
Volume 650, Issue 2, 29 February 2008
ISSN 1383-5718
ELSEVIER
MUTATION RESEARCH
PB
Genetic Toxicology and Environmental Mutagenesis
Editors M.D. SHELBY, R.A. BAAN & T. NOHMI
www.elsevier.com/locate/gentox

Available online at www.sciencedirect.com
ScienceDirect
Mutation Research 650 (2008) 210–216
Genetic Toxicology and Environmental Mutagenesis
www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Impaired swimming behaviour and morphology of sperm from barn swallows *Hirundo rustica* in Chernobyl

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Received 30 April 2007; received in revised form 20 November 2007; accepted 8 December 2007
Available online 23 December 2007

Abstract

We investigated the motility and morphology of live sperm from barn swallows *Hirundo rustica* breeding in radioactively contaminated areas around Chernobyl and control areas in Ukraine in order to test the hypothesis that swimming behaviour and morphology of sperm was impaired by radioactive contamination. We obtained sperm samples from 98% of sampled birds, thus avoiding sampling bias due to the fraction of males not producing sperm samples. Analyses of within- and between-sample repeatability revealed significant and intermediate to large estimates for all sperm parameters. There were significant differences between the Chernobyl area and the control area for two of 11 sperm behaviour parameters, and significant interactions between area and year for six of these parameters. The proportion of sperm with abnormal morphology was elevated in barn swallows from Chernobyl. A principal component (PC) analysis revealed four significant axes that explained 88% of the variance in sperm behaviour parameters. One of these principal components differed between areas, and three components showed significant year by area interactions. PC2 representing the frequency of slow sperm increased with increasing radiation in one year, but not another. PC3 representing sperm with high linearity, small amplitude of lateral head displacement and low track velocity decreased with increasing background radiation level. PC4 reflecting a large proportion of static sperm with high beat cross frequency increased with increasing background radiation level. Sperm behaviour as reflected by principal components was predictable among years from information on level of radiation, and it was predictable among sites in different years. These results are consistent with the hypothesis that sperm behaviour and morphology have been affected by radiation due to the Chernobyl accident.

Keywords: Abnormal sperm; Age; Barn swallow; *Hirundo rustica*; Radiation; Sperm tracking

1. Introduction

Fertilization is a major process affecting reproductive success in sexually reproducing organisms. Hence, factors that determine the performance of sperm are supposed to be under intense selection because of their close correlation with fertilization success. A number of different factors have been suggested to account for fertilization probability including sperm size, sperm motility and swimming speed [see, e.g., 1–6]. Given such evidence we may also expect that features of importance for sperm performance would be highly canalized, thus allowing males to produce sperm with a high probability of success under a range of different environmental conditions.

Sperm morphology and performance can be affected by radiation. Studies of the barn swallow *Hirundo rustica* have revealed a strong positive correlation between background level of radiation in different parts of Ukraine and the frequency of sperm with abnormal morphology [7], increasing from <5% in control regions to >40% in the most contaminated areas. In addition, that study revealed a possible mechanism underlying this effect of radiation on sperm morphology, because levels of antioxidants such as carotenoids and vitamins A and E in blood and liver showed strong, inverse correlations with the frequency of abnormal sperm. Since antioxidants may prevent damage caused

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1383-5718/$ – see front matter
doi:10.1016/j.mrgentox.2007.12.006

by free radicals in DNA and other molecules [8,9], this raises the possibility that sperm abnormality is caused by mutations arising from the action of free radicals. A few studies in humans have shown changes in ultra-structure of the sperm head when exposed to high levels of radiation [10], and the frequency of translocations in mouse spermatozoa increased with radiation dose rate [11–13]. In addition, the total number of sperm and the number of motile sperm has been shown to be lower in Chernobyl victims than in controls [14].

The objective of the present study was to test the extent to which sperm swimming behaviour and morphology were affected by background radiation around Chernobyl, using the barn swallow as a model system. Because sperm morphology may change in response to increased radiation levels [7,10], we also tested if sperm swimming behaviour changed in response to the level of radiation after controlling for differences in sperm morphology. Furthermore, we tested in two different ways whether sperm behaviour could be predicted from prior knowledge of the relationship between sperm performance and radiation. This was done by determining whether the relationship between sperm behaviour and radiation in one year could be used to predict sperm behaviour of different birds in another year. Furthermore, we tested explicitly whether mean sperm performance was consistent among years for particular sites with specific levels of radiation. In order to do so we captured adult male barn swallows around Chernobyl and in control areas, sampled ejaculates from these males and assessed sperm for swimming behaviour and morphological abnormalities. We adopted a computer assisted sperm analysis (CASA) approach because studies of sperm quality in humans and animals have recently shown that sperm swimming speed and motility are prime determinants of fertilization success [1–5]. Likewise, CASA studies of chicken sperm performance have indicated that sperm velocity is a major determinant of fertility [3].

Barn swallows are small, insectivorous, migratory passerines that breed commonly in farms and other human habitations [15]. Males and females are relatively similar in appearance, with the exception of the outermost tail feathers that on average are 20% longer in males than in females in our Ukrainian study populations [16]. Males with long tails enjoy several mating advantages including earlier reproduction and higher mating success [15]. Previous studies of barn swallows in Ukraine have shown that males in contaminated areas have paler color than males in control areas [17]. Partial albinism in the plumage is elevated in the Chernobyl region [18], and such albinism is associated with reduced survival prospects [19]. Furthermore, the phenotypic characters of males that are most suppressed in Chernobyl compared with control areas are those that are most important for mating success [16]. Male barn swallows in Chernobyl have elevated frequencies of abnormal sperm and greatly suppressed levels of antioxidants [7]. Finally, the frequency of non-reproducing adults is elevated, reproductive success is suppressed, and adult survival rate is reduced in Chernobyl compared with control areas [20]. Hatching failure due to infertile eggs increased from 1.4% in control areas to 5.9% in contaminated areas, a more than four-fold increase [20].

Fig. 1. Geographical location of the Ukrainian study sites in relation to background radiation level. Red-colored regions indicate radionuclide levels greater than 5 Ci/km^2 [27]. Green asterisks indicate contaminated sampling areas, while black asterisks indicate control sampling areas. The radioactivity symbol indicates the location of the Chernobyl reactor. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

2. Material and methods

2.1. General field procedures

We captured barn swallows in a number of different farms around Chernobyl, Borispil and Kanev in Ukraine during late May and early June 2005 to 2006 (Fig. 1). Using mist nets placed across windows and doors we captured wild birds at the peak of reproduction just before and during laying for most males. Birds were placed in cloth bags before being measured, weighed and assessed for ectoparasites. Then blood was sampled and feather samples were collected. Subsequently, we collected one or two sperm samples from all males, using a simple massage technique. Sperm were collected in micro-capillaries for easy handling.

Our field measurements of background radiation at the ground level using a hand-held Inspector dosimeter (Model: Inspector, SE International, Inc., Summertown, TN, USA) revealed levels of mainly γ radiation of on average 0.390 mR/h (S.E. = 0.317) at 14 breeding sites in the Chernobyl region. As control areas we chose Kanev, about 120 km southwest of Kiev, and Ghovtnere, about 35 km southeast of Kiev. Both had relatively low levels of radiation: mean levels were 0.025 mR/h (S.E. = 0.002) at five breeding sites near Kanev, and 0.006 mR/h (S.E. = 0.003) at Ghovtnere near Borispil. We cross-validated our radiation data with officially published measurements [21]. The latter were estimated as the mid-point of the ranges from this published source. This analysis revealed a very strong positive relationship (linear regression on log–log transformed data: $F = 159.46$, d.f. = 1,18, $r^2 = 0.89$, $P < 0.0001$, slope (S.E.) = 1.28 (0.10), suggesting that our field estimates of radiation were comparable with other estimates.

We have ringed barn swallows in Ukraine since 2000, and we assigned a minimum age to birds. When annual ringing effort is high, as it has been in our Ukrainian study sites [20], we can assume that unringed birds are yearlings. The reasons are that (1) adults hardly ever move from one site to another once they have chosen a breeding site [15], and (2) among 415 local recruits ringed as nestlings in Spain, Italy and Denmark 413 were first captured as breeders when 1 year old, with the two remaining birds first being captured breeding when 2 years old [15,22, A.P. Møller unpublished data]. Because annual survival rate of adult barn swallows is in the range 0.3–0.5 [20], very few reach an age of more than a couple of years.

We did not assign individual birds to nests and hence did not know their exact breeding status, although checks of nests revealed the contents to mainly be with feather lining but no eggs, or with eggs or small nestlings. An indirect measure of stage of breeding can be obtained from the presence or absence of mud on the beak. Male (and female) barn swallows build nests out of mud, with building in

Denmark taking place between 24 and 2 days before start of egg laying (A. P. Møller unpublished information on more than 200 pairs observed for 1 h daily during their entire breeding cycle in 1984 to 1990). This period coincides with the period when almost all copulations take place [23]. Barn swallows building nests have fresh or dried mud on their beaks, while birds that are not building do not. Therefore, any male with mud on its beak will be copulating intensely with its mate, while that is not the case for males without mud. Males that are more advanced in their breeding cycle do still copulate with their mates, but copulate more frequently with other females [23]. We recorded the presence of mud on the beak (22 of 101 males) and used this dichotomous character in the analyses as a proxy for stage in the breeding cycle.

2.2. *Sperm behaviour and morphology*

The first sperm sample was transferred to a microscope chamber, without the experimenter having any knowledge of the individual male. Hence, all measurements obtained from the video equipment were obtained blind with respect to identity of the individual male. For each male we quantified sperm motility within 2 min following extraction of the sperm.

The sperm sample was diluted in a one-step procedure, by placing an aliquot of undiluted sperm on a pre-warmed (37 °C) microscope slide with an 80-mm deep chamber (Hamilton Thorne Research, Beverly, MA, USA). Immediately afterwards, 9 ml Dulbecco's Modified Eagle Medium (D-MEM; Invitrogen, Carlsbad, California) was added and a cover glass was placed over the sample. D-MEM (Gibco) contains 4500 mg/l glucose, 4 mM L-glutamine and 110 mg/l sodium pyruvate. Sperm motility and images were recorded with a Sony CCD black-and-white video camera (XC-ST50CE PAL, Sony, Tokyo, Japan) at 50 Hz vertical frequency, mounted on an external negative phase-contrast microscope (Olympus CH30, Olympus, Tokyo, Japan) with a 10× objective. Video recordings were stored on a Sony TRV900 mini-DV camcorder. Several different video frames of sperm motility from each sperm drop were recorded, which allowed us to capture a larger number of sperm cells from each ejaculate. The recordings were later analyzed using computer-assisted sperm analysis (HTM-CEROS sperm tracker, CEROS version 12, Hamilton Thorne Research, Beverly, MA, USA). The image analyzer was set at frame rate of 50 Hz, number of frames 25, minimum contrast 30, and minimum cell size 20 pixels. Each motility measurement lasted 0.5 s. Several motility measurements were made for each ejaculate.

We recorded mean values of (1) VAP (smoothed path velocity; μm/s), VSL (straight line velocity; μm/s), (2) VCL (track velocity; μm/s), (3) ALH (amplitude of lateral head displacement; μm), (4) BCF (beat cross frequency; Hz), (5) LIN (linearity, VSL/VCL) and (6) STR (straightness, VSL/VAP) for each sample. Cells having a VAP < 10 μm/s and a VSL < 5 μm/s were excluded from the motility analysis, and counted as static. The measurements were used to estimate (7) the percentage of static sperm (the percentage of sperm that remained static), (8) the percentage of sperm with slow velocity (i.e. <10 μm/s), (9) the percentage of sperm with medium velocity (10–50 μm/s), (10) the percentage of sperm with high velocity (>50 μm/s) and (11) the percentage progressive sperm (the percentage of all sperm that moved with STR > 80 and VAP > 50 μm/s).

Images were downloaded from the video recordings for analysis to a PC via a firewire connection and stored in DV-AVI before conversion to MPEG-2 format for storage on DVD-ROM. Still images were grabbed from video files using Nero ShowTime software on a PC.

Sperm morphology was recorded using image analysis of still images. We measured head length and total length to the nearest 0.8 μm. These two measurements were used to estimate tail length as total sperm length minus sperm head length. Measurements were made from all males, with repeat measurements for ten males that were sampled twice. These measurements were conducted blind with respect to origin of the samples. The number of sperm measured per male ranged from 5 to 106, depending on availability of high-quality still images. The repeatability of measurements of the length of 126 individual sperm was 0.82.

2.3. *Statistical analyses*

Information on sperm behaviour and morphology was quantified as mean value per male based on repeat estimates. We tested for consistency in estimates from the same ejaculate, from different ejaculates for the same males collected during the same capture, and from different ejaculates for the same males collected in different years (within-sample repeatability, among-sample repeatability within years and among-sample repeatability among years, respectively). Repeatability estimates (standard errors) were obtained using variance components from one-way ANOVAs [24], with a repeatability of zero implying no consistency in estimates of sperm parameters among measurements or samples from the same individual, while a repeatability of one implies complete identity of estimates among measurements or samples from the same individual.

We tested for differences in sperm parameters between areas using one-way ANOVA. Relationships between sperm parameters and levels of background radiation were analyzed by linear regression with log-transformed radiation as the predictor variable. Because sperm behaviour may be affected by sperm morphology independent of levels of background radiation, we used multiple-regression models with log-transformed radiation level, mean sperm head length and mean sperm tail and mid-piece (hereafter sperm tail for simplicity) length as predictor variables. In these analyses we included mean and standard deviations for all sperm sampled.

We quantified sperm behaviour by using a principal component analysis of the 11-ejaculate behaviour parameters described above, by use of a varimax approach to the correlation matrix. This reduced the correlated variables to four statistically independent axes that reflected different aspects of overall sperm behaviour.

We randomly excluded one sample for the males that were sampled in both years to have each individual represented by a single observation in order to avoid pseudo-replication.

We tested for effects of age by adding age as an additional variable to these multiple-regression models.

We identified best-fit multiple-regression models, using the software JMP [25]. The best-fit model was determined with Akaike's information criterion as an estimate of the improvement in fit for addition of variables [26]. We started out by using the best-fit models and then deleting factors according to their delta AIC values, using the criterion that a change in AIC of more than 2.00 would be considered biologically meaningful [26].

Sample sizes differed among analyses depending on availability of data. All values reported are means (S.E.).

3. Results

3.1. *Determinants of sperm swimming behaviour*

We were able to obtain sperm from 190 of 193 male barn swallows (98.4%). Repeatability of sperm parameters within and among samples is reported in Table 1. Most variables show moderate repeatabilities and only one value was not statistically significant. Repeatability of sperm head length, sperm tail length and percentage abnormal sperm was highly significant. All among-sample repeatabilities were smaller than the within-sample repeatabilities (paired *t*-test, $t = 4.69$, d.f. = 10, $P = 0.0008$). Among-year repeatability of sperm swimming parameters based on a small sample of 18 males that survived from 1 year to the next and were sampled in both years, did not reach statistical significance ($F < 1.66$, d.f. = 17,18, $P > 0.15$). In contrast, the percentage abnormal sperm was highly repeatable among years (percentage abnormal sperm: $F = 901.52$, d.f. = 17,18, $P < 0.0001$, R (S.E.) = 0.99 (0.002)).

A principal component analysis of the 11 sperm-behaviour variables produced a model with four principal components that accounted for 88% of the variance (Table 2). PC1 tended to be positively correlated with all variables, with the strongest correlation with the proportion of rapid sperm (Table 2). PC2 reflected the proportion of slow and medium speed sperm, thus reflecting ejaculates with slow sperm (Table 2). PC3 represented

Table 1
Mean values (mean of means and S.E.) and within- and among-sample repeatability of sperm parameters from barn swallows from Ukraine

Variable	Mean (S.E.)	Within-sample F	Within-sample R (S.E.)	Among-sample F	Among-sample R (S.E.)
Pct. static	36.25 (1.67)	10.21***	0.48 (0.04)	9.81***	0.33 (0.02)
Pct. slow	5.10 (0.29)	1.73***	0.07 (0.02)	1.25	0.01 (0.01)
Pct. medium	24.29 (0.81)	4.74***	0.27 (0.04)	5.95***	0.22 (0.02)
Pct. rapid	34.36 (1.36)	9.89***	0.47 (0.04)	8.34***	0.29 (0.02)
Pct. progressive	29.84 (1.23)	8.98***	0.44 (0.04)	7.38***	0.27 (0.02)
LIN	64.75 (1.16)	7.82***	0.41 (0.04)	2.01*	0.05 (0.01)
STR	83.33 (1.02)	5.36***	0.30 (0.04)	3.69**	0.15 (0.02)
BCF (Hz)	21.48 (0.35)	3.50***	0.20 (0.03)	3.26**	0.14 (0.02)
ALH (μm)	3.85 (0.11)	5.30***	0.30 (0.04)	9.03***	0.31 (0.02)
VCL (μm/s)	78.85 (1.51)	7.92***	0.41 (0.04)	5.61***	0.21 (0.02)
VAP (μm/s)	57.15 (1.17)	8.25***	0.42 (0.04)	5.50***	0.20 (0.02)
Head length (μm)	14.04 (0.11)	–	–	7.72***	0.77 (0.05)
Tail length (μm)	71.98 (0.40)	–	–	10.82***	0.83 (0.04)
Pct. abnormal sperm	8.96 (1.17)	–	–	7.24***	0.76 (0.05)

Sample size is 166 males with repeat recordings for a single ejaculate, while 10 males were analyzed for two different ejaculates.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.0001$.

sperm with a high degree of linearity, small amplitude of lateral head displacement and low track velocity (Table 2). Finally, PC4 represented static sperm with high beat cross frequency (Table 2).

Two of the 11 sperm variables differed significantly between samples from Chernobyl and control areas, after controlling for year effects (Table 3). Mean values for these significant differences were larger in Chernobyl than in the control areas, with the exception of sperm tail length, which was shorter in Chernobyl than in the control areas. Seven variables differed significantly between years (Table 3). In addition, six of 11 variables showed significant area-by-year interactions (Table 3).

Table 2
Principal component analysis of 11 sperm parameters of barn swallows

	PC1	PC2	PC3	PC4
Eigenvalue	5.26	1.79	1.56	1.12
Percent	47.77	16.29	14.15	10.18
Cumulative percentage	47.77	64.07	78.22	88.40
Pct. static Sperm	−0.37	−0.18	−0.02	**0.40**
Pct. slow Sperm	0.05	**0.40**	0.37	−0.33
Pct. medium Sperm	0.16	**0.59**	0.11	−0.15
Pct. rapid Sperm	**0.40**	−0.15	−0.08	−0.25
Pct. progressive Sperm	0.39	−0.21	0.07	−0.21
LIN	0.32	−0.22	**0.42**	0.18
STR	0.35	0.04	0.26	0.37
BCF	0.15	0.38	0.12	**0.62**
ALH	0.16	0.34	**−0.58**	0.03
VCL	0.31	0.03	**−0.48**	0.17
VAP	0.38	−0.28	−0.11	0.08

Loadings exceeding 0.40 are highlighted in bold. Interpretation of PCs: PC1: loading on percentage of rapid sperm. This implies that PC1 represents an axis of increasing frequency of rapid sperm. PC2: loadings on percentage of slow and medium sperm. This axis represents the frequency of slow sperm. PC3: loadings on sperm with high LIN (linearity) and low ALH (amplitude of lateral head displacement) and VCL (track velocity). This implies that PC3 represents sperm with high linearity, small amplitude of lateral head displacement and low track velocity. PC4: loadings on static sperm with high BCF (beat cross frequency). Therefore, PC4 represents static sperm with high beat cross frequency.

Analysis of the principal components revealed a significant difference between areas for PC3, significant year effects for all four principal components, and a significant year-by-area interaction for PC1–3 (Table 3).

There was no significant difference in any sperm parameter between males with and without mud on their beaks (results not shown). Hence, there was no evidence that sperm parameters differed among males captured at different stages of the breeding cycle.

Age did not predict sperm morphology or swimming behaviour, with none of the 14 tests statistically significant ($F < 0.67$, d.f. = 1,137, $P > 0.41$).

3.2. Sperm behaviour and radiation

Analysis of the four principal components in relation to radiation level, year and morphology of barn swallows revealed three significant models that included background radiation levels (Table 4). PC2 scores increased significantly with radiation level, with additional effects of year, and a marginally significant interaction between radiation level and year (Table 4). PC3 scores decreased significantly with level of radiation (Fig. 2A), and this effect differed between years (Table 4). PC4 scores increased significantly with level of radiation (Fig. 2B), and this effect differed between years (Table 4). In addition, there was a marginal effect of tail length that differed between years, with long-tailed males having low PC4 scores (Table 4).

Analysis of the four principal components in relation to radiation level, year and sperm morphology revealed two significant models that included sperm morphology (Table 5). PC3 scores decreased significantly with tail length of sperm, accounting for 5% of the variance (Table 5). PC4 scores decreased significantly with the percentage of deformed sperm, and this effect accounted for 11% of the variance (Table 5). The models for PC1 and PC2 did not reach statistical significance.

Table 3
Tests for differences in sperm parameters between areas

Variable	F (area)	F (year)	F (area × year)	Mean (S.E.) Chernobyl 2005	Mean (S.E.) Chernobyl 2006	Mean (S.E.) controls 2005	Mean (S.E.) controls 2006
Pct. static	0.33	38.93***	1.81	44.98 (2.81)	29.81 (2.68)	47.36 (3.50)	23.85 (3.34)
Pct. slow	0.23	2.42	0.33	4.68 (0.53)	5.25 (0.51)	4.62 (0.66)	5.88 (0.63)
Pct. medium	1.20	13.91***	0.80	19.76 (1.45)	27.14 (1.38)	22.93 (1.80)	27.46 (1.72)
Pct. rapid	0.00	22.77***	3.84*	30.47 (2.38)	37.85 (2.26)	25.15 (2.96)	42.80 (2.82)
Pct. progressive	0.26	15.19***	7.95**	27.89 (2.17)	30.47 (2.07)	22.35 (2.70)	38.43 (2.58)
LIN	0.23	0.49	18.68***	68.48 (2.07)	60.22 (1.97)	59.71 (2.57)	71.15 (2.46)
STR	0.09	2.82	9.03**	84.89 (1.86)	82.16 (1.77)	78.10 (2.32)	87.73 (2.22)
BCF (Hz)	0.37	0.62	1.04	21.57 (0.66)	21.74 (0.62)	21.87 (0.82)	20.56 (0.78)
ALH (μm)	7.49**	19.47***	5.06*	3.36 (0.19)	4.73 (0.18)	3.26 (0.23)	3.70 (0.22)
VCL (μm/s)	10.42**	14.93***	0.06	76.28 (2.63)	88.21 (2.50)	67.64 (3.27)	78.13 (3.12)
VAP (μm/s)	2.45	6.08*	7.66**	58.83 (2.11)	58.13 (2.01)	60.93 (2.51)	48.75 (2.63)
PC1	0.59	18.51***	5.43*	-0.27 (0.31)	0.41 (0.30)	-1.34 (0.39)	0.96 (0.37)
PC2	0.01	5.21*	7.32**	-0.52 (0.19)	0.51 (0.18)	0.02 (0.23)	-0.07 (0.22)
PC3	9.00**	4.80*	9.70**	0.29 (0.17)	-0.69 (0.16)	0.27 (0.21)	0.44 (0.20)
PC4	2.54	17.31***	0.14	0.42 (0.15)	-0.19 (0.14)	0.22 (0.18)	-0.50 (0.17)
Head length (μm)	3.18	0.63	0.43	18.44 (0.19)	18.81 (0.26)	18.66 (0.24)	18.76 (0.25)
Tail length (μm)	4.84*	0.24	0.17	93.70 (0.62)	93.89 (0.84)	96.79 (0.77)	96.88 (0.81)
Pct. abnormal sperm	7.94**	2.78	2.34	27.19 (3.00)	19.06 (3.75)	28.26 (1.28)	15.44 (1.69)

Sample sizes are 101 for Chernobyl and 65 for control areas, with d.f. = 1,162.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.0001$.

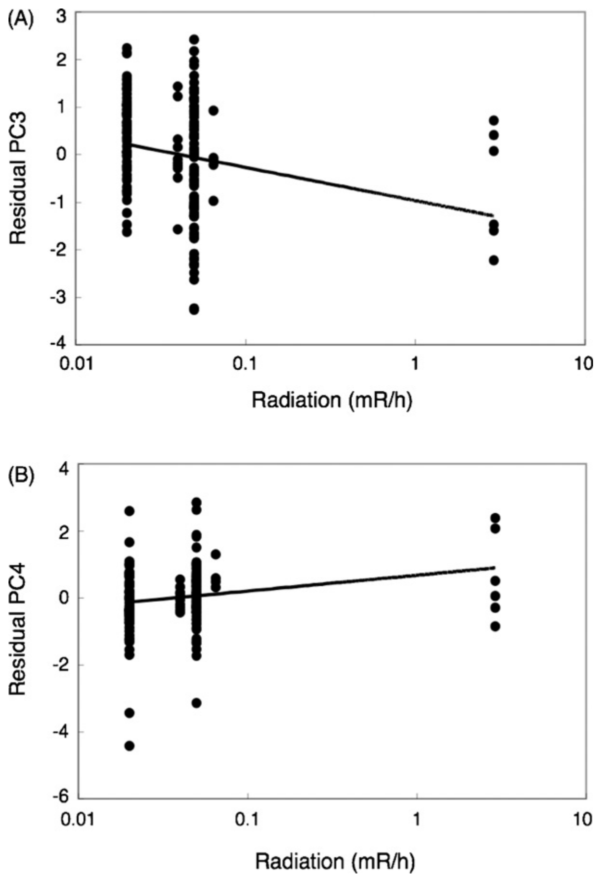

Fig. 2. The relationship between (A) PC3 score of ejaculates and background radiation level (mR/h), and (B) PC4 score of ejaculates and background radiation level (mR/h). The relationships are based on residuals after adjusting for the variables listed in Table 4.

3.3. Predictability of sperm performance

We investigated whether sperm behaviour could be predicted in two different ways. First, we tested whether we could predict sperm behaviour in 2006 based on levels of radiation and tail length, using the regression models based on principal components and levels of radiation and tail length in 2005. Only models for PC2, PC3 and PC4 reached the level of significance,

Table 4
Best-fit models of principal components 2, 3 and 4 (derived from a principal component analysis of 11 ejaculate parameters) in relation to background radiation level, year, tail length and their interactions

Variable	Sum of squares	F	P	Slope (S.E.)	Delta AIC
PC2					
Radiation	4.81	2.83	0.09	0.65 (0.39)	0.07
Year	18.17	10.71	0.0013	−0.35 (0.11)	8.10
Radiation × year	6.38	3.76	0.05	−0.75 (0.39)	1.81
PC3					
Radiation	32.26	26.21	<0.0001	−1.69 (0.33)	30.34
Year	24.53	19.93	<0.0001	0.40 (0.09)	38.04
Radiation × year	44.89	36.47	<0.0001	1.99 (0.33)	31.71
PC4					
Radiation	6.57	6.77	0.010	0.85 (0.33)	2.98
Year	9.41	9.70	0.002	0.26 (0.08)	17.70
Tail length	3.25	3.35	0.07	−0.015 (0.008)	3.00
Radiation × year	3.39	3.50	0.06	−0.61 (0.33)	1.59
Tail × year	4.88	5.03	0.026	−0.018 (0.008)	3.15

The three models had the statistics $F = 4.11$, d.f. = 3,162, $r^2 = 0.07$, $P = 0.0077$, AIC = 91.69; $F = 15.57$, d.f. = 4,158, $r^2 = 0.22$, $P < 0.0001$, AIC = 38.42 and $F = 6.05$, d.f. = 5,153, $r^2 = 0.17$, $P < 0.0001$, AIC = 1.07. Delta AIC was calculated as the difference in AIC between the best-fit model and the model excluding a specific variable.

Table 5
Best-fit models of principal components 3 and 4 (derived from a principal component analysis of 11 ejaculate parameters) in relation to background radiation level, year, sperm morphology and their interactions

Variable	Sum of squares	F	P	Slope (S.E.)	Delta AIC
PC3					
Tail length	9.53	6.46	0.012	−0.05 (0.02)	4.40
PC4					
Pct. deformed sperm	8.51	9.14	0.0034	−1.53 (0.51)	6.85

The two models had the statistics $F = 9.53$, d.f. = 1,161, $r^2 = 0.05$, $P = 0.012$, AIC = 58.58 and $F = 9.14$, d.f. = 1,157, $r^2 = 0.11$, $P = 0.0034$, AIC = 3.26. Delta AIC was calculated as the difference in AIC between the best-fit model and the model excluding a specific variable.

and hence we restricted the analyses to these three components. Regression of observed PC2, PC3 and PC4 against predicted values derived from the regression models from 2005 and radiation and tail length recorded in 2006, showed significant positive relationships (PC2: $F = 3.66$, d.f. = 1,85, $r^2 = 0.04$, $P = 0.046$; PC3: $F = 38.71$, d.f. = 1,85, $r^2 = 0.31$, $P < 0.0001$; PC4: $F = 8.40$, d.f. = 1,85, $r^2 = 0.10$, $P = 0.0048$). Thus, we were able to validate our regression models from 2005 by using data from 2006, showing some predictive power in the statistical models.

Second, we tested whether mean principal components were consistent for the nine different sites, using one-way analysis of variance. Indeed, there was a significant repeatability of sperm performance for the different sites among years for PC2, PC3 and PC4 (PC2: $F = 9.31$, d.f. = 8,9, $r^2 = 0.89$, $P = 0.0015$; PC3: $F = 5.63$, d.f. = 8,9, $r^2 = 0.83$, $P = 0.0091$; PC4: $F = 8.01$, d.f. = 8,9, $r^2 = 0.88$, $P = 0.0026$), while the analysis for PC1 did not reach statistical significance ($F = 3.04$, d.f. = 8,9, $r^2 = 0.73$, $P = 0.059$). Thus, mean swimming performance of sperm was consistent among years for specific sites with specific levels of radiation.

4. Discussion

The main findings of our study of sperm behaviour and morphology of barn swallows from Ukraine were that (i) there were highly significant differences in sperm behaviour and morphology among males; (ii) several sperm parameters differed between the Chernobyl region and uncontaminated control areas; (iii) principal component scores of sperm-behaviour variables were significantly related to level of background radiation, independent of morphology of males and male age; (iv) principal component scores of sperm-behaviour variables were related to sperm morphology and sperm abnormality; and (v) sperm performance in a given year could be predicted from information on sperm behaviour in a previous year and from levels of radiation. We will briefly discuss these results.

We found clear evidence of differences in swimming behaviour and morphology of sperm between male barn swallows from the Chernobyl region and control areas. Two out of 11 variables differed significantly between areas, which are more than the expected 0.7 variables under the null hypothesis of no difference (at 5% significance). The tests suggested that swimming behaviour of sperm was inferior for male barn swallows from Chernobyl compared with males from control areas. These differences could not be accounted for by differences in timing of reproduction because the mean laying date is similar in Chernobyl and in the control areas [20]. Likewise, there was no evidence of age effects on the conclusions. A larger fraction of sperm from birds at Chernobyl had abnormal morphology compared with sperm from birds in the control areas, as we have shown before [7]. In addition, tail length of sperm was significantly reduced among male birds from Chernobyl, the mean difference amounting to 3%, consistent with the hypothesis that radiation caused an increase in mutation rate. Ellegren et al. [19] showed for micro-satellites in the barn swallow that the frequency of germline mutations in Chernobyl was elevated by a factor two to ten compared with control areas.

If sperm have evolved a morphology that maximizes fertilization ability, a difference in mean tail length of 3% is significant. Consistent with this interpretation we found that sperm swimming behaviour as reflected by the third principal component decreased with increasing tail length of sperm. Therefore, male barn swallows with long-tailed sperm had reduced linearity, increased lateral head movement and high track velocity. In addition, the fourth principal component decreased as the percentage of deformed sperm increased. This implies that male barn swallows had more static sperm with high beat frequency when they had a large percentage of deformed sperm. These findings are consistent with the hypothesis that sperm swimming behaviour of barn swallows is reduced in the most contaminated areas because they have short-tailed sperm and a large fraction of sperm with abnormal morphology. Alternatively, selective mortality could differentially affect the composition of the population of males in the two regions. Because adult mortality rate is elevated in Chernobyl compared with the control area [20], birds from contaminated areas may be of higher phenotypic quality with less variance in sperm morphology than males from the control areas. If selection was important, old birds should have superior sperm morphology and behaviour compared with young birds, but that was clearly not the case.

Sperm behaviour and morphology showed evidence of statistical consistency among estimates obtained from the same sample and among estimates obtained from different samples from the same male barn swallow. The level of consistency was low to intermediate, raising the question whether sperm parameters are predictable. We explicitly tested whether this was the case by use of two different approaches. First, we developed regression models based on data on sperm behaviour and radiation from 2005 to predict the same variables in 2006. Although the degree of consistency between observed and predicted estimates in 2006 was not high, it was still statistically significant, implying that sperm behaviour was to some extent predictable from knowledge of background radiation levels. Second, we tested to which extent mean sperm behaviour for particular sites with specific levels of radiation was consistent among years. Again, we found a high and significant degree of consistency among years, suggesting that sperm behaviour could be predicted. In conclusion, we have shown that sperm behaviour is predictable based on information on levels of background radiation, despite the fact that individual males vary considerably in sperm behaviour among samples.

The implications of this study are that low-level radioactive contamination does affect ejaculate features and sperm morphology, with dose-dependent relationships. This may have consequences for fertilization success as suggested by reduced hatching of eggs of barn swallows from the Chernobyl zone [20].

Acknowledgements

We are grateful to G. Milinevski, A. Peklo and E. Pysanets for logistical help in Ukraine. APM and TAM received funding from the CNRS (France), the University of South Carolina School of the Environment, the Samuel Freeman Charitable Trust, the National Science Foundation and the National Geographic Society to conduct this research. GR received funding from the Norwegian Research Council (177744/V40).

References

[1] F. Lahnsteiner, B. Berger, T. Weismann, R.A. Patzner, Determination of semen quality in the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoal metabolism, Aquaculture 163 (1998) 163–181.

[2] D.R. Levitan, Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegates*, Proc. R. Soc. Lond. B 267 (2000) 531–534.

[3] D.P. Froman, A.J. Feltmann, M.L. Rhoads, J.D. Kirby, Sperm mobility: a primary determinant of fertility in the domestic fowl (*Gallus domesticus*), Biol. Reprod. 61 (1999) 400–405.

[4] Y. Kupriyanova, H. Havenhand, Variation in sperm swimming behaviour and its effect on fertilization success in the serpulid polychaete, *Galeolardia caespitose*, Invert. Reprod. Dev. 41 (2002) 21–26.

[5] M.J.G. Gage, C.P. Macfarlane, S. Yeates, R.G. Ward, J.B. Searle, G.A. Parker, Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success, Curr. Biol. 14 (2004) 44–47.

[6] G. Garcia Gonzales, L.W. Simmons, Sperm viability matters in insect sperm competition, Curr. Biol. 15 (2005) 271–275.

[7] A.P. Møller, P.F. Surai, T.A. Mousseau, Antioxidants, radiation and mutation in barn swallows from Chernobyl, Proc. R. Soc. Lond. B 272 (2005) 247–253.

[8] A.P. Møller, C. Biard, J.D. Blount, D.C. Houston, P. Ninni, N. Saino, P.F. Surai, Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? Poult. Avian Biol. Rev. 11 (2000) 137–159.

[9] P.F. Surai, Natural Antioxidants in Avian Nutrition and Reproduction, Nottingham University Press, Nottingham, 2003.

[10] A. Fischbein, N. Zabludovsky, F. Eltes, V. Grischenko, B. Bartoov, Ultramorphological sperm characteristics in the risk assessment of health effects after radiation exposure among salvage workers in Chernobyl, Environ. Health Perspect. 105 (1997) 1445–1449.

[11] V.A. Shevchenko, M.D. Pomerantseva, L.K. Ramaiya, A.V. Chekhovich, B.V. Testov, Genetic disorders in mice exposed to radiation in the vicinity of the Chernobyl nuclear power station, Sci. Total Environ. 112 (1992) 45–56.

[12] A.V. Chekovich, M.D. Pomerantseva, L.K. Ramaiya, V.A. Shevchenko, Genetic damages in laboratory mice exposed in the Chernobyl nuclear power station zone 4 years after injury, Genetika 29 (1993) 312–322.

[13] M.D. Pomerantseva, L.K. Ramaiya, A.V. Chekhovich, Genetic disorders in house mouse germ cells after the Chernobyl catastrophe, Mutation Res. 381 (1997) 97–103.

[14] I.Y. Sakharov, V.V. Evdokimov, E.A. Atochina, Sperm angiotensin-converting enzyme activity in Chernobyl victims and patients with chronic prostatitis, Andrologia 28 (1996) 259–260.

[15] A.P. Møller, Sexual Selection and the Barn Swallow, Oxford University Press, Oxford, 1994.

[16] A.P. Møller, T.A. Mousseau, Mutation and sexual selection: a test using barn swallows from Chernobyl, Evolution 57 (2003) 2139–2146.

[17] C. Camplani, N. Saino, A.P. Møller, Carotenoids, sexual signals and immune function in barn swallows from Chernobyl, Proc. R. Soc. Lond. B 266 (1999) 1111–1116.

[18] A.P. Møller, T.A. Mousseau, Albinism and phenotype of barn swallows *Hirundo rustica* from Chernobyl, Evolution 55 (2001) 2097–2104.

[19] H. Ellegren, G. Lindgren, C.R. Primmer, A.P. Møller, Fitness loss and germline mutations in barn swallows breeding in Chernobyl, Nature 389 (1997) 593–596.

[20] A.P. Møller, T.A. Mousseau, G. Milinevsky, A. Peklo, E. Pysanets, T. Szép, Condition, reproduction and survival of barn swallows from Chernobyl, J. Anim. Ecol. 74 (2005) 1102–1111.

[21] V.M. Shestopalov, Atlas of Chernobyl Exclusion Zone, Ukrainian Academy of Science, Kiev, 1996.

[22] A.P. Møller, T. Szép, Survival rate of adult barn swallows *Hirundo rustica* in relation to sexual selection and reproduction, Ecology 83 (2002) 2220–2228.

[23] A.P. Møller, Mixed reproductive strategy and mate guarding in a semi-colonial passerine, the swallow *Hirundo rustica*, Behav. Ecol. Sociobiol. 17 (1985) 401–408.

[24] W.A. Becker, Manual of Quantitative Genetics, Academic Enterprises, Pullman, WA, 1984.

[25] JMP, SAS Institute Inc., Cary, NC, 2000.

[26] K.P. Burnham, D.R. Anderson, Model Selection and Multi-Model Inference, Springer, New York, NY, 2002.

[27] UNDP/UNICEF, The Human Consequences of the Chernobyl Nuclear Accident: A strategy for Recovery, UNDP/UNICEF, New York, 2002.