Evolution of *P*-Element Copy Number in *Drosophila melanogaster*

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BACKGROUND

Transposable elements comprise a significant portion of the genomes of both plants and animals. Their presence in genomes leads to both positive and negative effects. The positive effects of transposable elements do not outweigh the negative effects, and, for this reason, transposable elements are recognized as genetic parasites. The parasitic nature of transposable elements can modify genome structure, gene expression, and genome stability. Transposable elements are responsible for their negative impact due to their inherent tendency to insert themselves into the genome of the host organism and replicate itself throughout the genome.

An example of a transposable element invasion is the invasion of the *P*-element transposon in *D. melanogaster* flies around 1950. *D. melanogaster* responded to the invasion via small RNAs known as piwi interacting RNA (piRNA). The piRNA pathway uses piRNAs that are transcribed in the germline in response to *P*-element copies located on piRNA clusters. The piRNA pathway evolved in *D. melanogaster* is a great model to study host tolerance and repression of transposable elements.

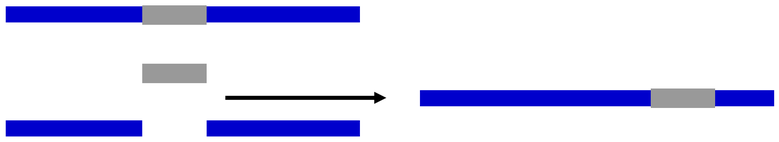

Figure 1. **Cut and Paste Mechanism of Transposition**. Excision of transposable element (gray) from original genomic location. The original transposon is then inserted into a different genomic location.

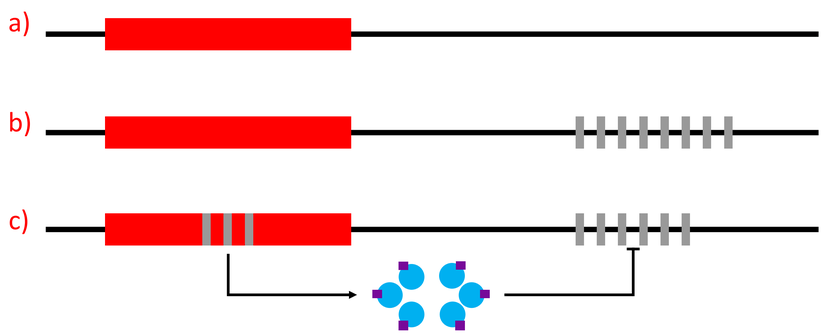

Figure 2. **piRNA regulation of P-elements**. **a)** Illustration of chromosome with no copies of *P*-elements (gray). **b)** Illustration of chromosome after *P*-element invasion. **c)** Illustration of chromosome after transposition of *P*-elements into the piRNA cluster (red). *P*-element copies in the piRNA cluster lead to production of piRNAs (blue), which coordinate with Piwi proteins (purple) to prevent transcription of *P*-element copies in germline cells.

METHODS

- Germline transformation techniques were used to introduce *P*-elements into a naïve strain of *D. melanogaster.*
- After germline transformation, populations of flies were maintained at 22C and 27C for multiple generations.
- QIAGEN DNeasy Blood & Tissue Kit was used to extract genomic DNA from flies from different generations.
- RNA is extracted from ovaries dissected from flies from different generations.
- RNA is reverse transcribed to create cDNA.
- DNA and cDNA are separately examined for number of *P*-element copies using quantitative PCR (qPCR).

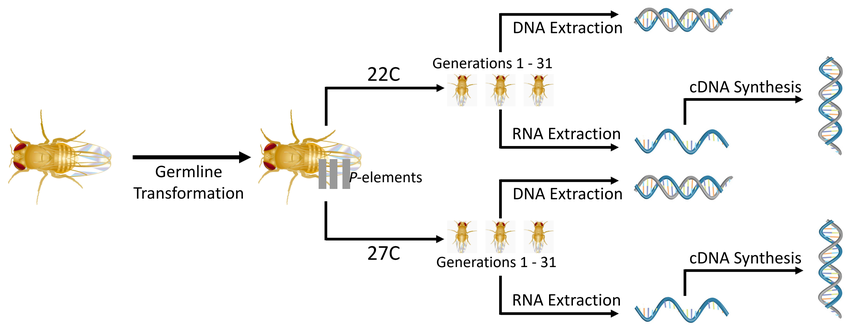

Figure 3. Illustration of Methods. After germline transformation of the *P*-element transposon into a naïve strain of *D. melanogaster*, two separate populations of flies were maintained: one at 22C and the other at 27C. For each population, there were 10 different replicates. Each replicate was maintained for 31 generations. The genome of flies from each set of generation-replicate were examined for *P*-element copy number.

OBJECTIVE

- Quantify the number of *P*-element copies in DNA and female ovarian mRNA across multiple generations of *D. melanogaster* populations.
- Analyze variations in *P*-element copy number across generations to understand *P*-element transposition rates.
- Examine changes in *P*-element transposition rates to understand the evolution of the piRNA mediated regulation of *P*-element activity.

EXPECTED RESULTS

Because the piRNA silencing pathways depends on *P*-element insertions into piRNA clusters, there should be a relationship between *P*-element copy number and evolution of the piRNA silencing pathway. As *P*-element copy number increases across successive generations, the probability of an insertion into a piRNA cluster should increase, which will lead to more production of piRNA and a more evolved piRNA silencing pathway. This relationship can be tested by examining the genome of experimental flies from successive generations for *P*-element copy numbers using relative standard qPCR.

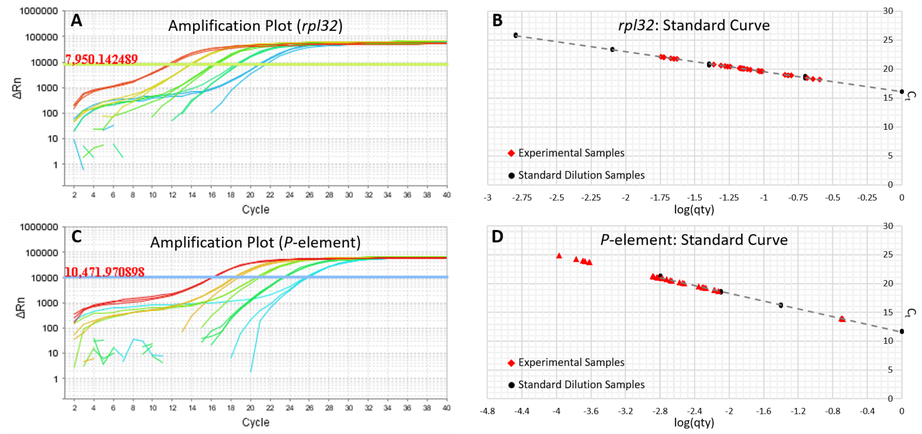

Figure 4. Analysis of qPCR Data. To quantify *P*-element copy number, a relative standard curve qPCR experiment is performed. The qPCR experiment is performed using *rpl32* as a reference gene and Harwich flies, a specific strain of *D. melanogaster*, as a reference genome. *rpl32* is used because its copy number is known in the Harwich and experimental fly genomes. The Harwich genome is used because it contains a large amount of *P*-element copies. The data obtained from the qPCR experiment (Panels A and C) are used to generate curves of relative abundance of *rpl32* (Panel B) and *P*-element (Panel D) in the Harwich genome. The standard curves are then used to calculate the relative abundance of *P*-element in the experimental fly samples.

REFERENCES

- Kelleher S. E., 2016 Reexamining the P-Element Invasion of *Drosophila melanogaster* Through the Lens of piRNA Silencing. Genetics 203: 1513-1531.
- Ayarpadikannan S., and H. Kim, 2014 The Impact of Transposable Elements in Genome Evolution and Genetic Instability and Their Implications in Various Diseases. Genomics Inform. 12: 98-104.

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