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## Evolutionary biology

# Patterns of sperm damage in Chernobyl passerine birds suggest a trade-off between sperm length and integrity

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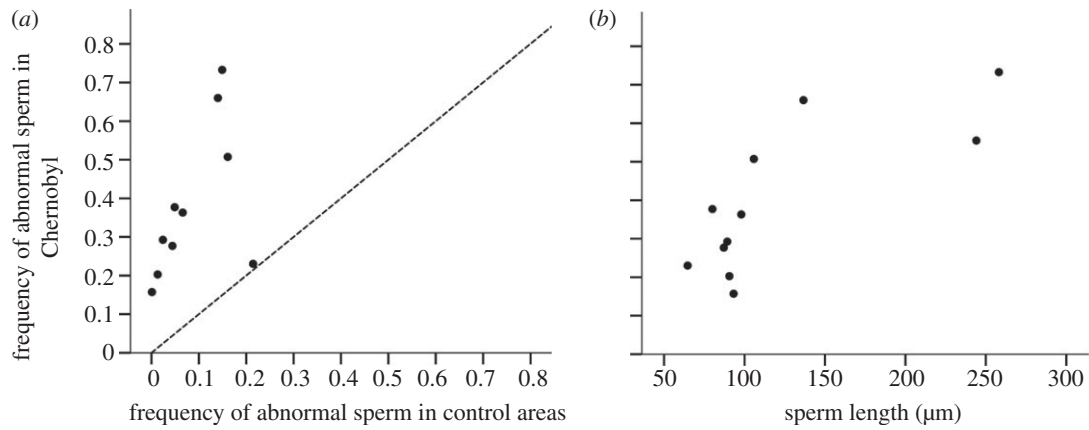
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Interspecific variation in sperm size is enigmatic, but generally assumed to reflect species-specific trade-offs in selection pressures. Among passerine birds, sperm length varies sevenfold, and sperm competition risk seems to drive the evolution of longer sperm. However, little is known about factors favouring short sperm or constraining the evolution of longer sperm. Here, we report a comparative analysis of sperm head abnormalities among 11 species of passerine bird in Chernobyl, presumably resulting from chronic irradiation following the 1986 accident. Frequencies of sperm abnormalities varied between 15.7 and 77.3% among species, more than fourfold higher than in uncontaminated areas. Nonetheless, species ranked similarly in sperm abnormalities in unpolluted areas as in Chernobyl, pointing to intrinsic factors underlying variation in sperm damage among species. Scanning electron microscopy of abnormal spermatozoa revealed patterns of acrosome damage consistent with premature acrosome reaction. Sperm length, but not sperm competition risk explained variation in sperm damage among species. This suggests that longer spermatozoa are more susceptible to premature acrosome reaction. Therefore, we hypothesize a trade-off between sperm length and sperm integrity affecting sperm evolution in passerine birds.

## 1. Introduction

Spermatozoa display tremendous diversity in size and shape across the animal kingdom [1]. Fertilization mode and post-copulatory sexual selection are two main factors assumed to shape this diversity among taxa [1–3]. Passerine birds have spermatozoa adapted to internal fertilization and sperm storage, with a sevenfold variation in length, i.e. approximately 40–290  $\mu\text{m}$  [2,4,5]. Post-copulatory sexual selection seems to promote the evolution of longer sperm [2,4,6] although the adaptive function of longer sperm in sperm competition is not well understood. Longer sperm have longer mid-pieces, which contain the fused mitochondrion and hence more energy resources [7]. However, energy does not seem to translate directly into higher swimming speeds [6,7], so other energy-dependent traits like longevity, could be important. Post-copulatory sexual selection also increases total investment in sperm production [8,9], which is manifested in a disproportionate increase in sperm number compared with the increase in sperm size across species [2]. This suggests that there is no clear trade-off between sperm size and number in



**Figure 1.** Frequency of sperm abnormality in Chernobyl passerine species plotted against (a) the frequency of sperm abnormality in uncontaminated areas and (b) mean sperm length for the species. The line in (a) is  $y = x$ .

**Table 1.** PGLS analyses of the proportion of abnormal sperm in relation to total sperm length and sperm competition risk ( $CV_{bm}$  of sperm length) among passerine species in Chernobyl, Ukraine and uncontaminated areas (Norway and Czech Republic). In all tests, the phylogenetic parameter ( $\lambda$ ) was approximately 0 and significantly different from 1, suggesting there was no strong phylogenetic signal in the relationships.

area	predictor	slope	s.e.	<i>t</i>	<i>p</i>
Chernobyl ( <i>n</i> = 11 species)	total sperm length	1.02	0.24	4.32	0.002
	sperm competition risk	0.08	0.09	0.89	0.40
uncontaminated ( <i>n</i> = 10 species)	total sperm length	0.16	0.29	0.55	0.60
	sperm competition risk	0.04	0.07	0.63	0.54

passerine birds, owing to some significant cost or constraint to the evolution of longer sperm.

Here, we report a comparative study of sperm abnormalities in passerine birds providing evidence for reduced functional performance of longer sperm. Radioactive contamination following the Chernobyl accident in 1986 had a series of negative environmental effects, including sperm damage in mammals [10] and birds [11]. We examined the frequency of abnormal sperm among 11 passerine species sampled around Chernobyl in 2010 and 2011. We also scored sperm abnormalities in samples from 10 of these species from uncontaminated areas elsewhere in Europe. We tested whether the patterns of sperm damage covaried with species-specific sperm lengths and sperm competition risk, as sperm competition enhances both sperm quantity and quality among passerine birds [9]. Thus, we predicted that species with a high risk of sperm competition should have more robust sperm, in this case lower frequencies of radiation-induced damage. As sperm length is positively correlated with sperm competition risk [2,4,6], we would also expect species with longer sperm to have less damaged sperm. Finally, we used scanning electron microscopy (SEM) to study the nature of sperm abnormalities.

## 2. Material and methods

We obtained sperm samples from 102 individuals belonging to 11 species of passerine birds in Chernobyl, Ukraine, during May–June 2010–2011, and 84 individuals from 10 of the same species in Norway and the Czech Republic (uncontaminated areas) during 2007–2013. A detailed sample list is given in the electronic supplementary material, table S1. Ejaculates were obtained by cloacal massage, diluted in saline and immediately fixed in 5% formaldehyde [6]. From each sample, an approximately 3  $\mu$ l aliquot

was spread on a microscope slide and air-dried, then gently rinsed with distilled water and air-dried again. Digital images of the spermatozoa were captured (160 $\times$  magnification) using a Leica DFC420 digital camera attached to a LEICA DM6000 B microscope, and analysed with Leica APPLICATION software. We typically examined 100 spermatozoa per sample, unless fewer sperm were available, and scored them as ‘normal’ or ‘abnormal’ (i.e. different head shape or reduced head length). All images were scored by one person (I.G.H.) to reduce observer error. Additional scoring of images from 10 samples (one sample randomly drawn from each species in uncontaminated areas), done blindly with respect to the first scorings, gave a high repeatability of the scored frequencies of abnormal sperm ( $R = 0.98$ ,  $F_{9,10} = 101.6$ ,  $p < 0.001$ ).

For SEM, we selected samples from three species with relatively high frequencies of abnormal sperm. Formalin-fixed spermatozoa were prepared and examined as in Liffield *et al.* [12]. Mean sperm lengths for each species were obtained from the avian sperm collection database at the Natural History Museum, Oslo, only using measurements of sperm with no sign of damage from the control populations (10 species) or Chernobyl (*Luscinia luscinia*). The coefficient of variation in mean sperm length ( $CV_{bm}$ ) among males from the same populations was used as an index of sperm competition risk [4]. Species-specific values for sperm length and sperm competition risk are listed in the electronic supplementary material, table S2.

Comparative analyses of the proportion of abnormal sperm were conducted using a generalized least-squares approach controlling for phylogenetic relatedness among species (i.e. PGLS; electronic supplementary material, methods). For all parametric tests, proportions were arcsine square-root transformed, and sperm lengths were  $\log_{10}$ -transformed to approach normality.

## 3. Results

Frequencies of abnormal sperm varied from 15.7 to 73.3% among Chernobyl species ( $N = 11$ ), with an overall mean

frequency of 39.6%. Among uncontaminated populations of the same species ( $N = 10$ ), frequencies varied from 0.1 to 21.1%, with an overall mean frequency of 8.6%. The frequency of abnormal sperm was always higher in Chernobyl than that in uncontaminated populations (paired  $t_9 = 7.68$ ,  $p < 0.001$ ; figure 1a), although for one outlier species, *Coccothraustes coccothraustes*, frequencies were quite similar. Sperm abnormalities tended to be positively correlated between contaminated and uncontaminated areas (Pearson  $r = 0.629$ ,  $p = 0.051$ ,  $n = 10$ ; figure 1a, with the outlier species excluded:  $r = 0.928$ ,  $p < 0.001$ ,  $n = 9$ ), which implies an intrinsic, species-specific factor underlying the consistent variation in sperm abnormalities between environments.

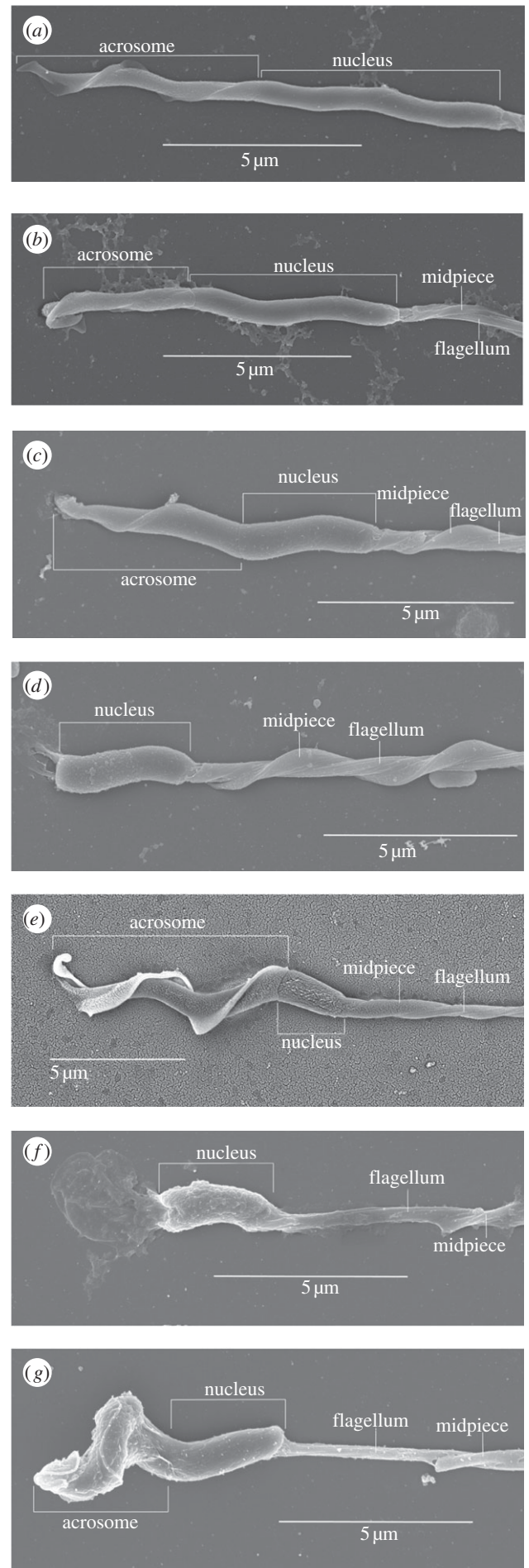
Variation in sperm abnormalities among Chernobyl populations was significantly associated with sperm length, i.e. species with longer sperm had more abnormal sperm (table 1 and figure 1b). By contrast, there was no significant association with sperm competition risk (table 1). Among uncontaminated populations, neither sperm length nor sperm competition risk explained significant variation in sperm abnormalities (table 1), but sperm length had a significant effect when the outlier was excluded (PGLS,  $t = 2.77$ ,  $n = 9$ ,  $p = 0.028$ ).

SEM images of spermatozoa (figure 2) revealed that abnormalities were restricted to the acrosome, i.e. the anterior part of the head. The types of damage varied between a bent acrosomal tip (figure 2b), a reduced acrosome and acrosomal microtubule helix (figure 2g), an 'emptied' acrosome with remnants of the plasma membrane (figure 2f) and an entirely missing acrosome (figure 2d). These changes reflect the various stages of the acrosome reaction, which normally takes place when the sperm penetrates the inner perivitelline layer of the ovum [13]. Hence, we interpret the sperm head abnormalities as evidence of premature acrosome reaction.

#### 4. Discussion

Our study documented three novel patterns of sperm damage. First, all studied passerine birds in the Chernobyl area have elevated frequencies of sperm abnormality, even 25 years after the radioactive fallout. Background radiation in this environment has had significant negative impact on many birds and other taxa [14,15]. Studies of local barn swallow *Hirundo rustica* populations have revealed a series of physiological and morphological defects [16,17], including increased sperm abnormalities and reduced sperm swimming speed [11,18]. Our results indicate that most passerine birds in the area are significantly affected by radiation-induced sperm damage.

Second, our SEM analyses identified premature acrosome reaction as the likely mechanism of the observed sperm damage. Correct timing of the acrosome reaction is crucial for fertilizing success of an ejaculate, and spermatozoa without an intact acrosome are unable to penetrate the inner perivitelline layer of the ovum [13]. Our sperm samples were fresh ejaculates, so the conditions causing the defect must be related to the physiology of the male. Several factors are known to induce premature acrosome reaction in birds and mammals, including mutations [19], extracellular calcium and lead [20,21], and oxidative stress [22]. Interestingly, a link between oxidative stress and poor sperm performance has already been indicated in barn swallows from Chernobyl [11,23], so



**Figure 2.** Scanning electron micrographs of normal and abnormal spermatozoa of three passerine species: (a,b) barn swallow *Hirundo rustica*, (c,d) common blackbird *Turdus merula* and (e–g) common chaffinch *Fringilla coelebs*.

we speculate that premature acrosome reaction is caused by oxidative stress and accelerated sperm senescence [24]. There was no seasonal trend in the proportion of abnormal sperm (see electronic supplementary material, methods).

Finally, species with longer sperm had more damaged sperm. This implies that longer sperm, under otherwise similar extracellular conditions, has a lower structural integrity of the acrosome, and may be more susceptible to lipid peroxidation of the plasma membranes [22]. Oscine passerine birds are characterized by the acrosome being longer than the nucleus [5], and longer acrosomes could be structurally less stable. However, it is not yet known whether species with longer

sperm also have longer acrosomes. Regardless of the actual mechanism underlying the inferred premature acrosome reaction of longer sperm, the pattern reflects a possible trade-off between sperm length and sperm integrity, and a possible explanation for why there is no run-away selection for longer sperm in passerine birds as compared with other taxa [2].

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