

and double heterozygotes – with both markers – collected. These are checked for equal male to female ratio after eclosion and males crossed to wild type females – again either on- or off-tet. Double-heterozygote females to wild type males crosses are used to test whether any observed effects are sex specific. Wild type control is included as the reference point for egg hatch rates. Eggs from the crosses are collected and counted. Three days later counting is repeated and numbers of un-hatched eggs assessed.

Figure 1. : Design of the egg hatch rate assay.

Results of a number of these crosses are shown below. All crosses presented here have followed the basic design described above.

OX4282 (*PB-HrIE-AmCyan-SV40-TurboGFP-teto14-ccTubulin-hsp83-tTAV-SV40*) x **OX4104** (*PB-YAFN-hsp83-tetO21-Hr5-IE1-Red*) (Figure 19)

Figure 2. OX4282-OX4104 male sterility on and off Tetracycline. Three independent, autosomal insertion lines of OX4282 transposon carrying tetracycline repressible transactivator (tTAV) driven by the β 2-tubulin promoter from *Ceratitis capitata* gene (I, L, G) were crossed to line ~~OX4014~~^{OX4104} carrying a tetO-3Zn-finger effector. Progeny of these crosses was reared and bred either on a diet with (100 μ g/ml; tet+) or without tetracycline (tet-). Males carrying both driver and effector alleles were crossed to the wild type females and the hatching rates of eggs obtained from these crosses were calculated (percentage of laid eggs that hatched). Two different egg collections of 100-150 eggs each were used for each cross. Wild type males crossed to wild type females in the presence or absence of tetracycline were used as controls. Crosses where highly significant male sterility was observed (chi-squared test, * represents $P < 0.01$, ** represents $P < 0.001$) are marked with asterisks.

Three OX4282 lines were crossed to a 3Zn-finger line (OX4104, Figure 19) which has previously shown promising results when crossed to two generic promoters (Hsp83 and OP). No adverse effects were recorded for flies containing both plasmids, indicating that basal expression of the effector in somatic tissue was not high enough to show an adverse effect. Only 14% egg hatching was observed from line L and 27% from line I. There was no significant reduction in egg hatching from line G. Both lines L and I contain a single copy of the transgene in contrast to line G, which contains two

CLAIMS:

1) An arthropod male germline gene expression system suitable for conditional expression of an effector gene in an Arthropod male germline, the system comprising:

- a first expression unit comprising an effector gene and a promoter therefor operably linked thereto;

- a second expression unit comprising a coding sequence for a transcription factor and an upstream regulatory element operably linked thereto, the transcription factor being capable of acting upon the promoter in the first expression unit to drive expression of the effector gene, the upstream regulatory element including:

- a promoter for the transcription factor; and

- a 5' UTR adjacent to a start site for the transcription factor coding sequence;

wherein the upstream regulatory element ~~drives~~ driving sufficient expression of the transcription factor ~~such that the~~ and said transcription factor ~~protein in turn~~ drives transcription of the effector gene before meiosis.

2) A gene expression system according to claim 1, wherein the transcription factor is a transcriptional activator, such as tTA, GAL4 or their variants.

3) A gene expression system according to any preceding claim, wherein the effector is endonuclease, most preferably a 3-Zn finger nuclease.

4) A gene expression system according to any preceding claim, wherein the promoter of the first expression unit is a minimal promoter.

5) A gene expression system according to any preceding claim, wherein the promoter of the upstream regulatory element in the second expression unit is ~~most from a~~ topi promoter, an aly promoter, or a Beta-2 Tubulin (B2T) promoter or a homologues thereof.

6) A gene expression system according to any preceding claim, wherein the 5' UTR in the upstream regulatory element of the second expression unit is that from hsp83,

preferably from Medfly or homologues of hsp83, particularly the homologue of hsp83 found in the target arthropod.

7) A gene expression system according to any preceding claim, wherein the Gal4-UAS system is used, such that the transcription factor is GAL4.

5 8) A gene expression system according to any of claims 1-6, wherein the system is an inducible system, where induction occurs by provision of a chemical entity, such as tetracycline or one of its analogues including doxycycline.

9) A gene expression system according to claim 8, wherein the transcription factor in the second expression unit is tTA or a variant thereof (tTAV, tTAV2, tTAV3).

10 10) A gene expression system according to any preceding claim, wherein the transcription factor of the second expression unit is tTA or a variant and the first expression unit includes the tet operator (tetO).

11) A gene expression system according to any preceding claim, wherein the arthropod is an insect, preferably a Dipteran, including a Tephritid, such as a Medfly or Olive fly.

15 12) A gene expression system according to any preceding claim, wherein the effector is a nuclease and confers or imparts paternal effect lethality.

13) A method of expressing an effector protein in a gonad or sperm, comprising transforming the gonad with the expression system according to any preceding claim.

20 14) A method of population control comprising expressing the effector protein of the expression system according to any of claims 1-12 in the gonads of a male arthropod.

15) A method of resistance management comprising use of the expression system according to any of claims 1-12.

16) A method of quality control, comprising including a reporter, such as a fluorescent protein as, or in addition to, the effector protein in the expression system according to

any of claims 1-12, such that individuals where expression from the system has been induced or de-repressed become visible under suitable wavelengths of light.

- 17) A method of determining the mating status of a female arthropod, comprising use of the expression system according to any of claims 1-12 in a transgenic male population, wherein
5 said system comprises a marker such as a fluorescent reporter protein; and where sperm is present, assaying for the presence of said marker in a female; the presence of the marker being indicative that the female has mated with a transgenic male carrying the system.