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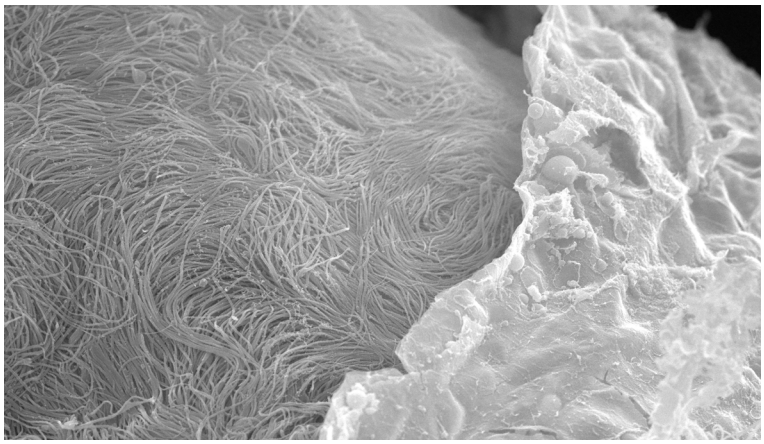
INSIDE JEB

# Female ants can protect live sperm for decades with targeted immune system



Sperm cells packed together in the female sperm storage organ, the spermatheca. Photo credit: Daniel Monteyne.

Selecting a mate is often a life-long commitment: for species that favour monogamy, the decision is irrevocable. However, female ants go a step further. Having mated in a single day early in life, a female ant never mates again. Instead, she stores the accrued live sperm in preparation for a lifetime of reproduction. 'I think this is pretty amazing', says student Sarah Chérasse, currently at Université Libre de Bruxelles, Belgium. However, Chérasse first became hooked on the question of long-term sperm storage while she was studying with Jacobus Boomsma and Morten Schiøtt at the University of Copenhagen, Denmark. 'From the point of view of the queen's immune system, sperm cells are foreign bodies, "non-self"', says Chérasse. So the queen must somehow tone down her immune system to prevent herself from damaging her precious cargo, yet she must also protect the sperm from fungal and bacterial infections, necessitating that she prime her immune system to provide antimicrobial protection. Intrigued by the paradox, Chérasse found herself travelling to Panama to collect virgin queens and male leaf-cutter ants (*Atta colombica*) to find out how the females protect their treasured sperm stores from destruction.

Describing the apprehension every time she excavated an ants' nest with Boomsma, Schiøtt and Boris Baer from the University of California, Riverside, USA, Chérasse says, 'We didn't know if they contained the virgin ants we were looking for'. While still in Panama, Baer cautiously collected semen from some of the virgin males and skilfully injected it into anaesthetised queens to artificially inseminate them. He then collected the organs through which the sperm passed after the queens were inseminated and where the sperm is stored (known as the spermatheca), in addition to the organs in which the males store sperm before mating. Back in Denmark, Chérasse then monitored the activity of two different immune system components in the female and male reproductive systems, focusing on two antimicrobial molecules (peptides) that fight bacterial and fungal infections, and on a more general antimicrobial response, where the immune system smothers invading entities with melanin ready for disposal. Impressively, the leaf-cutter queen ants mobilised both of the antimicrobial peptides in the spermatheca, probably to protect the precious gametes from infection. However, the insects had reduced production of the key enzyme prophenoloxidase, which triggers melanin production as part of the immune response that could, in theory, harm the hoarded sperm (doi:10.1242/jeb.173435).

Yet, no one had proved categorically that antimicrobial compounds produced by spermathecae actually destroy bacterial infections. Serge Aron, from the Université Libre de Bruxelles, Belgium, decided to focus on a common species of ant, the garden ant (*Lasius niger*), with Francisco Dávila and Anne Boteaux from the Université Libre de Bruxelles to measure how well *E. coli* bacteria could grow inside the structures. They collected minute quantities of fluid from inside the virgin male and female sperm storage organs and Aron admits that adapting the techniques that his team used to measure the antimicrobial power of the sperm storage organs was particularly challenging when handling such tiny volumes.

After tracking how well bacteria grew over a 4 week period in the fluid extracted from the spermathecae following mating, Aron, David Bauman, from the Université Libre de Bruxelles, and co-workers were impressed to see that the bacteria died within 2 days of mating, although the liquid lost its antibacterial powers after a week. In contrast, the fluid produced in the male's sperm storage organ prior to mating provided no antimicrobial protection (doi:10.1242/jeb.175158).

It seems that the queens' immune system purges sperm of bacteria in preparation for long-term storage and Aron is optimistic that the lessons learned could help us to devise more effective strategies for the long-term storage of live sperm for human *in vitro* fertilisation.

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**Chérasse, S., Baer, B., Schiøtt, M. and Boomsma, J. J.** (2018). Differential immune gene expression in sperm storage organs of leaf-cutting ants. *J. Exp. Biol.* **221**, doi:10.1242/jeb.173435.

**Dávila, F., Botteaux, A., Bauman, D., Chérasse, S. and Aron, S.** (2018). Antibacterial activity of male and female sperm-storage organs in ants. *J. Exp. Biol.* **221**, doi:10.1242/jeb.175158.

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