ORIGINAL ARTICLE

Sperm variation in Great Tit males (*Parus major*) is linked to a haematological health-related trait, but not ornamentation

Jana Svobodová[1] · Petra Bauerová[1] · Jiří Eliáš[1] · Hana Velová[2] · Michal Vinkler[2] · Tomáš Albrecht[2,3]

Abstract

The phenotype-linked fertility hypothesis (PLFH) proposes that both sexual ornaments and sperm traits are phenotypically plastic and co-affected by environmental factors through individual condition, resulting in a positive correlation between ornament expression and functional fertility. Ornaments may then serve females in the identification of the most fertile males. Despite intense research on the relationship between sexual characters and male ejaculate quality, published results are not consistent with the PLFH. The aim of our study was to test if sperm morphology is associated with sexual ornamentation and several health/condition-dependent traits in Great Tit males (*Parus major*). We evaluated the association between sperm morphology and two types of ornaments, carotenoid- and melanin-based ventral feather coloration, to evaluate predictions of the PLFH. As surrogates for condition and health/stress status, we used standardized male weight and the peripheral blood heterophil to lymphocyte ratio (H/L). Also, we used the immature erythrocyte frequency as a trait linked to the rate of haematopoiesis, and presumably metabolism and pace of life. Our results support an association of sperm traits with health-related traits: the within-male variability in total sperm length was negatively related to the H/L ratio. This either suggests that birds maintaining low sperm variability may afford to invest more into heterophil production or, in contrast to the PLFH, there could be a trade-off between individual investment in reproduction (ejaculate quality) and the avoidance of long-term physiological stress. Contrary to the predictions of the PLFH we were unable to identify any parameter of sperm morphology associated with either body condition or the expression of male sexual traits. Thus, our study contributes to evidence rejecting the hypothesis of ornamental involvement in fertility selection, while giving weak support to the sperm competition theory.

Keywords Carotenoid coloration · Melanin coloration · Condition-dependent sexual signalling · Haematology · Sperm flagellum · Sperm length

Zusammenfassung

Bei Kohlmeisenmännchen (*Parus major*) ist die Spermienvariabilität mit einem gesundheitsrelevanten hämatologischen Merkmal gekoppelt, nicht jedoch mit der Färbung.

Die Hypothese der phänotypgekoppelten Fertilität (Phenotype-linked Fertility Hypothesis; PLFH) besagt, dass sowohl die geschlechtstypische Färbung als auch die Spermienmerkmale phänotypisch plastisch sind und über die individuelle Körperkondition von Umweltfaktoren mitbeeinflusst werden, was zu einer positiven Korrelation zwischen äußerlicher Merkmalsausprägung und funktioneller Fertilität führt. Eine Schmuckfärbung kann dann den Weibchen dabei helfen, die fruchtbarsten Männchen zu erkennen. Trotz intensiver Erforschung der Zusammenhänge zwischen Geschlechtsmerkmalen und der Qualität der männlichen Ejakulate lassen sich die publizierten Ergebnisse nicht mit der PLFH in Einklang bringen. Ziel unserer Studie war es zu testen, ob die Spermienmorphologie bei Kohlmeisenmännchen (*Parus major*) mit der geschlechtstypischen Färbung und verschiedenen vom Gesundheitszustand beziehungsweise der Kondition abhängigen

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Merkmalen in Verbindung steht. Um die Vorhersagen der PLFH zu überprüfen, betrachteten wir den Zusammenhang zwischen der Spermienmorphologie und zwei Typen der Schmuckfärbung, nämlich der carotinoid- beziehungsweise melaninbasierten Färbung des ventralen Gefieders. Stellvertretend für die Kondition und den Gesundheits-/Stress-Status verwendeten wir das standardisierte Körpergewicht der Männchen und deren H/L-Verhältnis (Heterophile Granulozyten/Lymphozyten) im peripheren Blut. Außerdem nutzten wir die Häufigkeit unreifer Erythrozyten als Merkmal für das Ausmaß der Blutneubildung sowie vermutlich für die Stoffwechselrate und das Lebenstempo. Unsere Ergebnisse sprechen für einen Zusammenhang zwischen Spermieneigenschaften und gesundheitsbezogenen Merkmalen: Die Variabilität der Spermien-Gesamtlänge bei einem Männchen stand jeweils in einer negativen Beziehung zum H/L-Verhältnis. Entweder bedeutet dies, dass es sich Vögel mit einer geringen Spermienvariabilität leisten können, mehr in die Produktion heterophiler Granulozyten zu investieren, oder es könnte sich—im Widerspruch zur PLFH—um einen Kompromiss zwischen der individuellen Investition in die Fortpflanzung (Qualität des Ejakulats) und der Vermeidung langfristigen physiologischen Stresses handeln. Im Gegensatz zu den Voraussagen der PLFH konnten wir keinen Parameter der Spermienmorphologie ausmachen, welcher entweder mit der Körperkondition oder der Ausprägung männlicher Geschlechtsmerkmale im Zusammenhang stünde. Somit liefert unsere Studie einen weiteren Beleg für eine Widerlegung der Hypothese von der Beteiligung der Schmuckfärbung an der Fertilitätsselektion und unterstützt dagegen leicht die Theorie der Spermienkonkurrenz (Sperm Competition Theory; SCT).

Introduction

Health and the expression of secondary sexual traits both depend on an individual's condition. Only individuals in superior condition may be able to maintain the high expression of secondary sexual traits and produce high-quality gametes [the viability indicator hypothesis (Andersson 1994; Hill 2011)]. For example, the phenotype-linked fertility hypothesis (PLFH) proposes that the expression of secondary sexual traits reflects a male's reproductive performance, including ejaculate quality (Sheldon 1994), because pre- and post-copulatory traits (ornamentation and gametes) may share condition dependence [e.g. are affected by the same type of stressors (Blount et al. 2001)]. By selecting highly ornamented males, females may increase their chances of being successfully fertilized or even of having their sons inherit high-quality ejaculates (Pizzari and Birkhead 2002; Pizzari et al. 2004), leading to the evolution of pre-copulatory signals of sperm quality. However, a recent meta-analysis using a wide array of taxa found little support for a positive association between the expression of condition-dependent sexual signals and ejaculate quality (Mautz et al. 2013). An alternative scenario, originally outlined as a part of the sperm competition theory (SCT) (Parker 1998), assumes that the total amount of energy invested into reproduction is limited, resulting in a trade-off between investments into pre-copulatory and post-copulatory traits, and hence to a negative relationship between sexual male ornamentation and sperm quality. In this case, investments into costly sexual ornaments may compromise ejaculate quality [e.g. the ability to cope with oxidative challenge (Tomášek et al. 2017)].

Carotenoid-based ornaments may reflect individual quality because carotenoids are obtained exclusively from the diet and have been proposed to take part in anti-oxidative protection (Lozano 1994; von Schantz et al. 1999; Svensson and Wong 2011). In birds, carotenoids are responsible for the yellow to red coloration of skin derivatives (e.g. feathers, podotheca, rhamphotheca, etc.). Given the fact that carotenoids also occur in the testes and seminal fluid (Rowe and McGraw 2008; Rowe et al. 2012), they can directly influence sperm quality and quantity by quenching free radicals (von Schantz et al. 1999; Blount et al. 2001). For instance, their supplementation resulted in fewer sperm abnormalities in Zebra Finches (Tomášek et al. 2017). Although the PLFH may apply to carotenoid-based ornaments in birds (e.g. Helfenstein et al. 2010; Losdat et al. 2011), there might exist a trade-off between the use of carotenoids in maintaining either the high expression of sexual ornamentation or high sperm quality (Tomášek et al. 2017).

Recent studies have shown that similarly to carotenoid-based traits, melanin-based ornaments are frequently related to individual condition (Griffith et al. 2006; Guindre-Parker and Love 2014) and immune function (e.g. Gangoso et al. 2011; Jacquin et al. 2011). Therefore, the PLFH may apply to melanin ornaments as well. At present only a few studies have examined the relationship between sperm characteristics and melanin ornaments, and their results are inconsistent. For example, Calhim et al. (2009) found a positive relationship between sperm length and plumage blackness in Pied Flycatchers (*Ficedula hypoleuca*), whereas Lifjeld et al. (2012) found no such relationship in the same study population but with a much larger dataset. Similarly, Birkhead and Fletcher (1995) found no link between any sperm traits and melanin breast plumage area in Zebra Finches (*Taeniopygia guttata*).

Here we used Great Tits (*Parus major*) to evaluate the idea that sexually selected condition-dependent

ornamentation in males (carotenoid-based coloration of ventral feathers and the size of the melanin-based black stripe on the belly) reflects sperm phenotypes. Moreover, we evaluated possible links between sperm phenotypes and individual condition and health status. As general estimators of condition and health status we used standardized weight and two haematological parameters, the stress-linked peripheral blood heterophil to lymphocyte ratio (H/L) (Davis et al. 2008) and the immature erythrocyte frequency. The latter is positively linked to the rate of haematopoiesis, and presumably metabolism and the pace of life (Vinkler et al. 2010). Failures in spermatogenesis may lead to the production of morphologically diverse sperm including abnormal sperm cells (Opatová et al. 2016). We therefore calculated the coefficient of variation in sperm length for each ejaculate and tested this parameter with sexual ornamentation and condition estimates. Previous studies across passerine taxa have shown that increased sperm competition leads to longer sperm cells with a relatively longer midpiece (Kleven et al. 2009), and intra-specific analyses, based on experimental approach, have also demonstrated that longer sperm cells are faster and competitively superior than shorter sperm at least in some passerines (Bennison et al. 2015). We therefore assume that total sperm length and the midpiece/flagellum ratio may reflect ejaculate quality in our study species (also see Calhim et al. 2009; Mautz et al. 2013).

Methods

Field procedures

The study was carried out in the breeding seasons April–May 2011–2013, in a city forest in Prague (Czech Republic 50°8′10.591″N, 14°27′51.144″E, ~310 m a.s.l.). The forest was mainly dominated by oak (*Quercus* sp.), lime (*Tilia* sp.) and hornbeam (*Carpinus betulus*). Within the study area nest boxes were installed in a regular grid of 50×50 m on trees at a height of ~3 m. Nest boxes were regularly controlled during April to identify Great Tit breeding pairs. In total, 42 males were caught during the three breeding seasons with mist nets placed in front of their nest boxes.

Approximately 50 µl of blood was taken from the jugular vein of each individual, and a blood smear was prepared from a drop of blood. Then, body mass (measured by a Pesola PPS200, 200 g, $d = 0.02$ g) and tarsus length (measured by a digital calliper, accuracy 0.01 mm; Kinex, Prague, Czech Republic) were recorded. Two feather ornaments were chosen for further analyses: the area of the melanin breast stripe and coloration of the yellow breast carotenoid. To assess the area of the melanin breast stripe, standard digital images of the breast were taken of each male with a Perfection V30 scanner (Seiko Epson, Nagano, Japan). All images

were taken in a standardised position in a mobile dark tent, with grey and colour standard reference swatches equipped with a ruler (a GC 18 grey card and Q 14 colour and grey chart; Danes-Picta, Prague). Feather samples from the upper part of the yellow breast ornament were collected for spectrometry analysis (see below). Ejaculates were obtained by a gentle cloacal massage and stored in 10% formalin solution (Albrecht et al. 2013). Finally, every male was tagged by an aluminium ring with a unique code of the Czech Bird Ringing Centre (N Museum Praha) and released immediately after sampling. No male underwent any repeated sampling throughout the entire experiment. The study was approved by the Ethical Committee of the Czech Academy of Sciences (041/2011) and was carried out in accordance with the current laws of the Czech Republic.

Measurements of ornamental traits

The digital images of the melanin breast stripe were analysed using Adobe PHOTOSHOP CS.4 software version 11.0 (Adobe Systems, San Jose, CA). First, the image scales were equalised according to the rulers. From these standardised images, areas of the ornaments were measured.

Coloration of the yellow breast ornament was measured by an AvaSpec 2048 spectrometer with an AvaLight-XE light source (Avantes, the Netherlands). Samples of each individual were randomly arranged into two sets from ten feathers and fixed on microscope slides with a black background (Quesada and Senar 2006). In addition, feather quills were fixed with a black sturdy paper inset to measure just the yellow part of feathers. The spectrophotometer probe was placed in a plastic extender holding the same light condition, angle (45°) and distance (4 mm) of the probe from a measured sample. To obtain reliable measurements, each feather set was rearranged and the whole process was repeated a total of four times for each set, i.e. eight measurements were taken for each male. The spectrometer was standardized against the darkroom and a WS-2 white standard after measuring samples representing each ten individuals. Yellow chroma, which reflects the amount of yellow carotenoids (lutein and zeaxanthin) in breast feathers in the Great Tit (Isaksson et al. 2008), was calculated as R700 minus R450, divided by R700, (R700 is reflectance at 700 nm and R450 reflectance at 450 nm). In statistical analyses, the average values calculated from the eight measurements were used. The repeatability of yellow chroma measurements was $r = 0.76$, $n = 33$, $p = 0.003$ (Lessels and Boag 1987).

Measurements of sperm traits

Sperm morphometric data were obtained using light microscopy (Olympus BX41, camera UI-1540-C: Olympus, Tokyo) under 400× magnification and image ImageJ software

version 1.49. Slides were prepared by spreading a drop (approximately 15 µl) of the fixed sperm sample on a clean microscope slide and air drying it. We measured (to the nearest 0.1 µm) the length of three sperm components: the head, midpiece (containing fused mitochondria) and tail (also see Opatová et al. 2016). For each sperm, the total length was then calculated as a sum of these three components. Twenty spermatozoa per male were measured to obtain a representative estimate of the male's mean sperm total length (e.g. Immler et al. 2008). Within-male variability in total sperm length (CV_{wm}) was calculated as the coefficient of variation [$CV = (SD/mean) \times 100$ (Sokal and Rohlf 1981; Lifjeld et al. 2010)]. The accuracy of sperm measurements was high ($r_{head} > 0.72$, $r_{midpiece} = 0.96$, $r_{tail} = 0.87$, $r_{total} = 0.96$, $n = 10$, all $p < 0.001$ for all measured components).

Haematological assays

To analyse the differential leukocyte count and frequency of immature erythrocytes, the air-dried blood smears were stained with Modified Wright-Giemsa Stain (product no. WG128; Sigma-Aldrich) and scanned with an Olympus BX-41 microscope under 1000× magnification to count the proportion of lymphocytes and heterophils within a sample of 110–140 leukocytes per smear (e.g. Vinkler et al. 2010). We found two individuals with extremely high H/L ratios (> 4) in our data set. Since individuals with an H/L ratio > 4 have also been found in other Great Tit populations (e.g. Ots and Hõrak 1996) these individuals were included in subsequent analyses. The differential count of immature erythrocytes was estimated in five randomly chosen monolayer fields photographed at 100× objective magnification (ca. 500–1000 cells). The photographs were used to manually count the immature erythrocytes in the images using ImageJ software. The repeatability of the estimate was $r = 0.89$, $n = 10$, $p = 0.05$.

Statistical analyses

We first performed Sperman's correlation between individual sperm traits. We found strong significant positive correlations between average total sperm length and all other sperm traits except sperm CV_{wm} (Table S1). Therefore, only average total sperm length, midpiece/flagellum ratio and sperm CV_{wm} were included in the analysis. There was no correlation between the expression of melanin-based and carotenoid-based ornaments ($r = 0.156$, $p = 0.322$). Standardized weight and haematological traits were also uncorrelated ($r_{H/L} = -0.197$, $p = 0.211$; $r_{imEry} = -0.115$, $p = 0.468$). The relationships between sperm morphology, condition, ornaments and health status were analysed by using linear regression models (LM) in which either total sperm length, midpiece/flagellum ratio or sperm CV_{wm} were response variables (i.e. three LM models). The sperm

CV_{wm} was ln-transformed and logit transformation was used for the midpiece/flagellum ratio to obtain a normal distribution. The standardised weight, yellow chroma, area of the melanin ornament, H/L ratio, immature erythrocyte count, year and their meaningful two-way interactions (i.e. those between year, condition and haematological parameters, respectively) were included as explanatory variables. Similarly, relationships between ornaments and condition status were analysed by LM models. The yellow chroma and area of the melanin breast stripe were response variables and the standardised weight, H/L ratio, immature erythrocyte count, year and their meaningful two-way interactions were explanatory variables, resulting in two LM models. The significance ($p < 0.05$) of particular terms in the models was calculated based on the change in deviance between the full and reduced (null) models using the drop1 function. The statistic is reported for each corresponding step when terms were removed from the model (Crawley 2002). All analyses were performed in the software R.3.0.3 (R Development Core Team 2011).

Results

There were no associations between either the coloration of the yellow ornament (yellow chroma) or the melanin-based ornamentation and the selected condition-reflecting or haematological traits (Table 1).

Data on sperm trait variation (length differences in the sperm segments) are summarised in Table 2. Sperm morphological traits varied substantially in Great Tit males, but the most variable sperm characteristic was the within-male variation in total sperm length (CV_{wm}) and midpiece/flagellum ratio.

There was no association between the average total sperm length and either the condition or haematological traits (Table 3). Although there was substantial variation in the midpiece/flagellum ratio, no significant relationship was found between this sperm trait and the tested variables. There was, however, a significant relationship between the sperm CV_{wm} and H/L ratio (LM, estimate $\pm$ SE $= -0.135 \pm 0.058$, $F_{1,40} = 5.487$, $p = 0.024$; Table 3). Males with higher CV_{wm} had a lower H/L ratio (Fig. 1). It should be noted that when two data points with high H/L ratios (> 4; Cook's $D > 1$) are removed from the dataset, the negative relationship between CV_{wm} and H/L is not significant (LM, estimate $\pm$ SE $= -0.128 \pm 0.106$, $F_{1,40} = 1.204$, $p = 0.237$).

Discussion

In this study we evaluated the idea that condition-dependent carotenoid- and melanin-based feather ornamentation reflects sperm quality in Great Tits. Previous studies in this species have demonstrated that the ejaculates of more

Table 1 Results of linear models evaluating the association between male ornamentation and selected condition/haematological traits

	Yellow chroma			Breast strip		
	df	F	p	df	F	p
H/L	40	0.115	0.725	39	1.800	0.188
Immature erythrocytes	39	0.298	0.588	40	3.059	0.088
Year	37	1.687	0.199	36	0.593	0.588
Standardised weight	36	0.209	0.651	38	0.015	0.902
H/L×Tear	34	1.127	0.336	34	0.123	0.884
Immature erythrocytes×Year	32	0.203	0.818	32	0.513	0.604
Standardised weight×Year	30	0.473	0.628	30	0.060	0.942

In addition to the peripheral blood heterophil to lymphocyte ratio (*H/L*), frequency of immature erythrocytes and standardized weight, year was also included in the analysis as a response (explanatory) variable. Statistics for particular explanatory variables were found using a backward stepwise procedure, and correspond to the step when each term was removed from the model ($n = 42$)

Table 2 Variation in sperm characteristics among a sample of ten Great Tit males' ejaculates ($n = 42$)

	Mean	SD	Median	Minimum	Maximum	CV
Head length (µm)	14.7	0.6	14.6	13.4	16.6	4.4
Midpiece length (µm)	59.2	2.4	59.6	52.6	64.3	4.1
Flagellum length (µm)	23.9	2.3	24.3	17.5	28.3	9.8
Total length (µm)	97.8	3.5	96.9	91.5	105.5	3.6
Midpiece/flagellum proportion	2.5	0.3	2.4	2.1	3.7	11.9
CV_{wm}	2.5	1.1	2.1	1.2	6.4	–[a]

CV Coefficient of variation, CV_{wm} within-male variation in sperm length

[a]Not calculated

Table 3 Results of linear models, with the yellow chroma, breast stripe area, H/L ratio, frequency of immature erythrocytes, standardized weight and year as response variables to explain the variation in selected sperm traits

	Total sperm length			CV_{wm}			Midpiece/flagellum proportion		
	df	F	p	df	F	p	df	F	p
H/L	40	2.629	0.113	40	5.487	0.024	39	0.362	0.551
Yellow chroma	38	0.520	0.476	38	1.768	0.192	40	1.703	0.199
Breast strip	37	0.129	0.721	37	0.291	0.593	36	0.031	0.862
Immature erythrocytes	39	2.796	0.103	39	0.311	0.580	38	0.029	0.865
Year	34	2.318	0.114	34	0.948	0.398	34	1.410	0.258
Standardised weight	36	0.692	0.411	36	0.038	0.847	37	1.355	0.251
H/L×year	28	1.330	0.281	28	1.956	0.397	28	2.750	0.081
Yellow chroma×year	30	0.775	0.47	30	0.721	0.494	30	0.261	0.771
Breast strip×year	36	0.485	0.621	26	0.453	0.641	32	0.156	0.856
Immature erythrocytes×year	32	1.267	0.296	32	1.714	0.196	25	0.054	0.818
Standardised weight×year	25	<0.001	0.986	25	0.087	0.771	26	1.450	0.253

Statistics for particular explanatory variable were found using a backward stepwise procedure. All statistics correspond to the step when they were removed from the model ($n = 42$). For other abbreviations, see Tables 1 and 2

colourful males are more resistant to oxidative stress, and hence that male carotenoid-based coloration indicates ejaculate quality measured as sperm velocity. In our study we focused on other sperm traits that potentially reflect ejaculate quality: the sperm length, midpiece/flagellum ratio and within-male variation in sperm length. We found little support for an association between sperm traits and male ornaments or condition-dependent traits.

The SCT predicts a trade-off between sexual signals and ejaculate investment if resources available for allocation
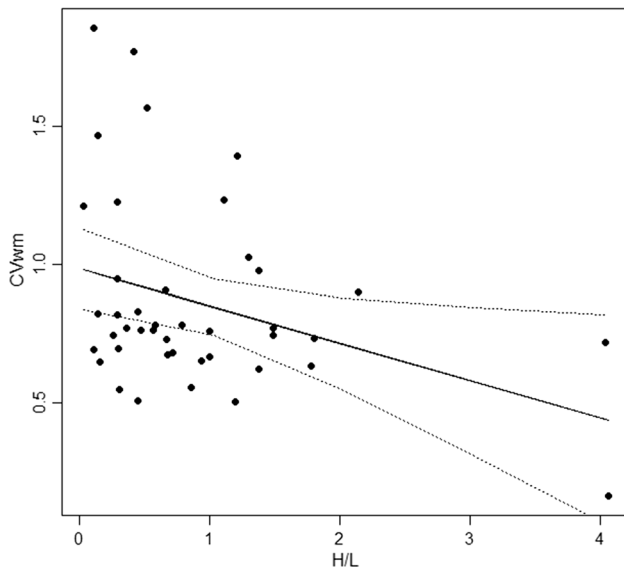
Fig. 1 Associations between the peripheral blood heterophil to lymphocyte ratio (H/L) and within-male variation in sperm length (CV_{wm}) in Great Tit males in 2011–2013 ($n=42$). The CV_{wm} is log transformed and *dashed lines* show the 95% confidence interval

to either trait are limited (Parker 1998). Interestingly, in accordance with this hypothesis we found that the within-male variability in sperm length [which may reflect variation in the size of the testicular seminiferous tubules (Aire 2007; Lüpold et al. 2009)] was negatively related to the H/L ratio, an indicator of general health and long-term stress (see e.g. Davis 2005; Davis et al. 2008). Alternatively, our findings could support the PLFH if males with enhanced H/L ratio were superior individuals defending nesting areas of high quality. Such males could be more stressed because they would be more involved in antagonistic interactions. However, since we found no correlation between the H/L ratio and standardised weight we are rather inclined to accept the SCT as a more likely explanation. The negative relationship between the within-male variability in sperm length and H/L ratio indicates that birds maintaining high ejaculate quality are not able to resist stress. Males that invest high energy into ejaculate quality may also not be able to invest into precopulatory signals of quality, such as carotenoid-based ornamentation (Tomášek et al. 2017). Similarly, immune activation of Great Tit males by lipopolysaccharide led to a reduction of sperm swimming velocity (Losdat et al. 2011) and males with enhanced H/L ratios produced longer spermatozoa in House Wrens (Cramer et al. 2012). The negative correlation between the H/L ratio and within-male variability in sperm length in our study may imply that the maintenance of proper spermatogenesis in Great Tit males, reflected by reduced within-ejaculate variation in sperm length, is only possible at some physiological costs reflected by an increased H/L ratio. Sperm competition in passerines

leads to a decrease in the sperm length variation within ejaculates (Kleven et al. 2008; Immler et al. 2008), possibly because selection favours optimal sperm length and acts against extremes. Male fertilization ability may to a large extent be determined by sperm numbers and hence the proportion of normal sperm in the ejaculate (Malo et al. 2005). Whether having less variable sperm is adaptive for Great Tits, a species with moderate levels of sperm competition (Griffith et al. 2002), remains unknown and requires further investigation. It should be noted that when two data points with extremely high H/L ratios (both > 4) were removed from the model, the negative relationship between CV_{wm} and H/L was not significant. It is worth noting, however, that we investigated sperm and condition traits of males in a free-living population, where it is difficult to catch enough males with a sufficient range of H/L ratios. Apparently, for a stronger test of the SCT (i.e. of the negative relationships among sperm traits and different levels of long-term stress) one would need an experimental approach to increase variation in the H/L ratio among individuals.

In our study, sperm morphology did not relate to any measurements of secondary traits, either carotenoid- or melanin-based feather ornaments, and we thus were unable to confirm the PLFH in our data set. This is in accordance with some previous studies on other passerine species (e.g. Lifjeld et al. 2012; but see Calhim et al. 2009). However, in contrast to our results, Helfenstein et al. (2010) showed that Great Tit males with paler carotenoid ornament and increased workload had a higher level of oxidative damage in their ejaculate and a lower proportion of motile spermatozoa than brighter males. Similarly, Losdat et al. (2011) also confirmed that more colourful males in a free-living population of the same species produced sperm with greater swimming motility. One possible explanation for this discrepancy may lie in the different sperm traits investigated (motility versus morphology). However, although we did not directly measure sperm motility as an indicator of ejaculate quality (Pizzari et al. 2008), we measured the midpiece/flagellum ratio, which has been associated with sperm speed/motility in some other studies on passerines (e.g. Laskemoen et al. 2010). In addition, total sperm length itself seems to reflect sperm speed and male fertilization success in at least some passerines (Bennison et al. 2015). The discrepancy could also be explained by the fact that there was no association between ornaments and condition traits in our study. This is not surprising because ornament function may differ not only among species but also among populations of the same species (Griffith et al. 2006; Dunn et al. 2010), and signalling may be context dependent (Vergara et al. 2012a, b). Taken together, we find it unlikely that Great Tit females could estimate ejaculate quality based on the expression of a male's secondary features in our Great Tit population.

In conclusion, based on our correlative study our data seem to partly confirm the idea that within-male variability in sperm length is negatively related to the H/L ratio in male Great Tits, which could indicate a trade-off between individual investment in ejaculate quality and immune capacity. However, there was no association between sperm traits and male ornamentation. Since other phenotypic sperm traits were not associated with any measured immunological and haematological traits, support for the trade-off between ejaculate quality and immunity needs further study, ideally involving an experimental approach.

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Affiliations

Jana Svobodová[1] [ORCID] · **Petra Bauerová[1] · Jiří Eliáš[1] · Hana Velová[2] · Michal Vinkler[2] · Tomáš Albrecht[2,3]**

✉ Jana Svobodová
    svobodovajana@fzp.czu.cz

[1] Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 1176, Prague 6 165 21, Czech Republic

[2] Department of Zoology, Faculty of Science, Charles University, Viničná 7, Prague 128 44, Czech Republic

[3] Institute of Vertebrate Biology, v.v.i, Czech Academy of Sciences, Květná 8, Brno 603 65, Czech Republic