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Leafcutter ant ejaculates; more than just a carrier for sperm

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Males transfer ejaculates consisting of sperm and other components such as seminal fluid, mating plugs and spermatophores to the female's reproductive tract during copulation. These ejaculate components are shaped by natural and sexual selection and have been shown to influence male fertility and competitiveness. Studying the biology of ejaculates, including their composition, the interaction between components, and their mode of transfer could thus help to understand factors affecting male fitness and paternity. We present a detailed account of the ejaculate biology of the leafcutter ant *Atta colombica*. Sperm is stored in the accessory testes (AT) prior to copulation, which starts with the appearance of clear pre-ejaculatory fluid (PEF) at the tip of the endophallus. This PEF likely originates from the accessory glands (AGs) located posterior to the ATs, as PEF and AG secretion have identical protein banding patterns on 1D gels, whereas those of AT secretion are different. The release of PEF is followed by the joint expulsion of sperm and AT secretions, along with a small mating plug. When studying the biological activity of these fluids, we could show that PEF, AG and AT secretion all have positive effects on sperm viability, but that PEF and AG secretion also reduce the survival of other males' sperm. Because PEF is transferred before sperm and AT secretion, it may have evolved to interact with rival sperm already present in the reproductive tract of potentially multiply mated queens (sperm competition), while at the same time providing a supportive biochemical environment for own transferred sperm. Finally, we could show that the protein fraction of the PEF is responsible for sperm incapacitation, warranting further proteomic studies to unravel their identity and biological function.