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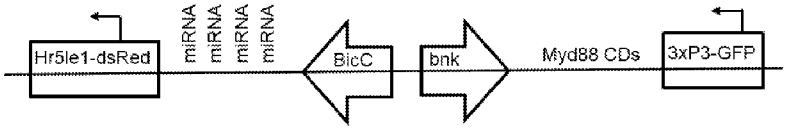

FIG. 2

(57) Abstract: An insect gene drive system for biocontrol of a population of an insect is provided. The gene drive system includes: a) a first DNA sequence encoding a toxin under the control of a maternal germline-specific promoter active in the insect, with the first DNA sequence being linked to b) a second DNA sequence encoding an antidote under the control of an early embryo-specific promoter active in the insect. The toxin is expressed in maternal germline cells of the insect and results in maternal-effect lethality in the insect, and the antidote is expressed in embryos of the insect and counters the maternal -effect lethality. In some embodiments, the insect is *Drosophila suzukii*.

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USE OF *MEDEA* ELEMENTS FOR BIOCONTROL OF *D. SUZUKII* POPULATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of Provisional Patent Application No. 62/286,946, filed on January 25, 2016, which is incorporated by reference herein.

REFERENCE TO SEQUENCE LISTING

[0002] A Sequence Listing is submitted electronically via EFS-Web as an ASCII formatted text file with the name "1279611XPCSequenceListing"; the file was created on January 23, 2017, is 9.45 kilobytes in size, and is incorporated herein by reference in its entirety.

BACKGROUND

FIELD OF THE INVENTION

[0003] The invention relates to a composition and method for a gene drive system.

RELATED ART

[0004] Spotted wing *Drosophila*, *D. suzukii,* is a pest of many small and soft fruits, including cherries, raspberries, blackberries, blueberries, strawberries, peaches, grapes, and others (Walsh et al. 2011). It damages these fruits by using its heavily sclerotized and serrated ovipositor to pierce fruits and lay eggs inside the fruit. Most of the damage caused by *D. suzukii* is a result of larvae feeding on fruit flesh. However, the insertion of the prominent ovipositor into the skin of the fruit can also cause physical damage to the fruit, as it provides access to secondary infections of pathogens – such as fungi, yeasts and bacteria – that may cause faster fruit deterioration and further losses. These damages can result in severe crop losses, and the implications for exporting producers may also be severe, depending on any quarantine regulations.

[0005] A native of eastern and southeastern Asia, *D. suzukii* has invasively spread in the last several decades, and has been recorded in China, India, Italy, Spain, Russia, and a number of other countries (Walsh et al. 2011). *D. suzukii* has also rapidly invaded the U.S. – initially found in California in 2008, it has spread to much of the Pacific Coast, to the East

Coast, and to north central and interior U.S. (Asplen et al. 2015) – and poses a significant threat to fruit industries there. For example, in 2008, *D. suzukii* caused over $38.3 million in cherry crop loss in California alone, and is predicted to result in up to $500 million in annual losses in U.S. Western fruit production areas (Goodhue et al., 2011).

[0006] Current method of control of *D. suzukii* rely heavily on the use of expensive non-insect specific insecticides (e.g., malathion), which have variable efficacy (Asplen et al. 2015) and also kill beneficial insects like pollinators (e.g., honeybees) and useful predator (e.g., green lacewings, which prey on various harmful insects like black cherry aphids and small caterpillars). As an alternative to insecticides, farmers can also attempt to trap *D. suzukii* using chemical attractants; however, no *D. suzukii* specific attractants are currently available (Walsh et al. 2011), and this approach is not particularly effective at preventing *D. suzukii* spread. And while biological control of *D. suzukii* may be possible via use of recently identified natural predators (Gabarra et al. 2015), no established means of *D. suzukii* biocontrol currently exists (Woltz et al. 2015). Overall, given the rapid spread and potential economic impact of *D. suzukii*, effective control measures are urgently needed.

SUMMARY

[0007] A *Medea* system is developed in *D. suzukii* that can be used as a form of biocontrol. Other similar systems can be built in *D. suzukii* and in related fly pests, such as in the Caribbean fruit fly, *Anastrepha suspense,* the Mexican fruit fly, *Anastrepha ludens*, the West Indian fruit fly, *Anastrepha oblique*, and other insect pests.

[0008] In one aspect, a gene drive system for biocontrol of a *Drosophila suzukii* population is provided. The gene drive system includes: a) a first DNA sequence encoding a toxin under the control of a maternal germline-specific promoter active in *D. suzukii*, with the first DNA sequence being linked to b) a second DNA sequence encoding an antidote under the control of an early embryo-specific promoter active in *D. suzukii*. In this *D. suzukii* gene drive system, the toxin is expressed in *D. suzukii* maternal germline cells and results in maternal-effect lethality in *D. suzukii*, and the antidote is expressed in *D. suzukii* embryos and counters the maternal-effect lethality.

[0009] In another aspect involving the *D. suzukii* gene drive system, a transgenic *D. suzukii* whose genome includes the *D. suzukii* gene drive system is provided.

[0010] In a further aspect involving the *D. suzukii* gene drive system, a method of manipulating a *D. suzukii* population is provided. The method includes releasing the transgenic *D. suzukii* into the population in sufficient numbers to spread the gene drive system through the population. In some embodiments, the gene drive system further includes an effector genetic element, which can result in inducible lethality in one or both sexes of *D. suzukii*, or in recessive sterility in one or both sexes of *D. suzukii*. The population can be a laboratory or wild population.

[0011] More generally, in another aspect, an insect gene drive system for biocontrol of a population of an insect is provided. The gene drive system includes: a) a first DNA sequence encoding a toxin under the control of a maternal germline-specific promoter active in the insect, with the first DNA sequence being linked to b) a second DNA sequence encoding an antidote under the control of an early embryo-specific promoter active in the insect. In this system, the toxin is expressed in maternal germline cells of the insect and results in maternal-effect lethality in the insect, and the antidote is expressed in embryos of the insect and counters the maternal-effect lethality.

[0012] In some embodiments involving the insect gene drive system, the insect is not *Drosophila melanogaster* or a flour beetle. In some embodiments, the insect can be *Drosophila suzukii*, *Anastrepha suspensa*, *Anastrepha ludens*, *Anastrepha oblique*, *Bactrocera oleae/Dacus oleae*, *Ceratitis capitata*, *Aedes aegyptii*, *Anopheles gambiae*, or any other insect in which a gene drive system may be of utility.

[0013] In a further aspect involving the insect gene drive system, a transgenic insect whose genome includes the insect gene drive system is provided. In some embodiments, the transgenic insect is not *Drosophila melanogaster* or a flour beetle. In some embodiments, the transgenic insect can be *Drosophila suzukii*, *Anastrepha suspensa*, *Anastrepha ludens*, *Anastrepha oblique*, *Bactrocera oleae/Dacus oleae*, *Ceratitis capitata*, *Aedes aegyptii*, or *Anopheles gambiae*.

[0014] In another aspect involving the insect gene drive system, a method of manipulating an insect population is provided. The method includes releasing the transgenic insect into a population of the same species in sufficient numbers to spread the gene drive system through the population. In some embodiments, the transgenic insect is not *Drosophila melanogaster* or a flour beetle. In some embodiments, the transgenic insect can be *Drosophila suzukii*,

Anastrepha suspensa, *Anastrepha ludens*, *Anastrepha oblique*, *Bactrocera oleae/Dacus oleae*, *Ceratitis capitata*, *Aedes aegyptii*, or *Anopheles gambiae*. In some embodiments, the gene drive system further includes an effector genetic element, which can result in inducible lethality in one or both sexes of the insect, or in recessive sterility in one or both sexes of the insect. The population can be a laboratory or wild population.

[0015] In any embodiment involving the *D. suzukii* gene drive system or the more general insect gene drive system:

a) the toxin can include one or more miRNAs, one or more RNA-guided endonucleases, or a combination thereof;

b) the toxin can target a gene for a maternally-deposited embryonic-essential RNA, a maternally-deposited embryonic-essential protein, a zygotically-expressed embryonic essential gene, and the like, of *D. suzukii* or another insect;

c) the target gene can be the myd88 gene of *D. suzukii* or another insect;

d) the antidote can be a toxin-resistant version of the target gene, or another gene that can substitute for/fulfill the biological role of the target gene; in some embodiments, the toxin-resistant version is a version not recognized by the toxin;

e) can further include an effector genetic element active in *D. suzukii* or other insect and linked to the first and second DNA sequences, with the effector genetic element encoding a gene conferring susceptibility to a chemical, a conditional lethal gene, a genetic element that disrupts a recessive fertility gene or recessive lethality gene, or a genetic element that disrupts a gene involved in *D. suzukii* pest behavior, such as ovipositor formation, olfaction, egg laying substrate preference and other such behaviors; or any other genetic element that reduces or eliminates the effect of *D. suzukii* pest behavior or the effect of pest behavior of another insect; or

f) any combination of a) - e).

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] For a more complete understanding of the present invention, reference is now made to the following descriptions taken in conjunction with the accompanying drawings, in which:

[0017] Figure 1 is a panel describing *Medea* genetics. (1A) Heterozygous females carrying *Medea* cause death of all offspring that fail to inherit *Medea*. (1B) Synthetic *Medea* elements contain two genes - maternally expressed miRNAs (the toxin) that silence the expression of a maternally expressed transcript (top line) that normally provides a product essential for early embryonic development, and a zygotic antidote consisting of the silenced maternal mRNA (resistant to the miRNA toxin) sufficient to rescue normal development (vertical line). Adapted from Akbari et al. 2012.

[0018] Figure 2 is a schematic drawing of a portion of a P transposable element vector including, 5' to 3', a Hr5Ie1-drived dsRed marker, miRNAs driven by the BicC promoter, recoded Myd88 CDs driven by the bnk promoter, and a 3xP3-GFP marker.

DETAILED DESCRIPTION

[0019] An alternative approach that would complement current control methods would be the use of genetically engineered *D. suzukii* as a biological control agent. Use of genetically modified insects for wild population manipulation was first suggested many decades ago (Serebrovskii 1940; Hamilton 1967; Curtis 1968), and has garnered a considerable amount of interest since (Burt 2014). Proposed methods typically rely on the use of engineered gene drives based on "selfish" genetic elements (SGEs) that function by forcing inheritance in a non-Mendelian fashion, allowing them to increase in frequency with each generation even without conferring a fitness advantage upon their hosts (Burt 2014; Alphey 2014; Bull 2015). Such methods can be utilized to spread desirable genes linked to the gene drive through a population or to suppress target insect populations (Sinkins and Gould 2006).

[0020] One type of gene drive system is the maternal effect dominant embryonic arrest (*Medea*). *Medea* was first discovered in the flour beetle (Wade and Beeman 1994), and multiple versions were later reverse engineered from scratch and shown to act as robust gene drives in the laboratory fruit fly, *Drosophila melanogaster* (Chen et al. 2007; Akbari et al. 2013). Such engineered *Medea* systems rely on a *Medea* element consisting of a toxin-

antidote combination. The toxin consists of a miRNA that is expressed during oogenesis in *Medea*-bearing females, disrupting an embryonic essential gene. A linked antidote is expressed early during embryogenesis and consists of a recoded version of the target gene that is resistant to the miRNA. This combination results in the survival of half of the embryos originating from a *Medea*-bearing heterozygote female, as those that do not inherit the *Medea* element die. If a heterozygous *Medea* female has mated with a heterozygous *Medea* male, the antidote from the male will also take effect in the embryo, resulting in 3/4 of the embryos surviving (Fig. 1). Therefore, *Medea* will rapidly spread through a population, carrying any linked genes with it.

[0021] In the case of *D. suzukii*, since elimination of the pest population is ultimately of interest, an engineered *Medea* system could spread a gene proffering susceptibility to a particular pesticide, or a conditional lethal gene that would be activated by some substance or environmental cue such as diapause (a state that allows insects survive periods of adverse conditions such as cold; Clark et al. 2008). For example, a *Medea* element can be used to spread a gene conferring sensitivity to a particular chemical that is normally innocuous, rendering such a chemical capable of being used as an environmentally-friendly, species-specific pesticide. Trigger-inducible transcription control elements – ones that turn on expression in the presence of a chemical such as tetracycline or vanillic acid (Urlinger et al. 2000; Gitzinger et al. 2012) – can be engineered to drive expression of an insect-specific toxin (e.g., Fu et al. 2007). A *Medea* element can also be used to spread a gene under the control of a diapause-induced promoter that will splice to produce a toxin in females only, so that, upon the onset of the diapause-inducing environmental cue, all of the females will perish, causing a population crash (Akbari et al. 2013). Further, if a *Medea* element is inserted into a fertility gene, it could cause a population crash by spreading through a population and making it infertile as it does. However, although transgenesis of *D. suzukii* has been established (Schetelig et al. 2013), no gene drive systems in this major pest have yet been engineered.

[0022] In embodiments of the *D. suzukii* gene drive system or the more general insect gene drive system described herein, a toxin can be an miRNA or an RNA-guided endonuclease.

[0023] MicroRNAs (miRNAs) mediate the RNAi pathway. The term "microRNA" or "miRNA" as used herein indicates a class of short RNA molecules of about 22 nucleotides in

length, which are found in most eukaryotic cells. miRNAs are generally known as post-transcriptional regulators that bind to complementary sequences on target mRNA transcripts, usually resulting in translational repression and gene silencing. miRNAs are encoded by miRNA genes and are initially transcribed into primary miRNAs (pri-miRNA), which can be hundreds or thousands of nucleotides in length and contain from one to six miRNA precursors in hairpin loop structures. These hairpin loop structures are composed of about 70 nucleotides each, and can be further processed to become precursor-miRNAs (pre-miRNA) having a hairpin-loop structure and a two-base overhang at its 3' end. In the cytoplasm, the pre-miRNA hairpin is cleaved by the RNase III enzyme Dicer. Dicer interacts with the 3' end of the hairpin and cuts away the loop joining the 3' and 5' arms, yielding an imperfect miRNA:miRNA duplex about 22 nucleotides in length. Overall hairpin length and loop size influence the efficiency of Dicer processing, and the imperfect nature of the miRNA:miRNA base pairing also affects cleavage. Although either strand of the duplex can potentially act as a functional miRNA, only one strand is usually incorporated into the RNA-induced silencing complex RISC where the miRNA and its mRNA target interact.

[0024] An RNA-guided endonuclease is a nuclease such as CRISPR-associated 9 (Cas9) or Cpf1, that is directed by guide RNAs to target and cleave specific nucleotide sequences either in DNA or RNA. Such an endonuclease may be used to cleave or silence a target gene RNA similarly to an miRNA. Examples of RNA-guided endonucleases include, but are not limited to, Cas9, C2C2, Cpf1, Cas13a, Cas13b, and any other suitable Cas-type proteins.

[0025] Other toxins include siRNAs and maternally supplied protein toxins (e.g. cell death genes, restriction endonucleases, insect toxins, e.g.,barnase).

[0026] The particular toxin that is used will depend on the selected target. A toxin can be prepared by standard molecular biological methods.

[0027] Examples of maternally-deposited embryonic-essential RNAs or proteins for use as toxin targets include, but are not limited to, myd88 (NCBI Gene database, Gene ID: 35956), Groucho (NCBI Gene database, Gene ID: 43162), DAH (NCBI Gene database, Gene ID: 32459), O-fut1 (NCBI Gene database, Gene ID: 36564), homologs of such promoters in other insects, and other *D. suzukii* and insect promoters identified by analyzing expression data. In some embodiments of the *D. suzukii* gene drive system or the general insect gene drive system, the toxin target is not myd88 but is another target.

[0028] The antidote can be a modified version of a target. The modified version can include nucleotide sequence changes that prevent an miRNA or RNA-guided endonuclease toxin from binding to the modified target sequence. The particular antidote can be prepared by standard molecular biological methods and will depend on the selected target. Examples of other types of antidotes include, but are not limited to, zygotically expressed RNAi, CRISPR, or siRNA to the toxin when the toxin is a protein.

[0029] Examples of maternal germline-specific promoters include, but are not limited to, bicoid, vasa, deadhead, zpg, and exu promoters, homologs of such promoters in insects, and promoters of other genes expressed specifically in the female germline.

[0030] Examples of early embryo-specific promoters include, but are not limited to, bottleneck, malpha, twi, ocho, and tin promoters, homologs of such promoters in insects, and promoters of other genes expressed specifically in the early embryo.

[0031] For a *D. suzukii* gene drive system or general insect gene drive system that contains an effector genetic element, the components of the system can be organized in various arrangements depending on the nature of the effector element. For example, the effector element can be located downstream of, or between, the first and second DNA sequences, or the first and second DNA sequences can be located within the effector genetic element. Other arrangements are possible as long as the first and second DNA sequences and the effector genetic element sequences are present.

[0032] The *D. suzukii* gene drive system or general insect gene drive system can be present, for example, in a DNA construct, in a nucleic acid vector such as a cloning vector or a P transposable element vector, or *in vivo* in an insect.

[0033] Although *D. melanogaster* and *D. suzukii* are closely related, in attempting to transfer the technology described here from the former to the latter, there was no reasonable expectation of success in preparing a *D. suzukii* gene drive system, and the results described here are unexpected. This is due to two primary reasons.

[0034] Firstly, although *D. suzukii* is a significant threat to agricultural output on a global level (Asplen et al. 2015), it is rather poorly studied compared to the extremely well-understood *D. melanogaster*, and few genetic tools that allow basic transgene construction and precise genome manipulation have been developed. A draft genome was assembled in

2013 (Chiu et al. 2013), and since then, transgenesis has been demonstrated once (Schetelig and Handler 2013) and a single study has shown that direct injection of CRISPR/Cas9 can result in mutation (Li and Scott 2015). This represents a very small genetic toolkit, especially when compared to other insects where gene drive is of interest, such as mosquitoes (e.g., Esvelt et al. 2014). *D. suzukii* can be more difficult to work with in the laboratory than *D. melanogaster* as it has different temperature and other environmental needs (Kinjo et al. 2014), making fewer researchers willing to work with it. The lack of genetic tools and the potential difficulties in rearing the insect possibly explain why no transgenic gene drive components, let alone functional gene drive systems, have yet been created in *D. suzukii*, despite the need for such elements.

[0035] Additionally, the *Medea* gene system itself is quite difficult to engineer in any organism, especially in one lacking a complete genetic tool kit, when compared to other gene drive systems. As outlined, for example, in Champer et al. (2016), *Medea* systems have not yet been developed in mosquitoes or other insects besides *D. melanogaster*, despite researcher efforts. This is due, in part, because a *Medea* system such as the one demonstrated by Chen et al. (2007) requires effective RNAi-mediated silencing in the germ line, which has been difficult to achieve in species other than *D. melanogaster*, and also because such a system depends on identification and functional characterization of maternal and zygotic promoters, embryonic essential target genes, and other genetic elements, that is lacking in many target species (including *D. suzukii*). Simply put, the type of Medea system developed in *Drosophila melanogaster* is not highly portable to different organisms.

[0036] Therefore, the success of such a system in a pest species is unexpected given the knowledge regarding said system in the field of gene drive.

[0037] Having demonstrated the transfer of the *Medea* system to *D. suzukii*, however, the transfer of the system to other insects is now expected to be successful because the requisite tools (germline RNAi, RNA CRIPSR, etc.) can now be made available, and the proof of principle for *D. suzukii* described herein shows that attempting this in other insects with similar conserved genes is now feasible.

[0038] The present invention may be better understood by referring to the accompanying examples, which are intended for illustration purposes only and should not in any sense be construed as limiting the scope of the invention.

EXAMPLE 1

Methods

Generation and testing of *D. suzukii Medea*

[0039] To create a *Medea*-like maternal-effect selfish genetic element in *D. suzukii*, synthetic *Medea* elements were engineered based on the same architecture used to generate the *Medea*^{myd88} system previously built in *D. melanogaster* (Chen et al. 2007). In *D. melanogaster*, maternal Myd88 is required for dorsal-ventral patterning in early embryo development, and germ-line loss-of-function myd88 mutant females produce embryos that fail to hatch (Kambris et al. 2003). Myd88 is highly conserved in *D. suzukii* (and in many other Drosophilids), and it was reasoned that Myd88 would likely be essential in this species, as well.

[0040] Briefly, a P transposable element vector was generated in which the predicted *D. suzukii* female germ-line–specific bicoid (bic) promoter drives the expression of a "toxin" containing three synthetic microRNAs (miRNAs) designed to target the 5' untranslated region (UTR) of *D. suzukii* myd88. (The synthetic miRNAs were generated using the mir6.1 backbone, as described by Chen et al. 2007.) The vector was generated by cloning DNA pieces generated by PCR into a standard Drosophila melanogaster P element vector via Gibson assembly (Gibson DG, Young L, Chuang RY, Venter JC, Hutchison CA 3rd, Smith HO (2009). "Enzymatic assembly of DNA molecules up to several hundred kilobases". *Nature Methods*. **6** (5): 343–345). The vector also contains an "antidote" transgene containing *D. suzukii* myd88, recoded to be insensitive to the miRNAs, expressed under control of the early embryo–specific bottleneck (bnk) promoter (Schejter et al. 1993). The vector also contained two separate transformation markers – GFP under control of the eye-specific 3xP3 promoter (Berghammer et al. 1999), and dsRed under control of the ubiquitous opie2 promoter (Theilmann and Stewart, 1992) (Fig. 2).

[0041] The sequences of the miRNAs used are as follows, with guide and passenger strands capitalized:

a) miRNA 1:

tttaaagtccacaactcatcaaggaaaatgaaagtcaaagttggcagcttacttaaacttaatcacagcctttaatgtAAAATTAA
AAAAAAATAGTACTAtaagttaatataccatatctaTATTACTATTTTTTTTTAATTTTgtacctaaag
tgcctaacatcattatttaatttttttttttttttggcacacgaataaccatgccgtttt (SEQ ID NO. 1)

b) miRNA 2:

ctttaaagtccacaactcatcaaggaaaatgaaagtcaaagttggcagcttacttaaacttaatcacagcctttaatgtTCCCGCGC
TTCATCGTTTTCTTtaagttaatataccatatctaAATAAAACGATGAAGCGCGGGAgtacctaaagtg
cctaacatcattatttaatttttttttttttggcacacgaataaccatgccgtttt (SEQ ID NO. 2)

c) miRNA 3:

atttaaagtccacaactcatcaaggaaaatgaaagtcaaagttggcagcttacttaaacttaatcacagcctttaatgtACGTCCCG
TTGATAAATACCTAtaagttaatataccatatctaTATGTATTTATCAACGGGACGTgtacctaaagtg
cctaacatcattatttaatttttttttttttggcacacgaataaccatgccgtttt (SEQ ID NO. 3)

[0042] The sequence of the miRNA-expressing promoter (predicted bicoid promoter) used is as follows:

CTGCTGAAACCATCGGCGTAAACCTCTAATTAAGGCTAGTAACCTTTGTAGAAAA
TTATTTAGTTTATATTTTTAAACATAAATTATTTTTGAAATTGTAACTAAAAATGT
ATGCCTATTTTAAAAATTCCTTCNTAAGAAAATTAGTTTAAGTAGTACACTTTTGA
CGCTCACTGTATCAAAATTTTTCTGGAGCGCCATCTGGGGAGCTTACTCAATTTC
AAAAGCTTTACTTTACTTAGGTAAAGGGCCGAAGATAAAAGCTGACGTAGGATT
TTAACTGAATGGGAGCTTTCTAAGGTTGTTTATGCACTGGAGAGATAGTAGATAA
ATGCACTTCCCACAGAACCCAGAGCTTTCGGATCTGAAGGTCAGTTGGGACTTGG
ACCCACAAGTCGAGCTTAGTTTAATAGTCAGCGCGCTTAAACGACGACAACTGC
ACGGCGCGCCCCCATTAATAATATATATTTTTCCAAAAATAAACTTAAAAAAAAT
AATTAAATAAAAAATAATATTAACATTGAACGCGCGGTGCACGATTTTTTGACAA
CAATTCAGTTTCGCTTTTCCTTAGATTTCCATATAATTTTTTCCTTTGTGTTTTCCA
CACACGTGGTGTTCTTCTGTTACTCGCACTTCGGTAGTGGTTATTTTTTTTGGGTT
ACATTGAAAAAAGTACATCGACTGCCAGCCGAATTCCCATTCGAACCTTAATTTG
ACCAATTGACGGAAAATTAATTGCGCACAAAATTAATTAGCAAGCGAATATATA
TTTTTTTTTTTACGTGAAACGAGCGTGTTATTTGTCAAAAAGATTTTACAACTGAT
TGTGTTTAAGTAAATTAACCTGAGATATATCTATGTGTTTGTGCTACGATCCGAA
ATTCCAATATTGCGGTAAGTGGTGTGATTTTTGGCGGGAATAAAATCGGTTTCCC
TTTGCGTCGTATTTCTCTCCCGATTTTTCTGGCGCCAAAATGAATGAAAACGGTTG
GCAACATTTTCAACTTAACATGGCAACATGCGAATCTAACCGCTGTTAGGGGAAC
TTGTAAGGGAACATGTTAGAAATTCTGGAACACCTCTCCAACTTTGTTTTGTTTGG
CGCTCTCTCTTTGAAATGTAGAATCTCTTATTTTGGAACGGGAGCGGTTCATTGAC

CCAGCAACAACAATATTCGCCATATCTTCTCCTTCCCAGCGATTTGCAGAATTCA
TCCGTACTACAGTTAGAAATAATTCTAGTCCAACAGTAAGCAAAGTCGATCGGCG
AAATCGGGAGCCGCGTTCAGTTGCCTTCGAGCCGTACTTAGAAGTGGTAGAGCCC
CCAGGAATCGGAGAGATTATCCCGTAACTTGATACTCTGACGAAATCCCCCAGAC
AGACCCCTCGCAATCACACACAGCTATATACTGATATATAGATCTATATATATTG
CGCAGATCACCCTTTAAATGCCTTTCGTCCAGAGATTCCAACAAATACGTAATTT
TGTTATGAACGTCTGCCTCGTCGAGGCCATCCAATTCGTGCAGGACCTCACGCAT
GCCCTCTGCCTGGACCTCCAGGGCCTGTTCAACCTCAAGTAGAGATTCCCGAGCG
ATCTCCCAGCGGTAAGTCACGCCATTTGGCCGCGGGCCGCGAAAAGTGAAAGTG
CGGGAGAAATTACGGTTATCGGGTTTTCGGGAGAAAGGGGAAAGTGGAAACCTC
ATGGCAGCAGCCCTCCATTTTGAGAGGTTATGTCAAAGGAAAGGAAGAGAAAGG
AAGTTAAAAAATGTTAGCAAGGAAAATATTAGCCTTCGTTTTTCATAAGTACACC
AATAAACCACATTTTACTGGGTGTCAAAAGTTAGAGAATTAAACAGCAAGTTAC
CAAAATTAGTTTGAAGGCAAAACTTTAGCTAGTTACGTTTTTAGAGGGTTCTGGA
ATTACAAATGCCCACAGCAGGAGAAATATAAATTTGTGTTTTCATTTTAAACAGA
TTATGTTTTAAAAAGTAATACATCAAAATTGACTTTAGCCGTTTTTTAAATAGGTA
ATTTGGTATATAAACATACATTTTTTAATAAAAATATGAACACTTTGTTTTTATTT
TTGTACTGGTAGGCAAACTACAAGATATAACTGTTAAATAAAAAAAGATTTTAA
GTGTAGGCAATAAAAAATCAAATCAAAAATATATTTTGAATAAAAGTGAAGGTC
AAAAATGAAAGACTTCAAAGTACCACTCTACTCATGAAGCTATAATAATAATGG
GTAAATAAACAGTATATTACATGTTCGTTTTAAATATTTTCAATGCAAAAATGCC
CTGCTTATATTAAGCTTTACTTTCACCACAACTGTTCCCATCATCTATTTTTAATAT
TATTGACCTCGGACTTCTTACATTTCATCGCCAGCTCATCCCATCAATCACCCAGA
TAAACTACCACCTTCATTAACACACCGCAAGCATCTCACAACGCTGACCAATAAA
CGCCTCGACATCCAGAATCCCCAACTGAGACGGACGTTTTGCTTGGTTTTTAAAA
CTAAACGACCCTTTGGTAGCCGCACCTATTGACCTTTCACACATTCATATCCCTCG
GTCCACAAGCCGACCCACTGATCCATTCATTCACCTCCCACG (SEQ ID NO. 4)

[0043] The antidote transgene sequence used, including the bottleneck promoter, MYD88 Coding Sequence, and SV40 3' UTR, is as follows (MYD88 coding region capitalized):

atgaaccctattaagatcccgacacctcttttcgcctttctgcataataatttcgccattccaccgctgtataattgcgtattcgccttcagtaa
atgaaaattgcagttgtattttataatttcgtttttcattttccctctctcattttctacgtctgtttggccagctgtcaattgccgagtgtcgactg
aattattggccattaattgcattgattttgattgtaatgaaccagctaatgaaacgtcaacagctaagagtgggccataaaacccggagga

attatcttctagaaaccttgaacatctgtcgcgaatttaagttttaagctgtttatgaattagtaccctcgctaatccttttcgaaaggaccttat
aaaagtgcgcggattagcaaaaagatttgtgtaaaattggtgcgcggaaaacacgcaaaattcgcgtgctcgggttgcaattaccggg
aaatgtgggccttttttacttgcagggcttaggtagtttcccggaatggagtcaagctaagctagccaggtacctgagttccccggacca
gctgtccggtgctgaaactagagcaggtagtccccagaatcccagctatataaggcctgccttcctggcaacagcatcacattcgttcat
cagccttcaaccctaggATGCGCCCTCGATTTGTATGCCATCAGCAGCACTCGGTGGCCCA
TTCCCACTTCCATCCCCTGTCTCACTTCCAGACCCATTCCCATACCCATACCCACT
TCCAGCGCCATCCCAATCCCCATCCCCATCCCCATCACTTTTACGACGCCACTGA
CGTCGGCTATCGGCGTTATCGCACCGCCAACATGGTGGTGGCCGAGGGAGTTATG
GACTCCGGATCGGGGATCGGTACGGGAATGGGAATGGGGCACTTCAACGAGACC
CCCTTATCCGAGCTGGGCGTGGAGACCCGCTCGCAGCTGTCCCGCATGCTGAACC
GCAAGAAGGTGCTGCGCTCCGAGGAGGGCTACCAGCGGGACTGGCGGGGCATCT
CGGAGCTGGCCAAGCAGAAGGGATTCGTCGACGAGAACGCCAACAATCCCATGG
ATCTGGTGCTGATCAGCTGGAGCCAGCGGAGCCCGCAAACCGCCAAGGTGGGCC
ATCTCGAGAACTTCCTGGGCATCATCGATCGGTGGGATGTCTGCGACGATATCCA
GGAGAATCTGGCCAAGGACACCCGGCGCTTCATCATAAAGCAGGAGCAGCGGCA
GACCTCTCTGGTGGAGGCGTGTCCCCCGCCCCCCAGCGACTGTTTCGAGACCAAC
AACAACTACAGCAGCAACAACAACATCACAGTGGGCCAAAGTGTCCAGATCCTG
AGCGACGAGGATCAGAGATGTGTGCAGATGGGCCAACCGCTGCCCAGATACAAT
GCCTGTGTCTTGTACGCAGAGGCAGACATCGATCATGCCACCGAGATCATGAATA
ACCTAGAGTCTGAGCGATACAATCTCAGGCTTTTCCTGCGCCATCGCGACATGCT
AATGGGCGTACCCTTCGAGCATGTCCAACTCTCCCACTTCATGGCCACCCGCTGT
AATCACCTGATCGTCGTGCTCACAGAGGAGTTTCTTCGGAGTCCGGAGAACACGT
ACCTCGTGAACTTCACCCAGAAGATACAAATCGAGAACCACACTCGCAAGATCA
TACCGATTCTGTACAAGCCAGACATGCACATACCCCAGACCCTGGGCATCTATAC
GCACATCAAGTACGCCGGGGACTCCAAGTTGTTCAACTTCTGGGACAAGTTGGCT
CGATCGCTGCACGATCTGGATGCCTGTTCCATTTACTCCACGCGCCAGGTGCAAA
CACCCTCGCCAGTGGAGGAATCGACTCCCCAGCGGGTGACCACGCCCAGCATTC
GGATACAGATCAACGACAAGGATGTGACCGACATGCCCAACTTCAAGGTGCCGG
AGGCGGAGACCACCATCGTTTCGGTATCCGGCGATACCGGTTCCCCTCTGCCGGA
ACACAAGCCGAAGAAAAAGGATCGCTTTCTGCGAAGAATCACGCACAGTTTCGC
CAAGACACCGAAGAATGAGGGGAGCAGTGCGAAGACCCTGCGACACGCGCACT
CCGTCAGCACCATAAATGTCACGGAACGGGAGAGGACACTCAGTGCCAGCAGCT
CCAATATCTCCACCACCTCGGAGAGCAAGAAGAGCTTCATCAAGTGGCAGCCGA
ATATCCTGAAGAAGGCCCTATTCTCCCGATCCAGCAACAAGCTACAGACGCCGG

GTTGAcatatggatctttgtgaaggaaccttacttctgtggtgtgacataattggacaaactacctacagagatttaaagctctaaggt
aaatataaaatttttaagtgtataatgtgttaaactactgattctaattgtttgtgtattttagattccaacctatggaactgatgaatgggagca
gtggtggaatgcctttaatgaggaaaacctgttttgctcagaagaaatgccatctagtgatgatgaggctactgctgactctcaacattcta
ctcctccaaaaaagaagagaaaggtagaagaccccaaggactttccttcagaattgctaagtttttgagtcatgctgtgtttagtaataga
actcttgcttgctttgctatttacaccacaaaggaaaaagctgcactgctatacaagaaaattatggaaaaatatttgatgtatagtgccttg
actagagatcataatcagccataccacatttgtagaggttttacttgctttaaaaaacctcccacacctcccctgaacctgaaacataaaa
tgaatggaattgttgttgttaacttgtttattgcagcttataatggttacaaataaagcaatagcatcacaaatttcacaaataaagcatttttt
cactgcattctagttgtggtttgtccaaactcatcaatgtatcttatcatgtctggttccaagcgctccgtacgcgtatcgataagctttaaga
tacattgatgagtttggacaaaccacaactagaatgcagtgaaaaaaatgctttatttgtgaaatttgtgatgctattgctttatttgtaaccat
tataagctgcaataaacaagttaacaacaacaattgcattcattttatgtttcaggttcagggggaggtgtgggaggttttttaaagcaagt
aaaacctctacaaatgtggtatggctgattatgatctagagtcgcgg (SEQ ID NO. 5)

[0044] The Genbank accession number for the *D. suzukii* MYD88 sequence is GenBank: KI419649.1 (incorporated by reference herein).

[0045] The Accession number for the *D. suzukii* genome sequence is Dsuzukii.v01 [GCA_000472105.1] (incorporated by reference herein).

[0046] The vector, along with a source of transposase, was injected into *D. suzukii* embryos using standard injection procedures as described in The Use of P-Element Transposons to Generate Transgenic Flies (Bachmann and Kust, Chapter 4) from *Methods in Molecular Biology:* Drosophila: *Methods and Protocols* Edited by: C. Dahmann © Humana Press Inc., Totowa, NJ, and the surviving G0 adults were individually outcrossed to wild type (WT) individuals. G1 progeny were screened for the presence of the *Medea* element (as evidenced by ubiquitous dsRed expression), and one G1 transformant male was recovered. When outcrossed to WT virgin females, the male produced 50% *Medea*+ and 50% WT individuals (consistent with Mendelian transmission ratios). The *Medea*+ G2 progeny were further individually outcrossed to WT individuals of the opposite sex. From these crosses, the males (n=3) gave rise to ~50% *Medea*+ progeny; the females (n=9) gave rise to 100% *Medea*+ progeny, which is consistent with the *Medea* element functioning as a maternal effect dominant embryonic arrest system. Of the G3 *Medea*+ progeny, nine males and 32 virgin females were further individually outcrossed to WT. The males have rise to 50% *Medea*+:50% WT individuals, and male outcrosses were discontinued at this stage. The females all gave rise to 100% *Medea*+ offspring (with a mean=9.34 G4 progeny recovered).

[0047] Fifty G4 heterozygous *Medea*+ virgin females were further individually outcrossed to WT males, and all of their progeny (n=603) were *Medea*+. Thirty-one resulting G5 heterozygous *Medea*+ virgin females were then outcrossed further. In this outcross, a small number of progeny that were negative for the *Medea* element were recovered for the first time, indicating that the system did not function at 100%. Of the 31 G4 crosses that gave rise to scorable progeny, eight G4 females produced a small number of *Medea*– offspring (ranging from one to seven per female), while 23 gave rise to 100% *Medea*+ progeny. Although a *Medea* system that works perfectly would be ideal, one that gives rise to mostly *Medea*+ progeny would still be expected to spread through a population. In this case, of the total G5 progeny, almost 97% (n=785) were *Medea*+, demonstrating that the system functions very efficiently, if not ideally.

[0048] Overall, when all generations were summed together, the percentage of *Medea*+ progeny arising from single heterozygous female outcrosses was nearly 99, with 1788 *Medea*+ progeny out of 1813 total (summarized in Table 1).

Table 1

Generation	Sex (# crossed out)	Number of Progeny	% Medea
G2	Female (9)	126	100%
	Male (3)	45	50%
G3	Female (32)	299	100%
	Male (9)	130	50%
G4	Female (50)	603	100%
G5	Female (31)	785	97%
Total Medea+ from females		1788/1813	99%

[0049] To assess whether the *Medea* system would function well in geographically distinct populations (which likely harbor genetic variability, especially in regions of the genome that canonically have less conservation, such as the 5'UTR regions), heterozygous *Medea*+ flies were individually introgressed with nine geographically distinct *D. suzukii* populations, and resulting *Medea*+ heterozygous virgins were outcrossed to males from the nine populations to determine whether the *Medea* element functioned appropriately. Although the rate of inheritance was not 100% (as would be expected if the *Medea* element functioned perfectly), it was an average of 96%, which is more than sufficient to bring about gene drive (data summarized in Table 2 below). Therefore, the developed *Medea* system is a gene drive tool for functioning globally, in diverse populations of the *D. suzukii* pest.

Table 2

Strain origin	# of progeny	% Medea +
Clayton, WA	152	88%
Watsonville, CA	62	100%
Brentwood, CA	251	94%
Enime, Japan	133	100%
Oahu, HI	167	99%
Beltsville, MD	100	98%
Oregon	155	100%
Tracy, CA	283	88%
Maryland	16	100%
Total	**1319**	**96%**

Population cage experiments

[0050] To determine whether the generated *D. suzukii Medea* is capable of spreading through populations, population cage experiments were set up as follows. Heterozygous *Medea* (*Medea*/+) males and WT (+/+) males were allowed to mate with WT (+/+) females in proportions of 25 *Medea*/+ males: 25 +/+ males: 50 +/+ females (for an allele frequency of ~12.5%) and 30 *Medea*/+ males: 20 +/+ males: 50 +/+ females (for an allele frequency of ~15%). Additionally, heterozygous *Medea* (*Medea*/+) males were allowed to mate with WT (+/+) females in proportions of 50 *Medea*/+ males: 50 +/+ females (for an allele frequency of ~25%), and homozygous *Medea* males (*Medea*/ *Medea*) were allowed to mate with WT (+/+) females in proportions of 50 *Medea*/ *Medea* males: 50 +/+ females (for an allele frequency of ~50%). The total number of flies for each starting population was 100. After being placed together, adult flies were removed after exactly seven days. After another seven days, progeny were collected and separated in half arbitrarily. One half was counted, while the other half was placed in a new bottle to continue the simulation, and this process continued throughout the duration of the experiment. All experiments were conducted in triplicate. All fly experiments were carried out at ~20°C with ambient humidity in 250 ml bottles containing a fly medium prepared based on a recipe from USDS.

[0051] These experiments are ongoing; however, when released at a higher threshold, the *D. suzukii Medea* system does appear to demonstrate gene drive (drive experiments continuing for several more generations should confirm these results). Given the observed genetic behavior of the present *Medea* system, it is anticipated that the *Medea* element will spread through the experimental populations in the predicted manner given sufficient time.

Theoretical framework

[0052] Previous theoretical analyses have shown that *Medea* elements can spread when introduced into the population at allele frequencies from 2.5% to 25%, depending on the fitness cost (Ward et al. 2011; Marshall and Hay 2013), and population cage experiments in *D. melanogaster* have practically shown *Medea* fixation with release frequencies of 25% (Chen et al. 2007; Akbari et al. 2012). These numbers, while high, are relatively small compared with those associated with classical sterile male release in other insects, e.g., up to ~10^9 in the case of Mediterranean fruit flies (Dyck et al. 2005).

[0053] Mathematical analyses have also shown that *Medea* elements can be used for purposes of population suppression. For example, Akbari et al. (2012) have demonstrated that a *Medea* system comprising a lethality cassette that kills females upon some environmental cue can be used to bring about population suppression by causing a cue-induced population crash.

[0054] Preliminary modeling as described herein has also indicated that a *Medea* element can induce a population crash when inserted into a male or female recessive fertility gene. In this scenario, heterozygous *Medea*-bearing individuals of one sex would be fertile, while homozygous individuals of that sex would be recessively sterile. Numerous male and female recessive fertility genes have been identified in *D. melanogaster* and are likely to be conserved in *D. suzukii*. A *Medea* element could be inserted into such a gene to disrupt it using a number of techniques, e.g., CRISPR-Cas9 homology directed repair (Mali et al. 2013). In this way, the *Medea* element would be inexorably linked to the mutation in the fertility gene, and individuals possessing two copies of the *Medea* element would necessarily be sterile.

Discussion

[0055] In brief, the engineered *D. suzukii Medea* element discussed here (and other similar elements) allow for manipulation of wild *D. suzukii* populations in a number of ways. Chiefly, such *Medea* elements enable the construction of a *D. suzukii* strain that could be released into the wild to suppress/eradicate the wild population of *D. suzukii*, by, for example, eliminating the production of females or rendering females or males sterile. A primary appeal of such an approach is that it is 100% insect specific, with only *D. suzukii* being targeted. Another benefit of this approach is that it is catalytic: modest numbers of engineered insects would need to be released into the wild population for the relevant transgenes to spread into the population and cause a population crash. An important consequence of the fact that the system relies on the engineered insects to do the work of suppression generation after generation is that it is cheap, with only a few releases resulting in suppression of the species on an ongoing basis, as compared to the use of insecticides, which need to be applied on a regular basis.

[0056] Although the system described here is based on the Myd88 gene, other *Medea* systems targeting a variety of genes can be constructed (e.g., Akbari et al. 2012), and should

function in the same manner. Second-generation *Medea* systems that can counteract the population suppression effects of the first system can also be generated, as discussed elsewhere (Chen et al. 2007; Akbari et al. 2012). Additionally, *Medea* systems can be developed using an RNA-guided endonuclease (instead of miRNAs) as the toxin designed to maternally target the mRNA of a maternally deposited embryonic essential gene, preferably in multiple places to reduce the generation of resistance alleles. And, *Medea* elements can also be engineered in other species of interest. For example, a *Medea* system of the kind described here can be generated in a number of fly pests related to Drosophila, such as in the Caribbean fruit fly, *Anastrepha suspensa,* the Mexican fruit fly, *Anastrepha ludens*, the West Indian fruit fly, *Anastrepha oblique*, the olive fruit fly, *Bactrocera oleae/Dacus oleae*, the Mediterranean fruit fly, *Ceratitis capitata,* and other insect pests.

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[0057] Although the present invention has been described in connection with the preferred embodiments, it is to be understood that modifications and variations may be utilized without departing from the principles and scope of the invention, as those skilled in the art will readily understand. Accordingly, such modifications may be practiced within the scope of the invention and the following claims.

[0058] Use of the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise.

[0059] Unless defined otherwise or the context clearly dictates otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

CLAIMS

What is claimed is:

1. A gene drive system for biocontrol of a *Drosophila suzukii* population, comprising

a first DNA sequence encoding a toxin under the control of a maternal germline-specific promoter active in *D. suzukii*, and linked to

a second DNA sequence encoding an antidote under the control of an early embryo-specific promoter active in *D. suzukii*,

wherein the toxin is expressed in *D. suzukii* maternal germline cells and results in maternal-effect lethality in *D. suzukii*, and the antidote is expressed in *D. suzukii* embryos and counters the maternal-effect lethality.

2. The gene drive system of claim 1, wherein the toxin comprises one or more miRNAs or RNA-guided endonucleases, or a combination thereof.

3. The gene drive system of claim 1, wherein the toxin targets a gene for a *D. suzukii* maternally-deposited embryonic-essential RNA or protein.

4. The gene drive system of claim 3, wherein the target gene is myd88.

5. The gene drive system of claim 3, wherein the antidote is a toxin-resistant version of the target gene.

6. The gene drive system of claim 5, wherein the toxin-resistant version is not recognized by the toxin.

7. The gene drive system of claim 1, wherein the maternal germline-specific promoter is a bicoid promoter.

8. The gene drive system of claim 1, wherein the early embryo-specific promoter is a bottleneck promoter

9. The gene drive system of claim 1, further comprising an effector genetic element active in *D. suzukii* and linked to the first and second DNA sequences, wherein the effector genetic element encodes a gene conferring susceptability to a chemical, a conditional lethal gene, a

genetic element that disrupts a recessive fertility gene or recessive lethality gene, or a genetic element that disrupts a gene involved in *D. suzukii* pest behavior.

10. Transgenic *D. suzukii* having a genome comprising the gene drive system of any one of claims 1-9.

11. A method of manipulating a *D. suzukii* population, the method comprising releasing a transgenic *D. suzukii* having a genome comprising the gene drive system of claim 9 into the population in sufficient numbers to spread the gene drive system through the population.

12. The method of claim 11, wherein the effector genetic element results in inducible lethality in one or both sexes of *D. suzukii* or results in recessive sterility in one or both sexes of *D. suzukii*.

13. A method of manipulating a *D. suzukii* population, the method comprising releasing the transgenic *D. suzukii* of claim 10 into the population in sufficient numbers to spread the gene drive system through the population.

14. A gene drive system for biocontrol of a population of an insect, comprising

a first DNA sequence encoding a toxin under the control of a maternal germline-specific promoter active in the insect, and linked to

a second DNA sequence encoding an antidote under the control of an early embryo-specific promoter active in the insect,

wherein the toxin is expressed in maternal germline cells of the insect and results in maternal-effect lethality in the insect, and the antidote is expressed in embryos of the insect and counters the maternal-effect lethality, and

wherein the insect is not *Drosophila melanogaster* or a flour beetle.

15. The gene drive system of claim 14, wherein the toxin is an miRNA or an endonuclease.

16. The gene drive system of claim 14, wherein the toxin targets a gene for a maternally-deposited embryonic-essential RNA or protein of the insect.

17. The gene drive system of claim 16, wherein the target gene is myd88.

18. The gene drive system of claim 16, wherein the antidote is a toxin-resistant version of the target gene.

19. The gene drive system of claim 18, wherein the toxin-resistant version is not recognized by the toxin.

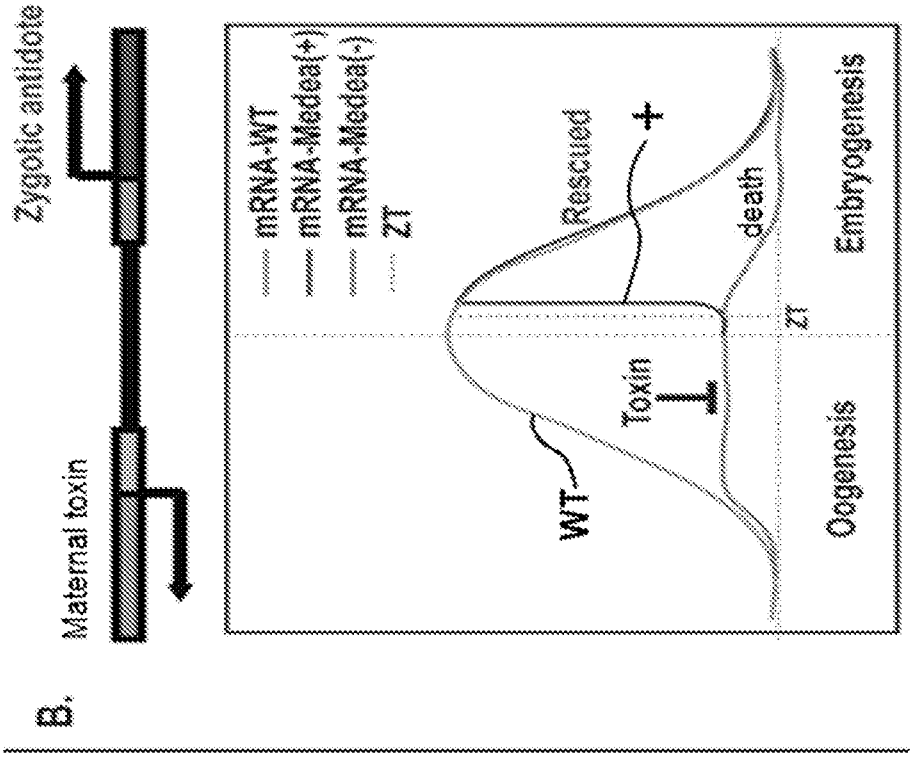
20. The gene drive system of claim 14, further comprising an effector genetic element active in the insect and linked to the first and second DNA sequences, wherein the effector genetic element encodes a gene conferring susceptibility to a chemical, a conditional lethal gene, a genetic element that disrupts a recessive fertility gene or recessive lethality gene, or a genetic element that disrupts a gene involved in insect pest behavior.

21. The gene drive system of claim 14, wherein the insect is *Drosophila suzukii*, *Anastrepha suspensa*, *Anastrepha ludens*, *Anastrepha oblique*, *Bactrocera oleae/Dacus oleae*, *Ceratitis capitata*, *Aedes aegyptii*, or *Anopheles gambiae*.

22. A transgenic insect whose genome comprises the gene drive system of any one of claims 14 - 21.

23. A method of manipulating an insect population, the method comprising releasing the transgenic insect of claim 22 into a population of the same species in sufficient numbers to spread the gene drive system through the population.

24. A method of manipulating an insect population, the method comprising releasing a transgenic insect having a gene drive system of claim 20 into a population of the same species in sufficient numbers to spread the gene drive system through the population, wherein the effector genetic element results in inducible lethality in one or both sexes of the species or results in recessive sterility in one or both sexes of the species.


B.
Zygotic antidote
Maternal toxin
mRNA-WT
mRNA-Medea(+)
mRNA-Medea(-)
ZT
Rescued
+
death
WT
Toxin
ZT
Oogenesis
Embryogenesis

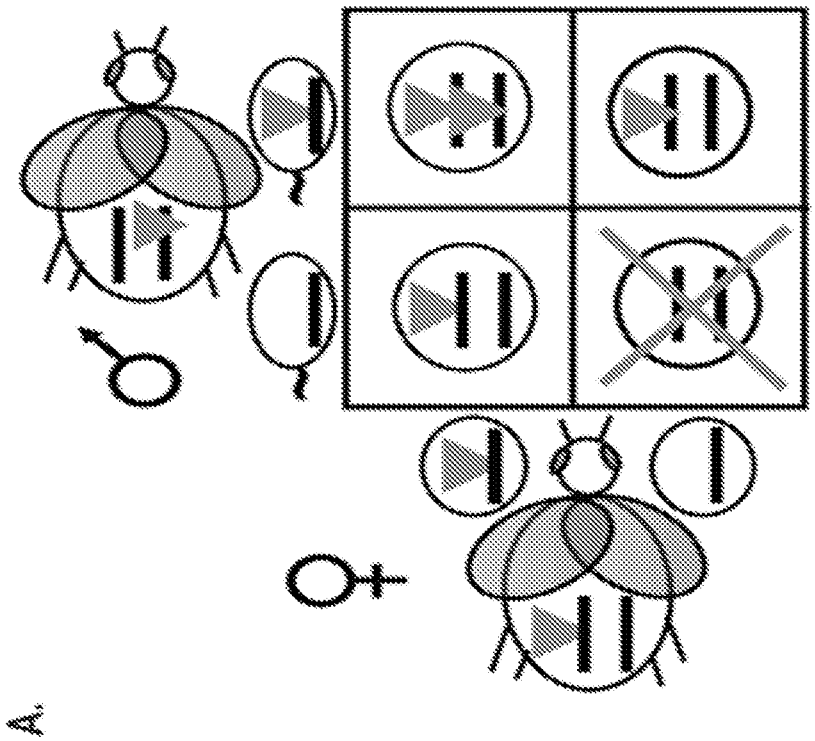

A.

FIG. 1

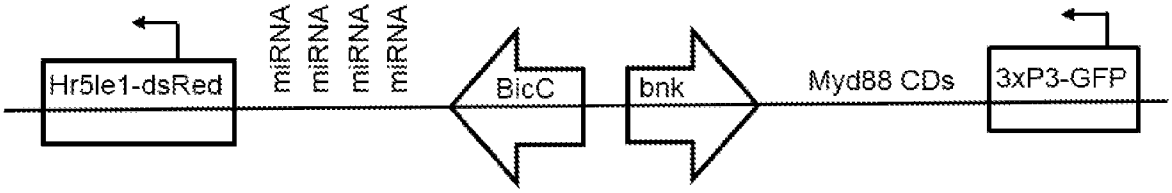

Hr5le1-dsRed
miRNA
miRNA
miRNA
miRNA
BicC
bnk
Myd88 CDs
3xP3-GFP

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/14846

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/14846

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A01K 67/033; C12N 15/85, 15/90 (2017.01)
CPC - A01K 67/0333, 67/0339; C12N 15/8509, 15/90

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	(AKBARI, OS et al.) Novel Synthetic Medea selfish genetic elements drive population replacement in Drosophila, and a theoretical exploration of Medea-dependent population suppression. ACS Synthetic Biology. 19 December 2014. Vol. 3, No. 12, pp. 915-928; DOI:10.1021/sb300079h; abstract; page 2, paragraphs 2-3; page 3, paragraph 1; page 4, paragraph 2; page 6, paragraph 4; page 9, paragraphs 2-3; page 10, paragraph 1	1-9, 10/1-9, 11, 12, 13/10/1-9, 14-21, 22/14-21, 23/22/14-21, 24
Y	US 2014/0283155 A1 (CALIFORNIA INSTITUTE OF TECHNOLOGY) September 18, 2014; paragraphs [0010], [0015]-[0016], [0062], [0067]	1-9, 10/1-9, 11, 12, 13/10/1-9, 14-21, 22/14-21, 23/22/14-21, 24
A	(MARSHALL, JM et al.) Medusa: A Novel Gene Drive System for Confined Suppression of Insect Populations. PLOS One. 23 July 2014. Vol. 9, Iss. 7; DOI: 10.1371/journal.pone.0102694	1-9, 10/1-9, 11, 12, 13/10/1-9, 14-21, 22/14-21, 23/22/14-21, 24
A	US 2003/0213005 A1 (ALPHEY, L et al.) November 13, 2003; entire document	9, 10/9, 11, 12, 13/10/9, 20, 22/20, 23/22/20, 24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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Date of the actual completion of the international search	Date of mailing of the international search report
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