The Effects of Tsetse Fly Beta 2 Tubulin on the Fruit Fly Axoneme

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Objective of Research

To determine the fertility of *D. melanogaster* (fruit fly) when the spermtail axoneme contains beta-2 tubulin from *G. moristans* (tsetse fly) and ultimately further our understanding of constraints during evolution, whether co-evolution or stabilizing selection is at work

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D.m
G.m.m

D.mel   PRAIL                                                    -
G.m.m   PRAILVDL                                                 -

D.mel   RKESEGCDCLQGFQL                    --          -
G.m.m   RKEAEGCDCFQGFQLTHS                 --          -

D.mel   EPYNATLSVHQLVENTDETYCIDN                              -
G.m.m   EPYNATLSVYQLMENTDETYCMDNEAL                           -

D.mel   RFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLT
G.m.m   RFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLTSRG

D.mel   AACDPRHGRYLTVAAIFRGRMSMKEVDEQMLNIQNKNSSFFVE      -
G.m.m   AACDPRHGRYLTVAAIFRGRMSMKEVDEQMLNIQQKNSSFFVEWIP   -

D.mel   LKMSATFIGNSTAIQELFKRVSEQFTAMFRRKAFLHWYTGEGMDEMEFTEAES  -
G.m.m   LKMSATFIGNTTAIQELFKRISEQFTAMFRRKAFLHWYTGEGMDEMEFTEAESSMN

D.mel   EYQQYQEATADEEGEFDEDEEGGGDE   446
G.m.m   EYQQYQEATAEEEGEFDEDEEGGADE   446
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Fig.1 Comparison of *D. melanogaster* and *G. morsitans* amino acid sequences; the highlighted sites indicate differences between the species

Fig.3 *D. melanogaster* with tsetse fly B2 phenotype (orange eyes).

Objective of Research

Some features evolve more rapidly than others from the beginning of life to present day. A big question that remains is why. Nature must have some constraints during protein evolution that impact their evolutionary rates.

The spermtail of *D. melanogaster* is the longest in the animal kingdom (2 mm) and is primarily made up of two proteins, alpha-1 and beta-2 tubulin that support the 9+2 microtubule configuration (Fig 2). The beta-2 protein has not evolved for over 60 million years, and this requires an explanation.

G. Moristans is the closest relative to the fruit fly that shows significant changes in the amino acid sequence of B2 (17 different amino acids).

Previous studies have shown the fruit fly axoneme to be very sensitive to change in its B2 sequence, making the spermtail immotile and nonfunctional.

The constraints for this protein to retain its function could be one of two sources, either the beta-2 tubulin protein co-evolves with its partner alpha-1 tubulin protein, which is a slow evolutionary process, or the beta-2 protein is undergoing stabilizing selection. Other sequences can work, but the present form works the most efficiently than the functional alternatives.

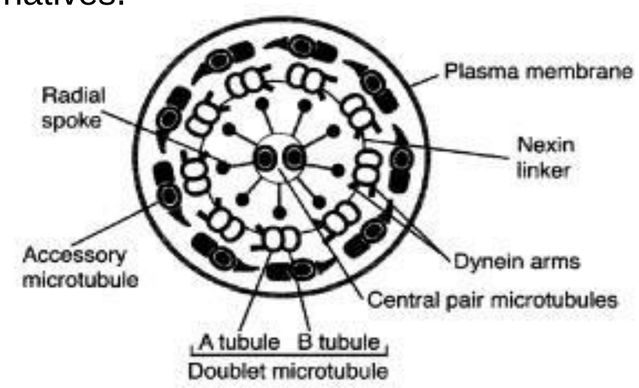

Fig.2 Schematic spermtail axoneme cross-section. Note the 9+2 microtubule configuration. (Nielsen et. al, 2002)

Methods

G. moristans B2 tubulin was chosen after searching other related species for similar amino acid sequences. *G. moristans* B2 tubulin was amplified using PCR and cloned into a vector that was injected in fruit fly larva. These flies are mated, creating two copies of Gm B2 with one copy of Dm B2 genotype. Now, matings are being done to generate flies with two copies of Gm B2 and no copies of Dm B2.

Once flies have been bred, their B2 tubulin will be analyzed via fertility tests, light microscopy, and transmission electron microscopy, to determine the sperm function, alignment within the testes, and B2 arrangement respectively.

Results

We generated a 2 Gm B2, 1 Dm B2 strain of flies and these had functional spermtails. The flies are fertile, the spermtails are full-length and Aligned properly in the testis, and have the normal 9+2 arrangement of microtubules in the spermtail axoneme.

This points to B2 tubulin protein being a stabilizing selection; the Dm beta 2 protein sequence is sufficient but not necessary to proper spermtail development. A second change does not need to occur in the axoneme in order for B2 tublulin sequence to evolve.

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