Newly discovered proteins in seminal fluid may affect odds of producing offspring

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Seminal fluid contains protein factors that, when transferred from a male to a female at mating, affect reproductive success. This is true of many different animals, from crickets to primates. In fruit flies, for instance, seminal fluid proteins influence the competitive ability of a male's sperm, and alter the female's post-mating behavior by dampening her interest in other males and cueing her to lay eggs. There is also some speculation, not yet proven, that having the wrong seminal fluid proteins might be one of several barriers to cross-breeding between closely related species.

Although several seminal fluid proteins have been characterized, little has been known about the exact kinds of transferred male proteins present in the female shortly after mating -- how many there are, their relative abundance, their structure, specific functions, and interactions with proteins from either the female or the seminal fluid of other males who mate with the same female. Gathering such information involves proteomics, the large-scale study of the nature and actions of proteins in living systems.

Using a new proteomic method, scientists at the University of Washington (UW) have discovered more than 80 proteins, previously not known to have a role in reproduction, that were transferred to female fruit flies in seminal fluids. Before this study, nearly 20 of the genes encoding these proteins were not even known to exist. The researchers also confirmed the presence of more than 70 additional proteins other scientists had predicted would be found.

The results were published in the July 29 issue of *PLoS Biology*. The authors were Geoffrey D. Findlay, a doctoral candidate in the UW Department of Genome Sciences; Xianhua Yi, formerly a postdoctoral researcher at the UW and now with Momento Pharmaceuticals, Cambridge, Mass.; Michael J. MacCoss, assistant professor of genome sciences whose lab designs and tests new proteomic technologies; and Willie J. Swanson, associate professor of genome sciences whose lab studies the evolution and function of reproductive proteins. The Swanson lab looks at how changes in these proteins may lead to male/female mismatches and infertility from incompatibility, analogous to rejecting an organ transplant.

MacCoss said that it was surprising to observe how rapidly seminal fluid proteins evolve in fruit flies.

"They change with the quickness we would expect for the immune system, which has to respond fast to new pathogens," MacCoss said. The rapid evolution of the proteins that chaperone sperm may be due to the high-stakes competition between the many males that mate with each female fly.
Each male fruit fly, Findlay said, has an evolutionary advantage if he can increase the competitive ability of his sperm. When the female retreats to lay eggs, he wants them to be his offspring.

To this end, the male's seminal fluid proteins aim to ensure that his sperm are successfully stored in the female's reproductive tract, cue the female to lay eggs immediately after receiving his sperm, and make the female less likely to mate again with another male. The proteins may also attempt to "disarm" the seminal proteins transferred to the female by other males. If a male's seminal proteins can outperform his competitors', he'll be more successful in passing on his genes to the next generation.

"Don't forget the female," Findlay added. "She's not a passive participant in the chemical struggle." The first male she mates with may not be the best father for her offspring, so it may not be in her interest to lay all of her eggs with his sperm. It is widely suspected that proteins in the female reproductive tract are co-evolving with their male counterparts to look out for the female's own reproductive interests.

"There is cooperation and conflict between the male and female," Findlay said. Each is pushing the envelop to serve his or her own reproductive interests. When the interests of males and females don't match, the sexes undergo an evolutionary struggle for control of the outcome. The competition among males, and the conflict between the sexes, may be driving the evolutionary patterns of their respective reproductive proteins.

This constant interplay between male and female proteins has caused seminal fluid content to differ between closely related species of fruit flies. The researchers showed this in two ways. First, by making DNA sequence comparisons, they found that when the same proteins appear in different species, the molecules often have different sequences and have diverged more quickly than would be expected by chance. Second, using proteomics, they identified proteins that are found only in certain species' seminal fluid.

Work in other labs has shown that female fruit flies that get too many shots of seminal fluid may pay with their lives. As the number of matings increase for a female, her behavior is more constantly under the influence of male seminal proteins and may move further away from the optimal for the female's physiological well-being. The males are not trying to kill the female, Findlay explained, but the toxic effect of mating is potentially a byproduct of protein manipulation.

Previous studies of seminal fluid proteins took years because each

protein had to be painstakingly culled out.

"Separating transferred proteins from an animal's own proteins is like searching for a needle in a haystack," said MacCoss. "We modified a technique to label the females' proteins by feeding them yeast carrying a stable isotope. This made the female proteins in the specimens invisible to our mass spectrometer. We then could pick out the transferred male proteins." This same isotope labeling method, MacCoss said, could be used to detect other proteins transferred from one organism to another, such as from a nursing mother to her baby or from a pathogen to the animal it infects.

How does knowledge about fruit fly seminal fluid proteins help improve understanding of fertility and infertility in other living things or in human couples?

"The specific genes and proteins might be different, but it's likely that other genes and proteins fulfilling similar reproductive functions will be found in other species. Seminal fluid proteins are of critical importance in reproductive fitness," said Swanson, "but it's not as easy as saying, 'If we find this in fruit flies, this means it's in people.'"

Source: University of Washington

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