ASSESSING THE POTENTIAL OF *HZTRANSIB* AS A GENETIC DRIVE IN *ANOPHELES* MOSQUITOES

By

YONG SOO PARK

YONG SOO PARK

A Thesis Submitted to The Honors College

In Partial Fulfillment of the Bachelors of Science in Health Science degree with Honors in

Physiology

THE UNIVERSITY OF ARIZONA

M A Y 2 0 1 2

Approved by:

Michael Riehle, Ph.D.
Department of Entomology

STATEMENT BY AUTHOR

I hereby grant to the University of Arizona Library the nonexclusive worldwide right to reproduce and distribute my dissertation or thesis and abstract (herein, the "licensed materials"), in whole or in part, in any and all media of distribution and in any format in existence now or developed in the future. I represent and warrant to the University of Arizona that the licensed materials are my original work, that I am the sole owner of all rights in and to the licensed materials, and that none of the licensed materials infringe or violate the rights of others. I further represent that I have obtained all necessary rights to permit the University of Arizona Library to reproduce and distribute any nonpublic third party software necessary to access, display, run or print my dissertation or thesis. I acknowledge that University of Arizona Library may elect not to distribute my dissertation or thesis in digital format if, in its reasonable judgment, it believes all such rights have not been secured.

Signed: ***YONG SOO PARK***

Date: *April 27th, 2012*

ABSTRACT

Malaria kills millions of people across the globe and measures to control this disease are not simple and definite. While anti-malarial effector genes have been identified and successfully engineered into the human malaria vector, *Anopheles* mosquitoes, a mechanism to drive these genes through wild *Anopheles* populations is needed. This study utilizes and assesses the *Hztransib* transposable element from *Helicoverpazea* as a potential candidate for the genetic drive mechanism. Specifically, this study tries to determine whether *Hztransib* is capable of duplicating and remobilizing the effector within the germ line of the *Anopheles* mosquitoes. To do that a helper line of DsRed representing *Transib* transgenic line is crossed with the ECFP/EYFP donor line. The progeny from this cross (G1 progeny) is examined for the eye expression of ECFP, EYFP and DsRed. Positive DsRed/ECFP/EYFP are then mated with wild-type mosquitoes and a large sample of individual progeny (G2 progeny) is screened for the expression of DsRed, EYFP and ECFP markers to determine the transpositional activity from *Hztransib*. The results from G2 progeny screens showed only Mendelian crossings suggesting that *Hztransib* might have been silenced or not sufficiently activated in the mosquito germ line.

INTRODUCTION

Malaria affects hundreds of people across the globe and kills over one million annually (1). The disease results from the multiplication of *Plasmodium* parasites within red blood cells which can cause symptoms of severe fever and headache. Preventing the development of the *Plasmodium* parasite in the obligate mosquito vector offers a novel means of blocking transmission and controlling malaria (2). The anti-malarial effector genes have been identified and genetically engineered into *Anopheles* mosquitoes to render them resistant to malaria (3). While considerable progress has recently been made in lowering vectorial capacity by introducing new genes into the mosquito germ line, little is known about how such genes could be introduced into the wild field populations (4). In other words, a mechanism for driving the effector genes through wild mosquito populations and increasing the inheritance rate of refractory genes is urgently needed. To successfully drive refractory genes to high frequency or fixation in wild populations, a gene drive must possess the capacity to increase its copy number and remobilize in the mosquito genome within the germ line tissue (5). One mechanism that could accomplish this is to utilize the transposable element, *Hztransib* which is a mobile genetic element (6). Since the transposable element's job is to change its relative position within the genome by a "cut and paste" mechanism, it is a potential gene driving mechanism where this enzyme can excise, duplicate and remobilize the target genes within the germ line of *Anopheles* mosquitoes (7). This thesis project will assess the suitability of the transposable element, *Hztransib* as a potential gene drive mechanism for driving anti-malarial effector genes through wild mosquito populations.

METHODOLOGY

Anopheles Mosquitoes

Mosquitoes were maintained at 37 °C and 70% Relative Humidity with 16:8 Day/Night. They were given 10% dextrose for maintenance. Human blood was provided for reproduction via a membrane feeding system.

Eye Color Mutants

Selectable markers are required to identify the transformants. The enhanced green fluorescent protein (EGFP) from the jellyfish has been used extensively as a transformation marker (8). EGFP is also readily detectable and suitable for use in a wide range of organisms. There are two distinguishable mutants of EGFP; a blue mutant variant, ECFP and a yellow mutant, EYFP that can be used as independent selectable markers (9). A fourth fluorescent marker, DsRed is completely separable from ECFP, EYFP and EGFP and is a highly efficient selectable marker for use in insect transgenesis (10). To assess the function of *Hztransib* in the *Anopheles* mosquitoes, these mosquitoes are engineered with an enhanced eye color fluorescent protein. Genetic cross between the helper line, *Hztransib* line which has a genetic marker of DsRed and the donor line of both ECFP and EYFP will bring the transposase protein together. Mosquitoes having both genes will have both blue and red fluorescent eyes when examined under the appropriate filters of the microscope. Figure 1 represents the diagram of how genes are constructed for the helper and donor lines. Figure 1A shows that the *Transib* helper construct, encoding the *Transib* transposase, will be stably integrated into the *Ae. aegypti* genome using the piggyBac transposable element. Expression of the *Transib* transposase will be driven by the *Drosophila* hsp70 promoter. The DsRed fluorescent marker will be expressed in the eyes and

nervous system using the 3XP3 promoter. Figure 1B shows that the *Transib* donor construct, containing the EYFP fluorescent maker flanked by the *Transib* inverted terminal repeats, will be stably transformed into a second *Ae. aegypti* line using piggyBac. The fluorescent marker ECFP will be used to identify this line. Figure 1C shows that the pBac helper plasmid, phsp-pBac, will provide the piggyBac transposase necessary to insert these constructs into the mosquito's genome.

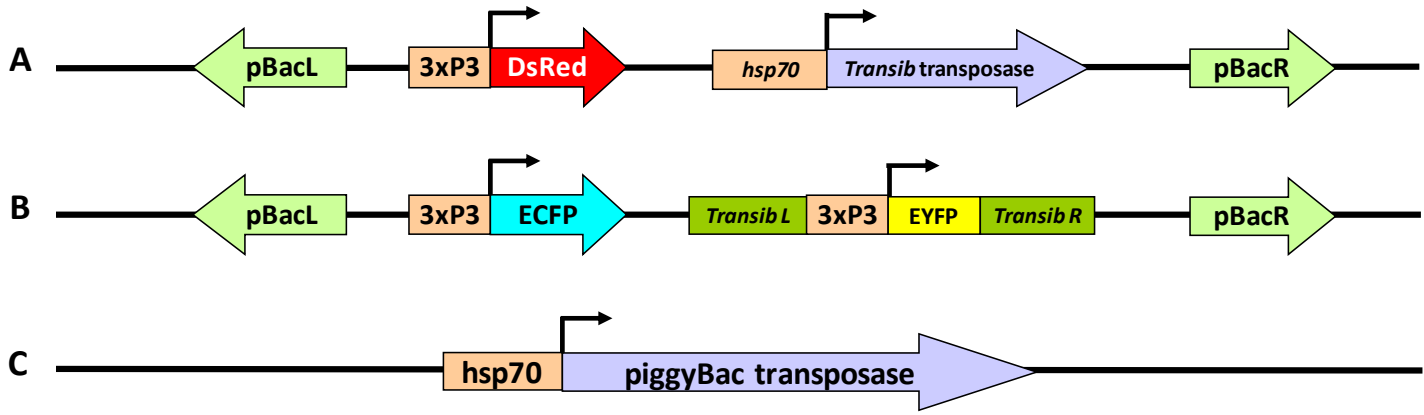

Figure 1. Diagram of the *Hztransib* and *piggyBac* helper and donor constructs used for germline transformation *An. stephensi*.

Rearing and Crossing Protocol

To determine the function of the *Hztrasib* in the *Anopheles* mosquitoes, the rate of excision, duplication and remobilization is to be determined. To do that, a helper line of DsRed representing *Transib* transgenic line is crossed with the ECFP/EYFP donor line. After crossing, the G1 progeny were examined for the eye expression of both EGFP and DsRed. When EGFP is expressed, it is suggested that both ECFP and EYFP are present. Individuals of pupae expressing both markers are heat shocked at 39 °C for an hour in a small plastic container in a hot water bath. Individuals of adult expressing both markers are heat shocked in the incubator at 37 °C for

an hour. These heat shocked adults that express all positives of ECFP/EYFP/DsRed are then mated with wild-type mosquitoes and a large sample of individual progeny (G2 progeny) is screened for the expression of DsRed, EYFP and ECFP markers. If no duplication, excision, and remobilization events of the *Hztransib-EYFP* donor element happen in the germ line tissues, the experimental crosses will, just like the control crosses, follow the Mendelian genetics of two loci, producing only four eye marker phenotypes; DsRed+/ECFP+/EYFP+, DsRed+/ECFP-/EYFP-, DsRed-/ECFP+/EYFP+ and DsRed-/ECFP-/EYFP-. ECFP and EYFP are tightly linked and thus behave as a single locus which suggests that there is no function of transposase seen in the germ line. But, if there is duplication, excision, and remobilization of the *Hztransib-EYFP* donor element, the ECFP and EYFP markers can become unlinked, resulting in up to four non-Mendelian eye marker phenotypes in addition to the four Mendelian phenotypes. The possible excision, duplication, or remobilization events controlling each of the eight eye marker phenotypes are summarized in Table 1 under the Results section. Figure 2 represents the summarized overview of which line to cross and all possible genotypes expected from the crosses.

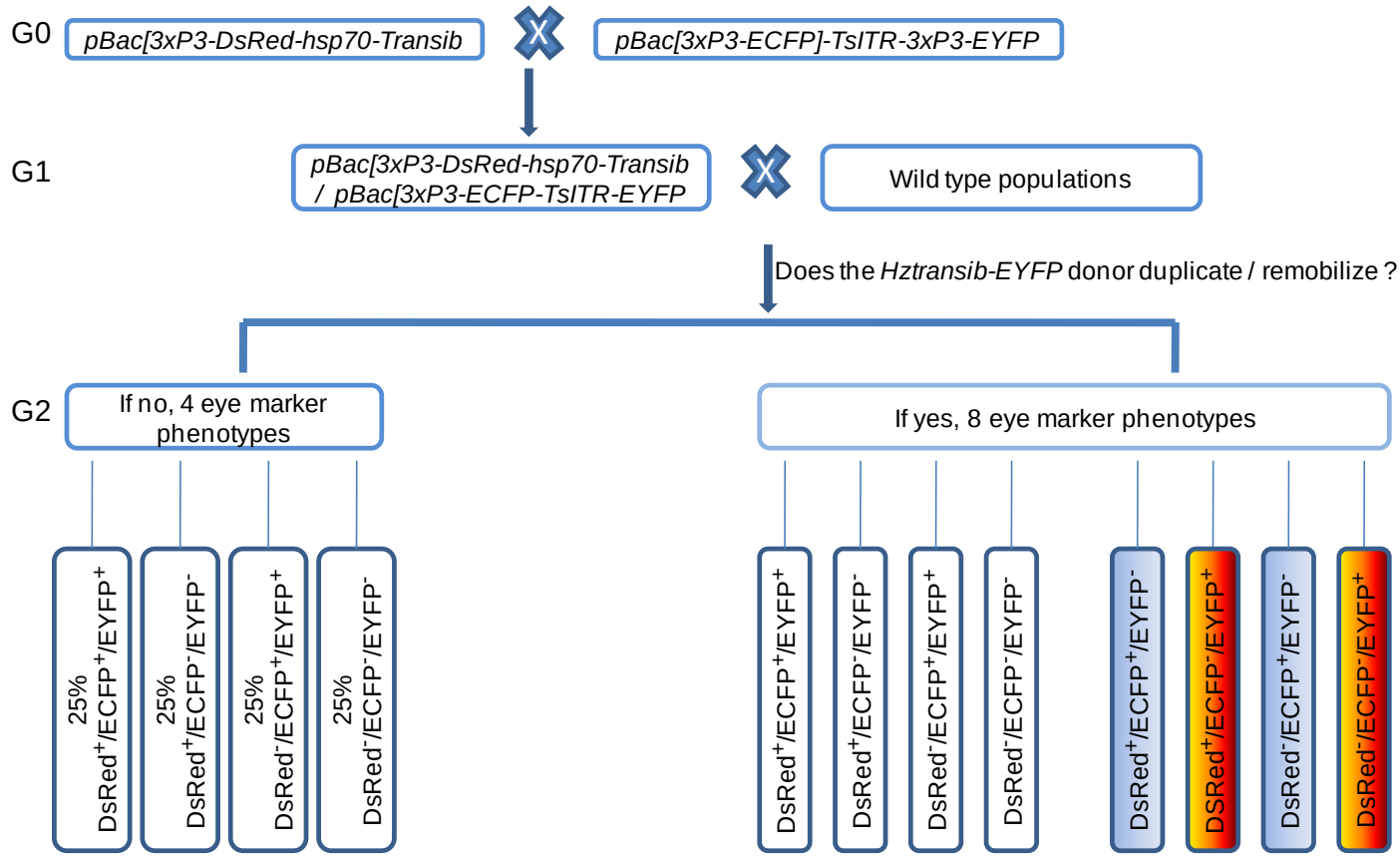

Figure 2. Genetic screening for post-integration excision, replication, and remobilization events of the *Hztransib-EYFP* donor element. After a minimum of 5 generations' outcrossing with wild population to reduce founder effects, the pBac[3xP3-*DsRed*]-hsp70-*Transib* transgenic (helper) line will be crossed with the pBac[3xP3-*ECFP*]-*TsITR*-3xP3-EYFP (donor) line. G1 individuals bearing the three markers (dsRed/ECFP-EYFP) will be heterozygous for both the helper construct (pBac[3xP3-*DsRed*]-hsp70-*Transib)* and the donor *(*pBac[3xP3-*ECFP*]-*TsITR*-3xP3-EYFP) constructs and will be crossed with wild populations. If the *Hztransib-EYFP* donor element in the donor construct remains inactive in the germline of those G1 individuals, there will be only four eye marker phenotypes (see the bottom left of the figure). Assuming there is no significant fitness cost for the two transgenes, each of the four eye marker phenotypes will be approximately 25% of the total G2 individuals. If *Hztransib-EYFP* will excise, duplicate, and remobilize in those G1 individuals, there will be four more possible eye marker phenotypes (see the bottom right of the figure) in the G2 progeny. The two blue boxes represent individuals with the *Hztransib-EYFP* being excised out from the original insertion site, whereas the two yellow/red boxes represent individuals with the excised intact *Hztransib-EYFP* being inserted into novel loci. Based on the number of the four additional phenotypes, the frequency of excision and remobilization can be estimated. Note: symbols "+" and "-" indicate having or not having the corresponding marker, respectively.

RESULTS

All positive DsRed/ECFP/EYFP lines are out-crossed with the wild-type mosquitoes for seven generations to minimize the founder effects. This is because each transgenic line originates from a single mosquito, thus it is likely that significant deleterious founder effects will occur, even if the transgene is not inserted into a deleterious position. In other words, founder effects should continue to go down as the lines will be continuously out-crossed with the wild-type populations over time. Table 1 summarized the eight possible eye marker phenotypes in the G2 progeny. First four phenotypes are results of Mendelian crossing where ECFP and EYFP are tightly linked and thus behave as a single locus which suggests that there is no function of transposase seen in the germ line. The latter four shows that there is duplication, excision, or remobilization of the *Hztransib-EYFP* donor element where the ECFP and EYFP markers can become unlinked due to "cut and paste mechanism" of transposase. Collaborators in Dr. Xianchun Li's lab confirmed that the *Hztransib* transposase transcript was expressed in all tissues and that in somatic tissue mobilization of the EYFP marker occurred. This verifies that the *Hztransib* transposase is active, at least in somatic tissue.

Table 1. Genotypes underlying the eight eye marker phenotypes in the G2 progeny

1	DsRed+/ECFP+/EYFP+
2	DsRed-/ECFP+/EYFP+
3	DsRed+/ECFP-/EYFP-
4	DsRed-/ECFP-/EYFP-
5	DsRed+/ECFP+/EYFP-
6	DsRed-/ECFP+/EYFP-
7	DsRed+/ECFP-/EYFP-
8	DsRed-/ECFP-/EYFP-

After the seven generations of crossing between the positive DsRed/ECFP/EYFP and the wild-type mosquitoes, the results and the description of G2 progeny are organized in Table 2. Clearly, it is easy to notice that there is none of EYFP or ECFP alone mosquitoes. This suggests that all the G2 progeny's eye phenotypes are limited to the first four options from Table 1. The grand total numbers of G2 progeny screened is 9138 and the percentage of DsRed+/ECFP+/EYFP+ out of all screened is 48.2% and 51.7% for DsRed-/ECFP-/EYFP- which is close to the expected outcome of 50% to 50% percentage distribution between positive and negative groups.

Table 2. Summary data of G2 progeny

Generation	YFP only	CFP only	CFP/YFP	None *	Total
1	0	0	931	1031	1962
2	0	0	849	827	1676
3	0	0	565	646	1211
4	0	0	644	738	1382
5	0	0	116	136	252
6	0	0	461	455	916
7	0	0	845	894	1739
TOTAL	0	0	4411	4727	9138

* None = DsRed-/ECFP-/EYFP-

DISCUSSION

To determine the gene drive potential of the *Hztransib*, I used fluorescent eye markers to detect excision and insertion of the transgene. Individuals expressing ECFP only, EYFP only, or both ECFP and EYFP are of particular interest in this study. Individuals expressing ECFP but not EYFP (#5 and #6 from Table 1) demonstrate that the *Hztransib-EYFP* donor element jumped from the original construct at the initial insertion site with no gap repair or with incomplete gap repair (thus no functional EYFP). In other words, *Hztransib-EYFP* is excised out but not replicated at the initial insertion site. Individuals expressing EYFP but not ECFP (#7 and #8 from Table 1) indicate that the excised EYFP is actually inserted into other loci, which represent successful remobilization events which this study ideally wanted to observe to confirm the function of *Hztransib*. Presence of EYFP only individuals or ECFP/EYFP individuals markedly beyond the expected Mendelian inheritance rate would have suggested that *Hztransib* is a sutiable drive mechanism. Individuals expressing both ECFP and EYFP (#1 and #2 from Table 1) represent three possible genotypes: original construct at the initial insertion site, the original construct with the *Hztransib-EYFP* donor being excised out and then being gap-repaired at the initial site or the original construct with the *Hztransib-EYFP* donor inserted in new loci. This study suggests that *Hztransib* may not be the best potential gene drive mechanism which can drive good anti-malaria effector genes in the wild-type mosquitoes. The next future direction of this study is to focus on identifying the possible silencing mechanism in germ tissue. The *Hztransib* might have been silenced or not fully activated to do its job by unknown mechanism. So, studying and identifying the possible reasons or mechanisms behind why *Hztransib* couldn't work as proposed in the *Anopheles* mosquitoes is next step.

ACKNOWLEDMENTS

I would like to specially thank Yan Mei Lee and Frank Ramberg to assist on scanning and managing the mosquitoes throughout this thesis work. Without their support and assistance, my thesis work would not have been completed on time.

REFERENCES

1. World Development Reports on Mosquitoes. April 27th, 2012.
2. Riehle MA, Garczynski SF, Crim JW, Hill CA, Brown MR. Neuropeptides and peptide hormones in Anopheles gambiae. *Science*. 2002 Oct 4; 298 (5591): 172-175.
3. Catteruccia F 2007. Malarai vector control in the third millennium: progress and perspectives of molecular approaches. *Pest Mang Sci* 63: 634-640.
4. James AA. 2005. Gene drive systems in mosquitoes: rules of the road Trends in *Parasitology* Volume 21, Issue 2, Feb. 2005: 64-87.
5. Holt RA, Subramanian GM, Halpern A, Sutton GG. The genome sequence of the malaria mosquito Anopheles gambiae. *Science*. 2002. Oct 4: 298(5591):129-149.
6. Grossman, G.L., Rafferty, C.S., Clayton, J.R., Stevens, T.K., Mukabavire, O., Benedict, M.Q., 2001. Germline transformation of the malaria vector, Anopheles gambiae, with the piggyback transposable element. *Insect Molecular Biolology* 10, 597-604.
7. Christophides GK, Zdobnov E, Barillas-Mury C, Birney E. Immunity-related genes and gene families in Anopheles gambiae. *Science*. 2002 Oct 4; 298(5591):159-165.

8. Tian B, Jun Hu, Haibo Zhang, Carol S. Lutz. 2005. A large-scale analysis of Mrna polyadenylation of human and mouse genes. *Nucleic Acids Research*, 2005. Vol. 33, No. 1 201-212.
9. Patterson G, Day RN, Piston D. Fluorescent protein spectra. *J Cell Sci*. 2001 Mar; 114 (Pt 5): 837-838.
10. Matz MV, Fradkov AF, Labas YA, Svitsky AP, Zaraisky AG, Markelov ML, Lukyanov SA. Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat Biotechnol*. 1999 Oct. 17 (10): 969-973.