Patterns of sperm storage in relation to sperm competition in passerine birds

Tom

- protocol OK?

- velocity ~ sperm traits – which ones + citations

Total length

Relative Midpeace a nebo relative flagellum

Lajkemoem et al 2010

velikost hlavy

flagellum

jiny spermie a stejnou rychlost

stejny spermie a jinou rychlost

pomery

- What is the normal temperature of ruffs?

- occurrence of aberrant/abnormal sperm

Dov

Send him link https://raw.githack.com/MartinBulla/ruff\_sperm/main/Protocols/protocol\_sperm.html

Can **concentration** be measured with meaning from electroejaculates?

Citation for “ruffs show no hint of assortative mating by morph

What citations do you have in mind for faeders having largest gonads? Jukema & Piersma 2006 and Kupper et al 2015?

What citations do you have in mind for faeders having least copulation opportunity?

I also wonder about the citation for “Satellites sit more towards faeders than independents with respect to gonad volume and copulation opportunity frequency” given the last Ruff’s and Bart’s paper showing that the copulation frequency of satellites and independents (after taking away the dominant resident) is same and driven by the amount of time spent on the lek.

Wolfgang

Note that all 3 morphs are under selection to optimize sperm, and a steeper selection gradient in Faders does not mean that a beneficial mutation will occur there rather than elsewhere. Note that chromosome 11 of independents has a 100 times higher allele frequency, so most beneficial mutations inside the inversion will arise in independents, not in Faders. I hence think that it only makes sense to argue in terms of trade-offs in investment, or in terms of pleiotropic effects (but whether the hormone levels of Faders are beneficial for spermatogenesis…?).

I would also argue based on known differences in testis size.

Thanks for checking the CV correction. I hope we will get measurements from > 5 sperm per individual and > 5 individuals per morph.

You note that we may estimate inbreeding from microsats, but correcting for this would only make sense for velocity. Why? Because inbreeding is expected to lead to abnormal sperm, but morphology of normal looking sperm shall be unaffected?

What is a good citation for weighing by sqrt(n-3)?

You note that the pedigree can be omitted, because velocity will have a low heritability. It is likely, but can we be sure?

GENERAL

Add housing section

Predicting velocity issues

many predictor (here 9 parameters) and their combined effects. One could do exploration, and control false-positives with a full-model test (8df which is quite demanding: effects must be large to reach significance).

Alternatively, one could try to confirm previous findings on other species (e.g. Knief et al. 2017 NEE).

Running models on individual sperm traits, averages or both – I am in favor of both.

How completene is the pedigree? To distinguishing real differences from chance effects due to bottlenecks,

* Shall we use discriminant function analyses on the two most different sperm traits to predict the sperm morph?

- Do we need to control for the pedigree, if residuals of the models are randomly distributed, i.e. there is no pedigree structure in residuals? WOLFGANG:This is a matter of heritability. I would say that the pedigree is important to control for analyses of sperm morphology, but not velocity or CV.

**Jasmin**

FSH only acts as the “on” signal for sperm production, but does not have a direct function associated with sperm quality traits per se…

Some other avenues to consider for your predictions could include:

How FSH treatment increases the number of sperm “without defects” (when the “before treatment condition” is infertility). It seems to increase the presence of “better sperm” due to the fact that overall more sperm is produced e.g. <http://www.asiaandro.com/archive/1008-682X/6/133.htm>

Many other genes, with known expression in specific parts of the sperm, could be associated to morphology, and this is unexplored territory in ruffs. I have no idea how conserved these genes are, but if you argue that the basis is genetic, then it is best to refer to genes known to be expressed in sperm specifically. E.g

<https://doi.org/10.1186/s12610-019-0083-9>

Dov

We have good reason to infer that the average number of sperm delivered per copulation will differ among morphs. Based on both gonad size [@Jukema2006; @Kupper2015] and multiple copulation opportunity frequencies in the wild (\*\*cit\*\*), 'faeders' should deliver the largest sperm packages per copulation (largest gonads, least copulation opportunity) and independents the smallest (smallest gonads, multiple copulations and multiple females). Satellites sit more towards faeders than independents with respect to gonad volume and copulation opportunity frequency.

Consequently: perhaps sperm do systematically face different morph-related competitive environments within the ejaculate of a single male when popped into females. And yes, there are times when females mate successively with multiple males, which we would also create larger sperm volume environments for competition for all morphs.

Sperm are initially racing towards female storage tubules primarily competing with others from the same male, and we don't know how limiting these tubules may be. Subsequent to their release from tubules, they will more often be in competition with sperm from different males, but the 'volume' or number of competitors may be similar per fertilization, at least during most of the season. At the ovum level, their success depends on both their arrival timing and their ability to penetrate membranes.

Are there cost benefit tradeoffs between these characteristics that would trace back to morph specific swimming speed performance differences?. It is not obvious to me why that should be so at this stage. .

PREDICTIONS

The following "no difference" predictions seem sound to me and I wonder whether you agree

1) Dov's prediction that total sperm length should be similar in the three morphs because the sperm length correlates with the length of sperm storage tubules and the ruffs do not mate assortatively according to morph.

2) Wolfgang's prediction that morphs have similar sperm traits because all three morphs are under selection to optimize sperm, and a steeper selection gradient in 'faeders' due to its low chance of copulating does not mean that a beneficial mutation will occur there rather than elsewhere.

The predictions of “no difference” are mirrored in a recent review (Kustra and Alonzo 2020). Across taxa there were no consistent differences in sperm traits between dominant and sneaker. Moreover, the authors highlight that despite the general expectation that sneaker males should produce sperm that are more competitive (e.g. higher quality or performance), the existing theory does not predict explicitly how sneaker males should differ in sperm traits.

The predictions for possible differences, if any, are trickier. Shall we go there? If yes,

A) Bart proposed (and Wolfgang agreed about) the prediction based on trade-offs in pre- and post-copulatory investment. We see gradient in pre-copulatory investment with independents investing the most on the lek and faeders the least, which is perhaps mirrored in the post copulatory investment, given that independents have the smallest and faeders the largest testes. We then also expect faster sperm and sperm optimized for speed in 'faeders' - a morph with the lowest pre-copulatory investment.

B)  Dov argued that given the differences in testes size and frequency and chance of copulation, 'faeders' are expected to deliver the largest ejaculat. Using the same logic, we could also expect them to have the fastest sperm (which however goes against Wolfgang’s expectation (1)). Faeder’s sperm maybe always or nearly always facing competition from sperm of other morphs, whereas this may not be the case for the other morphs. We do not know whether females that copulate with faeders always or mostly copulate also with one or bothof the other morphs. We know however that half of the females copulate only with a single resident or satellite (Lank *et al.* 2002).

The fastest sperm for faeders might be especially likely if females that

or never copulate only with faeders alone

Kustra MC, Alonzo SH. 2020. Sperm and alternative reproductive tactics: a review of existing theory and empirical data. Philos Trans R Soc Lond B Biol Sci. 375:20200075. 10.1098/rstb.2020.0075.

Lank DB, Smith CM, Hanotte O, Ohtonen A, Bailey S, Burke T. 2002. High frequency of polyandry in a lek mating system. Behav Ecol. 13:209-215. 10.1093/beheco/13.2.209.

BART

* Housing – aging of sperm, copulations
* Shall we keep males without females – three days before sampling?
* Counting abnormal sperm (I suggest to separate the photographing from counting)

go through each slide and consider each sperm that can be seen well (so no clumps), number consecutive sperm from 1-100, and then determine whether it looks normal:

* + If yes, determine whether it can be measured.

                                                               i.      If yes, make a picture for later measurement until 10 pictures are reached.

                                                             ii.      If no, move.

* + If no, note what is abnormal about it (e.g. odd-shaped head, no tail).
* Prediction based on varying levels of sperm competition
  + Is sperm competition higher for faeders?
  + Faeders seem to have larger testes, and this may imply different sperm allocation strategies. inds have a greater multiple copulation with the same female probability (and seem to even cease mating with a particular female after multiple copulations). May this imply that a female copulating with the independent is less likely to copulate with multiple males???
* Dov expects larger gonads in faeders, based on a tradeoff with relative copulation opportunity. This he then states “need not simply directly apply also to swimming speed… it is less clear to me what tradeoffs might be involved in the performance traits than it is in the copulation-frequency-versus-gonad-size traits.” Same tradeoffs may not operate, but could, no? As Tom replied Investments in ejaculate quality/competitive ability may involve not only ejaculate volumes or the volume of sperm transferred during the copulation but also sperm traits (many have been shown to be condition dependent, or costly to produce but at the same time associated with male fertility). But expectations regarding sperm traits and ARTs are not clearly confirmed by empirical data (research mostly done on fish sneakers, see Kustra and Alonso 2020)

From the discussion of Martin’s very nice proposal I took that the sampling set up will be crucial, when it comes to compare e.g. ejaculate size, density and/or viability(?). In the wild, there will be very different copulation rates based on a) hierarchy among Independents/Residents (perhaps also Satellites) and b) morphs (Faeders will have fewer opportunities for copulation). We should therefore carefully consider the housing conditions of the sampled birds and what that means for the sampling. Will males be exposed to females before sampling? If that’s the case then surely the dominance rank (specific male set up) needs to taken into account for the analysis too. Time of the day (or night) for sampling might also need to be standardized. Sampling from males that are kept in male-only groups would be perhaps analytically ‘cleaner’ but then one loses the interesting biological aspects of the different alternative reproductive tactics. On the other hand, it might be a very necessary first step. Perhaps even both is necessary & feasible.