Review of: "Post-Insemination Selection Dominates Pre-Insemination Selection in Driving Rapid Evolution of Male Competitive Ability"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

This study seeks to separate the effects of pre- and postcopulatory selection using experimental evolution and genetic manipulation of sperm production in *C. elegans*, and to assess the resulting changes across the genome. The system provides a unique and powerful tool for studying the evolution of reproductive success, and the incorporation of genome analysis is innovative and exciting. However, it seems to me that the data do not support the authors' main conclusions that "enhanced post-insemination competition increases the efficacy of selection" and that "enhanced pre-insemination competition hindered selection and slowed the rate of evolution."

Enhanced post-insemination competition refers to a treatment in which sterility was induced in the focal male worms after an initial period of mating (of ~12 hours? See comment below), followed by a further 48 hours of mating with no sperm transfer (Within-Strain Post Only, or WS-PO). Enhanced pre-insemination competition refers to a treatment in which focal males were not sterilized and fertile competitor males from a different strain were added 24 hours before the end of the mating period (n=2.5x as many as the focal males; Between-Strain Pre- & Post, or BS-P&P). A third treatment incorporated both focal male sterilization and the addition of competitors (Between-Strain Post Only, BS-PO), and a fourth, control treatment involved neither (Within-Strain Pre- & Post, or WS-P&P). I have three main concerns about this design and the conclusions drawn:

1. It is unclear to me why WS-PO, the sterilization treatment, corresponds to "enhanced post-insemination competition." While I agree with the authors that this treatment selects for sperm longevity, it would seem to in fact relax selection on sperm competitiveness rather than enhance it, since both the risk and intensity of sperm competition are reduced compared to the control WS-P&P treatment.

2. The competitor treatment, BS-P&P, is referred to as "enhanced pre-insemination competition," but in fact both mating competition and sperm competition were increased in this treatment, since the competitor males were not sterilized. Thus, the relatively slow rate of evolution in competitive reproductive success found in this treatment cannot be solely attributed to enhanced pre-insemination competition. As for the combined sterilization + competitor treatment, BS-PO, while it certainly increased premating

competition, its effect on sperm competition is unclear: within-strain competition was reduced, but the increased competition from the large number of added males likely more than made up for this.

3. Because competitive reproductive success increased over the course of evolution in all four treatments, the relevant comparison seems to be between the three experimental treatments and the control treatment (WS-P&P), not between the four treatments and the ancestral population. Only one experimental treatment differed significantly from the control: BS-P&P, the competitor treatment, which the authors refer to as enhanced pre-insemination competition but which seems to me entails an increase in both mating competition and sperm competition.

Thus, while I find the authors' question and the tool they use to address it very exciting, in my view the main conclusion that can be drawn from the data is that the increase in both pre-and post-insemination competition that occurred in BS-P&P, the competitor treatment, slowed the evolution of male competitive reproductive success. This may have been due to increased sexual conflict, as the authors note.

Minor points:

1. The method of assaying sperm competitive success (Lines 440-441) does not make sense to me. How does inducing sterility allow one to isolate the effect of fertilization success on total reproductive success? It is not surprising that only 4.1% of progeny were sired by ancestral males, since if I understand correctly they only transferred sperm for a short period of time relative to the competitors. Also, for any fertilizations occurring after the competitors were added, the ancestral males' sperm was older than the competitors' sperm, and thus more likely to be damaged by oxidative stress, etc. Finally, it's not clear how the 9.2% figure (contribution of post-insemination success to total reproductive success) was calculated (Line 155).

2. The authors suggest that the slower rate of evolution in the BS-P&P treatment may be due to decreased female fecundity, if "females altered egg-laying rates in response to the amount of sperm present as a result of a resource trade-off between reproductive and maintenance functions" (Lines 271-272). I don't follow this logic – if females are receiving more sperm, shouldn't they if anything be laying more eggs, barring any negative effects of seminal fluid components on their fecundity?

3. It would be useful to explain how long it takes an L1 worm to reach maturity, so that readers understand how long the "initial mating period" was.

4. Line 127: As this is the first mention of EMS, I would suggest defining it here.