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RESEARCH ARTICLE

Small and variable sperm sizes suggest low sperm competition despite multiple paternity in a lekking suboscine bird

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ABSTRACT

Sperm competition, whereby sperm from multiple males compete to fertilize an egg, selects for adaptations that increase fertilization success. Because fertilization success is related to sperm number, size, and quality, both interspecific and intraspecific variation in these traits are predicted to correlate with the level of sperm competition. Specifically, species and individuals that experience high sperm competition are predicted to produce more sperm per ejaculate, produce longer sperm, and exert higher quality control, resulting in reduced numbers of morphologically abnormal sperm and reduced size variation via selection for the most successful sperm phenotype. However, the causes of sperm morphological and size variation and its consequences for sperm competition remain poorly understood, especially within species. We quantified variation in sperm morphology, size, and number in the Lance-tailed Manakin (*Chiroxiphia lanceolata*), a Neotropical suboscine passerine with a cooperative lek mating system. Although alpha-status males sire almost all chicks, the numbers of sperm produced per ejaculate by betas, nonterritorial adults, and subadult males were similar to those produced by alphas. Sperm counts declined with age in alphas, which may explain the decreased siring success of older alphas. Most ejaculates contained both normal helical sperm and abnormal sperm with rounded heads. The proportion of morphologically normal sperm per ejaculate was unrelated to social status or age. The coefficients of variation in sperm component length (head, tail, and total) both between and within alpha males were comparable to variation reported in passerines with low sperm competition. Total sperm length was shorter than in the majority of avian species studied to date, and cloacal protuberance and relative testis size were small. These results indicate low sperm competition, despite evidence for multiple paternity, or that sperm number rather than sperm morphology may be a major postcopulatory mediator of male reproductive success in this species. This work represents the first thorough quantification of intraspecific sperm variability in a suboscine passerine.

Keywords: Chiroxiphia lanceolata, lek, manakin, multiple paternity, sperm competition, sperm morphology, spermiogenesis

Espermatozoides de tamaño pequeño y variable sugieren baja competencia espermática a pesar de la paternidad múltiple en un ave suboscina con apareamiento en arenas de cortejo

RESUMEN

La competencia espermática, en la que el esperma de múltiples machos compite para fertilizar un óvulo, selecciona adaptaciones que incrementan el éxito de fertilización. Dado que el éxito de fertilización se relaciona con el número, calidad y cantidad de los espermatozoides, se predice que la variación intra e interespecífica en estos rasgos se correlacione con el nivel de competencia espermática. Específicamente, se predice que las especies y los individuos que experimentan alta competencia espermática produzcan mayor cantidad de esperma en cada eyaculación, tengan espermatozoides más largos, ejerzan mayor control de calidad para reducir el número de espermatozoides morfológicamente anormales y reduzcan la variación en el tamaño debido a selección hacia el fenotipo espermático más exitoso. Sin embargo, las causas de la variación espermática en morfología y tamaño, y sus consecuencias para la competencia espermática aún son pobremente entendidas, especialmente dentro de las especies. Cuantificamos la variación en la morfología, el tamaño y el número de los espermatozoides en *Chiroxiphia lanceolata*, un suboscín neotropical con sistema de apareamiento cooperativo en arenas (*leks*). Aunque los machos alfa son los padres de la mayoría de los polluelos, el número de espermatozoides producidos por eyaculación fue similar en machos alfa, machos beta, adultos no territoriales y machos sub-adultos. El conteo de espermatozoides disminuyó con la edad en los machos alfa, lo que posiblemente explica la disminución del éxito de paternidad de los machos alfa viejos. La mayoría de las eyaculaciones tuvieron espermatozoides helicoidales normales y espermatozoides anormales con cabezas redondeadas. La proporción del esperma morfológicamente normal por eyaculación no estuvo relacionada con el nivel jerárquico o con la edad. Los coeficientes de variación en la longitud de los espermatozoides (cabeza, cola y total) entre y dentro de los machos alfa fueron comparables con la variación reportada en paserinos con baja competencia espermática. La longitud total de los espermatozoides fue corta comparada con la mayoría de especies de aves estudiadas a la fecha, y la protuberancia cloacal y el tamaño relativo de los testículos fue pequeño. Estos resultados indican baja competencia espermática a pesar de la evidencia de paternidad múltiple, o que el número de espermatozoides y no su morfología podría ser un mediador post-copulatorio del éxito reproductivo de los machos en esta especie. Este trabajo representa la primera cuantificación exhaustiva de la variabilidad espermática en un ave suboscina.

Palabras clave: Chiroxiphia lanceolata, competencia espermática, espermatogénesis, *lek*, morfología espermática, paternidad múltiple, saltarín

INTRODUCTION

Interspecific variation in sperm morphology and size is influenced primarily by the method of fertilization (Franzen 1970), phylogeny (Jamieson 1999), and the degree of sperm competition (Birkhead and Møller 1998, Birkhead and Pizzari 2002, Pizzari and Parker 2009). Within species, basic sperm morphology is considered to be under stabilizing selection and highly conserved (Morrow and Gage 2001, Calhim et al. 2007, Immler et al. 2008), showing high levels of heritability (Birkhead et al. 2005, Mossman et al. 2009). Finer-scale intraspecific variation in both the size of sperm components and morphology does occur, although its functional significance is relatively poorly understood (Kleven et al. 2008, Immler et al. 2010, 2012).

Generally, intramale variation in the size of morphologically normal sperm is expected to be lower than intermale variation, and both are predicted to be correlated negatively with the level of sperm competition (Morrow and Gage 2001, Birkhead et al. 2005, Immler et al. 2008). Specifically, fewer errors in spermatogenesis are expected when sperm competition is high, given the potentially high cost of ineffective sperm (Birkhead and Pizzari 2002, Hunter and Birkhead 2002, Birkhead et al. 2005). Indeed, strong negative correlations between variation in sperm length and sperm competition were found in recent comparative studies of passerines (Calhim et al. 2007, Immler et al. 2008, Kleven et al. 2008, Lifjeld et al. 2010). However, more studies from species with differing levels of sperm competition are needed to assess whether this is a general trend (Snook 2005, Kleven et al. 2008, Rowe and Pruett-Jones 2011). For example, although it is commonly predicted that tropical species experience lower sperm competition than temperate species—because of differences in life history traits such as life span, clutch size, breeding synchrony, migration, and promiscuity rates (Ricklefs and Wikelski 2002)—data on sperm variation in tropical passerines are rare, and a recent review found little evidence for such a distinction (Albrecht et al. 2013). Furthermore, although the level of sperm competition is often predicted to be relatively low in lekking systems, given that no pair bonds restrict females from all mating with the most attractive male, evidence for multiple mating and multiple paternity in lekking species indicates that postcopulatory sperm-competition levels may be higher than previously thought (Petrie et al. 1992, Lank et al. 2002, Hess et al. 2012).

A male's fertilization success depends on both the number and the quality of sperm produced (Parker 1982, Birkhead and Møller 1998, Birkhead and Pizzari 2002). Given honest signaling, functional fertility is predicted to vary with male phenotypic traits that influence female mate choice: the "phenotype-linked fertility hypothesis" (Sheldon 1994). The number of sperm per ejaculate may reflect dominance, with only those males in good condition able to invest in both status and sperm production (Pizzari et al. 2007, Chargé et al. 2010, Rowe et al. 2010, Lemaître et al. 2012). However, according to sperm competition theory, the opposite relationship is predicted if sperm characteristics represent tradeoffs with investment in mate acquisition, such as courtship display or plumage coloration (Birkhead and Møller 1998, Rowe et al. 2010). Furthermore, life history theory predicts that reproductive investment early in life is traded off against physiological function in later life; therefore, sperm number and quality may also be expected to decline with age (Preston et al. 2011). The expected relationship between social status, age, and sperm quantity and quality is therefore unclear, especially in species that are long-lived or those in which reproduction is age or status dependent (Pizzari et al. 2007). Further studies are needed linking sperm production to sperm competition via social status (Rowe and Pruett-Jones 2011).

With the goal of further elucidating the relationship of sperm quantity and quality with social status and age in highly polygynous species, we quantified variation in sperm number and morphology in male Lance-tailed Manakins (*Chiroxiphia lanceolata*; Figure 1). Lance-tailed Manakins are cooperatively displaying passerine birds with an exploded-lek mating system (DuVal 2007a). Alpha and beta adult males perform an elaborate courtship display, but, with rare exceptions, only the alpha males have the opportunity to mate (DuVal and Kempenaers 2008). Beta males delay reproduction until they attain alpha status in later seasons (DuVal 2007b). Nevertheless, females visit and may copulate with multiple alpha males, and alphas compete with each other to fertilize females. Given that

FIGURE 1. An adult male Lance-tailed Manakin on his display perch. Photo credit: Emily DuVal

more intense sperm competition is correlated with low within- and between-individual variation in other species, we measured intramale and intermale variation in sperm morphology and size as an estimate of the relative intensity of sperm competition in this species. Because male reproductive success is tightly linked to social status (DuVal 2012), we also tested the predictions that sperm morphology and size vary among status classes. In addition to providing important data on the correlation of sperm morphology with behavior and success in a high-skew mating system, we present the first thorough quantification of intraspecific sperm variability in a suboscine passerine.

METHODS

Study System

Lance-tailed Manakins are 15.5- to 22-g Neotropical birds in the family Pipridae. Males attain adult plumage in their 4th year after hatching, and dominant "alpha" individuals form cooperative alliances with subordinate "beta" males to perform 2-male courtship displays for females (DuVal 2005, 2007a). As a display progresses, the beta leaves and the alpha performs the solo display that always precedes copulation. Some males never attain alpha status, and not all alphas gain reproductive success, but others may be highly successful, resulting in strong sexual selection (DuVal and Kempenaers 2008, DuVal 2012). Betas, nonterritorial males (definitive-plumage males that are neither alpha nor beta), or males of indeterminate social status do occasionally sire offspring, but these represent <8% of chicks with assigned paternity (DuVal and Kempenaers 2008). Alphas have significantly higher androgen levels than betas and nondisplaying adult males (DuVal and Goymann 2011). Male social status is related to age, in that alphas tend to be older than their beta partners (DuVal 2007b), but alphas vary considerably in age, and alpha age is related to siring success, with evidence of senescence in the oldest individuals (DuVal 2012). For further details on the cooperative display and reproductive success, see DuVal (2013a) and DuVal and Kempenaers (2008).

Sample Collection

Fieldwork was conducted on 46 ha of Isla Boca Brava, Chiriquí, Panama (8°12′N, 82°12′W), in a color-marked population that has been studied since 1999. Males were mist netted for sperm sampling during the breeding seasons of 2012 and 2013 (mid-February until mid-June). In 2012, sperm was collected by cloacal massage (Rowe et al. 2010), followed by lavage with 7 μL of saline solution to collect the ejaculate. The sperm–saline mix was then diluted in further saline to ensure that samples did not dry out before examination. In 2013, sperm was also collected by cloacal massage, but the sample was collected via capillary tube and then diluted with 200 μL Lago avian semen extender (Hygieia Biological Laboratories, Davis, California, USA). Samples were first examined under a compound light microscope (Nikon Alphaphot) at 400× magnification. Sperm numbers were quantified using an "improved Neubauer" hemocytometer by pipetting 7 μL of diluted sperm onto the slide and counting the total number of sperm in each of 5 large squares diagonally across the grid. In 2012, each slide was counted by 2 observers, and counts for each individual were taken as the mean of these 2 measurements. Two readily identifiable sperm forms (with normal helical and abnormal rounded heads) were counted separately (Figure 2A). Repeatability (± SE) of counts of the same sample was 0.99 ± 0.00 ($P <$ 0.01) for normal sperm and 0.89 ± 0.08 ($P < 0.01$) for abnormal sperm, calculated by the method of Lessells and Boag (1987) and applied by the function rpt.poisGLMM.multi in the R-package rptR (Nakagawa and Schielzeth 2010; see below). In 2013, therefore, 2 separate aliquots were counted by the same observer and used to calculate the mean. Intra-observer repeatability of counts from different aliquots of the same sample, calculated as above, was 0.87 ± 0.08 ($P < 0.01$) for normal sperm and 0.96 ± 0.06 ($P < 0.01$) for abnormal sperm. The total number of sperm per ejaculate was calculated assuming that (1) the whole ejaculate was collected and (2) its volume was negligible compared with the volume of solution used to dilute the collected sample. When possible, the remaining sample was fixed in a final concentration of 5% formalin solution for later morphological quantification. Because we judged that collection techniques in 2012 were less reliable in collecting entire ejaculates (i.e. some saline–sperm mix clearly was not recollected following lavage) and, indeed,

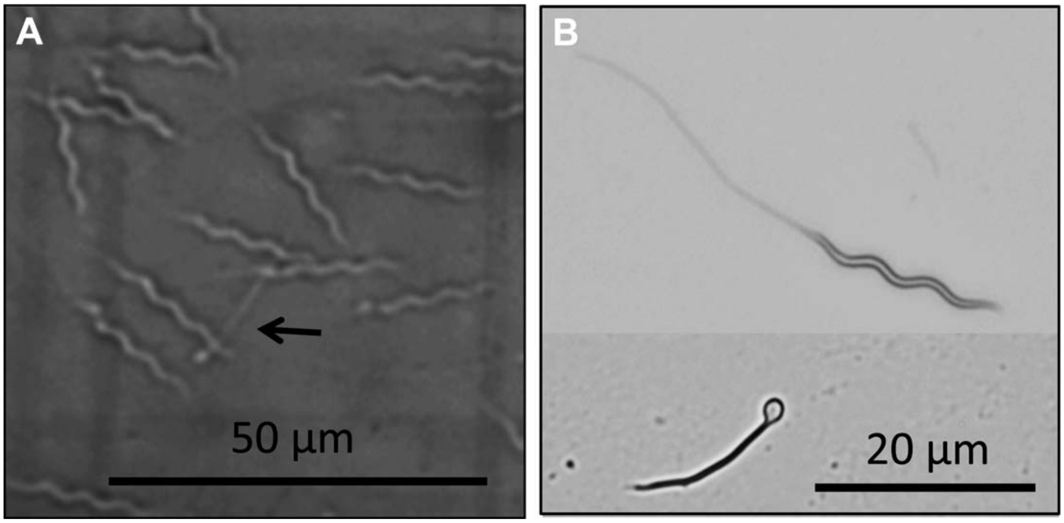

FIGURE 2. (**A**) A fresh sperm sample from an alpha male, visualized at 400× magnification in the field for total sperm counts immediately after collection. Two sperm morphologies are visible in one 0.001-mL grid division of a hemocytometer; the black arrow indicates the position of a single abnormal sperm with a rounded head. (**B**) Normal and abnormal sperm types from the same male, visualized in the laboratory at 1,000× magnification.

ejaculate sizes were estimated to be much smaller in that year, we restricted analyses of total sperm number to 2013 data. We have no reason to believe that collection techniques affected estimates of the proportion of normal sperm or sperm morphology, and so both years of data are included in those analyses.

To quantify fine-scale variation in sperm morphology, images were obtained by brightfield visualization on an Olympus BX61 microscope at 400–1,000× magnification with a DP71 camera (Figure 2B). Head and tail length of 5 randomly selected normal sperm were measured to the nearest 0.01 µm using ImageJ for all 9 alphas with preserved ejaculates (Schneider et al. 2012). Note that although 20 sperm samples were collected from 15 unique alpha males, problems with formalin preservation of some samples meant that only 9 unique alphas were available for sperm-size measurement. Only alpha males were used for fine-scale quantification of sperm morphology, because the goal of this quantification was to test predictions about variation in relation to sperm competition, and reproduction is essentially limited to the alpha social class. Including non-alpha individuals could artificially inflate variability measurements—for example, if individual males change ejaculate quality across their lifetimes or if males produce more consistent sperm when actively reproducing and ejaculating regularly. Measurement of sperm from 10 males was sufficient to quantify variation in cross-species comparisons of other taxa (Calhim et al. 2007, Immler and Birkhead 2007), and it has been shown that 95% of the variation in size can be captured by measuring 5 sperm male^{-1} (Calhim et al. 2007). In many cases, the midpiece of Lance-tailed Manakin sperm was indistinguishable from the head; we report those measurements from sperm in which the midpiece could be clearly identified, but we combine midpiece and head in other measurements of morphological variation. Straightened head length was measured as extending from the apex to include the midpiece and ending where the flagellum started, tracing the curvature of the head that results from the normal helical morphology of passerine sperm. The tail length was measured as the flagellum only. Scanning electron microscope (SEM) images were obtained by critical-point-drying samples and sputter coating with 4 nm of iridium metal, then visualizing with a FEI Nova 400 Nano SEM at 15 kV.

Testis Size

Relative testis size is one of the most widely accepted indicators of degree of sperm competition in wild birds (Møller 1991, Møller and Briskie 1995, Stutchbury and Morton 1995, Pitcher et al. 2005) and other taxa (Gage and Freckleton 2003). To put sperm measurements into perspective, we considered testis measurements from 4 alpha males collected in April or May, during the active breeding season for this species in the years 2000–2003 (UC Berkeley MVZ specimen nos. 181804, 181809, 181812, and 181813). Testis size was recorded as length (L) and width (W) of each testis separately; testis volume was calculated using the equation for the volume of an ellipsoid ($V = \pi[L \times W^2]/6$) and converted into mass using the estimate of 1.087 g cm^{-3} (Møller 1991). A reassessment of this assumption that testis density is consistent across species showed considerable interspecific variability, but no significant difference from this average value (Calhim and Birkhead 2007).

Analyses

Variation in sperm traits both among and within individuals was quantified by calculating the coefficient of variation ($CV = [SD/mean]*100$), adjusted for differences in sample size ($CV_{adj} = [1 + 1/(4n)]*CV$; Sokal and Rohlf 1981), which has been shown to correct effectively for small sample sizes (Lifjeld et al. 2010). For calculations of within-male CV_{adj}, we used all 5 sperm measured per male, whereas for among-male CV_{adj} we used the mean sperm size for each male. The within-male repeatability of size measurements for individual males (i.e. given that the proportion of variance explained by differences among males = among-male variance/[among-male variance + within-male variance]) was calculated according to Lessels and Boag (1987), using the function rpt.remlLMM from the R-package rptR (Nakagawa and Schielzeth 2010; also see Calhim et al. 2011). We quantified measurement error by measuring 10 individual sperm images 10 times each, and found repeatability (± SE) of 0.86 ± 0.06 for measurement of tail lengths and 0.92 ± 0.04 for lengths of head with midpiece.

Because of high levels of overdispersion, we used Kruskal-Wallis tests to determine whether sperm number and the proportion of abnormal sperm varied with social status (alpha, beta, nonterritorial adult, and subadult). The relationships of male age with sperm count and the proportion of abnormal sperm were analyzed using Spearman's rank correlations, considering only males with age precisely determined by prior capture in predefinitive plumage (DuVal 2005). We used only the first sample per male per year to avoid pseudoreplication. Results are presented as means ± SD unless otherwise noted.

RESULTS

Sperm Morphology

In 2012, sperm samples were collected from 28 males: 6 alphas, 6 betas, 8 nonterritorial males in definitive adult plumage (4th year+), and 8 subadults. In 2013, sperm samples were collected from 38 males: 12 alphas, 5 betas, 10 nonterritorial definitive males, and 11 subadults. Abnormal sperm with rounded heads were common, appearing in 54 of 73 individual ejaculates. Both normal and abnormal sperm were motile. Normal sperm were similar in morphology to those in other passerines and had long helical heads (22.56 ± 2.16 μm) and long tails (27.94 ± 0.98 μm), with a mean total length of 50.50 ± 2.82 μm ($n = 9$ alpha males; Figures 2 and 3). In the normal sperm of 3 alphas for which a distinct midpiece could be identified, this region measured 4.60 ± 0.28 μm.

Extent of Variation within and among Alpha Males

The average sperm measurements from 9 alpha males indicated that, among individuals, sperm head length was more variable than sperm tail length: CV_{adj} was 9.84% for heads, 3.62% for tails, and 5.74% for total length among alphas. Within individuals, males showed considerable range in variation of sperm component length, and values were roughly comparable to those between males: the average CV_{adj} among normal sperm from the same male was 6.01 ± 1.94% (range: 2.99–9.04%) for heads, 4.60 ± 1.58% (range: 2.62–7.26%) for tails, and 2.58 ± 1.46% (range: 1.01–5.81%) for total lengths. Repeatabilities (R ± SE) for helical-sperm head length, tail length, and total length were as follows: $R_{heads} = 0.72 \pm 0.13$, 95% confidence interval (CI): 0.38–0.87, $P < 0.01$; $R_{(tails)} = 0.27 \pm 0.15$, 95% CI: 0–0.55, $P = 0.03$; and $R_{total} = 0.80 \pm 0.11$, 95% CI: 0.49–0.91, $P < 0.01$.

Ejaculate Composition

In 2013, using the first ejaculate collected from each male, the mean total ejaculate size was $6.85 \times 10^6 \pm 9.02 \times 10^6$ sperm (range: 0.05×10^6 to 34.9×10^6, $n = 38$); the mean number of normal helical sperm was $6.79 \times 10^6 \pm 8.99 \times 10^6$ (range: 0.03×10^6 to 34.8×10^6, $n = 38$); and the mean number of abnormal sperm with rounded heads was $6.36 \times 10^5 \pm 11.94 \times 10^5$ (range: $0–57.50 \times 10^5$, $n = 37$; Figure 4) per ejaculate. The mean proportion of abnormal sperm per ejaculate was significantly higher in 2012 than in 2013 (Wilcoxon rank sums, $Z = 3.85$, $P < 0.01$; $n = 18$ males in 2012, $n = 37$ males in 2013). Combining both years but including each male only once, the mean proportion of abnormal rounded sperm per ejaculate was 0.03 ± 0.08 (range: 0–0.5) across 55 ejaculates collected (Figure 4). The numbers of both normal and abnormal sperm were highly correlated with the total number of sperm in an ejaculate (normal sperm: Spearman's rank correlation coefficient $r_s = 1$, $P < 0.01$, $n = 37$; abnormal sperm: $r_s = 0.46$, $P < 0.01$, $n = 37$), but the proportion of abnormal sperm per ejaculate was not correlated with the total number of sperm in the ejaculate ($r_s = 0.15$, $P = 0.38$, $n = 37$).

Small numbers of males were sampled more than once within or between years. For 8 males from which a second sperm sample was collected later in the same year, the mean proportion of abnormal sperm increased for 5 males, decreased for 2 males, and remained the same for 1 male that had no abnormal sperm; the total number of sperm decreased for all but 1 male. Differences in the number of days that elapsed between samples precluded a formal quantitative analysis of these data. Among 10 males from which samples were collected in both 2012 and 2013, the proportion of abnormal sperm decreased for 9 males and increased for 1 male. The total number of sperm increased between years for all 10 males, but this difference is thought to reflect a change in sperm collection methodology between 2012 and 2013 (see above).

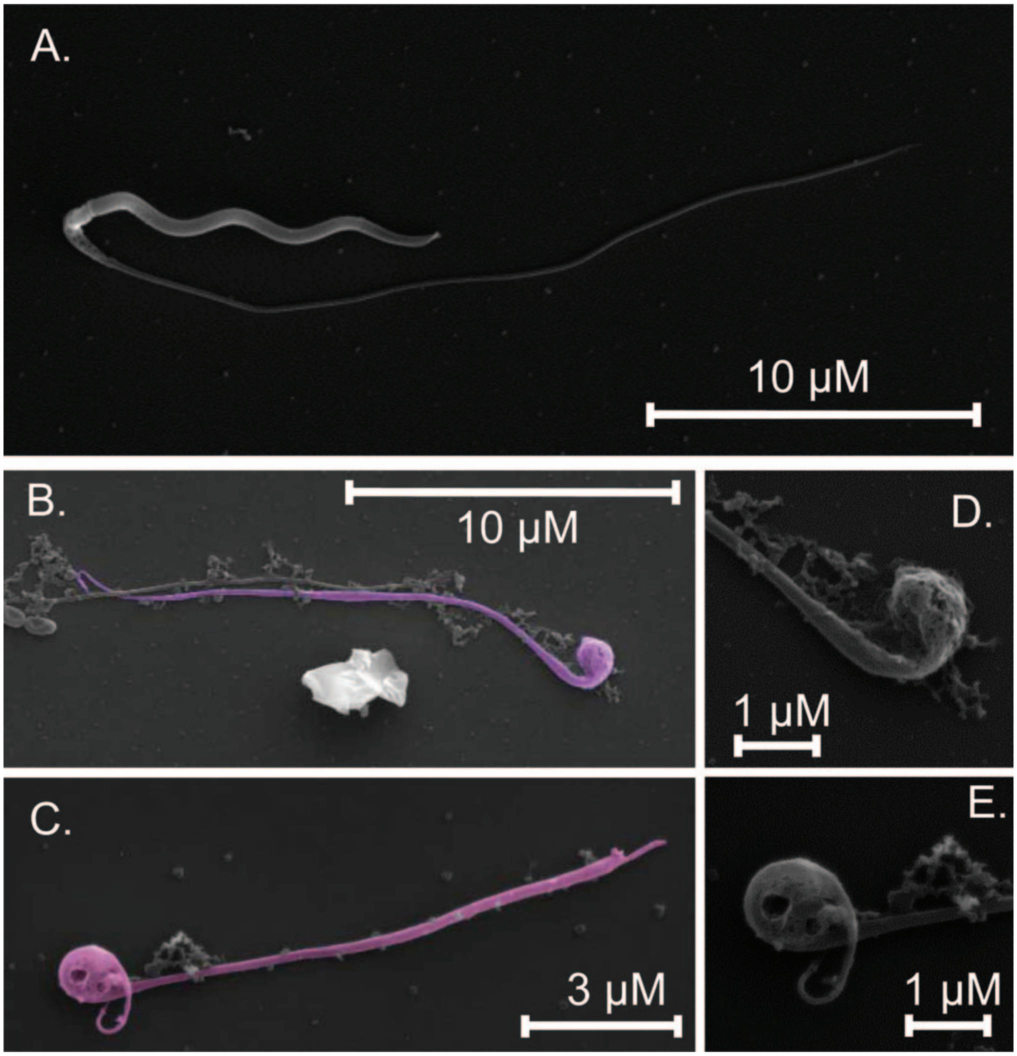

FIGURE 3. Scanning electron micrograph images illustrating the fine-scale morphology of normal helical sperm (**A**) and of abnormal sperm with rounded heads (**B** and **C**) in a single Lance-tailed Manakin ejaculate. Enlarged views of the heads of rounded morphotypes (**D** and **E**) show what is apparently retained cytoplasm, based on similarity to published images (du Plessis and Soley 2011).

Correlations with Male Status and Age

Male social status was unrelated to either the total number of sperm per ejaculate (Kruskal-Wallis, $\chi^2 = 0.03$, df = 3, $P = 1.0$, $n = 38$) or the proportion of abnormal sperm per ejaculate ($\chi^2 = 1.91$, df = 3, $P = 0.59$, $n = 55$; Figure 4).

Sperm counts of alpha males showed a significant negative relationship with individual age ($r_s = -0.66$, $P = 0.03$, $n = 11$ alphas of known age sampled in 2013; Figure 5A), but age was unrelated to the proportion of abnormal sperm ($r_s = -0.09$, $P = 0.77$, $n = 13$ alphas of known age sampled in 2012 and 2013; Figure 5B). Age was unrelated to sperm count or ejaculate composition in non-alpha males (sperm count: $r_s = 0.15$, $P = 0.49$; proportion abnormal: $r_s = 0.002$, $P = 0.99$; $n = 35$ non-alpha males, aged 2–8 yr; Figure 5). The significant relationship of alpha age with sperm count was clearly influenced by 2 outliers that were young alphas with high sperm counts. Removing these outliers reduced that relationship to a nonsignificant trend ($r_s = -0.36$, $P = 0.34$, $n = 9$), and so we note here that additional data will be required to know whether the relationship detected in the larger dataset is robust.

Testis Size

For 4 collected alpha males, the mass of the left and right testes averaged 0.068 ± 0.021 g and 0.039 ± 0.014 g, respectively, for a combined testis mass of 0.12 ± 0.03 g. Given the individual males' weights (range: 15.0–16.5 g), this combined testis mass represented 0.74 ± 0.25% of alpha males' total body weights (range: 0.60–1.11%).

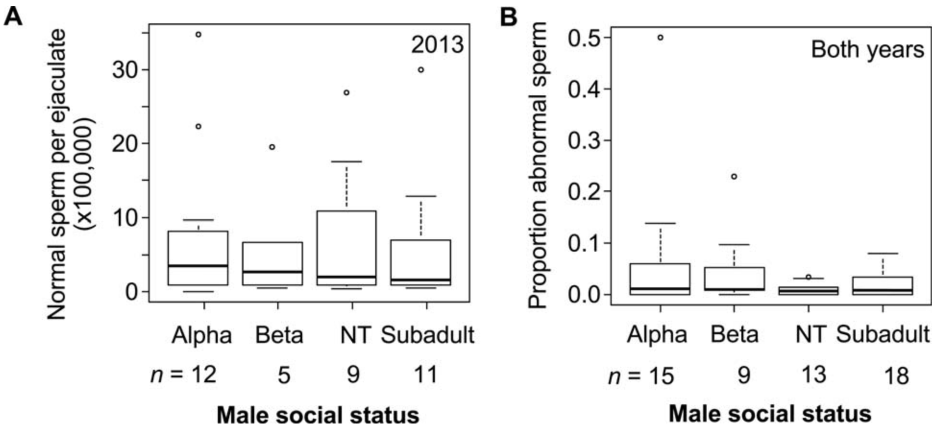

FIGURE 4. Male social status had no significant effect on either (**A**) the number of sperm per ejaculate or (**B**) the proportion of abnormal sperm. Sample sizes are given below each status category. Only data from 2013 are presented in (**A**) as collection methods were improved in that year leading to substantially higher estimates of ejaculate size than in 2012. Changes were not expected to affect values in (**B**), and so data from both years are pooled in that plot (see Methods). Status category "NT" denotes "nonterritorial" males, which are definitive-plumage adults that hold neither alpha nor beta status. Subadults are males in their 2nd or 3rd year after hatching. Neither NT nor subadult males display for females. Boxes indicate the 25th and 75th percentiles of the data, with median denoted by a horizontal bar, whiskers denoting data within 1.5 interquartile ranges, and points indicating data outside this range.

DISCUSSION

Lance-tailed Manakin sperm were highly variable, both in gross morphology and in the size of sperm components. Variation in the size of sperm components was at the upper range of that reported in other passerines, both within and among males. Furthermore, sperm length was short and testis size small in relation to the majority of species studied to date. This suggests low levels of sperm competition in the study population, despite some evidence of multiple mating, small clutch size, and small body size, which predict high sperm competition risk (Albrecht et al. 2013). One explanation for apparently low sperm competition could be that males of this species reproduce at a low rate over multiple years and, therefore, may be expected to invest more in advertisement and less in reproductive physiology. However, contrary to sperm competition theory (Parker 1998), there was no relationship between sperm number or the proportion of normal sperm and social status, indicating no obvious tradeoff between investment in sperm production and courtship display. Instead, the negative relationship of alpha age with number of sperm supported the hypothesis that investment in sperm production is traded off against physiological maintenance later in life (Preston et al. 2011), which suggests that sperm number may be an important factor in the decreased siring success previously reported for older alpha males (DuVal 2012). Sperm number, rather than

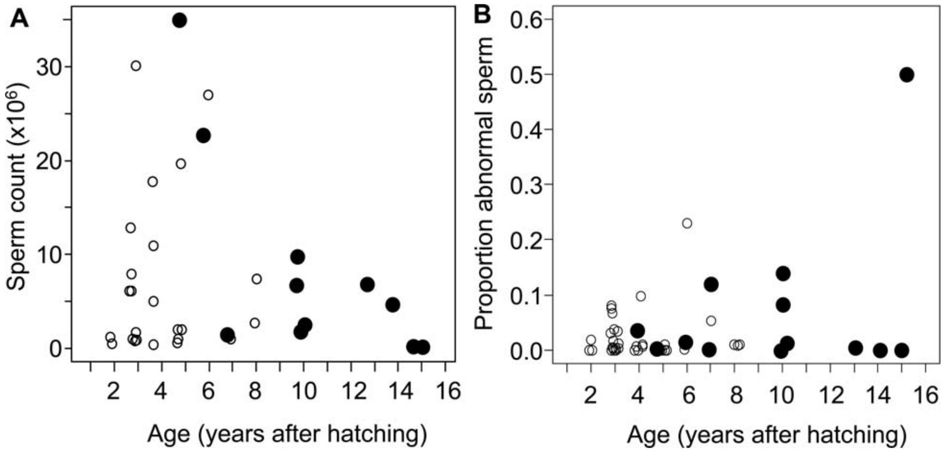

FIGURE 5. Age showed a significant negative relationship with (**A**) sperm count among alpha males (filled circles) but was unrelated to (**B**) the proportion of abnormal sperm. Age was unrelated to either sperm count or ejaculate composition in non-alpha males (open circles). Points are jittered on the *x*-axis for ease of visualization.

sperm morphology, may be a major postcopulatory mediator of male reproductive success in this species.

Sperm Size and Variability

Given that low sperm variability and long length are associated with high sperm competition, our data suggest that the level of sperm competition in Lance-tailed Manakins is relatively low. We found that Lance-tailed Manakin sperm were short (~50.5 μm) in relation to most other passerine species (range within 196 passerine species: 42–285 μm; Immler et al. 2011). Although relatively poorly studied, other species in the suborder Tyranni also have relatively short sperm (Lifjeld et al. 2010). Although short sperm tend to occur in species with low sperm competition, a quadratic relationship indicates that sperm size peaks in species with intermediate levels of sperm competition (Lifjeld et al. 2010, Immler et al. 2011). Further work identifying the frequency at which females mate multiply will help determine the true level of sperm competition in this species and may explain low sperm-size repeatability.

Variation in sperm component size is predicted to be negatively correlated with the risk of sperm competition (Birkhead et al. 2005). In the present study, most variation in sperm total length and head length occurred among males (rather than within males; for total length, repeatability = 0.80; for head length, repeatability = 0.72), whereas most variation in tail length occurred within males ($R = 0.27$). High repeatability of total sperm length was comparable to that reported for other passerines (e.g., Laskemoen et al. 2007) and corresponds with data showing larger coefficients of variation between than within males for most passerines (Lifjeld et al. 2010). However, coefficients of variation between male Lance-tailed Manakins were relatively high in all measured sperm components (for length of head with midpiece, $CV_{bm} = 9.84\%$; for tail length, $CV_{bm} = 3.62\%$; for total length, $CV_{bm} = 5.74\%$). This approximates levels of sperm variability between males reported for bullfinches (*Pyrrhula* spp.) and Zebra Finches (*Taeniopygia guttata*) ($CV_{bm} = 5.6$–9.6%), species in which sperm competition is low (Birkhead et al. 2005, Lifjeld et al. 2013). By comparison, in 5 recent comparative studies of both temperate and tropical passerines, mean CV_{bm} between males in sperm head length, tail length, and/or total length ranged between 0.5% and 6.2%, with many species having values $\leq 3.5\%$ (Immler et al. 2008, Kleven et al. 2008, Lüpold et al. 2009, Lifjeld et al. 2010, Albrecht et al. 2013).

Lance-tailed Manakins also showed coefficients of within-male variation in sperm measurements that were relatively high compared with those of many other species, but note that the sample size for sperm measurements was relatively small. Although 80% of variation in total sperm length could be explained by variation among males, mean variability in sperm measurements within alpha male Lance-tailed Manakins was also relatively high ($CV_{wm} = 6.01\%$ for head length, $CV_{wm} = 4.60\%$ for tail length, and $CV_{wm} = 2.58\%$ for total length). Within males of other passerines, CV_{wm} ranged between 0.8% and 6.7% (Immler et al. 2008, Kleven et al. 2008, Lifjeld et al. 2010, Albrecht et al. 2013) but was again markedly higher in species with low sperm competition (6.1–7.7% within males; Birkhead et al. 2005, Lifjeld et al. 2013). By contrast, in Superb Fairy-wren (*Malurus cyaneus*), a species with extreme promiscuity, the coefficients of variation in head, tail, and total lengths were 1.1–2.3% within males and 2.1–4.1% between males, with repeatability of 0.65–0.68 (Calhim et al. 2011). Measured variability within and between individuals includes, by definition, the combined quantification of phenotypic flexibility and measurement error (Nakagawa and Schielzeth 2010). We note that measurement error may have been introduced from slight variation in the viewing angle of individual sperm (because sperm cells are 3D structures visualized in a 2D image). However, this challenge is common to studies of sperm morphology and is not expected to unduly bias our results. Overall, our data on sperm-size variation suggest that selection on sperm morphology (and, hence, sperm competition) is relatively low in Lance-tailed Manakins.

The inference of low sperm competition inferred from variation in sperm morphology was supported by the relatively small combined testis mass given the body size of males (testes represent 0.74 ± 0.25% of the body mass of breeding males). This falls at the lower end of relative testis size in passerines (Møller 1991) and is comparable to values reported for lekking passerines in that study (0.94 ± 0.86%; $n = 14$ species; Møller 1991). Note that this range is also comparable to that reported for several nonlekking tropical birds (e.g., testes were 0.12–0.77% of body weight in 9 species of Tyrannidae; Stutchbury and Morton 1995). Despite these physical indications of low sperm competition, female manakins are known to sometimes mate with >1 male, creating the behavioral potential for sperm competition to exist. Multiple paternity was previously reported in 15% of 147 two-chick broods genotyped in the study population (DuVal and Kempenaers 2008). Given that not every copulation produces offspring, rates of multiple mating may be expected to be considerably higher than 15%. However, females are also frequently observed mating repeatedly with the same male in the same nesting cycle, which suggests that male sperm limitation may influence female copulation behavior. Furthermore, females are apparently free to assess males over several days before mating, and some females mate with the same male across nest cycles and years, which suggests a degree of mate fidelity that could also lead to low selection from sperm competition (DuVal 2013b). Indeed, all males we sampled had no swelling of the cloacal base (cloacal protuberance).

An enlargement of the base of the cloaca is associated with increased mating rate, number of stored sperm, testis size, and, hence, sperm competition in birds (Birkhead et al. 1993). Behavioral data on copulation rates and quantification of selection pressures from multiple mating are needed to determine whether sperm competition is driving the evolution of sperm morphology in this species.

Sperm Quantity

Ejaculate size varied substantially among males. Surprisingly, betas and nonterritorial adults (those that were neither alpha nor beta) produced numbers of sperm similar to alphas, despite having lower androgen levels (DuVal and Goymann 2011). Some variation is likely to reflect recent mating history in alphas and, hence, sperm depletion. Lower androgen levels in betas, despite their participation in elaborate courtship displays, were previously proposed to reflect either their younger age or their social environment (DuVal and Goymann 2011). Betas sired only 0.7% of chicks over 7 yr in this study population (DuVal and Kempenaers 2008), and they rarely encountered females without alpha accompaniment. However, males' ability to produce large ejaculates of motile sperm as betas, and even before attaining definitive adult plumage, indicates that males that do not sire chicks are nevertheless reproductively competent, and that reduced siring success is largely the result of a lack of social opportunity.

Age may also be an important factor determining sperm number. We found that sperm counts declined in the ejaculates of older alpha males. It is unclear from our data whether this trend is restricted to alphas, because we did not sample sperm from any non-alphas older than their 8th year after hatching, whereas the oldest alphas were in their 15th year. Decreased sperm counts may explain the decrease in siring success observed in the oldest age classes of alpha males (DuVal 2012). Decreasing sperm numbers are a common effect of advanced age across many taxa. Recent work in Houbara Bustards (*Chlamydotis undulata*) found that males that invested more in extravagant sexual displays experienced faster senescence in sperm function (Preston et al. 2011). However, decreased sperm counts in our study could also result from different social circumstances experienced by these older males, for example if females rarely visit to observe courtship displays. Future analyses separating out the effects of sperm quality and precopulatory female choice will further clarify this possibility.

Abnormal Sperm

We found no relationship between male social status or age and the proportion of abnormal sperm in ejaculates, which suggests that sperm abnormalities are independent of active reproduction. Abnormal sperm were more common in 2012 than in 2013. Abnormally shaped sperm in vertebrates are generally considered production errors (Wildt et al. 1986, Lindsay et al. 2009, Pitnick et al. 2009, du Plessis and Soley 2011) and may be variable in form, possessing multiple heads or tails, defects, or even no head or tail (Gage et al. 2006, Hayakawa 2007, Pitnick et al. 2009, du Plessis and Soley 2011, Preston et al. 2011). Instead, the gross morphology of the abnormal sperm described here was consistent among males and resembled published images of sperm with retained cytoplasm (e.g., immature sperm; du Plessis and Soley 2011) or immature sperm that failed to complete the final stages of spermiogenesis (Lifjeld et al. 2013). Other sperm forms—for example, helical sperm with 2 heads—occurred but were much less common. The prevalence of abnormal sperm may vary during the breeding season; 3 of 4 males sampled repeatedly in 2013 showed an increase in the relative number of abnormal sperm in the second sample (though sample size is clearly too low to draw conclusions from this observation). Variation in the proportion of normal sperm could reflect changes during and among breeding seasons in, for example, copulation activity, sperm production, or environmental stressors such as heat. Further work is required to confirm the developmental process that leads to the production of these abnormal sperm, including ultrastructure and fertilization ability.

The proportion of abnormal sperm detected here is interesting because sperm competition is thought to result in high "quality control" of sperm morphology, although detected levels of abnormality are well within the range of that reported in other species. The percentage of abnormal sperm varies: for example, 5–25% in ratites (du Plessis and Soley 2011) and <25% in mammals, but with examples of extremely high levels of abnormal sperm (85–95%) despite normal fertilization in both naked mole rats and humans, in which sperm competition is low (van der Horst et al. 2011). In fairy-wrens, the proportion of abnormal sperm varied with the level of sperm competition among species (5–30%; Rowe and Pruett-Jones 2011). In lekking Houbara Bustards, the percentage of abnormal sperm varied between 21% and 30% (Preston et al. 2011).

In conclusion, several well-established hallmarks of sperm competition (sperm size, sperm component variability, and testis size) suggest low sperm competition in Lance-tailed Manakins. However, nontrivial occurrence of multiple mating and paternity suggests that either (1) precopulatory mate choice by females is the major mediator of siring success, even when females mate with multiple mates; or (2) these classic metrics of sperm competition are not representative, and postcopulatory male success is mediated by sperm number (Levitan 2012). Indeed, sperm traits are likely to be constrained by tradeoffs (Snook 2005), with investment in sperm number favored over investment in sperm size in response to sperm competition in passerines (Immler et al. 2011). Why some females choose to mate multiply remains to be investigated, but the negative relationship of alpha ejaculate size with age suggests both a possible mechanism for previous observations of reduced alpha siring success at advanced ages (DuVal 2012) and that fertilization assurance may play an important role in female mating behavior. It is unknown whether decreased siring success of older alphas results from low sperm counts, or whether reduced visitation from females results in lower sperm production by these males. Details of the timing and number of female copulations in relation to fertilizations will be important for isolating these effects, and future work will examine whether sperm limitation influences female mate choice in this species. The present study provides the first detailed characterization of intraspecific variation in sperm morphology of any suboscine passerine and the necessary foundation for such future investigation.

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