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Sperm storage and immunity in leaf-cutting ants

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Leaf-cutter ant queens mate on a single day and store the sperm for the rest of their life, which may be up to 20 years. Sperm transits through the bursa copulatrix before entering the highly specialized spermatheca organ. Sperm storage creates a possible conflict with immune defences that generally target non-self cells, but queens also need to make sure that sexually transmitted bacteria and fungal spores are killed before sperm is stored. We therefore hypothesized that stored sperm is protected by immune mechanisms that target infecting microorganisms specifically, rather than by general innate non-self-recognizing immune defences. We used tissue-specific differential gene expression of two antimicrobial peptides (AMPs), abaecin and defensin, and we determined enzymatic activity levels and gene expression of phenoloxidase (PO) and its zymogen prophenoloxidase (proPO). The latter have a crucial role in the melanogenesis cascade that mediates a variety of non-specific insect immune responses. We investigated virgin and mated queens of both *Atta colombica*, a species where the bursa copulatrix has been reduced and sperm transfers directly to the spermatheca, and virgin queens and males of *Acromyrmex echinatior*, where the bursa is large and suitable for pre-storing sperm for several hours in between insemination and final storage. Queens of both species mate with multiple males, but the number of inseminations per *Acromyrmex* queen is ca. twice as high as in *Atta* queens. We found that PO/proPO responses were silenced in the spermathecae and bursae of both species, while abaecin was up-regulated in these organs and more so in *Acromyrmex* where queens mate with more males. Ant spermathecae thus appear to be analogous to sulphite-treated boxed wine that can be left alone for years because infections can never reach the interior of the bag, whereas normal body tissues require constant monitoring by standard non-self-recognizing immune defences.