Investigating the Structure, Function & Evolution of the External Genital Structures of *Drosophila* Species

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1.Introduction

Drosophila male genitalia exhibit incredible divergent morphology, representing one of the most striking examples of morphological evolution [1]. To understand the rapid evolution of these traits, ongoing research aims to characterize the genetic architecture underlying differences in genital morphology [2]. Sex-specific behaviours have been shown to potentially originate from differences in brain structure [3]. In most cases gene expression is restricted to small groups of neurons; this would potentially provide a starting point for circuit identification [3]. Focusing on how individual neurons or subsets of neurons contribute to the development and function of neuronal networks would provide an important perspective of the ways in which Drosopholids have evolved, and how this affects certain behaviours such as copulation. The exploitation of techniques that target sub-sets genital neurons will allow for greater understanding as to how such genes function. Furthermore, allow us to identify genetic markers for the neurons that will allow for protein expression to be mapped in important behaviours such as fly copulation.

Keywords: Evolution, male genitalia, *Drosophila*, divergence, development, Minos-mediated integration cassette (MiMIC).

2. Background: Male Genitalia

Despite being closely related species male *D. mauritiana* & *D. simulans* show great divergence among the external genitalia. Only through examining the male genitalia can the species of fly be identified [1]. The external genitalia consist of the claspers, posterior lobe, & the anal plate. Each structure has an important role in successful copulation (Fig 1).

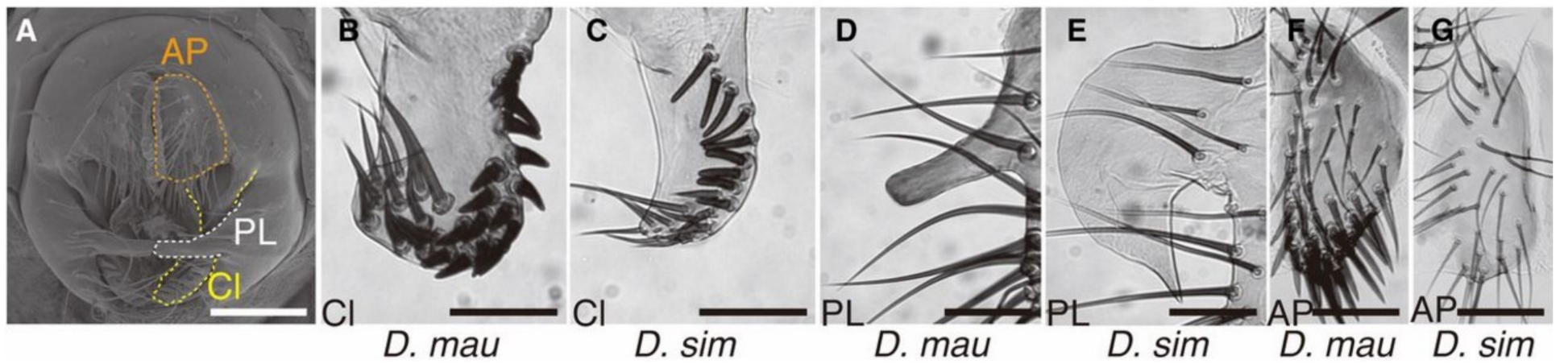

Figure 1*:* *Drosophila* male external genitalia and analia. (A) SEM image of a *D. mauritiana* male genital arch highlighting the right clasper (Cl), posterior lobe (PL), and anal plate (AP). Morphological divergence between *D. mauritiana* and *D. simulans* is shown, respectively, in B and C for claspers, D and E for posterior lobes, and F and G for anal plates. Bars, 100 µm (in A) and 50 µm (in B–G) (Adapted from Tanaka *et al.,* 2015).

2.Background: Neuroanatomy

Gene expression can often be restricted to small groups of neurons. By targeting smaller subsets of neurons, studies can begin to uncover the genes that are required to initiate certain behaviours[3]. One would prefer to have reproducible genetic access to defined populations for neural circuit analysis. Exogenous genes can be introduced through using random mobile transposon elements and genetic markers that insert around the genome[3,4]. In an enhancer trap the reporter gene is fused to a minimal promoter, typically containing a TATA box and transcription start site, that is unable to drive reporter gene expression alone but can be activated by neighbouring enhancer elements (Fig 2)[3].This can be a powerful tool, in combination with the split GAL4-UAS system, in understanding gene expression, that will begin to tease apart the neuroanatomical basis of neurons expressed in the genitalia, and the genes that are essential for this to occur.

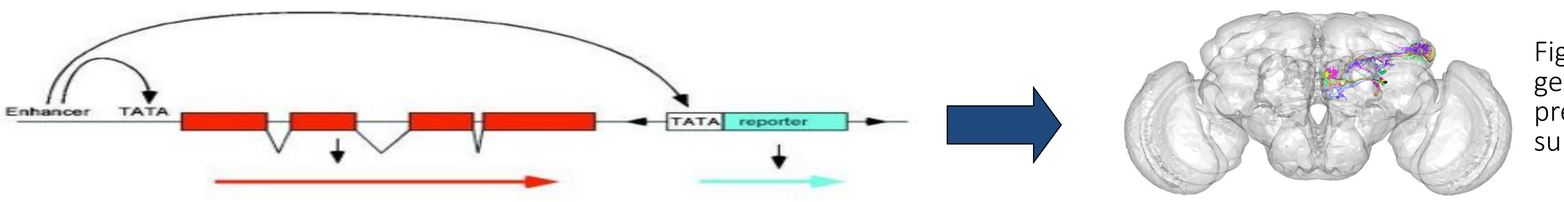

Figure 2*:* Enhancer trap construct. The minimal promoter of the reporter gene (TATA) is activated by a chromosomal enhancer element, resulting in expression of the reporter gene. This can be manipulated in order to target a subset of neurons. Such as the neurons associated in copulation.

3.Methods

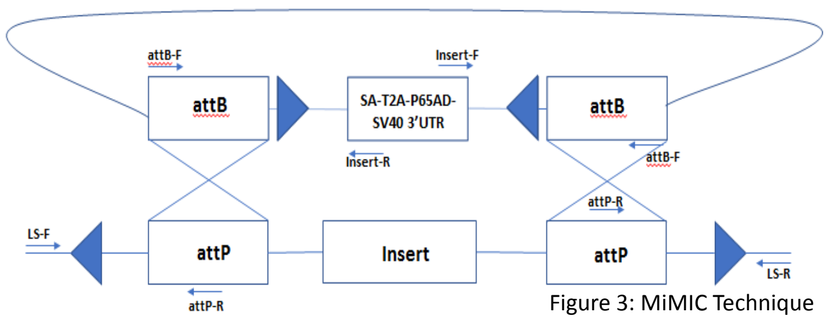

Figure 3: MiMIC Technique

The MiMIC transposon system was utilised to create gene trap constructs within candidate genes. MiMIC landing sites consist of two Minos inverted repeats (L and R) and two inverted phi-C31 integrase attP sites (P) flanking a yellow marker[4].Into this landing site we inserted a gene-trap cassette containing a splice acceptor site (SA) in all three reading frames, a T2A cleavage peptide & a cDNA encoding the p65 activation domain & a 3' UTR[4] .

Successful integrations could be identified via the loss of the yellow marker. To determine the orientation of the inserted cassette, we used combinations of landing site (genomic) and cassette-specific primers. Flies carrying the insertion in the 'sense' orientation were crossed to an elavDBD hemidriver line, used to drive eGFP and label subsets of cells.

4.Results: Using PCR to Verify MiMIC Line Orientation

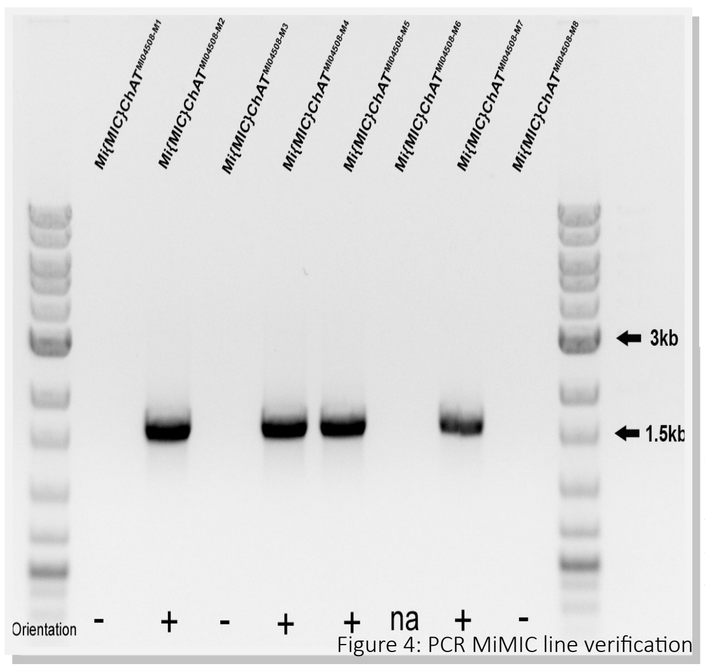

Figure 4: PCR MiMIC line verification

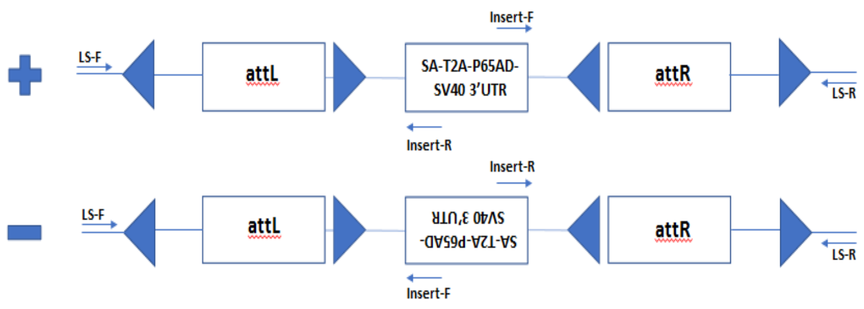

Figure 5: +/- MiMIC Lines indicating insert & insert specific primers

PCR was performed on 8 different MiMIC lines using insert and genomic DNA specific primers (Fig 4,5). The results were able to indicate that 4/7 MiMIC lines tested had a positive orientation with respect to the insert. Furthermore, 3/7 has a negative orientation with respect to the insert. The M6 line was uncharacterised. The lines the correct orientation can then be crossed to the elavDBD which will allow for expression in neurons.

4.Results: Confocal Microscopy

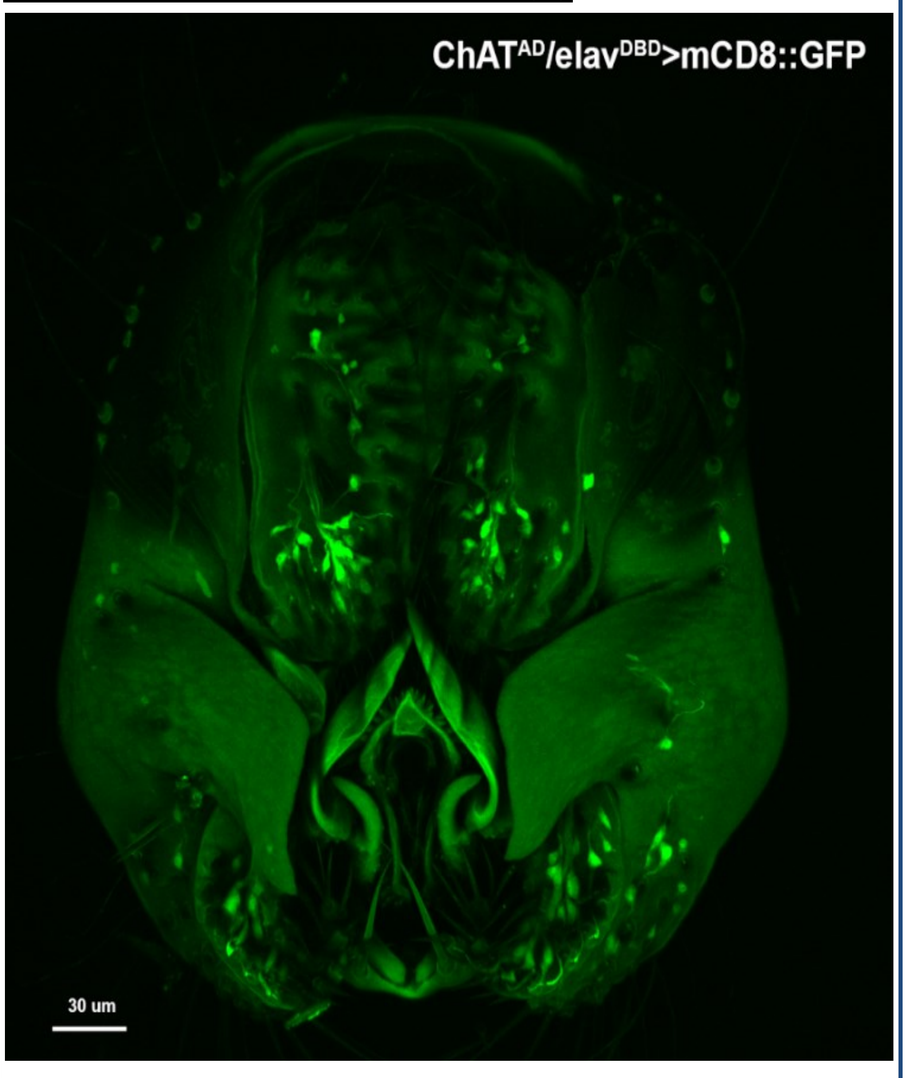

Figure 6: Confocal Microscopy used to identify where subsets of neurons were able to recapitulate expression.

Image of male *Drosophila* (Cantons line) expressing cholinergic neurons (Herbert, Unpublished).

5.Conclusions

MiMIC is a powerful tool that can be used to produce gene trap constructs, to begin to identify genes that play a prominent & functional role in the development of male genitalia. However, in order to utilise this tool, it is vital to first to determine the orientation of the cassette as it has inserted into the genome. Confocal microscopy can then be used to visualise expression of the neurons that are being expressed in the genitalia to determine expression pattern. Lastly, MiMIC insertion mutants can be reverted by recombinant mediated cassette exchange, due to the presence of attP recombination sites. The versatility of this method is thus paramount to begin to understand the differences that are exhibited among the neuroanatomy of different Drosopholids.

References:

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