Running head: *Fieldfare paternity and sperm length*

Extra-pair paternity and sperm length variation in the socially monogamous Fieldfare *Turdus pilaris*

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Abstract

Basic knowledge about the genetic mating system is lacking for the great majority of the approximately 10,000 extant bird species. Filling this knowledge gap is not only critical for a comprehensive understanding of the reproductive ecology of each particular species, but also for increasing the power of comparative approaches to uncover and explain interspecific patterns of variation in avian reproductive traits. Using six polymorphic microsatellite markers, we here present the first parentage study in the socially monogamous Fieldfare *Turdus pilaris*. In parallel, we also examined variation in sperm morphology and relationships between sperm traits and paternity loss of social males. Across two study years, extra-pair paternity was detected in 46.4% (95% CI: 28.9%−64.9%) of 28 broods, and on average 27.6% (95% CI: 16.8%−41.9%) of nestlings per brood were extra-pair offspring in a population in central Norway. The observed extra-pair paternity rates fall within the range of reported estimates of extra-pair paternity for four congeneric *Turdus* species (between 36% and 65% of broods and 27% and 46% of nestlings). Sperm total length was 87.0 ± 2.9 (SD) μm (range 79.7–96.8 μm) and 59.3% (95% CI: 37.1%−73.3%) of the total phenotypic variation in sperm total length was explained by differences between sperm samples collected from 17 different males. The among-sample coefficient of variation in mean sperm total length was 2.70% (95% CI: 1.99%−3.17%). We found no evidence for effects of sperm total length or relative midpiece length on loss of paternity among broods of 13 males.

Keywords Extra-pair copulation; passerine; paternity loss; social monogamy; sperm morphology

Introduction

Most passerine birds are socially monogamous, but the application of molecular genetic methods has revealed that social monogamy by no means implies genetic monogamy (reviewed by Griffith et al. 2002; Kempenaers and Schlicht 2010). Having copulations outside the social pair bond is widespread in birds and extra-pair paternity (EPP) has been detected in approximately 90% of bird species examined (Griffith et al. 2002). The incidence of EPP varies tremendously across species, ranging from zero to almost three quarters of all offspring in a population being sired extra-pair (Griffith et al. 2002). Adult mortality, fecundity and patterns of parental care appear to be important life-history traits associated with the interspecific variation in EPP observed across major avian lineages (Arnold and Owens 2002). Adaptive (e.g., ecological, genetic and social factors; Petrie and Kempenaers 1998; Arnold and Owens 2002; Griffith et al. 2002; Bonier et al. 2014) as well as non-adaptive (e.g., pathological polyspermy and genetic constraints; Forstmeier et al. 2014) hypotheses have been proposed for explaining variation in EPP between species as well as between populations within species.

Although much effort has been devoted to study EPP in birds, the taxonomic coverage of species is still very limited and this precludes a more comprehensive understanding of the evolution of avian reproductive traits and mating systems. Efforts to close this knowledge gap are relevant for better understanding the specific reproductive ecology of particular species but, importantly, also for increasing the power of comparative and meta-analytic approaches, for example when studying the effects of post-copulatory sexual selection on the evolution of sperm form and function (e.g., Kleven et al. 2009; Støstad et al. 2018). Particularly useful in this context are studies that examine rates of EPP as well as the reproductive traits of interest in the very same population as both, rates of EPP (e.g., Garcia-Del-Rey et al. 2012;

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69 Laskemoen et al. 2013a) and reproductive traits (e.g., Lüpold et al. 2011; Laskemoen et al.
70 2013a), may well vary, and covary, among populations of a species.
71
72 When females engage in extra-pair copulations, this will result in sperm from the social pair
73 male and the extra-pair male(s) competing for fertilisation (sperm competition; Parker 1970)
74 and/or provide females with an opportunity to choose among sperm from different males
75 (cryptic female choice; Eberhard 1996). These two fundamental mechanisms of post-
76 copulatory sexual selection may strongly influence the evolution of sperm traits (reviewed in
77 Birkhead et al. 2009). Comparative studies in birds have, for instance, revealed that the
78 strength of post-copulatory sexual selection is positively correlated with sperm total length
79 (Briskie and Montgomerie 1992; Kleven et al. 2009; Lüpold et al. 2009), sperm swimming
80 speed (Kleven et al. 2009) and patterns of sperm morphological variation (Calhim et al. 2007;
81 Immler et al. 2008; Kleven et al. 2008; Lifjeld et al. 2010; Støstad et al. 2018). Sperm total
82 length as well as components of spermatozoa (sperm head, midpiece and tail length) and their
83 proportions may also display considerable intraspecific variation, both between different
84 populations (Lüpold et al. 2011; Schmoll and Kleven 2011; Laskemoen et al. 2013a), between
85 different males (Laskemoen et al. 2013b; Schmoll et al. 2018; Edme et al. 2019) and between
86 different sperm samples of individual males (Lüpold et al. 2012; Schmoll et al. 2018; Edme et
87 al. 2019). Little is however known about possible relationships between sperm morphological
88 traits and competitive fertilisation success in birds (but see Laskemoen et al. 2010; Cramer et
89 al. 2013; Bennison et al. 2015; Sætre et al. 2018).
90
91 The aim of the present study was to examine, for the first time, the genetic mating system and,
92 in parallel, sperm morphological variation of the socially monogamous Fieldfare *Turdus*
93 *pilaris*. Fieldfares have large testes for their body size (Dunn et al. 2001) and as relative testes

size is assumed to be a reliable index of the degree of sperm competition (Møller and Briskie 1995), we expected to reveal moderate to high levels of EPP in our Fieldfare study population. Using a comparative approach across passerine birds, Lifjeld et al. (2010) demonstrated a negative relationship between variation in sperm length in a given population and the respective frequency of extra-paternity. More specifically, Lifjeld et al. (2010) suggested the coefficient of between-male variation in sperm total length as an index for the frequency of extra-pair paternity in passerine birds. Accordingly, we also expected moderate to low variation in mean sperm total length between sperm samples obtained from different males in our study population. Finally, we tested for associations between paternity loss and two selected sperm traits that have been shown to predict competitive fertilisation success in other passerine bird species.

Materials and methods

Study species and study population

The Fieldfare is a medium-sized (range 93-121 g, n = 74 adult individuals; own unpublished data), open-nesting, tree-breeding passerine species found in the Palearctic (Cramp 1988). Although Fieldfares may breed solitarily, they mainly breed in more or less dense colonies. Fieldfares are socially monogamous and females usually incubate the eggs alone while both parents feed the young (Cramp 1988). We studied Fieldfares in Graffmarka (63° 44' 8" N, 11° 19' 58 " E) in Levanger municipality in central Norway. The study site was located approximately 15 meters above sea level and consisted of a 5.3 hectare floodplain forest surrounded by a river and cropland. Approximately 120 and 50 pairs of Fieldfares were breeding semi-colonially in the forest in the study years 2017 and 2018, respectively.

Field methods

Fieldwork was carried out during the breeding seasons between May and June in 2017 and 2018. We trapped adults with mist nets mainly during the nestling feeding period. The adults were banded with a unique combination of three colour rings and one numbered aluminium ring provided by the Norwegian Bird Ringing Centre at Stavanger Museum (i.e. two rings on each leg). A small droplet (~10 µL) of blood was sampled and stored in 1 mL Queen's lysis buffer (Seutin et al. 1991) at 4°C until genetic analysis. We also collected tissue samples for molecular genetic analysis from two adult individuals found freshly dead as a result of predation. Adults were sexed molecularly (see below).

We gently massaged the cloacal protuberance of males to obtain a sperm sample as described in detail by Laskemoen et al. (2013b). The sample was first mixed with 10 µL standard phosphate-buffered saline (PBS) and immediately transferred into 250 µL of a 5% formaldehyde solution (equivalent to an approximately 12.5% formalin solution assuming a stock solution of 40% formaldehyde). Samples were stored at room temperature until sperm microphotography in autumn 2018 (differential storage duration appears not to affect avian sperm length; Schmoll et al. 2016).

We located nests, GPS-marked them using a Garmin GPSmap 62s GPS navigator and thereafter visited them approximately every third day to obtain information about hatching date. Nest predation, partial and complete, occurred in the study population but our monitoring regime was not suited to quantify this. Approximately 5-10 days after hatching, we collected a small (~10 µL) sample of blood from nestlings that was stored in 1 mL lysis

buffer at 4°C until parentage analysis. During the nestling feeding period, broods were observed from a distance of approximately 10-40 m using binoculars and telescopes; we considered adults to be social parents of a focal brood when they provisioned the nestlings with food.

Parentage analysis

Genomic DNA was extracted from blood using the QIAamp 96 Blood Kit (Qiagen, Hilden, Germany) and from tissue using the Maxwell 16 Tissue DNA Purification Kit (Promega, Madison, Wi, USA) following the manufacturers' protocols. Sex of adult birds was determined using the universal primers P2 and P8 (Griffiths et al. 1998). Genetic parentage was determined based on genotyping at five polymorphic autosomal microsatellite loci plus one Z-linked microsatellite locus (Table 1). All primers were combined into a single multiplex polymerase chain reaction (PCR) run using fluorescently-labeled forward primers and a multiplex PCR Kit (Qiagen). PCR products were separated on an ABI 3500xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and allele sizes were assigned using GENEMAPPER v5.0 software (Applied Biosystems). Marker polymorphism, exclusion probabilities and informativity were calculated using GenAlEx v6.5 (Peakall and Smouse 2012); for results see Table 1. None of the loci deviated significantly ($\alpha < 0.05$) from Hardy-Weinberg equilibrium; there was, however, indication of null alleles (i.e., non-amplifying alleles) at one locus (*Ase64*; see Table 1). For the five autosomal markers, the combined exclusion probability assuming the mother was known was 0.999 and the combined exclusion probability assuming the mother was unknown was 0.990. Adding the Z-linked marker and analysing males only, the combined exclusion probabilities were 0.999 and 0.992,

168 respectively, allowing reliable assignment of offspring paternity status as within-pair
169 offspring (WPO) *versus* extra-pair offspring (EPO).
170
171 Nestlings were considered WPO if their allele sizes matched those of the putative parents at
172 all loci or mismatched at a single locus (with either parent). Nestlings with two or more
173 mismatches with the putative father's alleles were considered EPO. There were 14 cases (all
174 at locus *Ase64*) with a single allelic mismatch between a nestling and one putative parent
175 (involving two different putative fathers and three different putative mothers). In all 14 cases
176 both offspring and putative parents were seemingly homozygous (for different alleles in
177 parents and offspring, respectively). Given the evidence for null alleles at locus *Ase64* (Table
178 1), we assumed these mismatches to be caused by null alleles and considered all 14 offspring
179 to have descended from their putative parents. Furthermore, in a brood of seven nestlings,
180 three nestlings mismatched both putative parents. The remaining four nestlings were genetic
181 offspring of the breeding pair observed providing parental care. The three nestlings could thus
182 result from intraspecific brood parasitism. However, we cannot rule out the possibility that the
183 female providing care at the nest had taken over a nest which already contained eggs from
184 another female due to e.g. partial depredation or desertion (all other brood sizes recorded
185 during our study ranged from three to six nestlings only). We therefore excluded these three
186 nestlings from all further analyses. We identified extra-pair sires among blood-sampled males
187 for only 21% of EPO and therefore refrained from comparisons of e.g. sperm traits between
188 social males and extra-pair sires.
189
190 Sperm morphology analysis
191

Approximately 3 µL of solution from each sperm sample were transferred onto a standard microscope slide and air-dried over-night. The slide was then carefully rinsed with distilled water in order to remove dirt and salt crusts and air-dried again. Slides were subsequently examined by light microscopy at 400 times magnification under light-field conditions using an Olympus BX50 microscope and all pictures were taken by the same person (Renate Feist) using a Canon EOS 600 digital camera. A micrometer scale was pictured for each sperm sample immediately before slides were screened for spermatozoa that showed no obviously artefactual morphology. Pictures of 20 intact spermatozoa per sperm sample were included for further analysis, as measuring 20 spermatozoa has been shown to provide a sufficiently precise estimate of a sample's mean sperm total length (Laskemoen et al. 2007). To ensure blind measurements with respect to sperm sample identity, all samples were anonymised before analysis by TS. Sperm head, midpiece and tail length were subsequently measured to a precision of 0.01 µm during a continuous measuring period by a single observer (Sonja Schindler) using ImageJ 1.52a (Rasband 1997-2018). Sperm total length was calculated as the sum of these components. On average 19.5 ± 1.5 (SD) spermatozoa were successfully measured per sperm sample (in one and two samples, respectively, pictures of only 19 and 17 intact spermatozoa were available and in each of two further samples a single spermatozoon could not be measured). All 331 spermatozoa were blindly measured twice to assess measurement error via repeatability analysis (see below). The mean of the two measurements was used for all subsequent analyses.

Statistical analysis

We used R 3.4.3 (R Core Team 2019) for all computations and the R package *rptR* (Stoffel et al. 2017) to calculate the repeatability of sperm length measurements for repeated

measurements of the same individual spermatozoa based on linear mixed effects models and including 95% confidence intervals (parametric bootstrapping, N = 10,000 replicates). We fitted the grand mean of the respective trait as the only fixed effect and sperm identity as the only random effect. Repeatabilities of measurements for sperm total length and sperm sections were high with the exception of sperm head length (see Table 2). In an analogous manner, in order to estimate variation in sperm total length between sperm samples, we fitted the grand mean of sperm total length as the only fixed effect and sperm sample identity as the only random effect.

We used generalised linear models (GLMs) with logit link and quasi-binomial errors to model i) the probability that a brood contained at least one EPO and for ii) estimating the proportion of EPO per brood (the latter using the R function *cbind* to create the independent variable as a column-bind matrix of the number of EPO and the number of WPO, respectively). Quasibinomial instead of binomial errors were assumed because inspection of dispersion parameters indicated moderate to substantial overdispersion). We estimated the population-level probability that a brood contained at least one EPO and population mean frequencies of EPO per brood including corresponding 95% Wald confidence intervals by fitting the grand mean as the only fixed effect.

To analyse the relationship between sperm traits and paternity loss, we *a priori* selected two sperm traits shown to predict competitive fertilisation success in a passerine bird species (sperm total length in the Zebra Finch *Taeniopygia guttata*, Bennison et al. (2015); and sperm midpiece/total length ratio in the Tree Swallow *Tachycineta bicolor*, Laskemoen et al. (2010)). No further sperm size traits or sperm proportions were tested for their effects on paternity loss.

To quantify variation among sperm samples in mean sperm total length per sample we used the coefficient of variation (CV) adjusted for small sample sizes as CV_{adj} = (1+1/4 N) × (SD × 100/mean) (Sokal and Rohlf 1995) with N = 17 (number of sperm samples). Confidence intervals (95%) for the among-sample CV_{adj} were obtained by non-parametric bootstrapping (N = 10,000 replicates).

Results

Patterns of extra-pair paternity

Across both study years, parentage data were obtained for a total of 123 nestlings from 28 broods of 28 different pairs. There were no allelic mismatches between nestlings and putative mothers. The probability for a brood to contain at least one EPO was 46.4% (95% CI: 28.9%−64.9%). Overall, 34 out of 123 nestlings were not sired by the male providing parental care (mean number of mismatches: 3.9; range: 2 to 6). On average 27.6% (95% CI: 16.8%−41.9%) nestlings per brood were EPO (Figure 1). There was no evidence for differences between study years in either the probability for a brood to contain at least one EPO (quasibinomial GLM, estimate ± SE on logit scale: −0.68 ± 0.82, t = −0.37, p = 0.71) or the proportion of EPO per brood (−0.30 ± 0.70, t = −0.43, p = 0.67).

Patterns of sperm morphological variation

Across both study years, morphometric data were obtained for 331 spermatozoa from 17 sperm samples of 17 different males. Patterns of variation in sperm length are detailed in Supplementary Table S1; 59.3% (95% CI: 37.1%−73.3%) of the total phenotypic variation in sperm total length was explained by differences between sperm samples of the 17 different males (linear mixed effects model with sperm sample identity as random effect: χ^2 = 230,

267 df = 1, p < 0.001, Figure 2). The among-sample coefficient of variation in mean sperm total
268 length per sperm sample was 2.70% (95% CI: 1.99%−3.17%).
269
270 Extra-pair paternity and sperm morphology
271 For 13 broods or males, respectively, paternity as well as sperm morphometric data were
272 available. We found no evidence for an effect of mean sperm total length or mean relative
273 midpiece length per sperm sample on paternity loss (i.e. proportion EPO per brood,
274 quasibinomial GLMs: estimate ± SE on logit scale: 0.34 ± 0.26, t = 1.27, p = 0.23, Figure 3a,
275 and −16.97 ± 21.00, t = −0.81, p = 0.44, Figure 3b, respectively).
276
277 **Discussion**
278 In our study of the socially monogamous Fieldfare, we found that mating outside the social
279 pair bond must have occurred frequently. While our sample sizes of broods and nestlings
280 were relatively low (cf. Griffith et al. 2002) and accordingly confidence intervals around our
281 estimates relatively wide, our results nevertheless clearly show extra-pair paternity to be
282 common in the study population: Almost half of the females had extra-pair offspring in the
283 nest and more than one quarter of all offspring were not sired by the putative father providing
284 parental care. Our findings thus further corroborate that extra-pair mating is a common
285 alternative reproductive strategy in passerine birds. Together with previously reported data on
286 comparatively large relative testes size (Dunn et al. 2001), our study suggests a high level of
287 sperm competition in the Fieldfare.
288
289 In four broods, parentage analysis revealed that all nestlings had been sired by an extra-pair
290 male. The fact that both, the putative father and the putative mother, were identified while
291 provisioning nestlings with food and that the putative mothers in our study turned out to be

292 the genetic mothers in all cases, suggests that the observed cases of broods with 100% EPO
293 represented part of the spectrum of natural variation in EPP rates rather than misassignments
294 of putative fathers in the field.
295
296 According to a recent compilation of EPP studies in birds, the median frequency of EPO per
297 brood among 132 passerine species with a predominantly socially monogamous mating
298 system was 15.3% (data extracted from supplementary material of Biagolini-Jr. et al. 2017).
299 The frequency of EPO in the Fieldfare (27.6%, this study) is in the upper level of the
300 interquartile range (spanning 6.1%−30.6%) among these species. Compared with four
301 congeneric species for which the frequency of EPO ranged from 26.7% in the Common
302 Blackbird *T. merula* (Hesler 2009), 31.8% in the White-necked Thrush *T. albicollis*
303 (Biagolini-Jr. et al. 2016), 37.8% in the Clay-colored Thrush *T. grayi* (Stutchbury et al. 1998)
304 to 45.9% in the American Robin *T. migratorius* (Rowe and Weatherhead 2007), the frequency
305 of EPO in the Fieldfare was below the average level (i.e. 35.6%).
306
307 Sperm was obtained from a single sample per male and during the early nestling feeding
308 period; thus the question arises to what degree the variation in sperm length reported here
309 represents variation that is biologically relevant, particularly in an among-male context. The
310 regular occurrence of replacement clutches due to for example nest predation in our study
311 population (own observations) may select for full sperm functionality (and sperm
312 competitiveness) well beyond the mean peak fertility of females in the population
313 (unfortunately we do not have information on the frequency of true second clutches in the
314 study population). Furthermore, besides documented short-term repeatability of sperm total
315 length across different sperm samples of individual males (Sætre et al. 2018), two recent
316 studies, in Collared Flycatchers *Ficedula albicollis* (Edme et al. 2019) and Great Tits *Parus*

317 *major* (Schmoll et al. 2018), also revealed substantial repeatabilities of mean sperm total
318 length per sperm sample across multiple sperm samples of individual males which were
319 obtained early *versus* late in the reproductive season. These results suggest that inference with
320 respect to mean sperm total length per sperm sample based on sperm from a single
321 experimental ejaculate sampled in the nestling feeding period may be valid for among-male
322 comparisons *within* populations.
323
324 While sperm total length varied substantially among sperm samples of different males, neither
325 sperm total length nor relative midpiece length affected paternity loss. Although associations
326 between sperm traits and paternity success (which includes paternity loss in the own broods)
327 have been established in two passerine species (e.g., Laskemoen et al. 2010; Bennison et al.
328 2015), our results are in line with recent studies in for instance the Bluethroat *Luscinia*
329 *svecica* (Sætre et al. 2018) and House Wren *Troglodytes aedon* (Cramer et al. 2013) that also
330 found no association between sperm traits and paternity loss. One of the reasons for why we
331 failed to reveal an association between sperm traits and paternity success could arguably be
332 the low sample size and thus a lack of statistical power. However, results by Bennison et al.
333 (2015) for sperm total length and Laskemoen et al. (2010) for relative midpiece length led us
334 to expect negative effect signs (less EPO with increasing sperm total length and less EPO
335 with increasing relative midpiece size); but we found an opposite sign for the former analysis.
336 It appears likely that other (maybe behavioural) determinants are relevant in shaping paternity
337 success in the study population. It has for instance been shown that frequent copulations
338 during the peak of the female fertile period represent a male strategy for securing paternity
339 (e.g., Crowe et al. 2009). Further research is required to obtain basic knowledge about
340 copulatory and extra-pair mating behaviour in the Fieldfare.

Besides the specific reproductive ecology of the Fieldfare, our study also provides valuable data for comparative analysis where studies that estimate both rates of EPP and the reproductive traits of interest in the very same population are especially useful. Using a comparative approach, Lifjeld et al. (2010) proposed a negative relationship between variation in sperm length and the frequency of extra-paternity across passerine birds. More specifically, Lifjeld et al. (2010) found that the among-sperm sample CV in mean sperm length per sperm sample predicted the (arcsin-square root transformed) overall proportion of EPO (note that Lifjeld et al. 2010 refer to this measure as between-male CV of mean sperm length). Assuming significant repeatability of mean sperm total length per sperm sample across multiple samples of the same males (as Lifjeld et al. (2010) did, and e.g. Schmoll et al. (2018) and Edme et al. (2019) demonstrated), the Fieldfare (27.6% EPO; this study) falls only just within the predicted 95% CI (5.9%–28.8%) for the frequency of EPO based on the regression shown in Figure 2 in Lifjeld et al. (2010); only the raw data from Lifjeld et al. (2010) was used to calculate the 95% CI, our estimate for the fieldfare was not included. As with three other *Turdus* species included in Lifjeld et al. (2010), the predicted value of the frequency of EPO in the Fieldfare (15.3% EPO) thus appears to be somewhat lower compared with the actually observed frequency of EPO.

In conclusion, we here provide the first estimate of extra-pair paternity in the socially monogamous Fieldfare. We found that extra-pair mating is a common reproductive strategy in a central Norwegian population of Fieldfares with 27.6% of the offspring not sired by the social father. We did not find support for the hypothesis that sperm morphology affects paternity loss among 13 males.

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522

TABLES

Table 1 Characteristics of microsatellite markers used for parentage analysis and molecular sex determination in the Fieldfare *Turdus pilaris*.

Locus	Reference	*n*	#A	Allele size range (bp)	H_O	H_E	P_{HWE}	$Freq_{NULL}$	P_{1p}	P_{2p}
Ase40	Richardson et al. (2000)	77	8	219–233	0.61	0.68	0.81	0.039	0.46	0.28
Ase64	Richardson et al. (2000)	77	27	373–437	0.86	0.93	0.25	**0.035**	0.85	0.74
Ltmr6	McDonald and Potts (1994)	77	16	209–253	0.87	0.88	0.13	0.007	0.77	0.62
Pat MP 2-43	Otter et al. (1998)	77	22	138–180	0.92	0.92	0.77	−0.003	0.84	0.72
Tgu06	Slate et al. (2007)	77	14	175–188	0.81	0.83	0.96	0.014	0.67	0.50
Z-054[1]	Dawson et al. (2015)	39	9	264–280	0.82	0.76	0.95	−0.033	0.57	0.39
P2/P8	Griffiths et al. (1998)	39♂	1	351	0	0				
		38♀	2	351; 388	1	1				

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n, number of presumably unrelated adult individuals; #A, number of alleles, bp, base-pairs; H_O, observed heterozygosity; H_E, expected heterozygosity; P_{HWE}, probability of deviation from Hardy-Weinberg equilibrium; $Freq_{NULL}$, estimated frequency of null alleles according to the Brookfield method implemented in MICRO-CHECKER (van Oosterhout et al. 2004) with numbers in bold indicating evidence (due to general excess of homozygotes for most allele size classes) for null alleles; P_{1p}; exclusion probability assuming the mother was known; P_{2p}; exclusion probability assuming the mother was unknown. [1]Z-054 is Z-chromosome-linked and therefore marker polymorphism was calculated for males only. Marker polymorphism and deviation from Hardy-Weinberg equilibrium was calculated using GenAlEx v6.5 (Peakall and Smouse 2012).

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Table 2 Repeatability for repeated measurements of sperm total length and the sperm components head, midpiece and tail length. All 331 spermatozoa originating from 17 sperm samples of 17 different Fieldfare males were blindly measured twice. Using the R package *rptr* (Stoffel et al. 2017), repeatabilities and 95% confidence intervals (CI) obtained by parametric bootstrapping were estimated based on linear mixed effects models with the grand mean as the only fixed effect and sperm sample identity as the only random effect.

Trait	**Repeatability**	**95% CI**	χ^2	**df**	**P**
Total length	0.974	0.968─0.979	981	1	<0.001
Head length	0.661	0.596─0.717	190	1	<0.001
Midpiece length	0.952	0.941─0.961	785	1	<0.001
Tail length	0.971	0.965─0.977	949	1	<0.001

FIGURE LEGENDS

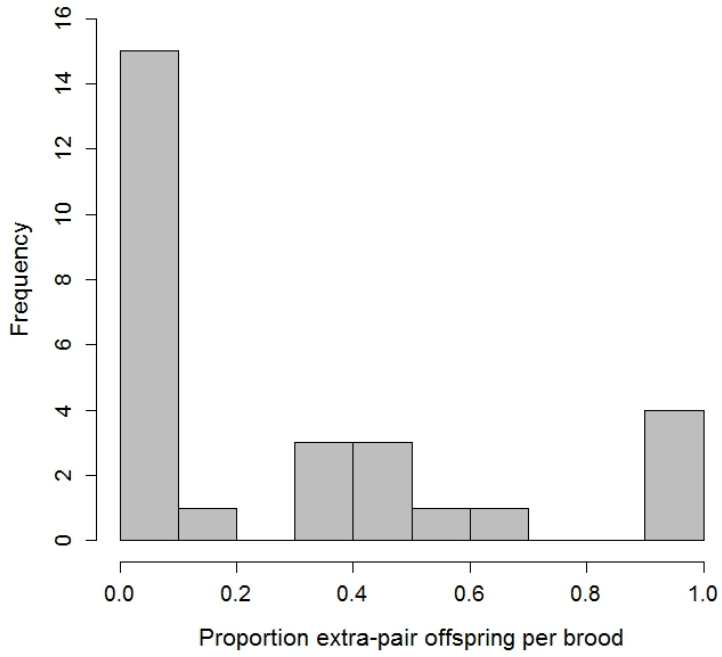
Fig. 1 Frequency distribution of the proportion of extra-pair offspring per brood for N = 28 broods of 28 different Fieldfare pairs.

Fig. 2 Variation in sperm total length within *versus* among 17 sperm samples obtained from 17 different Fieldfare males (N = 19.5 ± 1.5 spermatozoa per sample). Plots show medians, interquartile range (box) and data within 1.5 times the interquartile range (whiskers).

Fig. 3 Relationship between a) mean sperm total length (± SE) and b) mean relative midpiece (± SE) size per sperm sample and paternity loss measured as the proportion extra-pair offspring for 13 Fieldfare males.

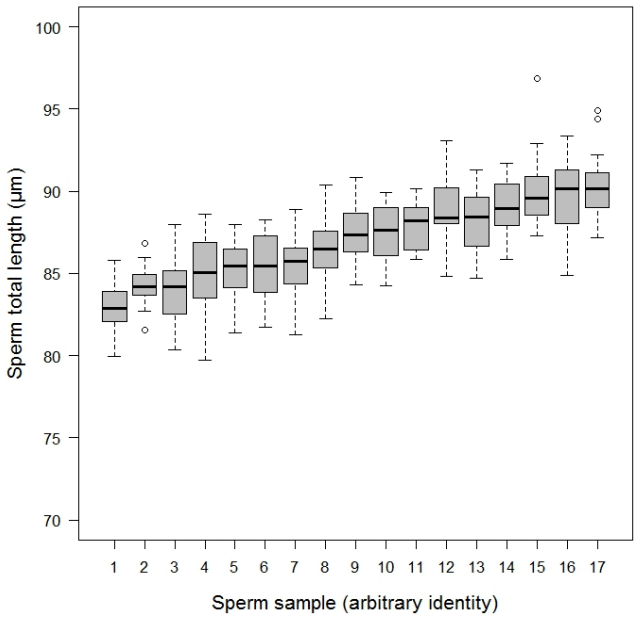
552 **FIGURES**

553 **Figure 1**



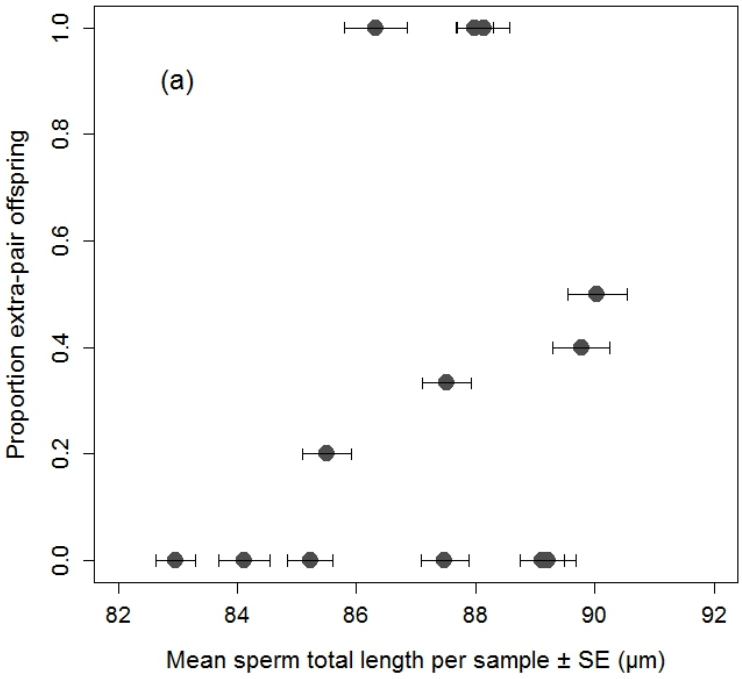
554

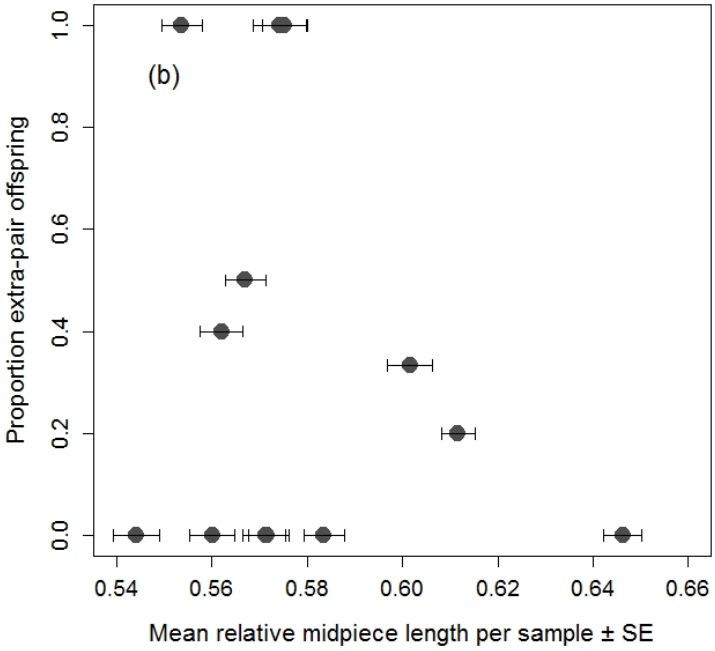
555 **Figure 2**



556

Figure 3





566 **Supplementary Table S1** Sperm morphometrics for N = 331 spermatozoa originating from
567 17 sperm samples obtained from 17 different male Fieldfares sampled in a Norwegian
568 population during the early nestling feeding period. Descriptive statistics are given for
569 population-wide estimates (*Population*, i.e. not accounting for sperm sample identity) as well
570 as based on mean values per sperm sample (*Sample means*).

571

Trait	Level of analysis	Mean ± SD (µm)	Range (µm)
Total length	Population	87.0 ± 2.9	79.7 – 96.8
	Sample means	87.0 ± 2.3	83.0 – 90.4
Head length	Population	12.7 ± 0.5	11.2 – 15.5
	Sample means	12.7 ± 0.5	12.4 – 13.5
Midpiece length	Population	50.2 ± 2.4	42.4 – 56.2
	Sample means	50.2 ± 1.8	47.1 – 53.6
Tail length	Population	24.1 ± 3.3	14.7 – 30.9
	Sample means	24.1 ± 2.7	16.8 – 27.1

572