



UNIFORM SPERM MORPHOLOGY IN THE LEK-BREEDING WIRE-TAILED MANAKIN (*PIPRA FILICAUDA*)

Emily R. A. Cramer^{1,2,3} · Tricia Rowlison⁴ · Pierre Comizzoli⁴ · T. Brandt Ryder¹

¹ Migratory Bird Center, Smithsonian Conservation Biology Institute, 3001 Connecticut Ave NW, Washington, DC 20008, USA.

² Cornell Lab of Ornithology, 159 Sapsucker Woods Rd, Ithaca, NY 14850, USA.

³ Current affiliation: Premedical Education Department, Weill Cornell Medicine - Qatar, Qatar Foundation - Education City, P.O. Box 24144, Doha, Qatar.

⁴ Center for Species Survival, Smithsonian Conservation Biology Institute, 3001 Connecticut Ave NW, Washington, DC 20008, USA.

E-mail: Emily R. A. Cramer · erc25@cornell.edu

Abstract · When females copulate with multiple males, selection on spermatozoa can reduce variation in sperm morphology. We describe sperm morphology for a polygynous lek-breeding suboscine, the Wire-tailed Manakin (*Pipra filicauda*). Total sperm length averaged $41.5 \pm 0.7 \mu\text{m}$ and the among-individual coefficient of variation in total sperm length was 1.8%. Variation was considerably lower than in the other manakin species with known sperm morphology, the Lance-tailed Manakin (*Chiroxiphia lanceolata*), despite similar promiscuity levels. This result highlights the need for further work on spermatozoa in lek-breeding species.

Resumen · Morfología uniforme en el esperma del Saltarín Uirapuru (*Pipra filicauda*), una especie con sistema de apareamiento de lek

Cuando las hembras copulan con más de un macho, selección actuando al nivel del espermatozoide puede reducir la variación en la morfología del esperma. Aquí describimos la morfología del esperma para una especie poligínica de suboscín con sistema de apareamiento de lek, el Saltarín Uirapuru (*Pipra filicauda*). Los espermatozoides tuvieron una longitud total promedio de $41.5 \pm 0.7 \mu\text{m}$, y el coeficiente de variación para la longitud total fue de 1.8%. El nivel de variación fue menor que en la otra especie de saltarín estudiada al respecto, el Saltarín Lanceolado (*Chiroxiphia lanceolata*), aunque ambas especies tienen casi el mismo nivel de promiscuidad. Estos resultados sugieren la necesidad de más estudios sobre especies de aves con este sistema de apareamiento.

Key words: Lek-breeding · Manakins · Post-copulatory sexual selection · Sperm competition · Sperm morphology

INTRODUCTION

Female birds of many species copulate with more than one male in a reproductive cycle, generating opportunity for spermatozoa to be under strong selection through sperm competition and/or cryptic (postcopulatory) female choice (Birkhead & Møller 1992). Studies on sperm biology in birds have recently focused heavily on socially monogamous oscine passerines with extra-pair paternity, and a robust pattern has emerged showing greatly reduced variation in sperm morphology in species with higher extra-pair paternity rates (Lifjeld et al. 2010). This pattern suggests that, when selection on spermatozoa is stronger, morphological variation is reduced (Lifjeld et al. 2010). To date, however, little work has been done in other taxonomic groups, or in groups with different mating systems, although oscines have different sperm structure compared to other avian taxa (Jamieson 2007).

In this study, we examined sperm morphology in the Wire-tailed Manakin (*Pipra filicauda*), a suboscine with a polygynous exploded-lek mating system where each lek consists of 4–12 territories, each approximately 40 m in diameter and separated by approximately 15 m from the nearest neighbor (Heindl 2002, Ryder et al. 2009). Territorial males perform complex courtship displays from their territories, and are sometimes joined in cooperative displays by other territorial or non-territorial floater males (Heindl 2002, Ryder et al. 2009). Females lay 1–2 eggs (mean \pm SE 1.79 ± 0.05) in cryptic nests away from the leks and perform all of the parental care (Ryder et al. 2009). Approximately 18% of females have mixed-paternity broods, indicating copulations with multiple males (Ryder et al. 2009). This level of multiple mating is similar to the average level in socially monogamous species (Griffith et al. 2002) and potentially generates opportunity for selection on spermatozoa and fertility. Spermatozoa from this species was previously undescribed, although sperm morphology has been described in another manakin species with a similar cooperative-lekking mating system (Sardell & DuVal 2014). Here we describe for the first time the morphology and variability of spermatozoa in Wire-tailed Manakins.

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METHODS

We captured males by mist netting at known territories within leks in the Tiputini Biological Station in Orellana, Ecuador (0°38'S, 76°08'W), between 22 February and 26 March 2016. Sperm samples were collected via cloacal massage, mixed gently in approximately 20 µL phosphate buffered saline and immediately transferred to 150 µL 5% formaldehyde for fixation and preservation. Males were banded and aged by plumage (first-cycle formative, < 1 year old; second-cycle basic, 2 years old; or definitive-cycle basic, ≥ 3 years old; Wolfe et al. 2010). Territorial status was assessed by re-sighting (see also Ryder et al. 2009).

Fixed spermatozoa (10–15 µL) were pipetted onto a microscope slide, dried, and stained by submersion in a bath of Coomassie Blue stain for 1.5 minutes. After rinsing off excess stain and allowing the slide to dry, we applied a cover slip with Permout and observed the slide at 1000X total magnification under oil immersion with an Olympus BX 41 microscope. Twenty morphologically normal cells were photographed (Spot Imaging software, Sterling Heights, Michigan, USA), and images were analyzed using Image J software (Schneider et al. 2012). We considered cells to have normal morphology if the head was generally straight with a helical super-structure; if the tail tapered rather than appearing blunt or looped at the tip; and if there was no evidence of cell breakage (Figure 1; see also Ålund et al. 2013). If fewer than 20 measurable cells were observed, additional slide(s) were made and examined from the male's sample.

We measured the length of the head (including acrosome), midpiece, and exposed flagellum (tail), following Kleven et al. (2008), then calculated total sperm length for each cell. One person (ERAC) took all measurements and was blind with respect to male characteristics such as age and territory status. We assessed measurement repeatability using the function rptGaussian (Stoffel et al. 2017), by measuring 16 cells (two cells for each male) four times each, with repeated measures taken on different days. Repeatability (\pm SE) was significant for all segments (all $p < 0.001$): 0.77 \pm 0.09 for head; 0.71 \pm 0.11 for midpiece; 0.98 \pm 0.01 for tail; and 0.99 \pm 0.01 for total length.

We calculated the coefficient of variation in the length of each sperm segment within individual males as standard deviation divided by mean times 100 (note that each male was represented by a single sample). A re-sampling study (Laskemoen et al. 2007) showed that measuring 10 spermatozoa per male gives accurate estimates for these measures. Further, we calculated the among-male coefficient of variation in sperm segment length using the mean for each male and applying a correction factor that avoids potential bias due to small sample sizes ((standard deviation / mean) * (1+1/(4n))); Sokal & Rohlf 1995, Laskemoen et al. 2007, Lifjeld et al. 2010). While Laskemoen et al. (2007) found that the uncorrected between-male variation in total sperm length may be biased when fewer than 10 males per species are sampled, the use of the adjusted value (CV_{BM_adj}) with sample sizes of four or more males per species is common practice in comparative studies (Lifjeld et al. 2010, Albrecht et al. 2013). We used bootstrapping to find 95% confidence intervals (CI) for coefficients of variation and for segment lengths (package boot, Canty & Ripley 2017).

We tested for differences in the length of sperm segments among males using ANOVA, using data on individual cells. Further analysis on correlates of sperm morphology were not attempted due to low sample sizes.

RESULTS

We captured 29 males and attempted to collect spermatozoa from all but a few individuals that showed signs of capture stress. For 22 males, fluid was produced following cloacal massage, but only 8 males (7 territorial males and 1 floater, all ≥ 3 years old; Table 1) produced samples with enough cells to measure 20 morphologically normal cells. For the remaining males, searching multiple microscope slides resulted in only one or two cells at most. Of the 14 males that did not produce sufficient sperm, 9 were territorial and 5 were floaters ($\chi^2_1 = 0.46$, $p = 0.5$); 12 were ≥ 3 years old, one was 2 years old, and one was < 1 year old (test comparing ≥ 3 to other ages, $\chi^2_1 = 0.12$, $p = 0.7$). Capture date did not differ between males that did and did not produce sperm (expressed as days since the first male was captured; $t_{12,9} = -0.3$, $p = 0.8$). The Coomassie blue stain stained acrosome, midpiece, and flagellum, but a large stretch of the head was not strongly stained (Figure 1). The region that did not stain with Coomassie blue did strongly stain with the DNA-binding stain DAPI, based on pilot work with one male (data not shown). Several types of abnormal morphologies were also observed (Figure 1).

Total cell length of normal cells averaged 41.5 \pm 0.7 µm (mean \pm SD, Table 1). Total sperm length varied significantly among males ($F_{7,151} = 9.6$, $p < 0.001$), as did the length of each component (all $F_{7,151} > 6.7$, all $p < 0.001$; Table 1). The single sampled floater male did not appear to be an outlier with respect to sperm morphology, as the 95% confidence intervals for his segment lengths overlapped with other males' (Table 1). The coefficient of variation in total sperm length within samples (CV_{WM}) varied across males (range 1.7–3.2, with some 95% confidence intervals not overlapping; Table 1). Within-male variation in head length ranged from 3.1–4.9%, in midpiece length from 9.1–21.7%, and in tail length from 2.4–5.3%. The between-male variation in total sperm length (CV_{BM_adj}) was 1.8% (95% confidence interval: 0.9–2.8); the coefficient of variation for head length was 3.5% (2.0–4.8); for midpiece was 9.0% (6.7–12.1), and for tail was 4.2% (2.2–6.4).

DISCUSSION

Wire-tailed Manakins in our study have short sperm cells with only a moderate level of variation. This result provides a striking contrast to the high variation found in the Lance-tailed Manakin (*Chiroxiphia lanceolata*; $CV_{BM_adj} = 5.7$; Sardell & DuVal 2014; 95% confidence interval for CV_{BM_adj} 3.9–8.5, calculated by us on their data). We had expected the two species to have similar levels of among-male variation because they have similar rates of multiple paternity (following the pattern observed in Lifjeld et al. 2010; Lance-tailed Manakins: 15% of broods with mixed paternity; DuVal & Kempnaers 2008), and similar relative testes mass (another proxy for sperm competition; Wire-tailed Manakin: 0.8%; Dunn et al. 2001; and Lance-tailed Manakin, 0.74%; Sardell & DuVal 2014). Although among-male variation differs be-

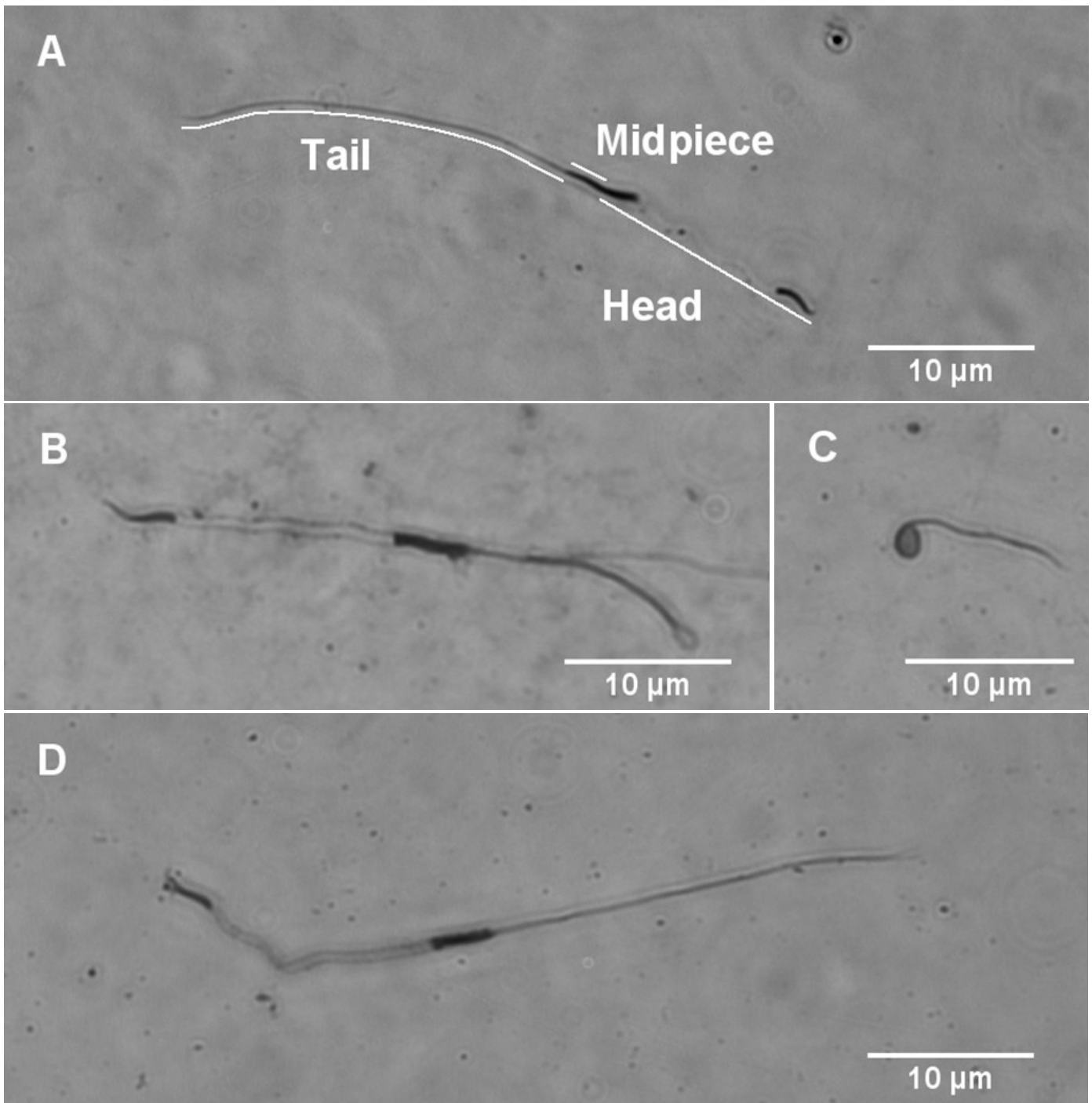


Figure 1. Wire-tailed Manakin (*Pipra filicauda*) spermatozoa under brightfield microscopy following Coomassie blue staining (1000x total magnification with oil immersion). A: Normal cell, with segments measured as the head, midpiece, and tail indicated. Measurements traced the curvature of the cell (approximated by the parallel white lines, though measurements were taken directly on the cell rather than in parallel, using the segmented line tool in ImageJ). B-D: Abnormal cells with heads lacking the typical helical structure and with the tail folded back on itself (B only; compare with Sardell & DuVal 2014, Ålund et al. 2013).

tween the two manakin species, the value for Wire-tailed Manakins is more similar to values predicted by the interspecific studies of oscines (Lifjeld et al. 2010). Patterns of selection may differ across taxa, although reduced variation in sperm morphology for species with higher selection on spermatozoa has also been found in rodents (Varea-Sánchez et al. 2014) and in hymenopteran insects (Fitzpatrick & Baer 2011), suggesting that it may be a broadly observed pattern. Differences in the CV_{BM_adj} between the two manakin species may alternatively reflect differences in the methods used to collect and measure sperm cells. Because we sampled each male only once, and over a fairly short time period, additional between-ejaculate, within-male variation in sperm size

and variability could also be present in Wire-tailed Manakins (e.g., Cramer et al. 2015). However, reliance on a single sample per male has been standard practice (e.g., Lüpold et al. 2009b, Calhim et al. 2011, Cramer et al. 2013, Rowe et al. 2015), and some species do not show seasonal changes in sperm morphology (Laskemoen et al. 2013). Further sampling may also reveal additional between-male variation in total sperm length, though we note that our sample included both territorial and floater males.

The functional impact of the length of different sperm components in birds is still under investigation. Males with longer sperm sired more offspring in an experimentally-controlled study on Zebra Finches (*Taeniopygia guttata*;

Table 1. Length of sperm segments (μm) in Wire-tailed Manakins (*Pipra filicauda*). Values are given as mean \pm SD (95% confidence interval) and are ordered by total sperm length (TSL). The coefficient of variation (CV) in TSL within males and between males, with 95% confidence intervals, is also given. Twenty cells were measured for all males except male 5154, for which only 19 cells were available. Where CV was calculated across males, it was adjusted for small sample size following (Sokal & Rohlf 1995). Males were categorized as territorial (T) or floater (F) based on re-sighting.

Male (Status)	Head	Midpiece	Tail	TSL	CV (%)
5126 (T)	14.2 \pm 0.4 (14.1–14.4)	3.4 \pm 0.3 (3.3–3.5)	22.9 \pm 1.2 (22.3–23.3)	40.5 \pm 1.3 (39.7–0.9)	3.1 (2.1–5.0)
5125 (T)	14.4 \pm 0.6 (14.1–14.6)	2.8 \pm 0.3 (2.6–2.9)	23.8 \pm 1.2 (23.3–24.3)	40.9 \pm 1.3 (40.4–41.4)	3.2 (2.6–4.6)
5165 (T)	13.8 \pm 0.6 (13.5–14.0)	2.9 \pm 0.5 (2.7–3.1)	24.7 \pm 1.2 (24.3–25.4)	41.4 \pm 1.1 (41.0–42.0)	2.7 (2.2–4.0)
5156 (F)	13.9 \pm 0.5 (13.7–14.1)	3.5 \pm 0.4 (3.3–3.7)	24.0 \pm 0.6 (23.8–24.3)	41.5 \pm 0.7 (41.2–41.8)	1.7 (1.4–2.2)
5154 (T)	15.1 \pm 0.6 (14.8–15.4)	3.3 \pm 0.5 (3.1–3.6)	23.1 \pm 1.0 (22.7–23.6)	41.5 \pm 1.0 (41.1–42.0)	2.3 (1.7–3.5)
1595 (T)	14.9 \pm 0.7 (14.7–15.3)	2.9 \pm 0.6 (2.6–3.1)	23.7 \pm 0.8 (23.4–24.1)	41.5 \pm 0.8 (41.2–41.9)	2.0 (1.7–2.4)
5000 (T)	14.4 \pm 0.7 (14.1–14.7)	3.3 \pm 0.5 (3.0–3.5)	24.2 \pm 1.0 (23.8–24.6)	41.8 \pm 0.9 (41.4–42.2)	2.3 (2.0–2.9)
5050 (T)	13.9 \pm 0.6 (13.6–14.1)	3.1 \pm 0.4 (2.9–3.2)	26.0 \pm 1.1 (25.5–26.4)	43.0 \pm 1.1 (42.5–43.4)	2.5 (2.1–3.4)
Combined	14.3 \pm 0.5 (14.1–14.73)	3.1 \pm 0.3 (2.30–3.3)	24.0 \pm 1.0 (23.5–24.8)	41.5 \pm 0.7 (41.1–42.1)	1.8 (0.9–2.8)

Bennison et al. 2014), and males with relatively longer midpieces sired more offspring in Tree Swallows (*Tachycineta bicolor*; Laskemoen et al. 2010), although other species show no relationships between sperm length and paternity success (Cramer et al. 2013, Sætre et al. 2018). Several studies find that longer spermatozoa and/or spermatozoa with a longer flagellum relative to head length swim at higher speeds (Lüpold et al. 2009a), while other studies find no relationship (Kleven et al. 2009) or even the opposite pattern (Cramer et al. 2015). Sperm morphology and swimming speed are influenced by the same genes or genomic regions in Zebra Finches (Kim et al. 2017). Wire-tailed Manakin spermatozoa are fairly short compared to passerine birds in general (mean \pm SD for oscines reported in Lifjeld et al. 2010: 147.8 \pm 71.9 μm). However, they are similar in length to the few other suboscines with published data (Lance-tailed Manakin 50.5 \pm 2.8, Sardell & DuVal 2014; Least Flycatcher *Empidonax minimus* 52.0 \pm 1.3, Lifjeld et al. 2010; and Eastern Phoebe *Sayornis phoebe* 48.0 \pm 1.3, Lifjeld et al. 2010). With previous work (Jamieson 2007), these results provide further evidence that sperm morphology in suboscines differs from morphology in oscine passerines. Broadening research to include more lek-breeding species, and taxa with other mating systems besides social monogamy with extra-pair paternity, will improve our understanding of the general patterns of selection on spermatozoa.

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REFERENCES

- Albrecht, T, O Kleven, J Kreisinger, T Laskemoen, TC Omotoriogun, J Reif, O Sedláček, D Horák, RJ Robertson, JT Lifjeld & D Hor (2013) Sperm competition in tropical versus temperate zone birds. *Proceedings of the Royal Society B* 280: 20122434.
- Ålund, M, S Immler, AM Rice & A Qvarnström (2013) Low fertility of wild hybrid male flycatchers despite recent divergence. *Biology Letters* 9: 20130169.
- Bennison, C, N Hemmings, J Slate & T Birkhead (2014) Long sperm fertilize more eggs in a bird. *Proceedings of the Royal Society B* 282: 20141897.
- Birkhead, TR & AP Møller (1992) *Sperm competition in birds: evolutionary causes and consequences*. Academic Press, San Diego, California, USA.
- Calhim, S, MC Double, N Margraf, TR Birkhead & A Cockburn (2011) Maintenance of sperm variation in a highly promiscuous wild bird. *PLoS One* 6: e28809.
- Canty, A & B Ripley (2017) *boot: bootstrap R (S-Plus) functions*. R package version 1.3-20.
- Cramer, ERA, T Laskemoen, O Kleven, K LaBarbera, IJ Lovette & JT Lifjeld (2013) No evidence that sperm morphology predicts paternity success in wild House Wrens. *Behavioral Ecology and Sociobiology* 67: 1845–1853.
- Cramer, ERA, T Laskemoen, E Stensrud, M Rowe, F Haas, JT Lifjeld, GP Sætre & A Johnsen (2015) Morphology-function relationships and repeatability in the sperm of *Passer* sparrows. *Journal of Morphology* 276: 370–377.
- Dunn, PO, LA Whittingham & TE Pitcher (2001) Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evolution* 55: 161–175.
- DuVal, EH & B Kempnaers (2008) Sexual selection in a lekking bird: the relative opportunity for selection by female choice and male competition. *Proceedings of the Royal Society B* 275: 1995–2003.
- Fitzpatrick, JL & B Baer (2011) Polyandry reduces sperm length variation in social insects. *Evolution* 65: 3006–3012.
- Griffith, SC, IPF Owens & KA Thuman (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* 11: 2195–2212.
- Heindl, M (2002) Social organization on leks of the Wire-tailed Manakin in southern Venezuela. *Condor* 104: 772–779.
- Jamieson, BGM (2007) Avian spermatozoa: structure and phylogeny. Pp 349–511 in Jamieson, BGM (ed). *Reproductive biology and phylogeny of birds*. Science Publishers, Enfield, New Hampshire, USA.
- Kim, KW, C Bennison, N Hemmings, L Brookes, LL Hurley, SC Griffith, T Burke, TR Birkhead & J Slate (2017) A sex-linked supergene controls sperm morphology and swimming speed in a songbird. *Nature Ecology and Evolution* 1: 1168–1176.
- Kleven, O, F Fossøy, T Laskemoen, RJ Robertson, G Rudolfson & JT Lifjeld (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.
- Kleven, O, T Laskemoen, F Fossøy, RJ Robertson & JT Lifjeld (2008) Intra-specific variation in sperm length is negatively related to sperm competition in passerine birds. *Evolution* 62: 494–499.
- Laskemoen, T, O Kleven, F Fossøy & JT Lifjeld (2007) Intraspecific

- variation in sperm length in two passerine species, the Bluethroat *Luscinia svecica* and the Willow Warbler *Phylloscopus trochilus*. *Ornis Fennica* 84: 131–139.
- Laskemoen, T, O Kleven, F Fossøy, RJ Robertson, G Rudolfson & JT Lifjeld (2010) Sperm quantity and quality effects on fertilization success in a highly promiscuous passerine, the Tree Swallow *Tachycineta bicolor*. *Behavioral Ecology and Sociobiology* 64: 1473–1483.
- Laskemoen, T, O Kleven, LE Johannessen, F Fossøy, RJ Robertson & JT Lifjeld (2013) Repeatability of sperm size and motility within and between seasons in the Barn Swallow (*Hirundo rustica*). *Journal of Ornithology* 154: 955–963.
- Lifjeld, JT, T Laskemoen, O Kleven, T Albrecht & RJ Robertson (2010) Sperm length variation as a predictor of extrapair paternity in passerine birds. *PLoS One* 5: e13456.
- Lüpold, S, S Calhim, S Immler & TR Birkhead (2009a) Sperm morphology and sperm velocity in passerine birds. *Proceedings of the Royal Society B* 276: 1175–1181.
- Lüpold, S, GM Linz & TR Birkhead (2009b) Sperm design and variation in the New World blackbirds (Icteridae). *Behavioral Ecology and Sociobiology*. 63: 899–909.
- Rowe, M, T Albrecht, ERA Cramer, A Johnsen, T Laskemoen, JT Weir & JT Lifjeld (2015) Postcopulatory sexual selection is associated with accelerated evolution of sperm morphology. *Evolution* 69: 1044–1052.
- Ryder, TB, PG Parker, JG Blake & BA Loiselle (2009) It takes two to tango: reproductive skew and social correlates of male mating success in a lek-breeding bird. *Proceedings of the Royal Society B* 276: 2377–2384.
- Sætre, CLC, A Johnsen, E Stensrud & ERA Cramer (2018) Sperm morphology, sperm motility and paternity success in the Bluethroat (*Luscinia svecica*). *PLoS One* 13: e0192644.
- Sardell, RJ & EH DuVal (2014) Small and variable sperm sizes suggest low sperm competition despite multiple paternity in a lekking suboscine bird. *Auk* 131: 660–671.
- Schneider, C, W Rasband & K Eliceiri (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675.
- Sokal, R & F Rohlf (1995) *Biometry: the principles and practice of statistics in biological research*. WH Freeman and Co, San Francisco, California, USA.
- Stoffel, MA, S Nakagawa & H Schielzeth (2017) rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution* 8: 1639–1644.
- Varea-Sánchez, M, LG Montoto, M Tourmente & ERS Roldan (2014) Postcopulatory sexual selection results in spermatozoa with more uniform head and flagellum sizes in rodents. *PLoS One* 9: e108148.
- Wolfe, JD, TB Ryder & P Pyle (2010) Using molt cycles to categorize the age of tropical birds: an integrative new system. *Journal of Field Ornithology* 81: 186–194.