Male age and its association with reproductive traits in captive and wild house sparrows

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Running title: Reproductive traits and male age in a passerine

Abstract

Evolutionary theory predicts that females seek extra-pair fertilisations from high-quality males. In socially monogamous bird species, it is often old males that are most successful in extra-pair fertilisations. Adaptive models of female extra-pair mate choice suggest that old males may produce offspring of higher genetic quality than young males because they have proven their survivability. However, old males are also more likely to show signs of reproductive senescence, such as reduced sperm quality. To better understand why old males account for a disproportionally large number of extra-pair offspring and what the consequences of mating with old males are, we compared several sperm traits of both captive and wild house sparrows, *Passer domesticus*. Sperm morphological traits and cloacal protuberance volume (a proxy for sperm load) of old and young males did not differ substantially. However, old males delivered almost three times more sperm to the female's egg than young males. We discuss the possibility that old males outcompeted young males after copulation and the consequences for females mated with old males.

Keywords: multiple mating; extra-pair paternity; internal fertilisation; sperm competition; polygamy; gamete selection

Introduction

In socially monogamous mating systems, mating outside the pair-bond (i.e. extra-pair mating) is adaptive for females if females gain direct (e.g. access to resources), or indirect (i.e. genetic) benefits [1]. In birds, male age is a robust predictor of extra-pair paternity [2]. Models of female choice support a preference for old males because old males have proven their viability, and female preference for old males could evolve if female preference is heritable and male viability is passed on to genetic offspring [3,4]. However, a recent study found no evidence that females preferred to mate with of old males [5]. Additionally, old males are ageing or senescent males, which means that their sperm – the only direct benefit passed on in an extra-pair mating – will be of lower quality [6,7]. An age-related reduction in sperm quality could incur direct (e.g. reduced fertilising efficiency) and indirect (e.g. decreased offspring fitness) costs to females mated to old males [7]. For instance, in insemination experiments in houbara bustards, *Chlamodytis undulata*, advanced paternal age was linked with inhibited post-hatching offspring growth [8]. Advanced paternal age was also associated with lower lifetime reproductive fitness in a wild house sparrow, *Passer domesticus*, population [9]. However, if old males achieve most extra-pair paternity [2], yet are not preferred by females in extra-pair matings [5], maybe it is post-copulatory traits that give old males the edge on young males?

Sperm quantity (e.g. sperm number) and sperm quality (e.g. morphology) are decisive for male reproductive success and scientific knowledge about the effects of male age on sperm traits is rapidly growing. Meta-analytical evidence showed that sperm quality decreases with increasing male age in humans, *Homo sapiens* [10] , and a similar trend has been found in brown Norway rats, *Rattus norvegicus*, [11] blue-footed boobies, *Sula nebouxii* [12], barn swallows, *Hirundo rustica* [13] and red junglefowl, *Gallus gallus* [14]. However, if sperm quality decreases with age, maybe other post-copulatory traits are at work for old males to sire a disproportionally large number of extra-pair offspring. What if old males, whilst producing

lower quality sperm, have increased sperm production? A higher number of sperm could give old males a numerical advantage over young males during sperm competition despite the overall lower quality of their sperm [15].

Increased sperm production by old males has been observed in internally and externally fertilising fish [e.g. 16–18]. In humans, male age and sperm number do not seem to be associated [10]. In birds, there are hints of sperm number being associated with male age when testes size is considered to be a proxy for sperm quantity [19,20] . Male birds in their first year of breeding have testes that are approximately 27% smaller than testes of older breeders [21]. Also, male passerines develop a cloacal protuberance indicative of their reproductive status [22], relative testes size and capacity to store sperm [23]. The larger a male's cloacal protuberance, the larger his relative testes size and hence sperm reservoir [23]. Again, old males have a larger cloacal protuberance. In two Australian fairywren species, *Malurus lamberti* and *splendens*, old males had larger cloacal protuberances than first-year breeders, and sperm number correlated positively with cloacal protuberance size [24] (but see [25]). Cloacal protuberances were also larger in old reed buntings, *Emberiza schoeniclus*, and increased in size with age within males [26]. Collectively, these findings provide support for age-related variation in reproductive traits and are consistent with the observation that old males robustly gain more extra-pair paternity across bird species [2].

In house sparrows it is unclear what sperm phenotype maximises fertilising capacity. One study concluded that sperm with relatively short heads swam fastest, and sperm length was positively associated with sperm longevity [27], but no such association was found in another study [28]. Sexual selection will favour both a sperm's capacity to outcompete rival's sperm and avoid being outcompeted [29], analogous to offence and defence strategies in sports. Therefore, multiple sperm traits will affect sperm performance and multiple sperm traits need to be analysed to understand differences in sperm competitiveness.

Here, we tested the hypothesis that post-copulatory competitiveness changes with age in captive and wild house sparrows. Our specific aims were to test: (1) whether sperm length is associated with male age, without predicting directionality; and (2) if the proportion of morphologically abnormal sperm is higher in old compared to young males. Further, to indirectly assess whether old males produce more sperm than young males, we studied (3) cloacal protuberance volume, and (4) the number of sperm trapped on egg membranes (i.e. perivitelline layers, hereafter PVL) [30]. In birds, the egg is surrounded by the PVL and the number of sperm at the PVL exemplifies the number of inseminated sperm, and the probability of an egg being fertilised [30–32].

Materials and Methods

Captive house sparrows

House sparrows were kept at the Max Planck Institute for Ornithology in Seewiesen, Germany, (47.9752° N, 11.2332° E) since 2005. The cohorts of 2005 and 2006 were wild-caught birds from rural Bavaria [33] and breeding took place in most of the subsequent years. All birds were fitted with a unique numbered metal ring and combination of colour rings for identification. The specific husbandry under semi-natural conditions has been described and illustrated previously [5,34].

Wild house sparrows

The wild house sparrows are resident on Lundy Island, approximately 19 km off the coast of Devon, England (51.1781° N, 4.6673° W). The population has been systematically monitored since 2000 allowing for individual identification and knowledge of precise individual ages, and social and genetic pedigrees. Annual resighting rates are 91-96% and migration to and from the mainland is almost absent [35,36].

Sperm collection techniques

Sperm were collected during the reproductive season of house sparrows (March until August) [37] in 2014 and 2015. Sperm were obtained using the standard techniques of faecal and abdominal massage sampling, which we have described and illustrated in depth previously [34]. Briefly, samples were stored in 200µl of 5% formalin before placing 10-µl aliquots onto microscope slides for morphological assessment of sperm. House sparrow males replenish their ejaculates overnight [38]. In captivity, we isolated males and females for at least two days before sperm collection to standardise samples for males' mating histories, which affect post-meiotic sperm senescence independent of male age [7,18]. In the wild, males could not be isolated from females, and we only applied abdominal massage to collect sperm.

Length of sperm components

Sperm linear measurements were as described [34]. Briefly, we took digital images of the first ten intact (i.e. no broken tails or heads), unobstructed (i.e. not covered by detritus), and morphologically normal sperm (see the abnormality section below for a definition). We always started in the upper left corner of the microscope slide using a Leica DFC450-C camera mounted on a Zeiss Axioplan-2 microscope at 400x magnification (40x objective) in bright field settings. Sperm components (i.e. head including acrosome, flagellum including midpiece) were measured from digital images using the Leica Application Suite (LAS) software v4.2. by one observer only (GC), who was blind regarding sample identities. Total length was calculated as the sum of the head and flagellum measures and mean observer repeatability was high for all sperm components ($R > 0.82$) [34] .

Proportion of morphologically abnormal sperm

Sperm were classified as abnormal if they deviated from the typical passerine (oscine) shape, which consists of an acrosome, a nucleus, and a flagellum, consisting of the midpiece whose mitochondria form a helix around the axoneme and the non-helical tail [39]. Abnormalities affected all sperm components, such as sperm heads (e.g. bends of more than 90°), midpieces (e.g. distal cytoplasmic droplets) and tails (e.g. coiled, stubbed or super

144 numerous). Sperm abnormality screening of the first 100 intact and unobstructed sperm was
145 done by one observer only (AG), always starting in the upper left corner of each microscope
146 slide. To establish observer repeatability, a subset of 20 microscope slides was randomly
147 selected using the function sample in R version 3.5.3 [40]. Sperm were then screened again,
148 following the same protocol, so that in principle the individual sperm measured were identical
149 on both occasions. However, the microscopes used differed between the two occasions. While
150 we mostly used the Zeiss Axioplan-2 microscope, we also relied on a substitute, Olympus BX
151 50, microscope. Observer repeatability (here and all following data) was calculated using the
152 R package rptR v. 0.9.2 [41] in R version 3.5.3 [40]. Because the second microscope introduced
153 variation to the data, we added it as a fixed effect to calculate adjusted observer repeatability
154 for abnormality scores. Adjusted observer repeatability was high: R = 0.78 ± 0.11 standard
155 error (SE) (95% CI (Confidence Interval): 0.50 to 0.94, $P < 0.0001$, (see the Supplements for
156 the unadjusted observer repeatability analysis). Further, the observer could guess the age of
157 some captive males from the sample descriptions but attempted to hide descriptions from
158 view when scoring abnormal sperm to be blind in the majority of the measurements.
159 ***Cloacal protuberance volume***
160 The diameter and height of the cloacal protuberance was measured with callipers to
161 the nearest 0.1 mm by one observer per population. Measurements took place before
162 abdominal massages were applied [25]. We used the cone formula($\frac{1}{3}\pi r^2 h$, r = cloacal
163 protuberance width/2, h = cloacal protuberance height) to calculate cloacal protuberance
164 volume because a cone best describes the shape of the cloacal protuberance of house sparrows
165 [22]. The observer remeasured 136 captive males, kept in single-sex aviaries within 48 hours,
166 expecting cloacal protuberance size to be stable during that period, and estimated observer
167 repeatability, which was high: R = 0.73 ± 0.04 SE (95% CI: 0.64 to 0.80, P <0.001). Observer
168 repeatability for the wild house sparrows could not be estimated because of insufficient repeat
169 measurements (e.g. six recaptures in 2015 with the shortest being 28 days apart). Both

observers measured the same 12 captive house sparrows once each to estimate between-observer repeatability, which was also high: (R = 0.76 ± 0.14 SE (95% CI: 0.38 to 0.92, P = 0.004).

Sperm on PVL

We collected unincubated eggs from captive females that were either held in aviaries with only old males (seven and eight years old), or young males (one and three years old). We did not collect eggs from the wild population. Our aviary set-up (N = 9 aviaries) ensured that eggs could only have been fertilised by males of one age group, dependent on the aviary in which the egg was laid. Note that three-year old house sparrows would be considered "mature" in the wild (e.g. less than 20% of wild house sparrows survive until three years of age) but can be considered young in captivity because mortality in captivity is comparably lower [42]. Aviaries held eight to nine pairs of birds, apart from one aviary with 13 pairs. We counted sperm on the PVL and examined the fertilisation status of 41 non-incubated eggs following an established protocol [43]. We did not count holes made by sperm hydrolysing the PVL because the number of sperm on the PVL correlates with the number of holes [44]. We carefully opened eggs with scissors, removed the germinal disc and washed it with phosphate-buffered saline (PBS). We put the germinal disc on a microscope slide, added a drop of DNA stain Hoechst 33342 (0.05 mg/mL) and searched for diploid cells as evidence of fertilisation [43] with the Zeiss Axioplan-2 microscope in fluorescent mode. Next, we removed the PVL from the yolk, washed it in PBS, and stretched the entire PVL onto a microscope slide. We again added a few drops of Hoechst and systematically counted fluorescent sperm nuclei using the same microscope and a tally counter. Eggs were prepared and examined by one observer only (AG), who was blind towards the experimental age treatment.

Statistical analyses

We ran statistical models using R version 3.5.3 [40] and the package lme4 version 1.1-21 [45]. We used the package arm version 1.10-1 and the function sim [46] to simulate values from

196 the posterior distributions (N = 2000 draws) of the model parameters. Throughout, we used
197 non-informative priors. From the simulated values, we extracted 95% Credible Intervals (CrI).
198 CrI not overlapping zero can be interpreted as a frequentist $P < 0.05$ [47]. In line with recent
199 calls to improve statistical inference, we decided to report our observed effects as continuous
200 measures of strength of evidence against the null hypothesis [48,49], using the language of the
201 "statistical clarity concept" [50], instead of emphasizing statistically significant results.
202 For all models, we followed recommendations to ensure that model assumptions were
203 met, including ruling out overdispersion in non-Gaussian models and multi-collinearity
204 between predictors [47]. In all models, continuous variables (e.g. male age, day of year) were
205 mean-centred and scaled, so that the variables were measured in the unit of standard
206 deviations (SD) from the mean. We specifically refer to either the captive or the wild house
207 sparrow dataset when describing our statistical model structure, unless the model structure
208 was identical for both populations.
209 ***a) Length of sperm components***
210 We fitted linear mixed models with the total length of single sperm components as the
211 response variable. We used individual data from all sperm measured per male (range 10 − 30
212 sperm per male) instead of using means or medians of sperm length. Male age in years was an
213 explanatory variable. Further, we estimated standardized multi-locus heterozygosity (hereafter
214 sMLH) as a proxy for degree of inbreeding from genetic marker data using the R package
215 inbreedR version 0.3.2 [51] to account for potential inbreeding affecting sperm morphology. The
216 identity and details of the genetic markers were published previously [5,52]. We added sampling
217 years (levels: 2014, 2015) and the method of sperm collection (captive house sparrow data
218 only) as explanatory variables (levels: abdominal massage, faeces). Further, captive male
219 house sparrows were either assigned or not to mixed-sex aviaries (N = 16 aviaries), which
220 created a sperm competition environment only for those males in mixed-sex aviaries. We
221 therefore added aviary set-up (levels: with, without females) as an explanatory variable to the

captive dataset. We included sample, male and aviary identities as random effects on the intercept to account for the non-independence of sperm from the same sample, repeated measurements of males and potential aviary grouping effects in the captive house sparrow dataset. We measured 3262 sperm from 127 captive male house sparrows, which were between one to ten years old. For the wild house sparrows, we had 672 sperm available from 34 males aged one to four years.

b) Proportion of morphologically abnormal sperm

Abnormality counts were fitted as a proportional two column matrix response variable using cbind in R (i.e. number of abnormal sperm, number of normal sperm) in generalized linear mixed models assuming a binomial error structure. Male age was modelled as an explanatory variable, as well as sMLH. We further fitted the following explanatory variables to the captive dataset: aviary set-up (N = 7 aviaries) (levels: with, without females), sperm collection method (levels: abdominal massage, faeces), and microscope used (levels: Zeiss, Olympus). Male identity was fitted as random effect on the intercept for the analysis of the captivity data to account for repeated measures. Year (levels: 2014, 2015) was added as an explanatory variable to the wild house sparrow data. Models for both populations were overdispersed [47], so we added an observation-level random effect. We had 87 samples available from 73 captive (between one and ten years old) and 23 samples from 23 wild house sparrows (between one to five years old).

c) Cloacal protuberance volume

To test for an association of the cloacal protuberance size with age, we fitted cloacal protuberance volume as a response variable in a linear mixed model. We accounted for potential seasonal and body size effects by adding day of the year (captivity: 14–21 June, wild: 6 May–17 August) and tarsus length as continuous explanatory variables. Additionally, a squared day of the year term was fitted for the wild house sparrow data because sampling took place during the whole breeding season, which could have led to nonlinear seasonal

changes in cloacal protuberance volume [37]. Further, we included the explanatory variable aviary set-up (N = 7 aviaries) (levels: with, without females) to the captive house sparrow analysis and year (levels: 2015, 2016) to the wild house sparrow analysis. Male identity was fitted as random effect on the intercept but the variance component was estimated as zero for the wild house sparrows. This may mean that we could not fully account for repeated measurements of males. To ensure that the model was robust, we re-ran it using only one randomly selected observation per male (function sample in R [40]; Table S3). We had 195 observations from 142 captive (between one to ten years old) and 56 observations from 46 wild house sparrows (between one to five years old).

d) Number of sperm on PVL

We show descriptive statistics for the number of sperm on the PVL (Figure 1b). We also ran an unequal variances *t*-test to compare the mean number of sperm (log-transformed) from old and young males. However, this approach should be treated cautiously because the male sperm donor and, therefore, the possibility of non-independence of data could not be established.

Data accessibility

All data and the R scripts are publicly available at the Open Science Framework (DOI 10.17605/OSF.IO/PKWSR).

Results

Length of sperm components

We did not find a statistically clear effect of male age on the length of sperm components. This was also the case for sMLH (Table 1, Table 2). As previously shown in the captive population [34], sperm sampled from faeces were shorter than sperm sampled by abdominal massage (Table 1). When the analysis was restricted to abdominal massage sampled sperm (2148 examined sperm from 116 males), the results were qualitatively similar

to the main dataset analyses, showing no statistical clear relationship between length of sperm components and male age (Table S1). Unexpectedly, and not among this study's original predictions, we further found that sperm were longer in males from mixed- than single-sex aviaries (Table 1). Additionally, there were both positive and negative statistical year effects on sperm length components in both populations (Table 1, 2).

Proportion of morphologically abnormal sperm

Captive house sparrows had on average 16.8% ± 12.9 (mean ± SD, N = 87 samples) morphologically abnormal sperm, compared to 5.3% ± 8.7 (N = 23 samples) morphologically abnormal sperm in the wild house sparrows, which was a substantial difference (χ^2 = 5.68, df = 1, P = 0.02). In neither dataset did the proportion of morphologically abnormal sperm and male age show a clear statistical relationship (Table 3). Because we interpreted our result as a lack of statistical association between the proportion of abnormal sperm and male age (Table 2), we can rule out the occurrence of a Type I error in the slightly overparameterized wild house sparrow dataset.

The Olympus microscope caused a statistical upward bias of abnormality scores in the captive population (Table 3). When we restricted the dataset to the main, Zeiss, microscope (51 samples of 38 males instead of 87 samples of 73 males), our interpretation of no clear statistical relationship between the proportion of morphologically abnormal sperm and male age remained qualitatively similar (Table S2).

Cloacal protuberance volume

There was no apparent statistical association between cloacal protuberance volume and male age in either population. This was also the case for sMLH (both populations), the aviary set-up (captive population), method of sampling (captive population) and the year sampling took place (wild population). We further found a large among-male variance in the captive population (Table 4). Cloacal protuberance volume showed a positive statistical association with tarsus size and day of the year in captivity (Table 4). In the wild, cloacal

protuberance volume showed a negative statistical association with the day of sampling, highlighting a seasonal decrease (Table 4).

Number of sperm on PVL

The number of sperm counted ranged from 0 to 1013 (Fig. 1 for an example of two sperm on a PVL). The mean number of old males' sperm reaching the eggs of females (mean ± SD: 147 ± 124, N = 28 eggs) was nearly three times higher than the mean number of young males' sperm (56 ± 53, N = 12 eggs, Fig. 2), which was a considerable difference (unequal variances t-test, $t_{16.73} = 2.36$, $P = 0.03$). We excluded an outlier egg with 1013 sperm (z-score = 7, so 7 SD above the mean value of all sperm counted) from the t-test (Fig. 2). Including it would have strengthened the result. Further, of 41 eggs examined, 39 were fertilised. The two unfertilised eggs originated from an aviary of each male age group.

Figure 1. Sperm on the perivitelline layer (PVL)

Two fluorescent house sparrow nuclei bound on the perivitelline membrane stained with Hoechst 33342.

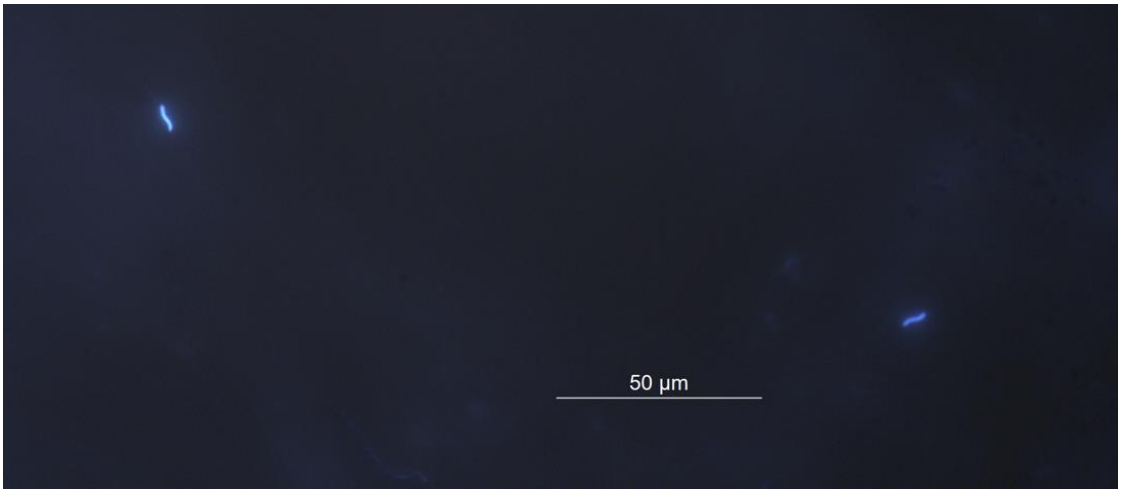
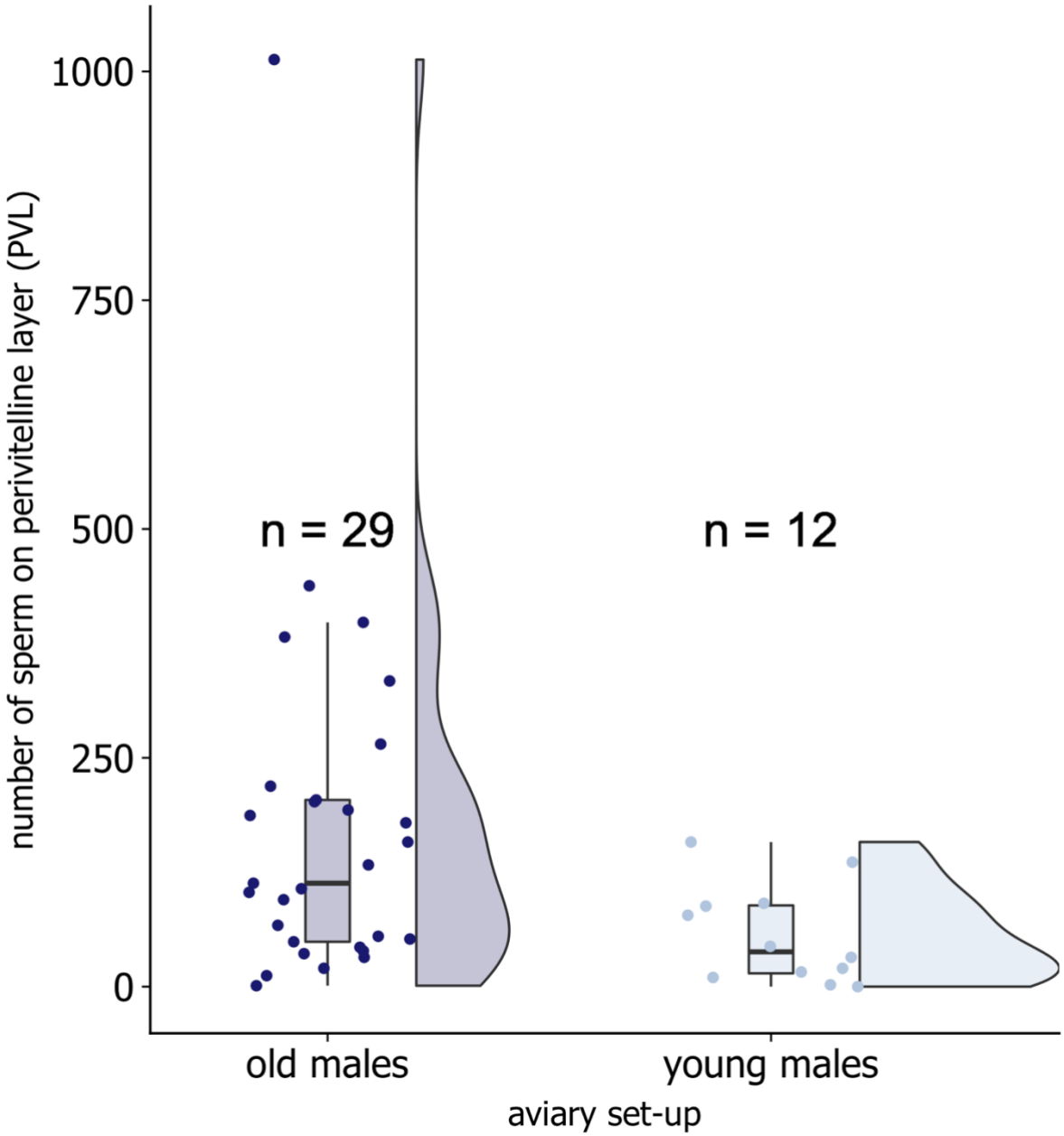


Figure 2. The effect of age treatment on the number of sperm on the PVL

The number of sperm on perivitelline layers (PLV) of 41 eggs was approximately three times higher in aviaries with old (> six years) than aviaries with young males (one to three years). We visualised the raw data including an outlier (one egg with 1013 sperm) using a Raincloud plot, combining box-, split violin- and scatter plots [53].



Discussion

Our overall aim was to elucidate the factors promoting a positive relationship between extra-pair paternity and male age. Specifically, we predicted a sperm quantity–quality trade-off related to male age. However, we found no evidence for such a trade-off in two populations of house sparrows. Specifically, we did not find a clear statistical association of sperm morphology or cloacal protuberance size with male age. Instead, we found that in captivity, the number of old males' sperm in the eggs of females was almost three times higher than the number of young males' sperm. Our result is intriguing because neither the number of mating attempts, the number of copulations nor female choice are explained by male age in this population. Hence, pre-copulatory differences do not seem to explain the age-related difference in extra-pair copulation success and it is tempting to suggest age-related post-copulatory differences between old and young males. Old males might have inseminated more sperm and/or there was cryptic female choice [55] of sperm from old males. Yet, our result is limited by a lack of information on the identities of the males that provided the sperm. For example, did all males in each aviary inseminate females? Also, whether more sperm on PVLs constitute a curse or a blessing remains to be seen too. This is because the more sperm are inseminated, the higher the probability that the egg gets fertilised [30–32] but the risk of embryo mortality caused by multiple sperm entering the egg (i.e. polyspermy) [56] might also be elevated. In our study, 95% of eggs were fertilised (N = 41 eggs total) pointing at two things. First, there was no difference in the fertilising ability of young and old males. Second, that infertility was rare [57]. Indeed, in house sparrows, the biggest cause of unhatched eggs is embryo mortality [58]. Under the assumption that old males inseminate more sperm, this could mean that they outcompete young males with numbers in sperm competition [15], at the cost of an elevated risk of unhatched eggs. Subsequent efforts could investigate the idea of such a double-sided effect of male age.

Cloacal protuberance volume was positively associated with tarsus size, as well as date of measurement in captive house sparrows, whereas it was negatively associated with the date of measurement in the wild house sparrows. In the wild, measurements included the end of the breeding season, so the decline in cloacal protuberance volume can be interpreted as the regression of male reproductive gonadal growth [20,37]. We also found a large among male variance in cloacal protuberance volume in the captive males, emphasizing that individual-level predictors other than age and body size must be at play. It would be worthwhile to analyse other individual-level predictors, such as individual mating status, in the future [20]. The lack of a clear statistical association between sperm length and male age in our data corroborates the results in other passerines with less precise age information [13,59,60].

Our results further revealed differences in sperm length in relation to the year of sampling (i), the social environment (ii), and the method of sperm sampling (iii). (i) The result of differences in sperm length across years might reflect an underlying seasonality. House wrens, *Troglodytes aedon*, [59] and male red-winged blackbirds, *Agelaius phoeniceus*, [61] show seasonal changes in sperm length. In the latter population, sperm length additionally varied across years [61]. (ii) We found that males kept with females had longer midpieces and flagella than males kept with males only. This could indicate a plastic male response to sperm competition, similar to that observed in Gouldian finches, *Erythrura gouldiae*, that increased their midpiece size in high competition environments [62]. Indeed, the social environment affects reproductive development in house sparrows, with males exhibiting declining sperm production and testes degeneration when caged individually [63]. Also, house sparrows' midpiece size shows only weak among male repeatability [27], which might support the idea of a plastic response to the social environment. What is unclear is how longer midpieces and flagella affect a sperm's fertilisation success because, whereas sperm with longer midpieces and flagella make the best swimmers with the highest fertilisation success in zebra finches, *Taeniopygia guttata*, [64] in house sparrows, midpiece length and sperm velocity seem to be

374 negatively correlated [28]. (iii) Additionally, sperm length varied within males in relation to
375 sperm collection method, which is discussed in detail elsewhere [34].

376 The proportion of morphologically abnormal sperm did not show a statistically clear
377 association with male age. This was surprising because we had relatively many old house
378 sparrows (47 captive males older than five years) available and these males are expected to
379 have more mutations in their germline than young males [6]. Yet, our sample size is modest
380 compared to a study using a breeding facility of 1080 houbara bustards, where male age and
381 the proportion of abnormal sperm were positively associated [65]. Whilst sperm morphology is
382 an important factor to evaluate a male's fertilisation efficiency [8], it is also a highly complex
383 trait that is difficult to standardize [66]. One reason is its sensitivity to an apparatus as simple as
384 a microscope, as evidenced in our results. It is thus possible that other analytical approaches
385 such as sperm DNA integrity or oxidative stress status assays [66], are better suited to detect
386 qualitative differences in the sperm of old and young males.

387 To conclude, sperm morphologies important for fertilisation success were unrelated to
388 male age in captive and wild house sparrow. Morphologically abnormal sperm, exemplifying
389 lower quality sperm [67], did not show a clear statistical relationship to male age either, and
390 male's cloacal protuberance sizes were suggestive of similar relative testes sizes and sperm
391 reservoirs in old and young house sparrows. Importantly, the number of sperm reaching the
392 site of fertilisation suggested that PVL sperm number and male age were positively
393 correlated, but sperm number did not translate into a higher number of eggs being fertilised.
394 Our study is an important step towards elucidating post-copulatory traits of old versus young
395 male passerines. Future data will reveal if conditions are met for adaptive interpretations of
396 female extra-pair mating with old males or if mating with old males bears a cost.

Acknowledgements

We thank Annemarie Grötsch and Natalie Fischer for animal care, Bart Kempenaers for access to Seewiesen sparrows, Helen Didsbury for help with sample preparation, Kim Teltscher for practical support, Natalie dos Remedios for molecular work and the Landmark Trust, Lundy Company and Lundy staff for facilitating field work. The captive house sparrow study was supported by the German research foundation (DFG grant SCHR 1147/1-1) to JS. The Lundy study and genotyping were supported by a Natural Environment Research Council grant (NERC Grant NE/N013832/1) to TB. Antje Girndt, Glenn Cockburn and Alfredo Sánchez-Tójar are members of the International Max Planck Research School (IMPRS) of Organismal Biology.

Author contributions

AG and JS conceived the study. AG and AST carried out sample collection, GC measured all sperm, MH supported the laboratory work and TB the molecular work, AG scored sperm abnormalities, performed fertilisation assays, statistical analysis and wrote the manuscript with the help of all co-authors.

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577 **Table 1.** Results from a linear mixed model of a) the total, b) the head, c) the midpiece and d)

578 the flagellum length of 3262 sperm from 127 captive male house sparrows of known age.

	Sperm length (µm)
Captive house sparrows	estimate (lower CrI to upper CrI)
a) total length	
(intercept)	99.48 (98.76 to 100.18)
age	0.36 (-0.10 to 0.86)
sMLH	-0.09 (-0.55 to 0.34)
aviary set-up (with females)	1.06 (0.42 to 1.66)
method (faeces)	-0.51 (-0.92 to -0.09)
year (2015)	-0.32 (-0.89 to 0.25)
Random effects	
male ID	7.15 (5.72 to 8.80)
aviary	0.04 (0.02 to 0.08)
sample ID	0.83 (0.70 to 1)
residual variance	2.88 (2.81 to 2.95)
b) head	
(intercept)	14.12 (13.82 to 14.43)
age	0.06 (-0.08 to 0.19)
sMLH	-0.08 (-0.18 to 0.03)
aviary set-up (with females)	0.15 (-0.15 to 0.42)
method (faeces)	-0.32 (-0.47 to -0.18)
year (2015)	-0.53 (-0.80 to -0.24)
Random effects	
male ID	0.25 (0.19 to 0.31)
aviary	0.03 (0.01 to 0.06)
sample ID	0.17 (0.15 to 0.219)
residual variance	0.86 (0.84 to 0.88)

579
580
581
582

Table 1. continued

	Sperm length (µm)
Captive house sparrows	estimate (lower CrI to upper CrI)
c) midpiece	
(intercept)	66.43 (65.86 to 66.99)
age	0.06 (-0.31 to 0.43)
sMLH	0.12 (-0.21 to 0.45)
aviary set-up (with females)	1.01 (0.53 to 1.51)
method (faeces)	-0.34 (-0.72 to 0.03)
year (2015)	0.98 (0.51 to 1.46)
Random effects	
male ID	4.19 (3.37 to 5.08)
aviary	0.02 (0.01 to 0.03)
sample ID	0.64 (0.53 to 0.76)
residual variance	2.71 (2.65 to 2.77)
d) flagellum	
(intercept)	85.45 (84.72 to 86.15)
age	0.24 (-0.21 to 0.70)
sMLH	0 (-0.44 to 0.41)
aviary set-up (with females)	0.86 (0.28 to 1.46)
method (faeces)	-0.19 (-0.55 to 0.18)
year (2015)	0.14 (-0.43 to 0.70)
Random effects	
male ID	7.40 (5.93 to 9.02)
aviary	0.07 (0.03 to 0.14)
sample ID	0.51 (0.42 to 0.60)
residual variance	2.80 (2.73 to 2.86)

583
584 We accounted for standardized multi-locus heterozygosity (sMLH), aviary set-up (levels: with,
585 without females), sperm collection method (levels: abdominal massage, faeces), and year
586 (levels: 2014, 2015) of sperm collection. Male age, as well as sMLH were centred and scaled.
587 We present posterior means and CrI (95% Credible Interval).

588 **Table 2.** Results from a linear mixed model of a) the total, b) the head, c) the midpiece and d)
589 the flagellum length from 672 sperm of 34 wild male house sparrows of known age.

	Sperm length (µm)
Wild house sparrows	estimate (lower CrI to upper CrI)
a) total length	
(intercept)	99.22 (98.06 to 100.35)
age	-0.07 (-1.03 to 0.90)
sMLH	0.52 (-0.51 to 1.58)
year (2015)	-2.81 (-4.44 to -1.22)
Random effects	
male ID	9.14 (7.26 to 11.80)
residual variance	2.60 (2.47 to 2.74)
b) head	
(intercept)	13.10 (12.82 to 13.39)
age	-0.05 (-0.30 to 0.19)
sMLH	0.13 (-0.11 to 0.37)
year (2015)	-0.29 (-0.73 to 0.17)
Random effects	
male ID	0.57 (0.47 to 0.70)
residual variance	0.82 (0.78 to 0.86)
c) midpiece	
(intercept)	68.02 (67.35 to 68.66)
age	0.40 (-1.08 to 0.07)
sMLH	-0.52 (-0.31 to 0.42)
year (2015)	-0.10 (-1.22 to 1.10)
Random effects	
male ID	2.64 (2.03 to 3.35)
residual variance	2.66 (2.52 to 2.81)

590

591

Table 2. continued

	Sperm length (µm)
Wild house sparrows	estimate (lower CrI to upper CrI)
d) flagellum	
(intercept)	86.06 (85.01 to 87.17)
age	0.05 (-0.83 to 0.91)
sMLH	0.38 (-0.52 to 1.26)
year (2015)	-2.33 (-3.82 to -0.87)
Random effects	
male ID	7.30 (5.71 to 9.28)
residual variance	2.57 (2.44 to 2.71)

592

593 We accounted for sMLH and year of sperm collection (levels: 2014, 2015). Male age, as well

594 as sMLH were centred and scaled. We present posterior means and CrI.

595
596

Table 3. Results from a generalized linear mixed model on the proportion of morphologically abnormal sperm in relation to male age in captive (87 samples of 73 males) and wild house sparrows (23 samples of 23 males).

Proportion of morphologically abnormal sperm (logit-link scale)	
a) Captive house sparrows	estimate (lower CrI to upper CrI)
(intercept)	-2.24 (-2.66 to -1.84)
age	0.16 (-0.06 to 0.38)
sMLH	-0.09 (-0.33 to 0.12)
aviary set-up (with females)	0.15 (-0.58 to 0.80)
method (faeces)	-0.09 (-0.56 to 0.37)
microscope (Olympus)	0.77 (0.11 to 1.44)
Random effects	
male ID	0.26 (0.18 to 0.36)
aviary	0 (0 to 0)
observation-level random	0.57 (0.43 to 0.73)
b) Wild house sparrows	
(intercept)	-3.84 (-4.50 to -3.16)
age	0.22 (-0.39 to 0.83)
sMLH	0.62 (-0.07 to 1.31)
year (2015)	0.44 (-1.01 to 1.90)
Random effects	
observation-level random	1.73 (1.14 to 2.49)

We accounted for sMLH in both populations, aviary set-up (levels: with, without females), sperm collection method (levels: abdominal massage, faeces), the microscope used (levels: Zeiss, Olympus) in the captive house sparrows and year (levels: 2014, 2015) in the wild house sparrows. Male age, as well as sMLH were centred and scaled. We present posterior means and CrI.

607 **Table 4.** Results from a linear mixed model on cloacal protuberance volume (mm^3) in relation
608 to male age in captive (195 observations of 142 males) and wild house sparrows (56
609 observations of 46 males).

Cloacal protuberance volume (mm^3)	
a) Captive house sparrows	estimate (lower CrI to upper CrI)
(intercept)	49.37 (42.05 to 57.03)
age	-1.07 (-4.43 to 2.34)
aviary set-up (with females)	2.57 (-7.91 to 13.90)
day of year	4.13 (0.60 to 7.49)
tarsus	2.86 (0.06 to 5.64)
Random effects	
male ID	222.69 (184.93 to 264.59)
aviary	15.12 (4.45 to 31.69)
residual variance	9.03 (8.19 to 9.97)
b) Wild house sparrows	
(intercept)	3.41 (3.12 to 3.68)
age	0.10 (-0.07 to 0.26)
day of year	-0.17 (-0.51 to 0.15)
day of $year^2$	-0.20 (-0.46 to 0.06)
tarsus	-0.04 (-0.21 to 0.12)
year (2016)	-0.04 (-0.54 to 0.47)
Random effects	
male ID	0 (0 to 0)
residual variance	0.61 (0.50 to 0.75)

610
611 We accounted for day of the year (captivity: 14–21 June, wild: 6 May–17 August) and tarsus
612 size in both populations. Aviary set-up (levels: with, without females) was added to the
613 analysis on captive house sparrows, and year (levels: 2015, 2016) was added to the analysis

614 on wild house sparrows. Cloacal protuberance volume of wild house sparrows was log-
615 transformed.
616
617

Supplements

Observer repeatability abnormality scores

Observer repeatability was calculated using the R package rptR v. 0.9.2 [41] in R version 3.5.3 [40]. When not adjusting for the second microscope (see main text) observer repeatability was moderate: R = 0.52 ± 0.16 standard error (SE) (95% CI (Confidence Interval): 0.17 to 0.79, *P* = 0.003).

Table S1. Results from a linear mixed model on a) the total, b) the head, c) the midpiece and d) the flagellum length from 2148 sperm of 116 captive male house sparrows of known age using only samples collected via abdominal massage.

	Sperm length (µm)
Captive house sparrows	estimate (lower CrI to upper CrI)
a) total length	
(intercept)	99.73 (99 to 100.45)
age	0.24 (-0.22 to 0.73)
sMLH	-0.19 (-0.63 to 0.25)
aviary set-up (with females)	0.81 (0.22 to 1.40)
year (2015)	-0.53 (-1.09 to 0.03)
Random effects	
male ID	6.92 (5.46 to 8.52)
aviary	0 (0 to 0)
sample ID	0.71 (0.56 to 0.89)
residual variance	2.97 (2.86 to 3.06)
b) head	
(intercept)	14.21 (13.90 to 14.51)
age	0.08 (-0.06 to 0.22)
sMLH	-0.05 (-0.16 to 0.05)
aviary set-up (with females)	0.13 (-0.17 to 0.43)
year (2015)	-0.62 (-0.92 to -0.32)
Random effects	
male ID	0.21 (0.16 to 0.27)
aviary	0.04 (0.02 to 0.08)
sample ID	0.16 (0.13 to 0.19)
residual variance	0.86 (0.83 to 0.88)

Table S1. continued

	Sperm length (µm)
Captive house sparrows	estimate (lower CrI to upper CrI)
c) midpiece	
(intercept)	66.75 (66.09 to 67.39)
age	-0.03 (-0.39 to 0.34)
sMLH	0.03 (-0.32 to 0.40)
aviary set-up (with females)	0.67 (0.14 to 1.19)
year (2015)	0.77 (0.19 to 1.34)
Random effects	
male ID	2.95 (2.34 to 3.63)
aviary	0 (0 to 0)
sample ID	1.24 (0.99 to 1.54)
residual variance	2.36 (2.29 to 2.43)
d) flagellum	
(intercept)	85.61 (84.86 to 86.34)
age	0.10 (-0.38 to 0.55)
sMLH	-0.13 (-0.57 to 0.30)
aviary set-up (with females)	0.65 (0.04 to 1.27)
year (2015)	0.03 (-0.61 to 0.62)
Random effects	
male ID	7.29 (5.76 to 8.97)
aviary	0.05 (0.02 to 0.10)
sample ID	0.47 (0.36 to 0.59)
residual variance	2.87 (2.78 to 2.96)

We accounted for sMLH, aviary set-up (levels: with, without females) and year of sperm collection (levels: 2014, 2015). Male age, as well as sMLH were centred and scaled. We present posterior means and CrI.

Table S2. Results from a generalized linear mixed model on the proportion of morphologically abnormal sperm in relation to male age in captive house sparrows (51 samples of 38 males) screened with one microscope only.

Proportion of morphologically abnormal sperm (logit-link scale)	
Captive house sparrows	estimate (lower CrI to upper CrI)
(intercept)	-2.12 (-2.57 to -1.65)
age	0.16 (-0.13 to 0.44)
sMLH	-0.13 (-0.44 to 0.17)
aviary set-up (with females)	-0.14 (-0.99 to 0.74)
method (faeces)	-0.27 (-0.77 to 0.25)
Random effects	
male ID	0.39 (0.23 to 0.59)
aviary	0 (0 to 0)
observation-level random	0.47 (0.31 to 0.66)

We accounted for sMLH, aviary set-up (levels: with, without females), and sperm collection method (levels: abdominal massage, faeces). Male age, as well as sMLH were centred and scaled. We present posterior means and CrI.

644 **Table S3.** Results from a linear mixed model on cloacal protuberance volume (mm^3) in

645 relation to male age in wild house sparrows (*N* = 46 males) excluding repeated measurements.

	cloacal protuberance volume (mm^3)
Wild house sparrows	estimate (lower CrI to upper CrI)
(intercept)	3.29 (3.06 to 3.54)
age	0.10 (-0.09 to 0.29)
day of year	-0.43 (-0.67 to -0.19)
tarsus	-0.04 (-0.23 to 0.13)
year (2016)	-0.31 (-0.80 to 0.19)

646

647 Using male ID as a random effect resulted in zero estimated variance signalling too few

648 repeated measurements from males. To ensure that our main model was robust, we re-ran it

649 using one randomly selected observation (function sample in R version 3.5.3 [40] per male only.

650 Cloacal protuberance volume was log-transformed. Male age, day of the year and tarsus

651 length were centred and scaled continuous input variables. We present posterior means and

652 CrI.

653