Saltella sphondylii is the exception: Multiple copulations do not reduce male longevity in Sepsidae.

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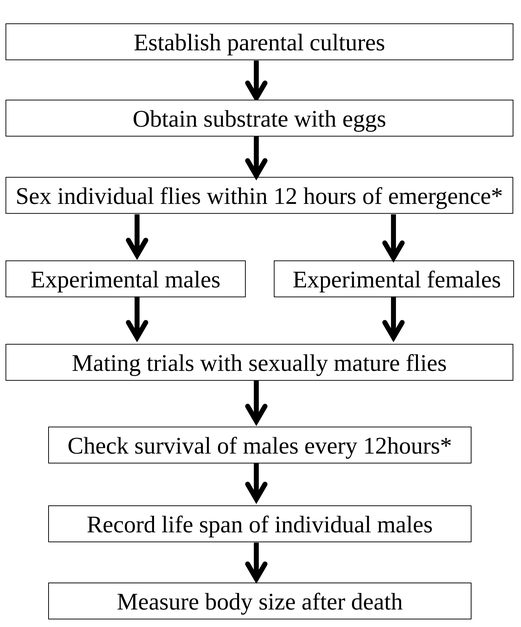
Introduction

An increasing number of research articles are addressing the cost of reproduction for males (as reviewed by Scharf *et.al*, 2012). However, few studies experimentally examine the effect of multiple copulations on male longevity. Only two studies have approached this topic in Sepsidae and they yielded conflicting results:

- Martin & Hosken (2003), in their widely cited study, demonstrated that male longevity was negatively correlated with number of copulations in *Saltella sphondylii*.
- Teuschl et al. (2009) reported that there was no correlation between number of copulations and male longevity in *Sepsis cynipsea*.

The aim of this study is to determine whether the observations made for *Saltella sphondylii* or *Sepsis cynipsea* are more common in Sepsidae. In order to draw conclusions for the sepsid ancestor, I selected three species (*Allosepsis indica*, *Sepsis fulgens* and *Themira superba*) representing three major clades within Sepsidae.

Materials and Methods



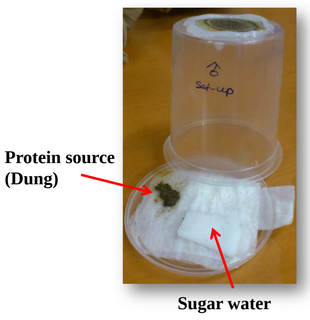


Figure 1. Rearing chamber for individual males.

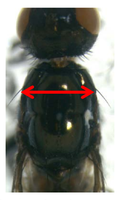


Figure 2. Body size is measured as the width of the scutellum.

- In order to obtain a large sample size, a continuous emergence of flies had to be ensured. All flies had to be sexed within the day to ensure virginity.
- Trials were performed using virgin females of the same age. Due to high mating success rates, *A. indica* males were provided with one female per trial. Two females were presented to males in *T. superba* and *S. fulgens* trials in order to increase copulation success rates.
- Trials for each species were carried out across three batches of emergence.
- As control, the longevity of 20 virgin males (without exposure to females) was also determined.
- Data were analyzed using multiple linear regression, with number of copulations, exposure to females, body size, and emergence batch as dependent variables.

*: 24 hours for *S.fulgens*

Allosepsis indica

Allosepsis indica

- High virgin mating success (> 95%).
  - Copulation generally occurs when males were provided with virgin females.
- Males were randomly assigned to different treatments (number of copulations).

Dependent variables		Pr(>|t|)
Number of copulations		0.240
Exposure to females		0.900
Body Size		0.484
Batch	dv1	0.233
	dv2	0.935

Table 1. Poisson regression.

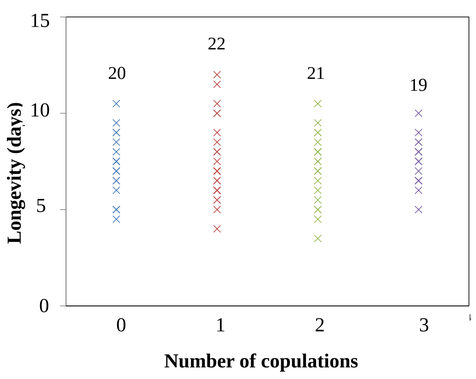


Figure 3. No longevity cost of multiple copulation in *A.indica*. Numbers above data points denote sample size.

- None of the dependent variables investigated affect male longevity.

Multiple copulations do not reduce male longevity in *Allosepsis indica.*

Sepsis fulgens

Sepsis fulgens

- This species was chosen because phallus morphology is similar to *Saltella sphondylii* males. Phallus morphology may affect longevity (Fig. 4).
- Very low virgin mating success rate (<35%), and males do not re-mate more than once in a five hours time frame.
- Males were not assigned to treatments. Instead, repeated mate trials were used to segregate them into classes; leading to unequal sample sizes.

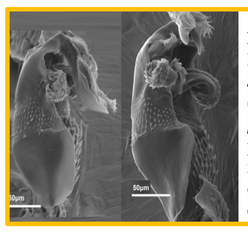


Figure 4. Male genitalia of *Saltella sphondylii* (left) and *Sepsis fulgens* (right). A large proportion of the penis is membranous which could be damaged during copulation.

Dependent variables		Pr(>|t|)
Number of copulations		0.899
Exposure to females		0.904
Body Size		0.909
Batch	dv1	0.500
	dv2	0.773

Table 2. Poisson regression. None of the dependent variables affect longevity.

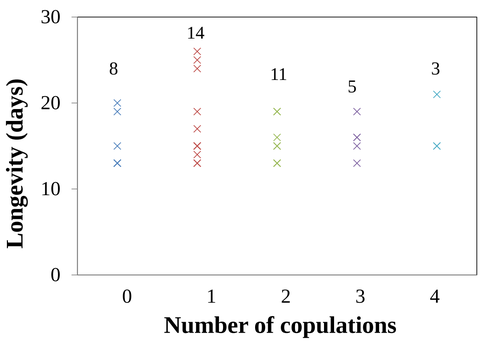


Figure 5. No longevity cost of multiple copulation in *S. fulgens*. Numbers above data points denote sample size.

- Number of copulations do not affect longevity.
- *S. fulgens* males do not display any size preference for females in mate trials regardless of whether male body size is controlled (paired t-test, p = 0.176; not controlled: p = 0.120).

Multiple copulations do not reduce male longevity in *Sepsis fulgens* and males do not have a preference for female size.

Themira superba

Themira superba

- This species was chosen because virgin mating success rate is usually high (> 90%), and hence I am able to control the number of copulations for each male (such as in the case of *A.indica*).
  - However, trials involving flies from “Batch 1” had very low virgin mating success (< 10%).
- Therefore, males were not assigned to treatments. Instead, repeated mating trials were used to segregate them into classes.

Dependent Variables		Pr(>|t|)
Number of copulations		0.508
Exposure to females		**0.041**
Body Size		0.676
Batch	dv1	0.639
	dv2	0.407

Table 3. Quasipoisson regression. Exposure to females affect males longevity.

Dependent Variables	Pr(>|t|)
Exposure to females after removing problematic batch	0.255

Table 4. Quasipoisson regression. Exposure to females does not affect male longevity after the removal data from “Batch 1”.

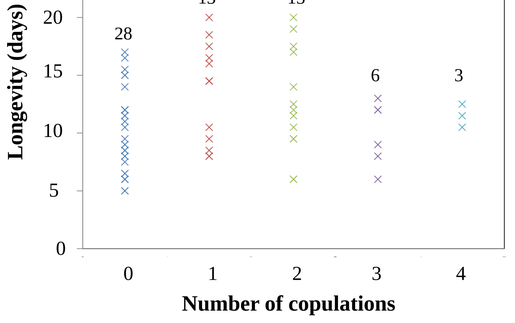


Figure 6. No longevity cost of multiple copulation in *T.superba*. Numbers above data points denote sample size.

- Number of copulations does not affect male longevity.
- Exposure of females seems to impact male longevity, but the result is driven by flies from “Batch 1”. This effect disappears after the exclusion of data from “Batch 1”.
- *T. superba* males have no preference for larger or smaller females, regardless of whether male body size is controlled (paired t-test, p = 0.690; not controlled: p = 0.707).

Multiple copulations do not reduce male longevity in *Themira superba* and males do not have a preference for female size.

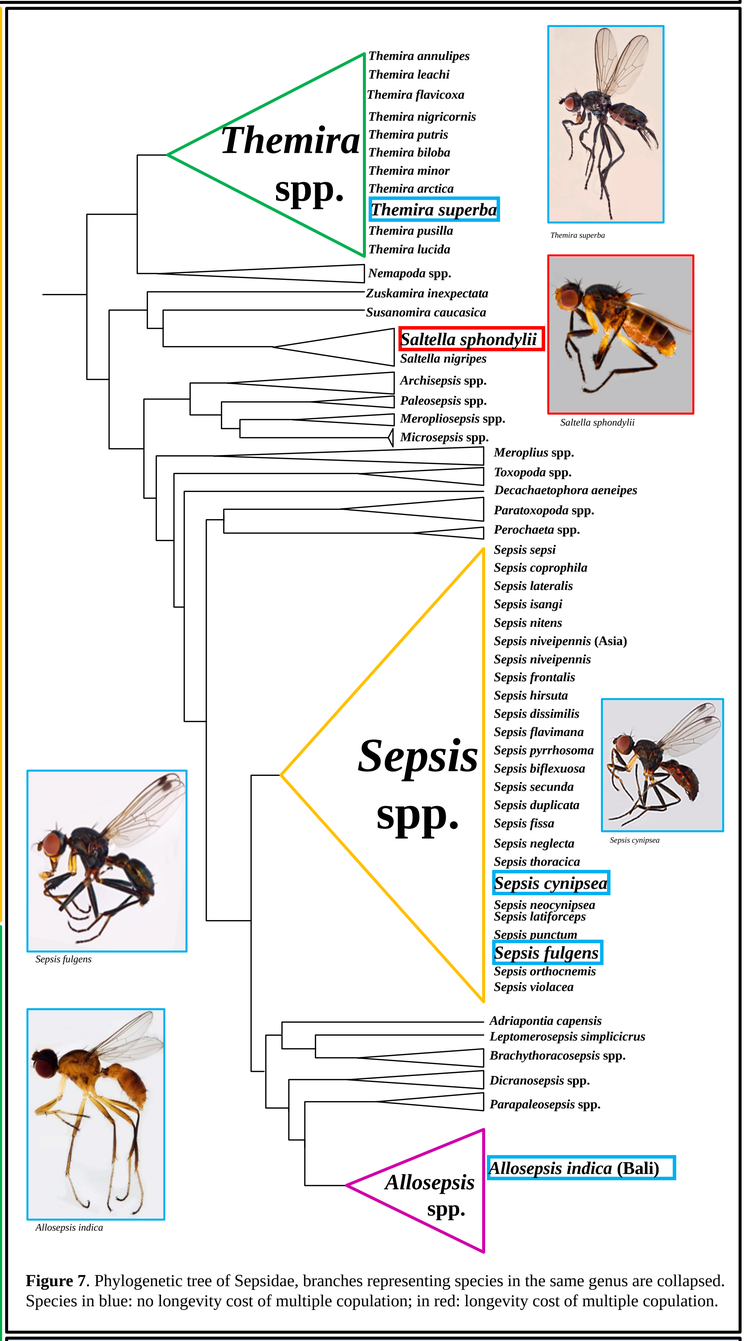


Figure 7. Phylogenetic tree of Sepsidae, branches representing species in the same genus are collapsed. Species in blue: no longevity cost of multiple copulation; in red: longevity cost of multiple copulation.

Body size and longevity in control males

- Using data from control males, body size was found to be correlated to longevity in: (A) *T. superba* and (B) *S. fulgens*, but not in (C) *A. indica*.

Discussions and Conclusions

Multiple copulations do not reduce male longevity in most of Sepsidae: *Saltella sphondylii* is the exception.

- In a resource-rich environment, the cost of sperm production and sperm replenishment is low.
- It appears that longevity costs of multiple mating in males evolved in Saltella. Based on parsimony, a longevity cost for multiple copulations was most likely to be absent in the sepsid ancestor (Fig. 8).
- Differences observed between the species may be due to differences in mating behaviour.
  - *Saltella sphondylii* males can copulate up to six times in a short period of time (Martin and Hosken, 2004).
  - In the three other species studied, male re-mating frequency is much lower.
  - *Saltella sphondylii* males invest more on reproduction than survival.
- Male body size is positively correlated with longevity in virgin *Themira superba* and *Sepsis fulgens* males.
- Is there a longevity advantage for larger males?
  - Larger control males live longer in *Themira superba* and *Sepsis fulgens*, but this relationship is not significantly after exposure to females. This is not relevant in a natural environment where males are in close contact with females

Figure 8. Based on parsimony, longevity cost of multiple copulation in males evolved in *Saltella* spp (red), reflecting the origin of a new longevity cost in the species.

Suggestions for future studies:

1) More species should be included, especially in the major clades that were not studied.
2) Increase the number of treatments (document longevity for higher numbers of copulations).
3) Investigate the relationship of longevity and number of copulations under resource-limited conditions.

References

- Martin O.Y., and D.J. Hosken. 2004. Copulation reduces male but not female longevity in *Saltella sphondylii* (Diptera: Sepsidae). *J . Evol. Biol*. **17**: 357–362.
- Scharf I., F. Peter, and O.Y. Martin. 2012. Reproductive trade-offs and direct costs for males in Arthropods. *Evol Biol.* Springer US.
- Teuschl, Y., C. Reim and W.U. Blanckenhorn. 2009. No size-dependent reproductive costs in male black scavenger flies (Sepsis cynipsea). *J.Behav. Ecol.* **21**: 85-90.