

# No evidence that sperm morphology predicts paternity success in wild house wrens

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**Abstract** Postcopulatory sexual selection (PCSS) in internally fertilizing vertebrates is a topic of great interest, yet relatively little is known about the characteristics of sperm and ejaculates that confer an advantage in PCSS. In this study, we investigated several measures of sperm morphology that potentially contribute to fertilization success under PCSS. We tested whether sperm morphology related to success in PCSS (via extra-pair paternity) in house wrens (*Troglodytes aedon*). We found no evidence that sperm morphology differed between extra-pair sires and the within-pair males they cuckolded, nor that sperm morphology correlated with the proportion of within-pair offspring sired, the number

of extra-pair offspring sired, or the total annual reproductive success. Male behavioral strategies may affect the probability that their sperm compete with other males' sperm and that their sperm succeed under competition. Effects of these behavioral strategies, as well as differences between males in sperm number, could mask the effects of sperm morphology on the outcome of PCSS. Despite moderate levels of extra-pair paternity, selection on sperm may be relatively weak in house wrens. Further work is needed to understand general patterns in how sperm morphology relates to fertilization success within species.

**Keywords** Passerine · Extra-pair paternity · Postcopulatory sexual selection · Sperm competition · Sperm morphology · *Troglodytes aedon*

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## Introduction

When females copulate with multiple males, sperm from those males compete, and females may exert cryptic choice for certain sperm traits, generating postcopulatory sexual selection (PCSS; Parker 1970; Eberhard 1996). While in some species it is clear that sperm characteristics affect fertilization success (reviewed in Snook 2005; Pizzari and Parker 2009), in most species, we know relatively little about how between-male variation in sperm characteristics affects the outcome of PCSS. Furthering this knowledge is important to understand how PCSS occurs and how it relates to precopulatory sexual selection (e.g., Sheldon 1994; Andersson and Simmons 2006; Kvarnemo and Simmons 2013). It is perhaps particularly important for passerine birds, since PCSS occurs in many species (generally via extra-pair (EP) paternity; Griffith et al. 2002), but most studies focus only on somatic characteristics involved in precopulatory processes such as mate choice and/or male–male competition.

Several ejaculate and sperm traits affect competitive fertilization success in controlled experiments in various taxa: success correlates with sperm number (e.g., insects: Gage and Morrow 2003; Pattarini et al. 2006; but see Gage et al. 2004), sperm swimming speed or mobility (birds: Birkhead et al. 1999; Donoghue et al. 1999; Denk et al. 2005; fishes: Gage et al. 2004; Liljedal et al. 2008; Gasparini et al. 2010; Rudolfson et al. 2008), sperm size (mice: Firman and Simmons 2008; insects: Gage and Morrow 2003; Pattarini et al. 2006; García-González and Simmons 2007; but see Simmons et al. 2003; Gage et al. 2004; Denk et al. 2005; Gasparini et al. 2010; Simmons and Fitzpatrick 2012), and combinatorial ejaculate traits (Lüpold et al. 2012). The importance of these traits to PCSS specifically in passerine birds is corroborated by comparative studies. Passerine species with more frequent multiple mating likely produce more sperm, as they have larger testes (Møller and Briskie 1995; Lifjeld et al. 2010; Rowe and Pruett-Jones 2011), and they have faster-swimming sperm (Kleven et al. 2009a), sperm cells with longer total lengths (Kleven et al. 2009a; Lüpold et al. 2009a; Lifjeld et al. 2010; but see Immler and Birkhead 2007), and a higher proportion of motile, viable, and morphologically normal cells (Rowe and Pruett-Jones 2011). Higher PCSS (estimated using relative testes size and/or the rate of EP paternity) also correlates with lower variability in sperm length across passerines, likely reflecting stronger stabilizing selection for optimal sperm morphology in these species (Calhim et al. 2007; Immler et al. 2008; Kleven et al. 2008; Lifjeld et al. 2010).

Sperm morphology may be under direct selection, as suggested in insects by Pattarini et al. (2006), or because morphology correlates with functional sperm characteristics. In passerines, total sperm length correlates with the length of females' sperm storage tubules, perhaps indicating direct selection (Briskie et al. 1997). Total sperm length may also correlate with sperm longevity (positive correlation in Helfenstein et al. 2008, but see Kleven et al. 2009b; negative correlation in Helfenstein et al. 2010; no correlation, Kleven et al. 2009a). Studies in passerines show that the length of the sperm midpiece (which contains fused mitochondria and wraps around the flagellum), or its length relative to total sperm length, correlates positively with swimming speed (Lüpold et al. 2009a; Laskemoen et al. 2010), as does relative flagellum length (Lüpold et al. 2009a; Mossman et al. 2009; Helfenstein et al. 2010; Immler et al. 2010). However, other studies find no relationship between sperm morphology and velocity (Kleven et al. 2009a; Lüpold et al. 2009b; Immler et al. 2010 in the postexperimental dataset, Lifjeld et al. 2012). The relationship between swimming speed and midpiece size may explain why passerine species with higher PCSS have relatively longer midpieces (Lüpold et al. 2009a; though swimming speed did not correlate with the strength of PCSS in that study, and see Immler and Birkhead 2007).

Although theoretical work suggests that sperm numbers may be the most critical determinant of male fertilization success in passerines (Immler et al. 2011), the above studies suggest that variation in sperm morphology and swimming speed within a species could affect a male's sperm competitive ability. To our knowledge, only two papers have tested these predictions in wild passerines. Laskemoen et al. (2010) found that male tree swallows (*Tachycineta bicolor*) that sired a higher total number of offspring (within pair (WP) and EP) had larger cloacal protuberances (likely indicating higher sperm production) and higher midpiece/total sperm length ratios, when controlling for cloacal protuberance size. Sperm swimming speed did not predict success (Laskemoen et al. 2010). Calhim et al. (2011) found in superb fairy-wrens (*Malurus cyaneus*) that a shorter flagellum or lower flagellum/head ratio correlated with increased success in siring EP offspring in other nests, but decreased success in maintaining WP paternity. They found no patterns with cuckoldry and midpiece/flagellum ratios or total midpiece length (Calhim et al. 2011). Given the complexity of the above results, and the paucity of studies, more work is needed to understand what sperm traits confer a fertilization advantage in PCSS in passerines.

Here, we examined how sperm morphology relates to fertilization success in wild house wrens (*Troglodytes aedon*). The house wren is a passerine species with a moderate level of EP paternity (13.7–20 % of offspring, e.g., Forsman et al. 2008; LaBarbera et al. 2010; Cramer 2013). The potential for PCSS is therefore substantial. Based on results from the above studies, we predicted that males whose sperm were more successful (in terms of WP paternity, EP paternity, and total reproductive success) should have longer sperm and/or a longer relative midpiece or flagellum length. This prediction assumes that longer midpieces and/or flagella promote faster swimming and that faster swimming confers an advantage in PCSS. Alternatively, we predicted that males with more successful sperm might have sperm total lengths that were less divergent from the population mean total length, based on the comparative evidence for stronger stabilizing selection on sperm total length in species with higher PCSS. Finally, because within-male variability in sperm morphology possibly arises due to errors during sperm production (Cohen 1967; Knudsen 2009), we further predicted successful males to have lower intra-ejaculate variation in sperm total length.

## Methods

### Field methods and study system

This study was conducted on two field sites in Ithaca, NY (42°31'N, 76°28'W), that are 3 km apart, with about 75 nest boxes on each site. For details on the study sites, see

Llambías et al. (2012). Adults were captured between April and August in 2009–2011 using either mist nets or box traps. We banded and bled all birds and collected sperm samples by cloacal massage (e.g., Kleven et al. 2008; see Cramer et al. 2013 and Cramer 2013 for further details). Nestlings were banded when approximately 8 days old, and all banded nestlings were assumed to fledge unless there were signs of nest depredation. Annual reproductive success was the total number of genetic offspring fledged in the year, with paternity evaluated as described below.

We measured the head, midpiece, and exposed flagellum lengths of 30 morphologically intact sperm cells per ejaculate sample using brightfield microscopy, image capture, and on-screen image analysis (details in Cramer et al. 2013: magnification  $\times 320$  and  $\times 400$  in 2009 and 2010–2011, using Leica Microsystems (Heerbrugg, Switzerland) and Zeiss Axiover AxioCam MRm (Zeiss Inc), respectively). For two males, only 29 sperm cells were available for measurement. From these measurements, we calculated total sperm length, flagellum/head ratio (where the flagellum is the sum of the midpiece and the exposed flagellum), midpiece/total sperm length ratio, and variability (estimated by the coefficient of variation in the total length of the sperm cells,  $CV_{wm}$ ). To test the hypothesis concerning the divergence of sperm length from the population mean, we took the absolute value of the difference of sperm length from the mean sperm length of the year (to control for microscope effects). Hereafter, we call this variable “length deviance.”

For paternity analysis, we used the seven microsatellite loci and genotyping conditions described in LaBarbera et al. (2010), compared the genotypes of offspring and their putative parents in Cervus 3.0 (Kalinowski et al. 2007), located Mendelian mismatches between social parents and offspring using GenoPed (Z. Zhang), and confirmed those mismatches by re-genotyping (see also Cramer 2013). To conservatively estimate EP paternity, we attributed a chick to EP paternity if it had more than one mismatch with its WP father that could not be attributed to a null allele. We considered a candidate EP sire with a single null allele mismatch to be the genetic father if there was no alternative sire that lacked mismatches (Dakin and Avise 2004). Because we collected blood from all known territorial males, unassigned EP fathers could have been territorial males breeding just off site (the study sites are surrounded by suitable house wren habitat) or in undetected natural cavities on site (we typically found one to two natural cavities per breeding season and sampled the males when possible). Males that bred on site may have sired EP offspring off site, which we did not detect, but these events should be random with respect to sperm morphology and therefore should not bias our results. Data reported here are a subset of the paternity data presented in Cramer (2013), focusing on nests where sperm had been measured for either the WP sire or for an EP sire.

## Statistical analysis

In total, we measured sperm and paternity success for 57 males in 1 year only, 12 males in 2 years, and two males in all 3 years. Only sperm samples taken in the year where paternity was analyzed were used. For males with paternity success and sperm data in more than 1 year, we randomly chose 1 year to include for each male. 9 males had 2 sperm samples collected in the same year, and for correlational analyses, we randomly chose a sample to include (main results are the same if we instead used the sample date closest to clutch initiation, but doing so produced a bias in capture date with success measures, not shown). Males were sampled at various points during the season, which could add noise to some analyses. That is, while the total sperm length, the midpiece/total sperm length ratio, and length deviance do not correlate with capture date, capture date correlates with other measures of sperm morphology in this population (Cramer et al. 2013 and unpublished data). The flagellum/head ratio increases from an average of approximately 5 to 5.5 over the season (due to a tendency for head length to decrease and a significant increase in flagellum and midpiece lengths across the season: Cramer et al. 2013 and unpublished data). Within-male variation in sperm total length is quadratically related to date, with a mean  $CV_{wm}$  of about 5 % at the beginning of the season, declining to about 3 % in the middle of the season (Cramer et al. 2013). It is unclear whether these seasonal affects reflect within-individual changes in sperm or between-male differences in sperm morphology coupled with a bias in capture timing (Cramer et al. 2013). We therefore find it more conservative in the current analyses to compare actual sperm measurements rather than performing a statistical control for date (though including statistical control for date produced the same results). Additionally, between-male variation in both flagellum/head ratio and  $CV_{wm}$  was relatively high compared to the seasonal changes (Cramer et al. 2013), so there should be sufficient variation to detect effects of sperm morphology on success in PCSS.

We conducted paired *t* tests to compare sperm morphology of EP males to the WP males they cuckolded. Because we did not have sperm samples from all males, the final data set for paired comparisons included 25 unique pairs of EP and WP sires, encompassing 34 males. Ten males were included in the data set multiple times (for cuckolding multiple males, being cuckolded by multiple males, or both cuckolding and being cuckolded): 7 males appeared twice, 1 appeared 3 times, 1 appeared 4 times, and 1 appeared 5 times. There was one instance of reciprocal cuckoldry, where each member of the pair cuckolded the other. Each paired comparison, however, was unique, so we treated these 25 pairs as independent data points. For males captured multiple times in 1 year, we used the capture time closest to clutch initiation,

which did not produce a bias in capture dates (mean difference =  $6.76 \pm 5.38$  days,  $t_{24} = 1.26$ ,  $p = 0.22$ ).

We tested for correlations between sperm traits and the proportion of social offspring that a male sired: 27 males sired all offspring in their own nest, while 28 lost paternity of  $2.0 \pm 1.3$  (mean  $\pm$  SD; range, 1–5) offspring in their own nests. We included males that maintained complete WP paternity in these analyses because experimental work in two other passerine species indicates that many more females perform EP copulations than have EP chicks (Michl et al. 2002; Fossøy et al. 2006), suggesting that complete WP success may reflect success in sperm competition rather than an absence of sperm competition. Excluding males that maintained complete WP paternity did not result in any changes to the main conclusion of these comparisons (Table S1). We also tested for correlations between sperm traits and the number of EP offspring a male sired. Twenty-one males sired  $1.8 \pm 1.1$  (1–5) EP offspring, and 48 sired no EP offspring, after excluding males that left the study site immediately after capture without siring EP offspring (as these males likely moved off site to breed (pers. obs.) and may have sired EP offspring there). We further tested for correlations between sperm traits and total annual reproductive success ( $n = 49$  males). We conducted additional categorical tests to further investigate these patterns (Supplementary Materials 2, Table S2).

For each of these analyses, we also investigated models that controlled for potential confounding effects such as sample date, male age, size, and body condition, but results were unchanged (not shown). We also tested for differences in date with measures of success, to assess the possibility that date effects on sperm could have biased our results. The date of sperm sampling did not correlate with continuous measures of success (proportion of WP young sired, Spearman's  $\rho = -0.21$ ,  $p = 0.12$ ; number of EP young sired, Spearman's  $\rho = 0.17$ ,  $p = 0.17$ ; annual reproductive success, Spearman's  $\rho = -0.13$ ,  $p = 0.38$ ).

Our a priori predictions concerned sperm total length, length deviance, within-male variability in sperm morphology, the flagellum/head ratio, and the midpiece/total sperm length ratio. For completeness, we also tested for

correlations with the length of individual sperm components. Annual proportion of WP young sired, number of EP young sired, and reproductive success could not be transformed to fit any known distributions, so we used nonparametric Spearman rank correlations when testing for associations with sperm traits. For the paired  $t$  tests, the distribution of differences was normal for all variables. These statistical tests were performed in JMP 7.0 (SAS Institute, Cary, NC). Correction for multiple testing was conducted using false discovery rate (Benjamini and Hochberg 1995), implemented in R version 2.15.1 (R Development Core Team 2012).

## Results

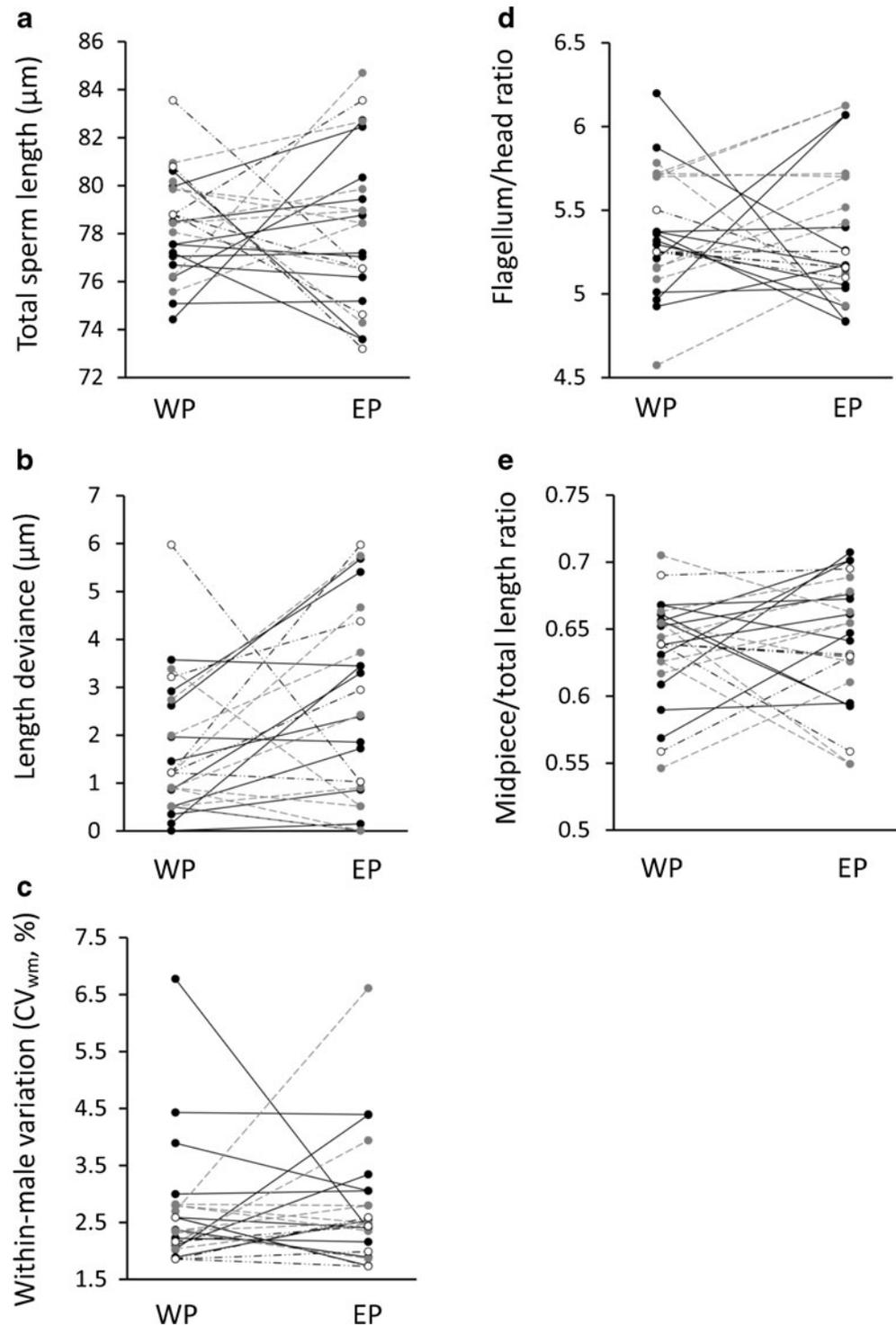
In this house wren population, 13.5 % (116/857) of offspring in 37.6 % (68/181) of broods were EP, and 83.6 % (97/116) of the EP offspring could be assigned an EP father (values from the larger data set reported in Cramer 2013, including males for which sperm was not sampled). Of the 68 broods with EP offspring, 54 (79 %) broods had a single EP sire, and the remaining 14 broods had 2 to 5 EP sires (overall mean  $\pm$  SD,  $1.26 \pm 0.64$  EP sires per nest). All but 2 identified EP sires (47/49, 96 %) also attracted a mate on site that year; the remaining 2 males were observed in territorial encounters on site but disappeared shortly thereafter.

Sperm measures did not differ significantly between EP males and the WP males they cuckolded in paired comparisons, though there was a trend for EP males to have sperm that were more divergent from the population mean than the WP males they cuckolded (Table 1; Fig. 1). Sperm morphology did not correlate with the proportion of WP young a male sired, the number of EP offspring a male sired, or with total annual reproductive success (Table 2; Fig. S1). Midpiece length negatively correlated with number of EP offspring sired, but this relationship was not robust to correction for multiple testing.

**Table 1** Mean values of sperm traits and paired comparisons of EP males to the WP males they cuckolded ( $n = 25$  unique pairs, 34 males)

Sperm trait	EP mean	WP mean	Mean difference $\pm$ SE	Test statistic ( $p$ )
Total sperm length	78.15	78.36	$-0.21 \pm 0.85$	$t_{24} = -0.25$ (0.81)
Length deviance	2.49	1.63	$0.85 \pm 0.42$	$t_{24} = 2.04$ (0.052)
CV <sub>wm</sub>	0.028	0.027	$0.001 \pm 0.003$	$t_{24} = 0.44$ (0.66)
Flagellum/head	5.36	5.36	$-0.00 \pm 0.11$	$t_{24} = -0.00$ (1.00)
Midpiece/total length	0.64	0.64	$0.004 \pm 0.01$	$t_{24} = 0.39$ (0.70)
Head length	12.36	12.39	$-0.02 \pm 0.25$	$t_{24} = -0.10$ (0.92)
Midpiece length	49.96	49.80	$0.16 \pm 0.82$	$t_{24} = 0.20$ (0.85)
Flagellum length	65.79	65.97	$-0.19 \pm 0.73$	$t_{24} = -0.26$ (0.80)

**Fig. 1** Paired comparisons of the sperm traits of WP and EP males that cuckolded them. Years are indicated as follows: *black points and solid line*, 2009; *gray points and simple gray dashed line*, 2010; *white points and dot-dashed black line*, 2011. See Table 1 for statistical tests



## Discussion

Our prediction that sperm morphology would relate to paternity success was not supported. Sperm morphology did not differ in paired comparisons between EP males and the males they cuckolded. It also did not correlate with success in maintaining WP paternity, in gaining EP paternity, nor

with annual realized reproductive success (the sum of WP and EP paternity). We had based the prediction largely on the results of comparative studies, where some aspect of sperm morphology typically correlates with the level of PCSS. However, the relationship between sperm morphology and PCSS depends on the species studied (Immler and Birkhead 2007; Kleven et al. 2008; Lüpold et al. 2009a), suggesting

**Table 2** Correlations between sperm traits and male fertilization success. Male sperm traits were analyzed in relation to the proportion of social offspring that a male sired (Prop. WP;  $n=55$  males), the number of extra-pair young sired (N EP,  $n=70$  males), and annual reproductive success (RS,  $n=49$  males). The  $p$  values are uncorrected for multiple testing; no tests were significant after correction

Sperm trait	Spearman's $\rho$ ( $p$ )		
	Prop. WP	N EP	RS
Total sperm length	-0.16 (0.25)	-0.12 (0.33)	0.03 (0.82)
Length deviance	-0.07 (0.59)	-0.09 (0.46)	-0.01 (0.94)
CV <sub>wm</sub>	0.09 (0.51)	-0.11 (0.35)	0.02 (0.87)
Flagellum/head	-0.08 (0.55)	0.01 (0.91)	-0.08 (0.57)
Midpiece/total length	0.22 (0.10)	-0.19 (0.12)	-0.01 (0.96)
Head length	0.05 (0.74)	0.03 (0.80)	0.20 (0.16)
Midpiece length	0.07 (0.64)	-0.24 (0.05)	-0.01 (0.94)
Flagellum length	-0.15 (0.27)	-0.10 (0.43)	0.02 (0.89)

that selective pressures may vary among lineages. Moreover, interspecific patterns commonly are not apparent in intraspecific studies (e.g., Read and Weary 1992; or compare Lüpold et al. 2009a, b).

Two previous studies found complex effects of sperm morphology on fertilization success in passerines (Laskemoen et al. 2010; Calhim et al. 2011). However, both studies were in species with very high levels of EP paternity (48 % of offspring in tree swallows, Delmore et al. 2008, and 72 % of offspring in superb fairy-wrens, Dunn and Cockburn 1998), where selection on sperm is presumably much stronger than it is in the house wren, which has a more moderate level of EP paternity (on average, 13.7 % of EP offspring in our study site across 4 years; Cramer 2013). Additionally, house wrens may be under weaker stabilizing selection than would be expected even given their rate of EP paternity. Across passerine species, EP paternity rate is strongly and negatively correlated with between-male variability in sperm length (CV<sub>bm</sub>), putatively due to stronger stabilizing selection in species with higher PCSS (Calhim et al. 2007; Kleven et al. 2008; Lifjeld et al. 2010). Based on the correlation in Lifjeld et al. (2010), the CV<sub>bm</sub> for house wrens is predicted to be 2.8 %, but the observed value is 4.0–5.0 % (Cramer et al. 2013). This deviation from the expected value is relatively large compared to the range of CV<sub>bm</sub> values observed across species (1.13–6.2 % across 55 species in Lifjeld et al. 2010). While the high between-male variance in sperm length should increase the statistical power for finding relationships between sperm measures and paternity success, it could also reflect weakened selection on sperm in house wrens. If selection on sperm morphology is sufficiently weak, a relationship between sperm morphology and paternity success may not be detectable without extreme sample sizes, even with high between-male variation. It is unclear why selection should be so weakened in house wrens. The trend for EP males to have more deviant sperm lengths than the WP males they cuckolded is consistent with this heightened CV<sub>bm</sub>, though we did not find further evidence that length deviance affected success.

Successful EP males may use behavioral strategies that enhance fertilization success, and these behavioral effects could easily mask effects of sperm morphology. The last male to copulate may sire a disproportionate number of offspring (Birkhead and Møller 1992), and copulating at the peak of female fertility may also improve fertilization success (e.g., Crowe et al. 2009). Studies on passerine copulation are rare, but where studied, EP males do not appear to time their copulations better than WP males (e.g., Johnsen et al. 2012). In domestic fowl (*Gallus gallus*), males strategically invest large numbers of sperm into EP copulations (Pizzari et al. 2003), although this is not the case in the only passerine studied (Birkhead and Fletcher 1995). Relatively little is known about copulation behavior in house wrens specifically (Brylawski and Whittingham 2004).

While sperm morphology does affect competitive fertilization success in some experiments (Gage and Morrow 2003; Pattarini et al. 2006; García-González and Simmons 2007; Firman and Simmons 2008), sperm number, swimming speed, and/or mobility may be the most important factor for many species (e.g., Birkhead et al. 1999; Donoghue et al. 1999; Gage and Morrow 2003; Gage et al. 2004; Denk et al. 2005; Snook 2005; Pattarini et al. 2006; Liljedal et al. 2008; Gasparini et al. 2010; Immler et al. 2011). We did not measure sperm number, but if females perform WP copulations more frequently than EP copulations, WP males may have a substantial numerical advantage over EP males (Birkhead et al. 1987; Fossøy et al. 2006), which could obscure effects of sperm morphology. Rather than measuring sperm swimming speed, we used two aspects of morphology (the flagellum/head ratio and the midpiece/total sperm length ratio) that correlate with sperm swimming speed in some other passerines (Lüpold et al. 2009a; Mossman et al. 2009; Helfenstein et al. 2010; Immler et al. 2010; Laskemoen et al. 2010). However, these morphological measures may not correlate with sperm swimming speed in house wrens: several studies in other passerines report no correlation between swimming speed and morphology (e.g., Kleven et al. 2009a; Lüpold et al. 2009b; Lifjeld et al. 2012). If sperm swimming speed is the target of selection in

house wrens, but neither of these morphological traits correlates with it, we would not expect to find a difference in sperm morphology between EP and WP males.

As with most studies on wild birds, we assessed EP behavior only by the paternity of nestlings rather than by directly observing copulations. Using paternity outcomes to measure success in PCSS, without knowing about precopulatory processes, may be problematic. Males with the least successful sperm—those that copulated with females but failed to fertilize their eggs—were not identified, though they would be the most informative group to compare against successful males. Moreover, both pre- and postcopulatory processes could explain variation in paternity outcomes. Males could have maintained complete paternity in their own nests if their sperm outcompeted the sperm of all EP males their mates copulated with or if their females did not mate multiply. Males that did not sire EP offspring on site could include males that copulated with EP female(s) without achieving fertilizations as well as males that failed to attract EP females for copulation. We could partially overcome this issue by performing tests within subsets of the data that putatively reflect postcopulatory effects more strongly (namely, by focusing on males that were known to face PCSS because they sired offspring in mixed-paternity broods; Table S2; also, see Table S1). While these comparisons necessarily had reduced sample size and statistical power, the mean sperm values for successful and unsuccessful males were still very similar, suggesting that any effects of sperm morphology on EP fertilization success, if present, must be quite small. Furthermore, this approach to testing for a relationship between sperm morphology and success in PCSS is the most biologically relevant approach for a socially monogamous passerine, since PCSS largely occurs through EP copulations in such species.

In conclusion, we did not find support in house wrens for the hypothesis that sperm morphology affects PCSS, despite evidence from comparative studies that sperm characteristics relate to PCSS in passerines (e.g., Kleven et al. 2009a; Lüpold et al. 2009a; Lifjeld et al. 2010). Males may use behavioral strategies to enhance reproductive success, effects of variation in sperm number or swimming speed could mask effects of sperm morphology, and within-species variation may be too slight to detect effects on reproductive success without very large sample sizes (but see Laskemoen et al. 2010; Calhim et al. 2011). Additional work in more species is needed to understand how sperm traits affect PCSS.

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**Ethical standards** This study complied with the current laws of the countries in which it was performed. Animal use was approved under Cornell Institutional Animal Care and Use Committee (Protocol 2007–0123), and the appropriate state and federal bird banding permits were obtained (numbers 1231 and 20954, respectively).

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