Changes in sperm traits over the breeding season in passerine bird - barn swallow *(Hirundo rustica)*

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Introduction

Post-copulatory sexual selection is thought to be one of the evolutionary forces responsible for the rapid and divergent evolution of sperm design. Sperm cells are highly diversified across taxa, however, little is known about **intraspecific variation** in sperm traits in birds. In temperate zone, many reproductive traits show remarkable seasonal variation, nevertheless, the potential for such dynamics in sperm traits has been overlooked (but see Lüpold et al. 2012). Several studies have revealed high within-male repeatability in sperm morphology (e.g. Birkhead and Fletcher 1995), but samples have typically been collected within a short period, and the consistency of sperm morphology over the whole season remains unexplored. Here, we tested whether ejaculate traits exhibit **seasonal phenotypic plasticity** in a long-lived seasonal breeder, the barn swallow *(Hirundo rustica)*. Three ejaculate parameters were studied repeatedly during a three-month-long breeding season: sperm number, sperm morphology (size of sperm components) and sperm motility.

Methods

We captured the birds repeatedly in a two-week interval into the mist nets at their nesting site. Every bird was marked with a ring having the unique number of National Museum in Prague.

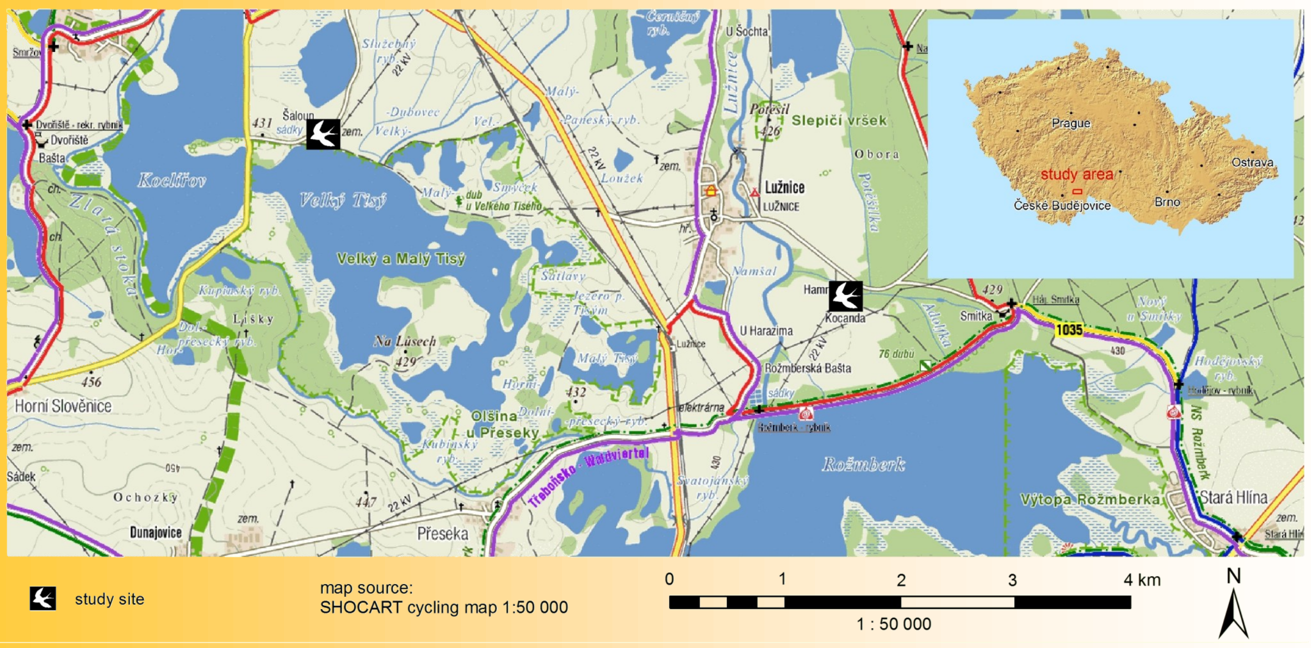

Figure 1. Map showing locality of the study population of barn swallows

We measured the dimensions (length, width, height) of the male cloacal protuberance (CP), which is swollen as a result of the growth of the seminal glomera - the coiled distal end of the *vas deferens* where sperm are stored before ejaculation. This variable was used as a measure of sperm number (Laskemoen et al. 2010).

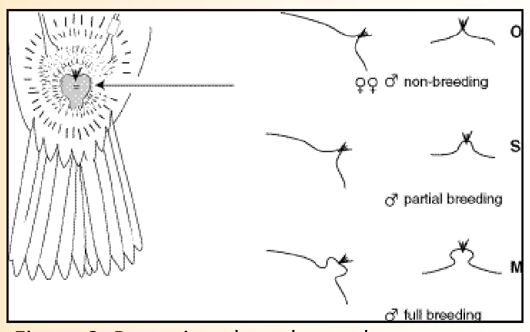

Figure 2. Passerine cloacal protuberance

Ejaculate samples were collected using a cloacal massage technique (Wolfson 1952). The sample was collected by a preheated micro capillary and immediately diluted in about 50 µl of DMEM (Dulbecco's Modified Eagle Medium, Advanced D-MEM, Invitrogen, Carlsbad, CA). The amount of about 2 µl of the diluted sperm was put onto a preheated standard microscopic count slide (Leja, The Netherlands). The sperm movement was recorded with a digital camera (UI-1540-C, Olympus) attached to the microscopic set (microscope CX41 with the phase contrast, magnification 100x). The recordings were analysed using the CASA software (Computer Assisted Sperm Analysis, CEROS, Hamilton Thorne, Inc. USA). The rest of the diluted sperm sample was put into 10% formalin and was used for morphology analyses later.

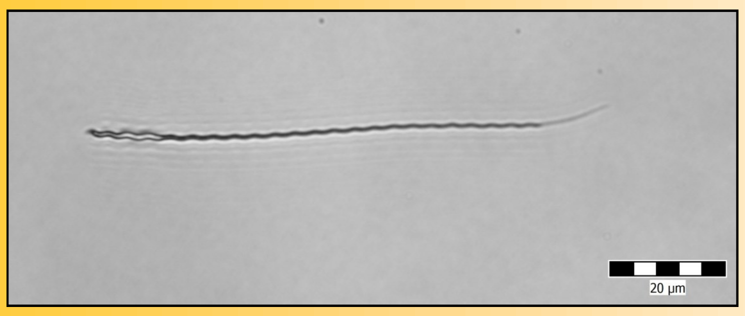

Figure 3. Barn swallow sperm cell

For statistical analyses of seasonal effect on sperm traits we applied linear and nonlinear mixed effects models and a method of maximal likelihood. Male identity was used as a random effect. Models were compared according to the AIC value. All statistics were conducted in R 2.15.2 software (R Development Core Team 2011).

References

BIRKHEAD T.R. & FLETCHER F. (1995) "Male phenotype and ejaculate quality in the zebra finch *Taeniopygia guttata*." Proc R Soc B, **262**, 329-34.

LASKEMOEN T., KLEVEN O., FOSSØY F., ROBERTSON R.J., RUDOLFSEN G. & LIFJELD J.T. (2010) "Sperm quantity and quality effects on fertilization success in a highly promiscuous passerine, the tree swallow *Tachycineta bicolor*." Behav Ecol Sociobiol, **64**, 1473-83.

LÜPOLD S., BIRKHEAD T.R., WESTNEAT D.F. (2012) "Seasonal variation in ejaculate traits of male red-winged blackbirds (*Agelaius phoeniceus*). " Behav Ecol Sociobiol, **66**, 1607-17.

WOLFSON A. (1952) "The Cloacal Protuberance: A Means for Determining Breeding Condition in Live Male Passerines." Bird-Banding, 23, 159-65.

Aims

To evaluate seasonal effect on ejaculate parameters: sperm number, sperm morphology (size of sperm components) and sperm motility in a passerine bird.

Results

In two years (2011 and 2012) we collected over 450 ejaculate samples from 180 males. We found a linear seasonal effect on sperm head length and total sperm length (TSL) and nonlinear seasonal effect on sperm number, coefficient of variance in TSL (CV), sperm tail length and sperm motility.

Table 1. Ejaculate traits in relation to time of a season: AIC comparison of linear and nonlinear mixed effect models.

variable	model	AIC	Δ AIC	p-value
sperm number	null	4074.87	-	-
	linear	4049.79	-25.08	0.0001
	nonlinear	4007.18	-67.69	0.0001
total sperm length	null	1660.75	-	-
	linear	1629.99	-30.76	0.0001
	nonlinear	1630.33	-30.42	0.0001
CV	null	638.24	-	-
	linear	624.21	-14.03	0.0001
	nonlinear	606.25	-31.99	0.0001
head length	null	454.11	-	-
	linear	440.56	-13.55	0.0001
	nonlinear	441.82	-12.29	0.0003
midpiece length	null	1630.3	-	-
	linear	1631.24	0.94	0.304
	nonlinear	1629.97	-0.33	0.115
tail length	null	1626.74	-	-
	linear	1606.066	-20.674	0.0001
	nonlinear	1592.84	-33.9	0.0001
motility	null	3732.5	-	-
	linear	3729.04	-3.46	0.019
	nonlinear	3720.45	-12.05	0.0003

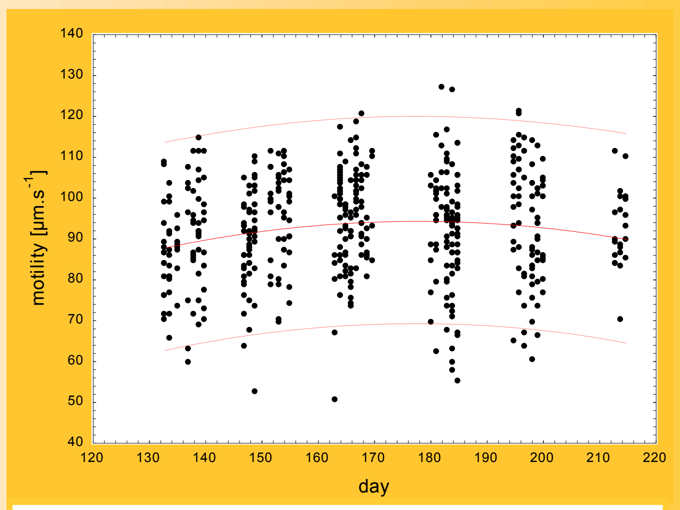

Figure 4. Nonlinear relationship between sperm motility and day in season (1st January ~ day 1)

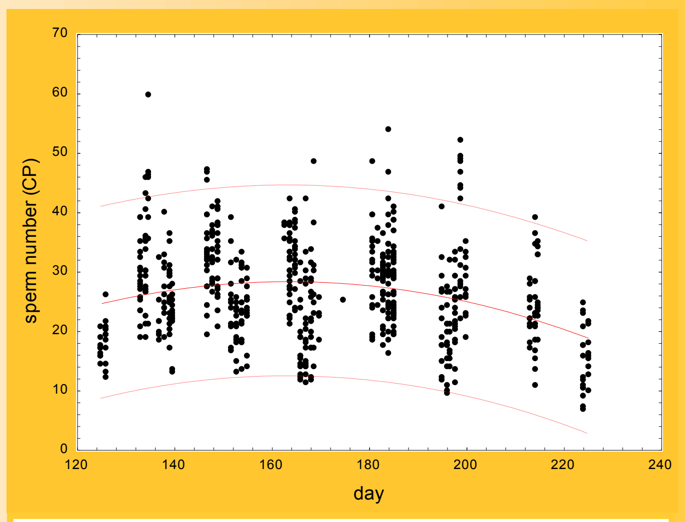

Figure 5. Nonlinear relationship between sperm number and day in season (1st January ~ day 1)

Conclusion

Sperm phenotypic traits are regarded as stable characteristics, but our results imply a trend of seasonal changes. For further studies we recommend to take into consideration the effect of time in the season.

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