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Eip74EF may promote sperm elongation at the cost of fecundity in *D. melanogaster*

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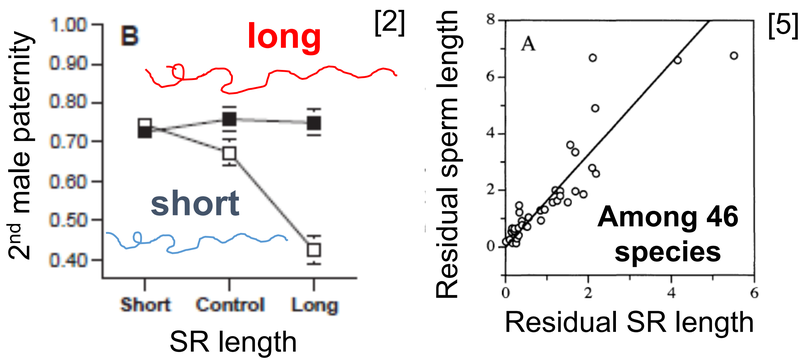
Introduction

Drosophila sperm are very long[1], evolve rapidly under sexual selection due to a long-sperm advantage during sperm competition[2-4], and coevolve with the female's sperm storage organ (seminal receptacle or SR)[5-6].

We found that the gene *Eip74EF* (*Ecdysone-induced protein 74EF*) is evolving very rapidly across 22 *Drosophila* species, and we asked if this gene is involved in male-female co-evolution in *Drosophila*.

Eip74EF is expressed post-meiotically during spermatid elongation[7], but it is not expressed in the sperm proteome[8]. It is an "early response" gene in the ecdysone signaling pathway, with spikes of expression associated with larval molts and puparium formation[9]. The ecdysone pathway is also involved in spermatogenesis[10-11], but the role of *Eip74EF* in testis has not been studied.

D. bifurca sperm are 5.8 cm long[1]



Question: Is *Eip74EF* involved in male-female co-evolution in *Drosophila*?

Methods

We used PAML v4.6[12] to estimate the rate of positive selection (M8 vs. M8a) across 22 *Drosophila* species using DNA sequences downloaded from GenBank.

Only the protein isoform E74A is expressed in adults. We used a hypomorphic mutant with partial knockdown that retains viability (BDSC #12619). We crossed the mutant with its wildtype genetic background (w^{1118}) to produce control flies. Competitor males and standard females came from a transgenic Canton-S stock expressing GFP in eye ocelli and sperm heads.

We examined sperm length, SR length, male fecundity, female fecundity, and male mating success including P_2. All analyses were performed in R v3.5.3[13].

Measurement, fecundity, and copulation duration were analyzed using lmer with Anova to calculate *P*, remating was analyzed using chi-square, and P_2 required logistic regression with a logit link function and binomial error distribution (with no overdispersion) using glm.

Results

Eip74EF is rapidly evolving under positive selection, with an M8a model estimate of -20882.5, M8 of -20875.6, and a χ^2 of 13.80, well above the 1% critical threshold of 5.41.

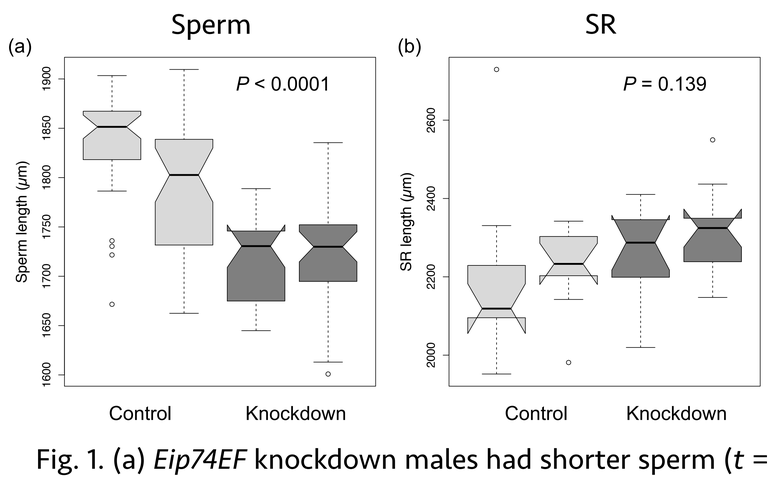


Fig. 1. (a) *Eip74EF* knockdown males had shorter sperm (t = -5.341; P < 0.0001), and (b) knockdown females had no change in SR length (t = 1.132; P = 0.258).

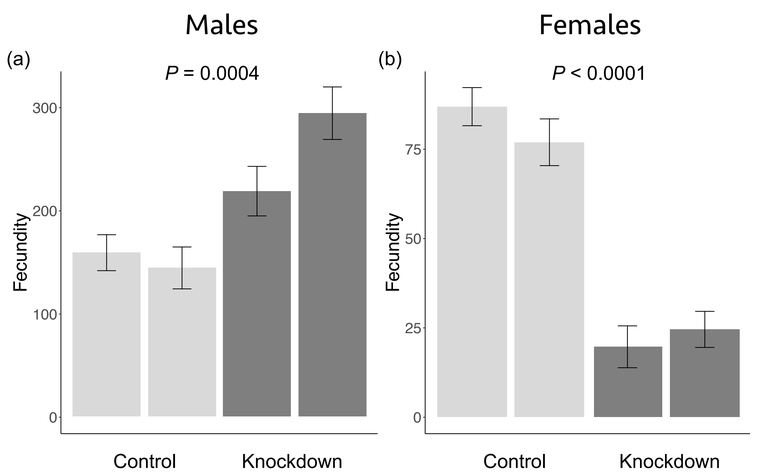


Fig. 2. (a) Knockdown males had increased fecundity (t = 3.529; P = 0.0004), while (b) knockdown females had decreased fecundity (t = -10.17; P < 0.0001).

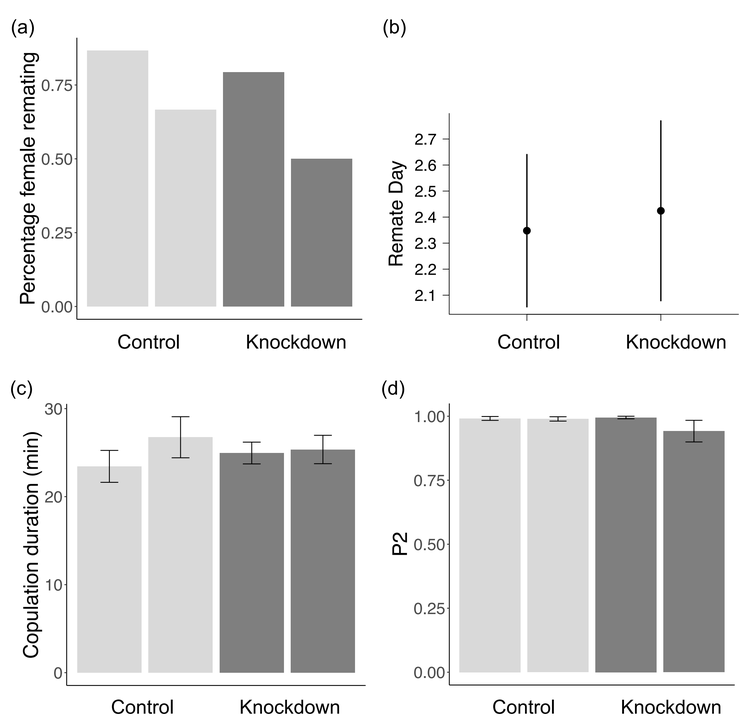


Fig. 3. Male knockdown had no effect on (a) female remating rate (χ^2_3 = 3.143; P = 0.323), (b) female remating latency (t = 0.336; P = 0.737), illustrated as effect sizes of the statistical model, (c) copulation duration (t = 0.109; P = 0.913) (c), or (d) P_2 (z = 0.163; P = 0.871).

Discussion

Eip74EF controls sperm length but not sperm function *per se*, as knockdown males actually have increased fertility. Most sperm gene knockdowns lead to infertility, suggesting that *Eip74EF* can be considered to be a sperm competition gene. However, it does not seem to be involved in male-female coevolution, since knockdown had no effect on female SR lengths.

While P_2 was no different in knockdown males, the effect of sperm length on sperm competitive success depends on both sperm length of the competitor male and SR length of the female[2-3,14]. Future work should re-examine P_2 while also controlling competitor sperm lengths and female SR lengths.

Eip74EF knockdown leads to shorter sperm but higher fecundity, suggesting a trade-off between sperm length and sperm number. Future work should test this hypothesis.

Ecdysone signaling is involved in spermatogenic cyst differentiation[10-11], but *Eip74EF* has not been implicated directly, and its function is unknown in testis. It is an ETS domain transcription factor with little known about the downstream genes it regulates.

Eip74EF is also required for oocyte maturation[15], which explains why female knockdowns have decreased fecundity.

It is surprising that *Eip74EF* is rapidly evolving, given its roles in larval molting and metamorphosis, which are conserved processes. Most rapidly evolving reproductive proteins directly interact with female-derived molecules[16], but *Eip74EF* may control spermatid morphogenesis.

Acknowledgements

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