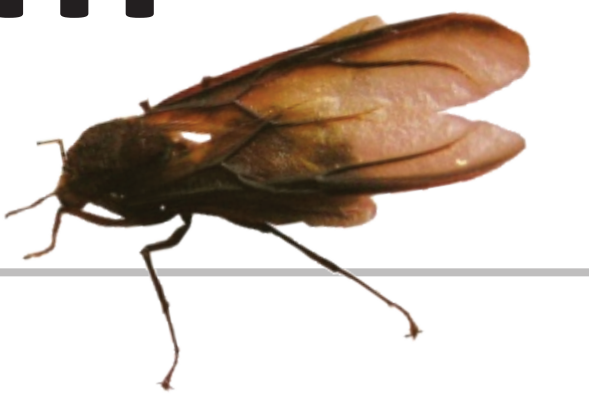


Leafcutter ant ejaculates; more than just carriers for sperm

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BACKGROUND

The mating systems of social insects have some unique characteristics; males complete the production of sperm at the pupal stage, mating takes place at the start of life, queens store sperm for years and use it sparingly to produce thousands of workers without ever replenishing their sperm stores. In addition, in many species queens mate multiply and sex ratios can be extremely male-biased. This means that males likely need to compete for insemination and egg fertilization. Selection on male reproductive traits to outcompete other males is thus expected.

We studied the ejaculate biology of leafcutter ant males (*Atta colombica*) in detail to uncover some of the mechanisms that can influence paternity.

THE STUDY

- 1 We examined what happens during ejaculation, how is an ejaculate transferred?
- 2 For the first time, we tested the effects of separate ejaculate components on own and rival male's sperm viability.
- 3 We examined whether these effects are mediated by proteins as has been found in other insects.

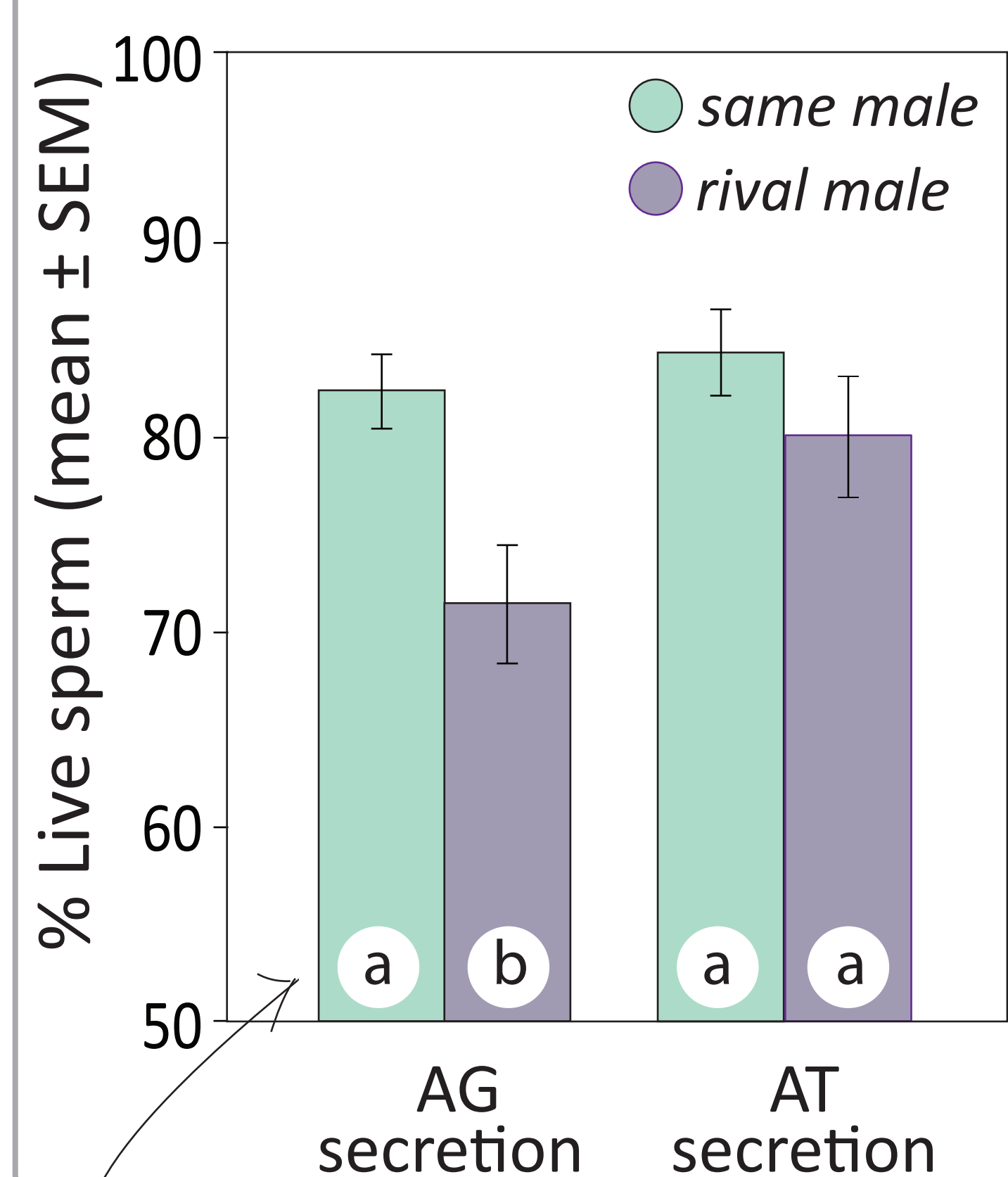
WHAT WE DID & WHAT WE FOUND

1 We watched males ejaculate

- When males ejaculate, they first transfer a clear liquid to the queen (A). This liquid is produced by the males' accessory glands (AG).
- After this liquid, sperm is released (B+C) along with secretion from the accessory testes (AT), the organs where sperm is stored prior to ejaculation.
- A small mating plug (originating from the AGs) is transferred along with the sperm.

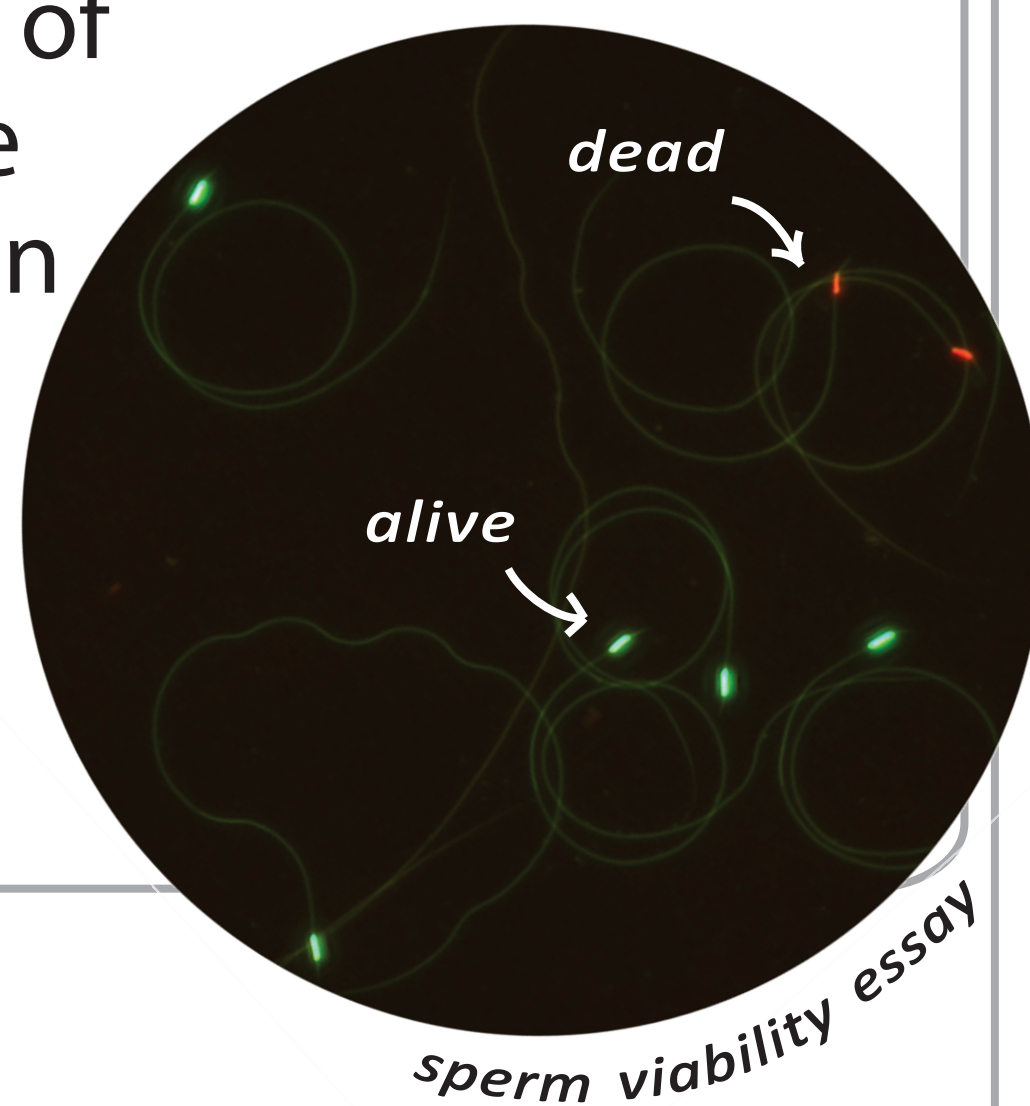


Close-up of the endophallus of an ejaculating male, from the start of ejaculation (A) until the end (C)

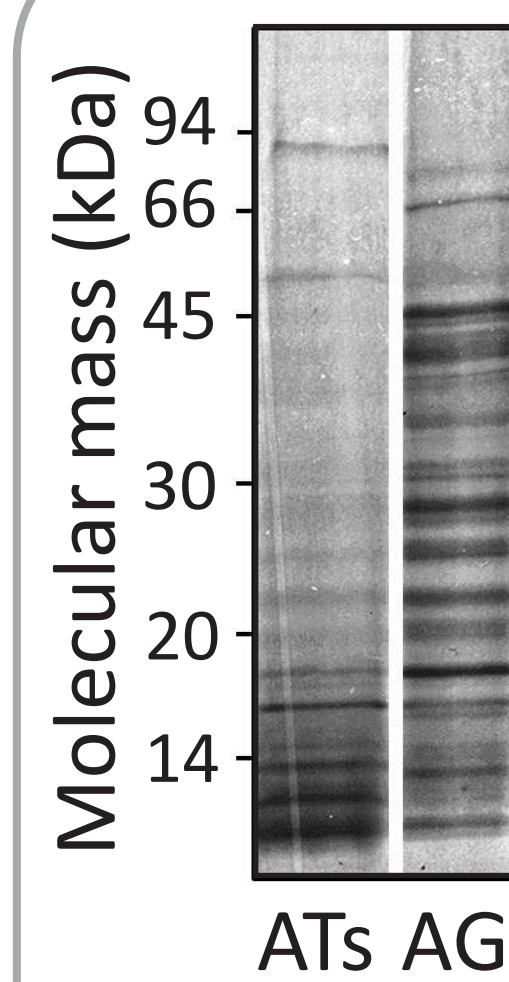


2 We examined survival of sperm when exposed to AG or AT secretion collected from the same male or an unrelated ('rival') male

AG secretion, the fluid that is transferred first, has a negative effect on sperm survival of other males, while AT secretion has an equal effect on own and rival sperm



Different letters indicate significant differences between groups ($p < 0.05$)

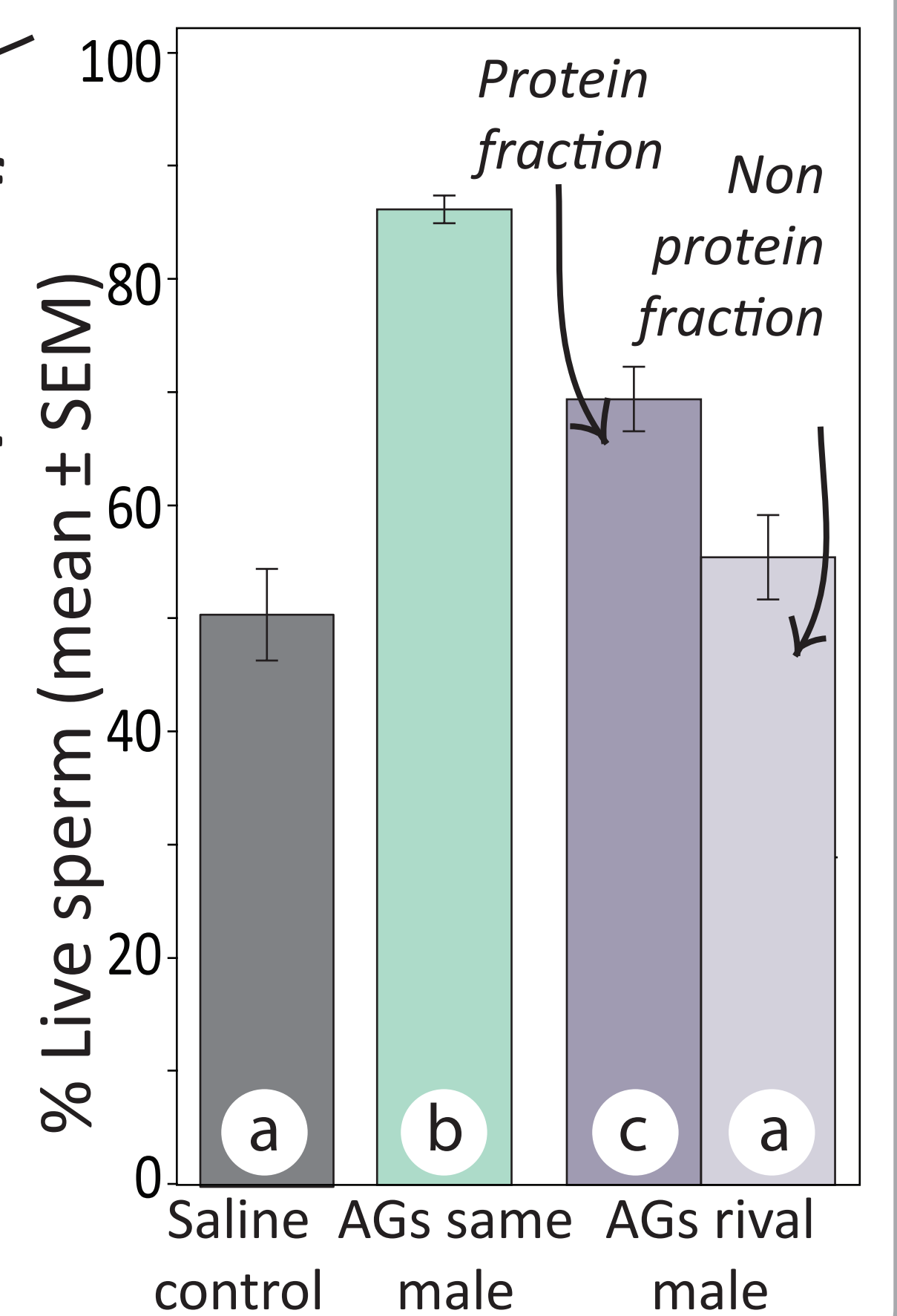


3 We studied the composition of AG and AT secretion

A gel (1D) shows that there are differences in protein profiles between AT and AG secretions.

We split the AG secretion of unrelated males into a protein and non protein fraction and separately examined their effects on sperm survival

- Proteins in general have a positive effect on sperm survival (middle bars vs outer bars)
- Some proteins seem involved in incapacitation of sperm, if that sperm comes from a different male (significantly less sperm alive in 3rd bar than in 2nd bar)



TAKE HOME MESSAGES

- 1 Ejaculates are composed of several components that are shaped by natural and sexual selection and can each have their own function.
- 2 The accessory gland secretion that is transferred to the queen ahead of sperm has a dual function: it can protect a male's own sperm upon its arrival in the queen (against rival ejaculates and maybe also the queen's reproductive environment), but it is harmful to rival sperm already present in a queen's reproductive tract: SPERM COMPETITION. Proteins seem to be mediating this.
- 3 Accessory testes secretion is transferred simultaneously with sperm and is not involved in sperm competition. Its main function seems to be ensuring sperm survival during storage in the male and during transfer to the queen.

