. V., Montgomerie, R., & Birkhead, T. R. (1997). The Evolution of Sperm Size in Birds. *Evolution*, *51*, 937. https://doi.org/10.1111/j.1558-5646.1997.tb03674.x

Byrne, R. J., & Avise, J. C. (2012). Genetic mating system of the brown smoothhound shark (*Mustelus henlei*), including a literature review of multiple paternity in other elasmobranch species. *Marine Biology*, *159*, 749–756. https://doi.org/10.1007/s00227-011-1851-z

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

Cardullo, R. A., & Baltz, J. M. (1991). Metabolic regulation in mammalian sperm: Mitochondrial volume determines sperm length and flagellar beat frequency. *Cell Motility and the Cytoskeleton*, *19*, 180–188. https://doi.org/10.1002/(ISSN)1097-0169

Carrier, J. C., Pratt, H. L., & Martin, L. K. (1994). Group reproductive behaviors in free-living nurse sharks, *Ginglymostoma cirratum*. *Copeia*, *1994*, 646–656. https://doi.org/10.2307/1447180

Eberhard, W. G. (1996). *Female control: Sexual selection by cryptic female choice*. Princeton, NJ: Princeton University Press.

Fitzpatrick, J. L., & Baer, B. (2011). Polyandry reduces sperm length variation in social insects. *Evolution*, *65*, 3006–3012. https://doi.org/10.1111/j.1558-5646.2011.01343.x

Fitzpatrick, J. L., Kempster, R. M., Daly-Engel, T. S., Collin, S. P., & Evans, J. P. (2012). Assessing the potential for post-copulatory sexual selection in elasmobranchs. *Journal of Fish Biology*, *80*, 1141–1158. https://doi.org/10.1111/j.1095-8649.2012.03256.x

Fitzpatrick, J. L., & Lüpold, S. (2014). Sexual selection and the evolution of sperm quality. *Molecular Human Reproduction*, *20*, 1180–1189. https://doi.org/10.1093/molehr/gau067

Fitzpatrick, J. L., Montgomerie, R., Desjardins, J. K., Stiver, K. A., Kolm, N., & Balshine, S. (2009). Female promiscuity promotes the evolution of faster sperm in cichlid fishes. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 1128–1132. https://doi.org/10.1073/pnas.0809990106

Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic analysis and comparative data: A test and review of evidence. *American Naturalist*, *160*, 712–726. https://doi.org/10.1086/343873

Gage, M. J. G., & Freckleton, R. P. (2003). Relative testis size and sperm morphometry across mammals: No evidence for an association between sperm competition and sperm length. *Proceedings of the Royal Society B-Biological Sciences*, *270*, 625–632. https://doi.org/10.1098/rspb.2002.2258

Gage, M. J. G., Macfarlane, C. P., Yeates, S., Ward, R. G., Searle, J. B., & Parker, G. A. (2004). Spermatozoal Traits and Sperm Competition in Atlantic Salmon. *Current Biology*, *14*, 44–47.

García-González, F., & Simmons, L. W. (2007). Shorter sperm confer higher competitive fertilization success. *Evolution*, *61*, 816–824. https://doi.org/10.1111/j.1558-5646.2007.00084.x

Gomendio, M., & Roldan, E. R. (1991). Sperm competition influences sperm size in mammals. *Proceedings of the Royal Society of London Series B*, *243*, 181–185.

Gomendio, M., Tourmente, M., & Roldan, E. R. S. (2011). Why mammalian lineages respond differently to sexual selection: Metabolic rate constrains the evolution of sperm size. *Proceedings of the Royal Society B-Biological Sciences*, *278*, 3135–3141. https://doi.org/10.1098/rspb.2011.0275

Hamlett, W. C. (2005). *Reproductive biology and phylogeny of Chondrichthyes: Sharks, batoids and chimaeras*. Plymouth, UK: Science Publishers.

Hansen, T. F. (2016). On bias and precision in meta-analysis: the error in the error. *Journal of Evolutionary Biology*, *29*, 1919–1921. https://doi.org/10.1111/jeb.12947

Hansen, T. F., & Bartoszek, K. (2012). Interpreting the evolutionary regression: The interplay between observational and biological errors in phylogenetic comparative studies. *Systematic Biology*, *61*, 413–425. https://doi.org/10.1093/sysbio/syr122

Hemmings, N., Bennison, C., & Birkhead, T. R. (2016). Intra-ejaculate sperm selection in female zebra finches. *Biology Letters*, *12*, 20160220. https://doi.org/10.1098/rsbl.2016.0220

Humphries, S., Evans, J. P., & Simmons, L. W. (2008). Sperm competition: linking form to function. *BMC Evolutionary Biology*, *8*, 319. https://doi.org/10.1186/1471-2148-8-319

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

Immler, S., & Birkhead, T. R. (2007). Sperm competition and sperm midpiece size: no consistent pattern in passerine birds. *Proceedings. Biological sciences*, *274*, 561–568. https://doi.org/10.1098/rspb.2006.3752

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

Immler, S., Pitnick, S., Parker, G. A., Durrant, K. L., Lüpold, 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*, 5325–5330. https://doi.org/10.1073/pnas.1009059108

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*, 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*, 494–499. https://doi.org/10.1111/j.1558-5646.2007.00287.x

Lüpold, S., Calhim, S., Immler, S., & Birkhead, T. R. (2009). Sperm morphology and sperm velocity in passerine birds. *Proc. Biol. Sci.*, *276*, 1175–1181. https://doi.org/10.1098/rspb.2008.1645

Lüpold, S., & Fitzpatrick, J. L. (2015). Sperm number trumps sperm size in mammalian ejaculate evolution. *Proceedings of the Royal Society B-Biological Sciences*, *282*, 20152122. https://doi.org/10.1098/rspb.2015.2122

Lüpold, S., Manier, M. K., Berben, K. S., Smith, K. J., Daley, B. D., Buckley, S. H., … Pitnick, S. (2012). How multivariate ejaculate traits determine competitive fertilization success in *Drosophila melanogaster*. *Current Biology*, *22*, 1667–1672. https://doi.org/10.1016/j.cub.2012.06.059

Lüpold, S., & Pitnick, S. (2018). Sperm form and function: What do we know about the role of sexual selection? *Reproduction*, *155*, R229–R243. https://doi.org/10.1530/REP-17-0536

Lüpold, S., Wistuba, J., Damm, O. S., Rivers, J. W., & Birkhead, T. R. (2011). Sperm competition leads to functional adaptations in avian testes to maximize sperm quantity and quality. *Reproduction*, *141*, 595–605.

Malo, A. F., Gomendio, M., Garde, J., Lang-Lenton, B., Soler, A. J., & Roldan, E. R. S. (2006). Sperm design and sperm function. *Biology Letters*, *2*, 246–249. https://doi.org/10.1098/rsbl.2006.0449

Miller, G. T., & Pitnick, S. (2002). Sperm-female coevolution in *Drosophila*. *Science*, *298*, 1230–1233. https://doi.org/10.1126/science.1076968

Mundry, R. (2014). Statistical issues and assumptions of phylogenetic generalized least squares. In L. Garamszegi (Eds.), *Modern phylogenetic comparative methods and their application in evolutionary biology* (pp. 131–153). Berlin, Germany: Springer.https://doi.org/10.1007/978-3-662-43550-2

Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence intervals and statistical significance: a practical guide for biologists. *Biological Reviews*, *82*, 591–605. https://doi.org/10.1111/j.1469-185X.2007.00027.x

Orr, T. J., & Brennan, P. L. R. (2015). Sperm storage: Distinguishing selective processes and evaluating criteria. *Trends in Ecology & Evolution*, *30*, 261–272. https://doi.org/10.1016/j.tree.2015.03.006

Orr, T. J., & Zuk, M. (2014). Reproductive delays in mammals: An unexplored avenue for post-copulatory sexual selection. *Biological Reviews*, *89*, 889–912. https://doi.org/10.1111/brv.12085

Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, *401*, 877–884. https://doi.org/10.1038/44766

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

Parker, G. A. (1982). Why are there so many tiny sperm ? Sperm competition and the maintenance of two sexes. *Journal of Theoretical Biology*, *96*, 281–294. https://doi.org/10.1016/0022-5193(82)90225-9

Parker, G. A. (1993). Sperm competition games: Sperm size and sperm number under adult control. *Proceedings of the Royal Society B-Biological Sciences*, *253*, 245–254.

Parker, G. A. (1998). Sperm competition and the evolution of ejaculates: Towards a theory base. In T. R. Birkhead, & A. P. Mèller (Eds.), *Sperm competition and sexual selection* (pp. 3–54). London, UK: Academic Press. https://doi.org/10.1016/B978-012100543-6/50026-X

Pattarini, J. M., Starmer, W. T., Bjork, A., & Pitnick, S. (2006). Mechanisms underlying the sperm quality advantage in *Drosophila melanogaster*. *Evolution*, *60*, 2064–2080. https://doi.org/10.1111/j.0014-3820.2006.tb01844.x

Pitnick, S., Hosken, D. J., & Birkhead, T. R. (Eds.) (2009). Sperm morphological diversity. In *Sperm biology* (pp. 69–149). New York, NY: Academic Press.https://doi.org/10.1016/B978-0-12-372568-4.00003-3

Pitnick, S., Markow, T., & Spicer, G. S. (1999). Evolution of Multiple Kinds of Female Sperm-Storage Organs in *Drosophila*. *Evolution*, *53*, 1804. https://doi.org/10.1111/j.1558-5646.1999.tb04564.x

Pizzari, T., & Parker, G. A. (2009). Sperm competition and sperm phenotype. In T. R. Birkhead, D. J, Hosken, & S. Pitnick (Eds.), *Sperm biology: An evolutionary perspective* (pp. 207–245). New York, NY: Academic Press.https://doi.org/10.1016/B978-0-12-372568-4.00006-9

Pratt, H. L. (1993). The storage of spermatozoa in the oviducal glands of western North Atlantic sharks. *Environmental Biology of Fishes*, *14*, 139–149.

R Core Team (2017). *R: A language and environment for statistical computing. R foundation for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rasband, W. (1997). *ImageJ*. Bethesda, MD: National Institutes of Health.

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Rowley, A., Daly-Engel, T. S., & Fitzpatrick, J. L. (2019). Testes size increases with sperm competition risk and intensity in bony fish and sharks. *Behavioral Ecology*, *30*, 364–371. https://doi.org/10.1093/beheco/ary174

Simmons, L. W., & Fitzpatrick, J. L. (2012). Sperm wars and the evolution of male fertility. *Reproduction*, *144*, 519–534. https://doi.org/10.1530/REP-12-0285

Simpson, J. L., Humphries, S., Evans, J. P., Simmons, L. W., & Fitzpatrick, J. L. (2014). Relationships between sperm length and speed differ among three internally and three externally fertilizing species. *Evolution*, *68*, 92–104. https://doi.org/10.1111/evo.12199

Stein, R. W., Mull, C. G., Kuhn, T. S., Aschliman, N. C., Davidson, L. N. K., Joy, J. B., … Mooers, A. O. (2018). Global priorities for conserving the evolutionary history of sharks, rays and chimaeras. *Nature Ecology and Evolution*, *2*, 288–298. https://doi.org/10.1038/s41559-017-0448-4

Tomkins, J. L., & Simmons, L. W. (2002). Measuring relative investment: a case study of testes investment in species with alternative male reproductive tactics. *Animal Behavior*, *63*, 1009–1016. https://doi.org/10.1006/anbe.2001.1994

Varea-Sánchez, M., Montoto, L. G., Tourmente, M., & Roldan, E. R. S. (2014). Postcopulatory sexual selection results in spermatozoa with more uniform head and flagellum sizes in rodents. *PLoS ONE*, *9*, e108148. https://doi.org/10.1371/journal.pone.0108148

Whitney, N. M., Pratt, H. L., & Carrier, J. C. (2004). Group courtship, mating behaviour and siphon sac function in the whitetip reef shark, *Triaenodon obesus*. *Animal Behavior*, *68*, 1435–1442. https://doi.org/10.1016/j.anbehav.2004.02.018

SUPPORTING INFORMATION

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How to cite this article: Rowley A, Locatello L, Kahrl A, et al. Sexual selection and the evolution of sperm morphology in sharks. *J Evol Biol*. 2019;00:1–9. https://doi.org/10.1111/jeb.13501