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Pre- and post-copulatory traits working in concert: sexual dichromatism in passerines is associated with sperm morphology

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Passerine birds produce costly traits under intense sexual selection, including elaborate sexually dichromatic plumage and sperm morphologies, to compete for fertilizations. Plumage and sperm traits vary markedly among species, but it is unknown if this reflects a trade-off between pre- and post-copulatory investment under strong sexual selection producing negative trait covariance, or variation in the strength of sexual selection among species producing positive covariance. Using phylogenetic regression, we analysed datasets describing plumage and sperm morphological traits for 278 passerine species. We found a significant positive relationship between sperm midpiece length and male plumage elaboration and sexual dichromatism. We did not find a relationship between plumage elaboration and testes mass. Our results do not support a trade-off between plumage and sperm traits, but may be indicative of variance among species in the strength of sexual selection to produce both brightly coloured plumage and costly sperm traits.

1. Introduction

Observations of avian sexual dichromatism were fundamental to Darwin's [1] theory of sexual selection. Colourful, elaborate male plumage provides a classic example. Evidence of costly signalling of quality via elaborate plumage provided a mechanism for female choice to drive plumage evolution [2]. Across species, elaborately coloured males are thought to be sexually selected by females, even though other forms of natural selection might favour crypsis and/or efficient thermoregulation in both sexes [3,4]. Sexual selection occurs in two phases, pre- and post-copulation; each phase may require different traits to allow a male to compete successfully. While plumage traits experience pre-copulatory sexual selection, sperm and ejaculate traits evolve under post-copulatory sperm competition for fertilizations [5,6].

Under intense sperm competition, multiple costly traits evolve, such as larger testes [7], longer or more numerous sperm [8] or specialized ejaculate components [9]. Spermatozoa are highly morphologically variable [10]. Responses to differing intensities of sexual selection are observed in sperm morphology, especially in passerines [11,12]. Across taxa, morphometric traits such as total sperm length, midpiece length and/or volume and tail length appear to respond strongly to post-copulatory selection, with highly competitive environments giving rise to the longest or largest traits, while sperm head length is largely unaffected [11,13,14] but see [15]. Longer sperm may be more successful because they reach the egg first, although across passerines the relationship between sperm length and swimming speed is unclear [16,17].

Two studies have shown that passerine species with the most elaborate and sexually dichromatic male plumage are generally under strong sexual selection pressure [3,4]. Exceptions to this pattern come from species with tropical life histories (breeding in the tropics, low seasonality, small clutches), where females are brightly coloured [3]. The intensity of sexual selection estimations came from the observed social mating system and relative testes size [4], and social mating system, sexual size dimorphism and paternal care [3]. While these studies described the evolutionary consequences of pre-copulatory sexual selection for plumage, neither considered whether these responses were related to traits influencing sperm competition.

Sperm competition theory predicts a trade-off within species between pre-copulatory mating expenditure and post-copulatory ejaculate expenditure [6,18]. In various taxa, many studies have demonstrated a negative relationship [19–21], but others found a positive relationship [22–24]. A positive relationship has been taken as evidence for the phenotype-linked fertility hypothesis [25] whereby condition-dependent male quality is expressed by investment in both secondary sexual characters and ejaculate traits.

Trade-offs *within* species do not necessarily lead to negative relationships between traits *among* species. If sexual trait variation among species is caused mostly by changes in the strength of sexual selection, positive correlations between traits under pre- and post-copulatory sexual selection could arise [26]. However, while diverse taxonomic groups show positive correlations [27], others show no correlation, or a negative relationship, suggestive of among-species trade-offs [28–31]. A key predictor of among-species relationships appears to be the ability of males to monopolize females: as the frequency of monopolization increases, the relationship between the two types of trait shifts from positive or neutral to negative [27]. However, the degree of monopolization is difficult to measure for wild passerines, unlike other bird groups (e.g. phasianids).

The investment relationship between passerine pre- and post-copulatory traits is unclear. There are few large-scale studies across passerine species, but Australasian wrens (Maluridae) demonstrated significant positive correlations between song traits and testes mass [32]. Since passerines probably include species where males can monopolize females and those where they cannot, patterns of trait investment should depend on local sexual selection intensity and monopolization opportunities.

Extensive datasets on sperm traits [8,33] and sexual dichromatism [3,4] make it possible to analyse the relationship between plumage and sperm morphology traits under differing intensities of sexual selection across species. We analysed these data to answer the question: do species with the most elaborate and/or most sexually dichromatic male plumage also show signs of investment in competitive sperm morphology, or is there a trade-off across species?

2. Material and methods

A sperm morphometric dataset for 278 passerine species was created from published (232 species [8,33]) and unpublished data (46 species, K. Durrant). Measurements (micrometer) of four sperm traits were used: total sperm length, head length, midpiece length and tail length (see electronic supplementary material). Potentially, the tail length indicates swimming speed [16] but see [17], the head length indicates fertilizing capacity [10] and midpiece length indicates available mitochondria energy [34]. Testes mass data were obtained for 232 species [8,33].

Plumage data derived from two studies [3,4] (see electronic supplementary material). Dale *et al.*'s [3] data included 278 species for which we had sperm data. These provided a measure of plumage 'maleness', where males are expected to have more elaborately colourful plumage that is very different from females. We analysed both raw plumage scores for both sexes (henceforth 'elaboration'), and sex differences in scores (henceforth 'dichromatism'). Because plumage coloration is a complex trait characterized in many ways, we considered an alternative dataset [4], including 153 species for which we had sperm data. Dunn *et al.* [4] assessed dichromatism by calculating the difference in brightness and hue between spectrophotometric measures of male and female feathers. Here, we consider both the signed and un-signed (modulus) values of this colour difference. We also used body size and tropical life-history data from Dale *et al.* [3]. Full methods are in the electronic supplementary material.

To meet the assumption of normality, sperm tail length and testes mass were $\log_{10}$-transformed, while midpiece length was square-root transformed. Sperm head length and total length were not obviously skewed, and were not log transformed. Subsequently, all reproductive morphology traits were *z*-standardized, as were the plumage elaboration and dichromatism traits from Dale *et al.* [3]. To quantify associations between traits, while controlling for phylogeny, we used phylogenetic least-squares regression (PGLS) using multiple phylogenies for inference (details in electronic supplementary material). Reproductive morphology traits were response variables, with plumage elaboration and sexual dichromatism scores as predictors. While we think it more likely that selection on pre-copulatory traits drives selection on post-copulatory traits, alternative patterns of causation are possible [30], and hence partial correlation (which does not formally assume causality) might be suitable for the analysis. However, since partial regression *t*-values are effectively the same as partial correlation *t*-values (with only minor variation due to the phylogenetic structure of the model residuals), the qualitative conclusions are unaffected. Body size and tropical life-history score were included as covariates, because both strongly influence plumage coloration and potentially other sexually selected traits [3].

Relationships between sperm traits and other sexually selected traits may be nonlinear [12]. Consequently, we used likelihood-ratio tests to examine whether models that also included a quadratic predictor term for the plumage trait provided a better fit. In no case was the polynomial model a significantly better fit, consequently, we present the results only from the simpler linear models.

3. Results

Passerine bird species with more elaborate and dichromatic male plumage had longer sperm with longer midpieces. Averaging across 100 PGLS models, using 278 species [3], plumage elaboration in males, and dichromatism, were significantly positively associated with sperm midpiece and total sperm length (table 1; figure 1). Other results indicate that species with more elaborate female plumage had significantly longer sperm heads. No measures of plumage elaboration were significantly associated with testes mass, although plumage dichromatism shows a positive tendency ($p = 0.064$).

Covariates previously reported to be associated with plumage elaboration showed significant relationships with sperm and testes traits in most of our models for Dale *et al.*'s [3] data (table 1). Body size was negatively associated with midpiece length, head length and total sperm length,

Table 1. PGLS estimates of relationships between three different passerine plumage characteristics and sperm traits. Covariates: body size and tropical life history. s.e., standard error. Significant results in italics.

response variable	coefficient	male plumage elaboration			female plumage elaboration			sex difference in elaboration		
		estimate (s.e.)	t	p	estimate (s.e.)	t	p	estimate (s.e.)	t	p
		$R^2 = 7.40\%; \lambda = 0.949$			$R^2 = 5.14\%; \lambda = 0.947$			$R^2 = 8.21\%; \lambda = 0.954$		
midpiece length	plumage characteristic	0.107 (0.038)	2.813	*0.005*	−0.029 (0.029)	−1.026	0.306	0.100 (0.030)	3.309	*0.001*
$n = 278$	body size	−0.181 (0.062)	−2.934	*0.004*	−0.144 (0.062)	−2.327	*0.021*	−0.153 (0.060)	−2.538	*0.012*
	tropical life history	−0.131 (0.042)	−3.150	*0.002*	−0.105 (0.043)	−2.436	*0.016*	−0.100 (0.041)	−2.432	*0.016*
	intercept	−1.285 (0.428)	−3.007	*0.003*	−1.282 (0.432)	−2.967	*0.003*	−1.259 (0.433)	−2.907	*0.004*
		$R^2 = 5.60\%; \lambda = 0.868$			$R^2 = 5.76\%; \lambda = 0.872$			$R^2 = 4.42\%; \lambda = 0.860$		
head length	plumage characteristic	0.111 (0.059)	1.886	0.060	0.095 (0.047)	2.024	*0.044*	0.009 (0.050)	0.175	0.861
$n = 278$	body size	−0.256 (0.091)	−2.800	*0.006*	−0.251 (0.091)	−2.759	*0.006*	−0.226 (0.090)	−2.509	*0.013*
	tropical life history	−0.183 (0.066)	−2.758	*0.006*	−0.194 (0.067)	−2.895	*0.004*	−0.165 (0.066)	−2.490	*0.013*
	intercept	0.291 (0.550)	0.529	0.597	0.279 (0.554)	0.504	0.615	0.293 (0.545)	0.536	0.592
		$R^2 = 1.65\%; \lambda = 0.901$			$R^2 = 1.31\%; \lambda = 0.895$			$R^2 = 1.70\%; \lambda = 0.899$		
tail length	plumage characteristic	−0.045 (0.045)	−1.020	0.309	0.007 (0.035)	0.212	0.832	−0.040 (0.037)	−1.074	0.284
$n = 278$	body size	0.134 (0.070)	1.918	0.056	0.121 (0.070)	1.738	0.083	0.125 (0.069)	1.817	0.070
	tropical life history	0.034 (0.050)	0.683	0.495	0.025 (0.051)	0.489	0.625	0.023 (0.049)	0.456	0.649
	intercept	1.074 (0.442)	2.431	*0.016*	1.075 (0.438)	2.455	*0.015*	1.070 (0.441)	2.427	*0.016*
		$R^2 = 7.75\%; \lambda = 0.944$			$R^2 = 5.32\%; \lambda = 0.942$			$R^2 = 8.06\%; \lambda = 0.948$		
total sperm length	plumage characteristic	0.120 (0.043)	2.806	*0.005*	−0.023 (0.033)	−0.706	0.481	0.104 (0.034)	3.032	*0.003*
$n = 278$	body size	−0.204 (0.070)	−2.930	*0.004*	−0.165 (0.070)	−2.364	*0.019*	−0.174 (0.068)	−2.548	*0.011*
	tropical life history	−0.158 (0.047)	−3.345	*0.001*	−0.133 (0.049)	−2.708	*0.007*	−0.125 (0.047)	−2.674	*0.008*
	intercept	−1.017 (0.478)	−2.126	*0.034*	−1.016 (0.482)	−2.106	*0.036*	−0.990 (0.483)	−2.050	*0.041*
		$R^2 = 32.15\%; \lambda = 0.498$			$R^2 = 32.02\%; \lambda = 0.480$			$R^2 = 32.78\%; \lambda = 0.488$		
testes mass	plumage characteristic	0.079 (0.057)	1.389	0.166	−0.030 (0.051)	−0.594	0.553	0.094 (0.051)	1.863	0.064
$n = 232$	body size	0.700 (0.075)	9.329	*<0.001*	0.731 (0.074)	9.818	*<0.001*	0.716 (0.073)	9.816	*<0.001*
	tropical life history	−0.263 (0.065)	−4.052	*<0.001*	−0.240 (0.065)	−3.680	<0.001	−0.247 (0.064)	−3.877	*<0.001*
	intercept	−0.192 (0.293)	−0.657	0.512	−0.183 (0.287)	−0.639	0.524	−0.173 (0.288)	−0.601	0.548

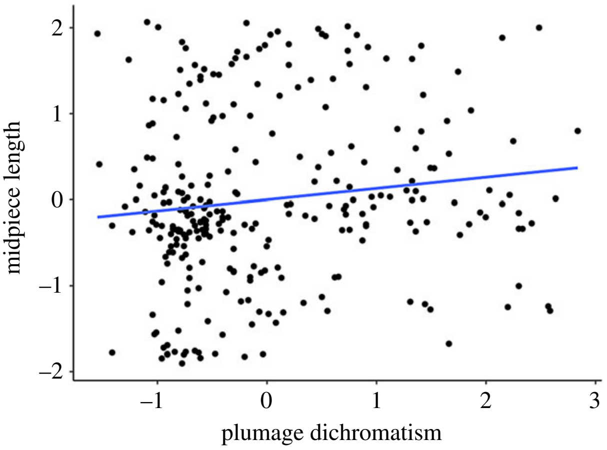

Figure 1. Relationship between sperm midpiece length and plumage dichromatism (*z*-standardized), with best-fit linear regression line. (Online version in colour.)

and positively associated with testes mass. Tropical species had shorter sperm and smaller testes: tropical life history was significantly negatively associated with midpiece length, head length, total sperm length and testes mass. All models had relatively low predictive power, explaining less than 9% of the variance, except for testes mass ($R^2 \approx 32\%$).

PGLS models of alternative plumage data ($n = 153$; [4]) did not show any significant relationships with sperm or testes traits (electronic supplementary material, table S1). The only significant predictor was body size, which was negatively associated with measures of sperm length, and positively associated with testes mass. The models had low predictive power ($R^2 < 12\%$), except for testes mass ($R^2 \approx 45\%$). This analysis included fewer species and had lower statistical power than that derived from Dale *et al.* [3]. When we re-fitted the first set of models, using Dale *et al.*'s [3] data for only the 153 species considered by Dunn *et al.* [4], the significant relationships with sperm traits disappeared (electronic supplementary material, table S2).

4. Discussion

We found a significant relationship between sexual dichromatism and the length of the sperm midpiece in passerines: increasing male-biased dichromatism is generally associated with greater midpiece length. If having a longer midpiece means that sperm are more competitive and successful at fertilization [35], this suggests that the dominant pattern of investment in pre- and post-copulatory sexual traits is not a trade-off. Instead, the result suggests that the interspecific strength of sexual selection varies markedly, and species under strong sexual selection invest more in both pre- and post-copulatory traits.

The assumption that midpiece length is a costly trait that conveys post-copulatory advantages lacks direct evidence, and our data are consistent with a lack of cost. However, considerable indirect evidence exists: longer sperm are generally produced under intense sperm competition and are more successful at fertilizing ova [36]. Longer sperm may swim at higher speeds [16] but see [17], leading to greater paternity-share in zebra finches [36]. Longer sperm are made up of longer flagella and midpieces [12]. In most passerines, longer midpieces have a single mitochondrion wound around the flagellum [37,38]. Longer midpieces produce more energy via adenosine triphosphate (ATP) [39], but see [40], which is correlated with increased sperm mobility and fertilizing capacity in Galliformes [41,42], although this relationship is unknown for passerines.

In passerines, although sperm ATP concentration is not associated with faster swimming or rates of extra-pair paternity [39], it may be associated with sperm longevity. If females store sperm, as most birds do [43], spermatozoa that can remain both viable and motile over time should have a fertilizing advantage. Stored sperm reduce ATP consumption but still require it upon release [44]. A comparative study of passerine sperm length, swimming speed, rate of extra-pair paternity and clutch size (used to estimate female sperm storage duration) found there was no relationship between sperm speed and morphology, and a positive relationship between sperm length and extra-pair paternity rates, but no relationship with clutch size [17]. However, clutch size is only weakly positively correlated with sperm storage duration [45]. Mechanisms of sperm storage are not fully understood [44,46] and improved measures or proxies for female sperm storage capacity across passerines are needed before conclusions are drawn.

Tropical life history was negatively associated with midpiece length and testes mass, indicative of reduced sperm competition. Lower levels of sperm competition have been suggested for tropical-zone species [47,48], but see [49]. Body size was positively associated with testes mass, probably reflecting allometric scaling [7]. However, there was no equivalent relationship between body size and sperm size: body size was negatively associated with midpiece and head length. This is consistent with other studies of vertebrates that have failed to find positive relationships (e.g.: mammals [13]; birds [11]; frogs [14]).

Interestingly, there was no correlation between sexual dichromatism and testes mass. Dunn *et al.* [4] found an association between testes mass and dichromatism in hue, but not in brightness, while there were no significant associations between any sperm traits and these measures of dichromatism or testes mass in our analysis. However, Dunn *et al.*'s [4] large sample size ($n = 977$), and high statistical power meant they were able to detect a relatively weak correlation. This relationship may be weak due to the competing ecological demands on plumage for female crypsis.

There are many ways of measuring plumage colour and dichromatism, all subject to error, and caution is required in interpreting the results of analyses of these data. We found correlations, but in contrasting ways, between Dale *et al.*'s [3] and Dunn *et al.*'s [4] measures of sexual dichromatism for the species in our study. Dale *et al.*'s [3] measure of dichromatism had a positive correlation of ($r = 0.412$, d.f. = 151, $p < 0.001$) with Dunn *et al.*'s [4] measure of hue dichromatism, but no correlation ($r = -0.065$, d.f. = 151, $p = 0.424$), with their measure of brightness dichromatism. These measures actually estimate subtly distinct aspects of dichromatism; plumage elaboration in the case of Dale *et al.* [3], and plumage coloration in the case of Dunn *et al.* [4]. The datasets were produced by different methods: digital scoring of illustrations [3], and spectral reflectance measurements of museum specimens [4]. The lack of correlation implies that methodological differences have significant effects on the biological meaning, and/or measurement error, of resulting variables.

The evolution of elaborate male plumage appears to be driven by intense pre-copulatory sexual selection. When we examined plumage in the context of post-copulatory selection, the strongest relationship was with sperm midpiece size. Understanding the function of enlarged midpieces requires investigation of the cost of producing midpieces and their role in fertilizing capacity. The observed relationship may reflect ecologically driven variation in the strength of sexual selection across taxa, with species under intense sexual selection investing heavily both in pre- and post-copulatory traits, while species under weaker sexual selection invest less in sexually selected traits. If resources are invested along an 'allocation tree' [50], any trade-off between different sexually selected traits could be masked by the higher-level trade-off between sexually selected traits and non-sexually selected traits. A separate point is that data are lacking on the ability of wild passerine males to monopolize females. Other studies have suggested that trade-offs between pre- and post-copulatory traits are most likely to arise in taxa where males can monopolize [27]; hence, if monopolization is generally rare in passerines, we might not expect a trade-off anyway. A greater understanding of the evolutionary interplay between pre- and post-copulatory traits is required to improve understanding of the ability of sexual selection to influence phenotypes.

Data accessibility. Data are provided as electronic supplementary material.

Authors' contributions. K.L.D. and T.R. conceived the study and K.L.D. drafted the manuscript, M.R.E.S. performed the analysis and all authors interpreted the analysis and contributed to the final version of the paper. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

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