Using CRISPR Mutagenesis to study Butterfly Wing Pattern Development

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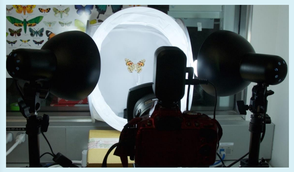
Summary

What genes are deployed during development to specify complex animal morphologies, and how? Butterfly wings are a promising model system for the study of the genetic basis of pattern formation. However, there has been a cruel lack of techniques allowing the manipulation of gene function in butterflies.

CRISPR genome editing techniques, which can induce specific mutations in genes of interest in a large number of organisms, and in fact, recent research has shown CRISPR's ability to create mutations that modify the size and shape of color patterns on butterflies[1,2]. We have established a protocol to analyze gene function during the development of a non-traditional model organism, the Painted Lady butterfly (*Vanessa cardui*): we implemented CRISPR-mediated mutagenesis to invalidate the functions of genes involved in cell-cell signaling, which are generally involved in pattern formation during animal development. To establish the technique in our new laboratory, **we first reproduced known *WntA* phenotypes** (Martin ***in prep.***), which result in wing pattern modifications. Then, we tested the function of four new candidate genes in butterfly wing formation and patterning: *SFL*, *TTV*, *ARR*, and *STAT92E*. While none of these genes produced pattern phenotypes *per se,* ***SFL* mutants displayed wing margin defects and *STAT92E* mutants showed reduced wings and antennae, consistent with the known roles of these two genes in *Drosophila*[3,4].**

These preliminary results reveal that CRISPR is a novel technique particularly well-suited to the study of fundamentals questions of developmental biology in butterflies. We will actively continue to use this method to invalidate gene function and shed light on the mechanisms that underlie pattern formation and diversity.

Materials and Methods



1) Rearing of *Vanessa* wild type population

2) Egg collection

3) Eggs placed in petri dish and flipped upright

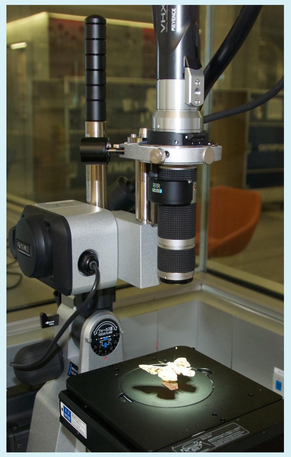
4) sgRNA and Cas9 mix is prepared

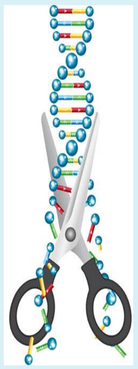
5) Nanoliter injections in 3-5hrs embryos

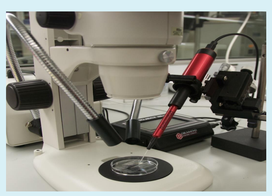
6) Placed in individual cups and reared in incubator

7) Emerged butterflies are examined and spread

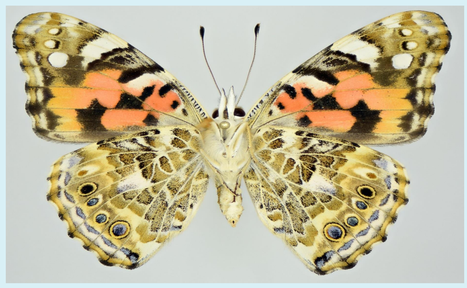
8) Microscopy and photos





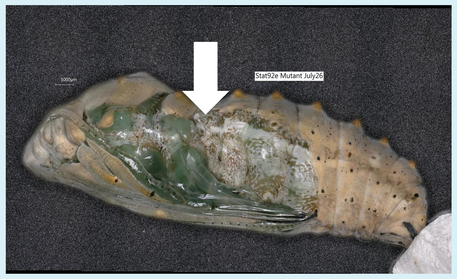


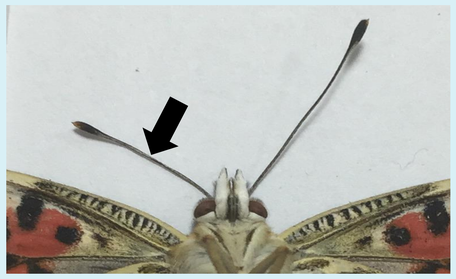
Results



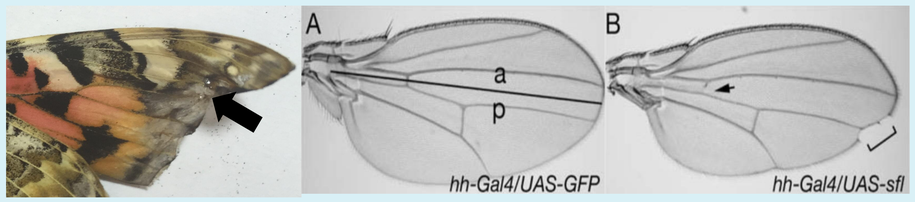
Wild Type

WntA mutant





STAT92E: missing wings in pupae; reduced antennae in adults



SFL : wing margin defect ; similar to *Drosophila* phenotype (reproduced from ref. 3)

Gene	Total Injected	Indivs. with mutant phenotypes
WntA *Morphogen: Ligand of the Wnt family*	50	6
SFL *Enzyme involved in ECM signal transport*	1053	number TBD – effect on wing margin
TTV *Enzyme involved in ECM signal transport*	524	No Defect
ARR *Co-receptor of the Wnt ligand*	191	No Defect
STAT92E *Transcription factor of the JAK/STAT pathway*	281	4 (missing wing) + 11 (reduced antennae)

Discussion

We obtained morphological phenotypes for 2/4 new candidate genes tested, suggesting the CRISPR approach allows the study of gene function in butterflies. **FUTURE DIRECTIONS:** we will continue targeting more genes and analyzing their role in pattern development, while also optimizing the injection conditions that will improve the rate of mutant phenotypes obtained. We will focus our analysis on more genes of the Wnt pathway, to better decompose the striking effects of *WntA* Knock-Outs.

Acknowledgements

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References:

1: Zhang et al. Nat Commun. 2016
2: Perry et al. Nature. 2016
3: Kamimura et al. Glycobiology. 2011
4: Ayala-Camargo et al. Dev Dyn. 2007