Selection on sperm morphology under relaxed sperm competition in a wild passerine bird

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Theories regarding the role of sexual selection on the evolution of sperm traits are based on an association between pre-copulatory (e.g. female preference) and post-copulatory (e.g. ejaculate quality) male reproductive traits. In tests of these hypotheses, sperm morphology has rarely been used, despite its high heritability and intra-individual consistency. We found evidence of selection for longer sperm through positive phenotypic associations between sperm size and the two major female preference traits in the pied flycatcher, *Ficedula hypoleuca*. Our results support the sexually selected sperm hypothesis in a species under low sperm competition and demonstrate that natural and pre-copulatory sexual selection forces should not be overlooked in studies of intraspecific sperm morphology evolution.

Keywords: sperm design; avian reproduction; postcopulatory sexual selection

1. INTRODUCTION

Sexual selection ultimately favours male traits that influence reproductive success in pre- and/or post-copulatory processes (Birkhead & Pizzari 2002; Andersson & Simmons 2006). Trivers (1972) proposed that elaborate sexual ornaments in males might signal sexual competence, i.e. a positive association between secondary (somatic or behavioural) and primary (gametic or gonadal) sexually selected traits (Birkhead & Pizzari 2002; Andersson & Simmons 2006). More recently, two non-exclusive hypotheses suggest that a positive relationship between male primary and secondary sexual phenotypes can explain why females copulate with more than one male. The phenotype-linked fertility hypothesis proposes that extra-pair copulations in birds provide a mechanism to overcome (temporary) infertility of the pair male (e.g. sperm depletion or suboptimal sperm; Sheldon 1994). The sexually selected sperm hypothesis (reviewed in Pizzari & Birkhead 2002) states that

females mate with multiple males to increase the probability of producing sons that inherit their sires' superior fertilization efficiency (the 'sexy sperm' hypothesis) or more viable offspring (the 'good sperm' hypothesis). In contrast to the phenotype-linked fertility hypothesis, the sexually selected sperm hypothesis assumes additive genetic variance in fertilization-efficiency traits and/or genetic benefits to promiscuous females (Evans & Simmons 2007). Previous tests of these hypotheses have been conducted in several taxa with mixed results (reviewed in table S1 in the electronic supplementary material). However, the latter were largely based on non-morphometric sperm traits (table S1 in the electronic supplementary material), despite high heritability of sperm morphology across species (Simmons & Moore 2008). Theory predicts that a longer flagellum allows for faster sperm (Katz & Drobnis 1990) and that a larger midpiece, through more or larger mitochondria, provides sperm with greater potential energetic output (Cardullo & Baltz 1991). Across birds, species under more intense sperm competition have sperm that is longer, faster and/or with relatively longer midpieces (e.g. Briskie *et al.* 1997; Immler & Birkhead 2007; Lüpold *et al.* submitted). There is also evidence for these associations between sperm form and function at the intraspecific level (e.g. Froman & Feltmann 1998; Froman & Kirby 2005; cf. Malo *et al.* 2006 for mammals). We therefore assume in this study that gametic quality (and hence post-copulatory fertilization efficiency) is positively associated with (i) sperm size and (ii) relative mid-piece size.

The aim of this study was to test whether an association exists between gametic morphology and two well-established secondary sexually selected traits, using natural variation in a wild pied flycatchers (*Ficedula hypoleuca*).

2. MATERIAL AND METHODS

(a) Sperm traits

Males were captured in 2006 in a study area near Oslo, Norway, when defending an empty nest-box or mist-netted in their own territory. Sperm samples for morphometric analysis were collected non-invasively, from the faeces of individuals in reproductive condition, and stored in 5 per cent formalin solution (Immler & Birkhead 2005). Sperm morphometric data were obtained using digital image analysis software (Leica IM50 IMAGE MANAGER) and light microscopy. Two sperm morphometric traits were used in this study (measured to the nearest 0.1 µm): (i) sperm total length (flagellum length plus head length) and (ii) straight midpiece length (Birkhead *et al.* 2005). Five sperm per male were measured (see table S2 in the electronic supplementary material). Sperm total length and relative size of the midpiece (controlling for sperm size) were used as indices of sperm morphometry (size and design, respectively).

(b) Male traits

Two well-established female preference traits were used as measures of male quality: (i) plumage blackness and (ii) date of first egg laid by the (primary) social female, hereafter referred to as breeding date. Mate choice in the pied flycatcher (*F. hypoleuca*) is female driven (Dale & Slagsvold 1994) and influenced by several inter-correlated male traits. Date of arrival (and consequently territory quality) is the major predictor of female settlement (i.e. pre-copulatory choice) across all populations studied (Alatalo *et al.* 1986; Potti & Montalvo 1991; Dale & Slagsvold 1996). We used breeding date as a proxy of arrival date since early breeding directly reflects male quality (e.g. Alatalo *et al.* 1986). In the breeding season, the sexes are dichromatic: males have a white forehead patch and dorsal plumage that varies in conspicuousness from female-like brown to black (Lundberg & Alatalo 1992). When

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Figure 1. Negative associations between sperm size (mean $\pm$ standard deviation) and two secondary sexually selected traits in the pied flycatcher: (a) plumage blackness ($n=17$; $p<0.001$) and (b) breeding date (i.e. calendar date in May, $n=13$; $p<0.02$). Note that higher quality/preferred males have lower plumage scores and breed earlier.

territory quality is controlled experimentally, plumage blackness is the strongest male phenotypic trait that predicts reproductive success (e.g. Slagsvold & Drevon 1999), whereas forehead patch size is not a sexually selected trait in the Norwegian study population (Dale et al. 1999). Plumage was scored by experienced researchers (HML and TS) using the Drost (1936) 7-point scale where 1 is conspicuous black and white, and 7 is brown and female-like, so that blacker males have lower score values. There were only two first-year males with disparate blackness scores (2 and 6.75) and so the confounding effect of age on plumage is negligible in our study. Thus we consider higher values of both colour score and breeding date to be associated with lower male quality.

(c) Statistical analyses

All statistical analyses were conducted in R v. 2.3.1 (R Development Core Team 2006). Data were log-transformed and sperm total length was included as covariate in models with midpiece size as the dependent variable. Breeding date and plumage blackness were analysed in separate generalized linear models, since these two predictors had different sample sizes and were uncorrelated (Spearman $r_s=0.24$, $p=0.426$). Effect size (partial r from model term t statistic) and its non-central 95 per cent confidence intervals are reported (Nakagawa & Cuthill 2007).

3. RESULTS

There were significant associations between sperm size and sexually selected male traits: males with blacker plumage (GLM: $t=-4.16$, d.f.=1,15, $p=0.00084$, effect size r (95% CI) = -0.73 (-1.50 , -0.36); figure 1a) and earlier breeding males (GLM: $t=-2.74$, d.f.=1,11, $p=0.0194$, effect size r (95% CI) = -0.63 (-1.43 , -0.07); figure 1b) had longer sperm. There were no significant associations between midpiece size (controlling for sperm total length) and either male plumage score or breeding date ($p>0.2$). See table S3 in the electronic supplementary material for full statistical results.

4. DISCUSSION

Male pied flycatchers that had blacker plumage and bred earlier in the season (i.e. occupied better quality territories) had longer sperm. Our findings contrast with previous work on passerines, where no associations were found between sperm morphology and female pre-copulatory choice traits (*Taeniopygia guttata*, Birkhead & Fletcher 1995; *Acrocephalus schoenobaenus*, Birkhead et al. 1997a; table S1 in the electronic supplementary material). In fact, the only other species where such a link has been found, and only

in some populations, is the guppy (*Poecilia reticulata*; Pitcher et al. 2007; table S1 in the electronic supplementary material).

Females might gain direct fitness benefits from copulating with darker and/or early arriving males through (i) improved paternal care of the brood, i.e. natural selection (Sætre et al. 1995) and/or (ii) insured fertility of their, often single (Lundberg & Alatalo 1992), clutch by copulating with males with longer, thus higher quality, sperm (i.e. the phenotype-linked fertility hypothesis, Sheldon 1994). Alternatively, females might incur indirect benefits through the production of sons that inherit higher fertilization efficiency, both at the pre- and post-copulation stages (i.e. the sexy sperm hypothesis, Keller & Reeve 1995). It is difficult to assess the relative role of the three different (and non-exclusive) hypotheses, and the current data do not allow the rejection of either one. However, three of four major aspects of pied flycatcher reproductive life history are more consistent with the premises of the sexually selected sperm hypothesis than with the phenotype-linked fertility hypothesis or natural selection *per se*. First, extra-pair paternity is uncommon and particularly low in the study population (EPP=4%, Lifjeld et al. 1991). Second, sperm storage ability of females is relatively short (ca 7–9 days; Birkhead et al. 1997b), reducing the likelihood of sperm competition and/or selection for sperm longevity. This short sperm storage reflects the importance of pre-copulatory sexual selection processes in this species. Moreover, it might explain the observed non-significant results of midpiece size, if sperm energy potential is not critical to male reproductive success. Third, the occurrence of polygyny and unpaired (i.e. zero reproductive fitness) males (Lundberg & Alatalo 1992) further suggests that pre-copulatory sexual selection might be a stronger force than natural selection. Fourth, both the gametic and secondary sexually selected traits used in this study show relatively weak condition-dependent expression: there is strong genetic (cf. environmental) determination of sperm morphology in passerines (Birkhead et al. 2005), a considerable heritable component and across-year consistency in plumage blackness in this species (Slagsvold & Lifjeld 1992),

and, in the closely related collared flycatcher (*F. albicollis*), there is additive genetic variance in breeding date (Sheldon *et al.* 2003), making it possible that the same is true in the pied flycatcher. In order to conclusively infer a genetic correlation from the observed phenotypic correlations, a larger sample or multigenerational data are needed in order to apply quantitative evolutionary approaches, such as the animal model (e.g. Kruuk 2004). Nevertheless, the pattern at the phenotypic level is rather strong, considering the relatively small sample size.

This study highlights importance of considering pre-copulatory sexual selection and/or natural selection forces when studying sperm design evolution, particularly at the intraspecific level and in species under relaxed sperm competition.

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