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(54) Title: COMPOSITIONS AND METHODS FOR CONTROLLING MOSQUITOES

(57) Abstract: This invention relates to polynucleotides, compositions, and methods for mosquito control.



COMPOSITIONS AND METHODS FOR CONTROLLING MOSQUITOES

CROSS-REFERENCE TO RELATED APPLICATIONS AND INCORPORATION OF SEQUENCE LISTINGS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/370,543, filed August 3, 2016, which is incorporated by reference herein in its entirety. The sequence listing contained in the file “P34300WO00_SEQ.txt” which is 1,624,790 bytes (measured in MS-Windows®) and created on August 2, 2017, is filed electronically herewith and is incorporated by reference in its entirety.

FIELD

[0002] This invention relates to polynucleotides, compositions, and methods for mosquito control. Polynucleotides and methods of use thereof for modifying the expression of genes in a mosquito, particularly through RNA interference are disclosed. The polynucleotides, compositions, and methods of the invention are useful for controlling vectors of human diseases including the mosquito genera *Aedes* sp., *Culex* sp., and *Anopheles* sp.

BACKGROUND

[0003] Mosquitoes are the insect vectors for a number of important pathogen-caused diseases of humans (Table 1). These vector mosquito species require a blood meal by the female as part of the egg-laying cycle, during which transmission of the causative pathogen to the human can occur.

Table 1

Insect vector	Causative pathogen	Disease
<i>Aedes aegypti</i> (yellow fever mosquito)	dengue virus	dengue fever
	Chikungunya virus	Chikungunya
	yellow fever virus	yellow fever
	Zika virus	Zika
<i>Culex quinquefasciatus</i> (southern house mosquito)	West Nile virus	West Nile fever
	<i>Wuchereria bancrofti</i>	lymphatic filariasis
<i>Anopheles gambiae</i> (or <i>Anopheles gambiae</i> species complex)	<i>Plasmodium</i> spp., including <i>Plasmodium falciparum</i>	malaria

[0004] Compositions for controlling mosquito infestations have typically been in the form of chemical insecticides. However, there are several disadvantages to using chemical insecticides. For example, chemical insecticides are generally not selective, and applications of chemical insecticides intended to control mosquitoes can exert their effects on non-target insects and other invertebrates as well. Chemical insecticides often persist in the environment and can be slow to degrade, thus potentially accumulating in the food chain. Furthermore, the use of persistent chemical insecticides can result in the development of resistance in the target mosquito species. Thus, there has been a long felt need for environmentally friendly methods for controlling or eradicating mosquito infestations, *i. e.*, methods

which are species-selective, environmentally inert, non-persistent, and biodegradable, and that fit well into pest resistance management schemes.

[0005] RNA interference (RNAi, RNA-mediated gene suppression) is an approach that shows promise for environmentally friendly pest control. In invertebrates, RNAi-based gene suppression was first demonstrated in nematodes (Fire *et al.*, (1998) *Nature*, 391:806–811; Timmons & Fire (1998) *Nature*, 395:854). Subsequently, RNAi-based suppression of invertebrate genes using recombinant nucleic acid techniques has been reported in a number of species, including agriculturally or economically important pests from various insect and nematode taxa. The enzymes involved in canonical RNAi pathways (*e. g.*, Dicers, Drosha, Argonaute proteins) and various classes of small silencing RNAs are known to exist in these vector mosquito species; see, *e. g.*, Barnard *et al.* (2012), *Genes*, 3:702-741. Attempts to control mosquito pests or to silence target genes in mosquitoes through the use of RNA interference or RNA-mediated gene suppression have been reported. See, *e. g.*, Boisson *et al.* (2006) *FEBS Lett.*, 580: 1988 – 1992; Pridgeon *et al.* (2008) *J. Med Entomol.*, 45: 414 – 420; Zhang *et al.* (2010) *Insect Mol. Biol.*, 19:683 – 693; Gu *et al.* (2011) *PLoS ONE*, 6:e21329; and Coy *et al.* (2012) *J. Applied Entomol.*, 136:741 – 748.

SUMMARY

[0006] The present embodiments relate to control of mosquitoes. The compositions and methods disclosed herein include recombinant polynucleotide molecules, such as recombinant DNA constructs and polynucleotide agents, such as RNAs that are useful for controlling or preventing mosquito infestation. Several embodiments described herein relate to a polynucleotide-containing composition (*e. g.*, a composition containing a dsRNA for suppressing a mosquito target gene) that is provided to a mosquito species or is applied to an environment to be protected from infestation by a mosquito species. Other embodiments relate to methods for selecting mosquito target genes that are likely to be effective targets for RNAi-mediated control of mosquito species.

[0007] Several embodiments relate to methods of mosquito control, including (a) contacting a mosquito with a polynucleotide comprising a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene, or an RNA transcribed from the target gene; (b) contacting a mosquito with an effective amount of a polynucleotide, one strand of which is complementary to at least 21 contiguous nucleotides of a mosquito target gene, or an RNA transcribed from said target gene; (c) providing in the diet of a mosquito a polynucleotide comprising a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a mosquito target gene, or an RNA transcribed from said target gene; (d) causing mortality or stunting in larvae of a mosquito by providing in the diet of said larvae at least one polynucleotide comprising at least one silencing element, wherein said silencing element comprises at least 21 contiguous nucleotides that are complementary to a mosquito target gene, or an RNA transcribed from said target gene; (e) contacting a mosquito with a polynucleotide comprising at least one segment that is identical or complementary to at least 21 contiguous nucleotides of a mosquito target gene, or an RNA transcribed from said target gene; or (f)

contacting an environment infested by said mosquito with a composition comprising a polynucleotide comprising a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a mosquito target gene, or an RNA transcribed from said target gene; in embodiments of the above methods of mosquito control the target gene is at least one gene selected from the group consisting of

(a) a gene identified by name in Tables 2 and 3; (b) a gene encoding a proteasome protein selected from the group consisting of: Proteasome Alpha 2, Proteasome Beta 5, Proteasome regulatory subunit 2, Proteasome regulatory subunit 3, Proteasome regulatory subunit 8, Proteasome regulatory subunit 9, Proteasome regulatory GTPase SAR1, 26S protease regulatory subunit 6; (c) a gene encoding an ATPase protein selected from the group consisting of: V-ATPase subunit A, V-ATPase subunit B, V-ATPase subunit D, V-ATPase subunit E, Actin 6; (d) a gene encoding a ribosomal protein selected from the group consisting of: Ribosomal protein Large subunit 3, Ribosomal protein Large subunit 7, Ribosomal protein Large subunit 19, Ribosomal protein Large subunit 40, Ribosomal protein Small subunit 21, Ribosomal protein Small subunit 4; (e) a gene encoding a vesicle protein selected from the group consisting of: Vesicle Sorting protein 2, Vesicle Sorting protein 4, Vesicle Sorting protein 16A, Vesicle Sorting protein 20, Vesicle Sorting protein 24, Vesicle Sorting protein 27, Vesicle Sorting protein 28, Vesicle Sorting protein Shrb (Snf7), Vesicle Sorting protein Chmp1, Secretory vesicle protein 6, Secretory vesicle protein 23, Coatomer Protein Alpha, Coatomer Protein Beta, Coatomer Protein Beta Prime, Coatomer Protein Delta, Coatomer Protein Epsilon, Coatomer Protein Zeta; (f) a gene encoding an actin; (g) a gene encoding a protein related to behaviour selected from the group consisting of: Bendless, scribbler, capa receptor, diapause hormone receptor, Eb1, Ether-a-go-go, flightless-I, foraging, juvenile hormone epoxide hydrolase, neuropeptide F, pheromone biosynthesis activating neuropeptide receptor, semaphorin 1a, shaker, slowmo, stress-sensitive B, turtle, twitchin, myosin heavy chain, nervana, N-synaptobrevin, nuclear hormone receptor 25, syntaxin, and bent; (h) a gene encoding a glycolysis and energy metabolism protein selected from the group consisting of: BiP, citrate synthase, glycogen synthase, hexokinase, isocitrate dehydrogenase, pyruvate dehydrogenase, phosphofructokinase, GTPase activator, GTPase Ran, sarcoplasmic Ca²⁺ ATPase, Ca²⁺ ATPase, hydrogen exporting ATPase, NADH dehydrogenase, F1F0 ATPase, spermatogenesis ATPase, beta-ketoacyl synthase, pyruvate kinase, and knickkopf; (i) a gene encoding a protein turnover and mitosis protein selected from the group consisting of: cyclins including cyclin A, cyclin B, cyclin B3, cyclin C, cyclin D, cyclin E, cyclin G, cyclin H, cyclin J, cyclin K, cyclin T, and cyclin Y, Ubiquitin conjugating enzyme E1, chromosome segregation protein, CDC5, COP9 signalosome, and ketel; (j) a gene encoding a gene expression protein selected from the group consisting of: cleavage polyadenylation specificity factor, E1A/CREB-binding protein, RNA helicases, RNA polymerases, Arf1, Bx42, and eft2; (k) a gene encoding a protein-folding, stress, and heat shock response protein selected from the group consisting of: protein disulfide isomerase, heat shock cognate 70, chaperonin TCP1, chaperonins, carrier protein, and HSP70; and (l) a gene encoding a miscellaneous protein selected from the group consisting of: spermatogenesis ATPase, pitchoune, and insect proteins.

[0008] Several embodiments relate to a mosquitocidal composition including a mosquitocidally effective amount of a recombinant polynucleotide comprising a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a mosquito target gene, or an RNA transcribed from the target gene. In some embodiments, the target gene is selected from the group consisting of (a) a gene identified in Tables 2 and 3; (b) a gene encoding a proteasome protein selected from the group consisting of: Proteasome Alpha 2, Proteasome Beta 5, Proteasome regulatory subunit 2, Proteasome regulatory subunit 3, Proteasome regulatory subunit 8, Proteasome regulatory subunit 9, Proteasome regulatory GTPase SAR1, 26S protease regulatory subunit 6; (c) a gene encoding an ATPase protein selected from the group consisting of: V-ATPase subunit A, V-ATPase subunit B, V-ATPase subunit D, V-ATPase subunit E, Actin 6; (d) a gene encoding a ribosomal protein selected from the group consisting of: Ribosomal protein Large subunit 3, Ribosomal protein Large subunit 7, Ribosomal protein Large subunit 19, Ribosomal protein Large subunit 40, Ribosomal protein Small subunit 21, Ribosomal protein Small subunit 4; (e) a gene encoding a vesicle protein selected from the group consisting of: Vesicle Sorting protein 2, Vesicle Sorting protein 4, Vesicle Sorting protein 16A, Vesicle Sorting protein 20, Vesicle Sorting protein 24, Vesicle Sorting protein 27, Vesicle Sorting protein 28, Vesicle Sorting protein Shrb (Snf7), Vesicle Sorting protein Chmp1, Secretory vesicle protein 6, Secretory vesicle protein 23, Coatamer Protein Alpha, Coatamer Protein Beta, Coatamer Protein Beta Prime, Coatamer Protein Delta, Coatamer Protein Epsilon, Coatamer Protein Zeta; (f) a gene encoding an actin; (g) a gene encoding a protein related to behaviour selected from the group consisting of: Bendless, scribbler, capa receptor, diapause hormone receptor, Eb1, Ether-a-go-go, flightless-I, foraging, juvenile hormone epoxide hydrolase, neuropeptide F, pheromone biosynthesis activating neuropeptide receptor, semaphorin 1a, shaker, slowmo, stress-sensitive B, turtle, twitchin, myosin heavy chain, nervana, N-synaptobrevin, nuclear hormone receptor 25, syntaxin, and bent; (h) a gene encoding a glycolysis and energy metabolism protein selected from the group consisting of: BiP, citrate synthase, glycogen synthase, hexokinase, isocitrate dehydrogenase, pyruvate dehydrogenase, phosphofructokinase, GTPase activator, GTPase Ran, sarcoplasmic Ca²⁺ ATPase, Ca²⁺ ATPase, hydrogen exporting ATPase, NADH dehydrogenase, F1F0 ATPase, spermatogenesis ATPase, beta-ketoacyl synthase, pyruvate kinase, and knickkopf; (i) a gene encoding a protein turnover and mitosis protein selected from the group consisting of: cyclins including cyclin A, cyclin B, cyclin B3, cyclin C, cyclin D, cyclin E, cyclin G, cyclin H, cyclin J, cyclin K, cyclin T, and cyclin Y, Ubiquitin conjugating enzyme E1, chromosome segregation protein, CDC5, COP9 signalosome, and ketel; (j) a gene encoding a gene expression protein selected from the group consisting of: cleavage polyadenylation specificity factor, E1A/CREB-binding protein, RNA helicases, RNA polymerases, Arf1, Bx42, and eft2; (k) a gene encoding a protein-folding, stress, and heat shock response protein selected from the group consisting of: protein disulfide isomerase, heat shock cognate 70, chaperonin TCP1, chaperonins, carrier protein, and HSP70; and (l) a gene encoding a miscellaneous protein selected from the group consisting of: spermatogenesis ATPase, pitchoune, and insect proteins. In some embodiments, the mosquitocidal composition is provided as a solid, liquid, powder, suspension, emulsion, colloid, spray, encapsulation,

microbeads, carrier particulates, granules, film, gel, or matrix. In some embodiments, the
mosquitocidal composition is provided in the form of a bait, a trap, a sprayable liquid, a concentrate,
dunks, dispersible granules, or ingestible particulates or cells. In some embodiments, the mosquitocidal
composition further comprises one or more components selected from the group consisting of a carrier
5 agent, a surfactant, an organosilicone, a lipid, a sugar, a non-polynucleotide mosquitocide, a dipterocidal
Bacillus thuringiensis toxin, a safener, a mosquito attractant or pheromone, and an insect growth
regulator. In some embodiments the composition further comprises a cationic carbohydrate. In some
embodiments the composition further comprises a cationic starch. In some embodiments the
composition further comprises a cationic guar. In some embodiments the composition further comprises
10 a branched Polyethylenimine.

[0009] Several embodiments relate to a recombinant DNA construct comprising a heterologous
promoter operably linked to DNA encoding an RNA transcript including: (a) a sequence of about 95%
to about 100% identity or complementarity with a mosquito target gene; (b) at least 21 contiguous
nucleotides that are complementary to a mosquito target gene, or an RNA transcribed from the
15 mosquito target gene; (c) at least 21 contiguous ribonucleotides of a mosquito target gene RNA
sequence; or (d) a strand comprising a RNA sequence complementary to at least 21 nucleotides of a
mosquito target gene. In some embodiments, the RNA transcript is dsRNA, which can be encoded on a
single RNA strand or on two essentially complementary RNA strands. In some embodiments, the
heterologous promoter is functional for expression of the RNA transcript in a bacterium, or in a
20 eukaryotic cell. Various other embodiments include a recombinant vector, chromosome, plasmid, or
transgenic cell including the recombinant DNA construct.

[0010] Several embodiments relate to man-made compositions comprising one or more
polynucleotides as described herein, such as dsRNA formulations useful for topical application to an
area or surface in need of treatment for or protection from a mosquito infestation; recombinant
25 constructs and vectors useful for making transgenic eukaryotic or prokaryotic cells and transgenic
plants; and formulations and coatings useful for treating a surface or area. Several embodiments relate
to polyclonal or monoclonal antibodies that bind a peptide or protein encoded by a sequence or a
fragment of a sequence selected from the group consisting of SEQ ID NOs.:1 – 105 and 897 - 1132.
Several embodiments relate to polyclonal or monoclonal antibodies that bind a peptide or protein
30 encoded by a sequence or a fragment of a sequence selected from the group consisting of SEQ ID
NOs.:106 – 896 and 1133 – 2087 or the complement thereof. Such antibodies are made by routine
methods as known to one of ordinary skill in the art. In some embodiments the composition further
comprises a cationic carbohydrate. In some embodiments the composition further comprises a cationic
starch. In some embodiments the composition further comprises a cationic guar. In some embodiments
35 the composition further comprises a branched Polyethylenimine.

[0011] Other aspects and specific embodiments of this invention are disclosed in the following
Detailed Description and Examples.

DETAILED DESCRIPTION

[0012] Unless defined 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. The terms “to include” or “to include” are understood to mean “to include, but not to be limited to”. Where a term is provided in the singular, the inventors also contemplate aspects of the invention described by the plural of that term. Where there are discrepancies in terms and definitions used in references that are incorporated by reference, the terms used in this application shall have the definitions given in this application. The inventors do not intend to be limited to a mechanism or mode of action. Reference thereto is provided for illustrative purposes only.

[0013] Unless otherwise stated, nucleic acid sequences are given, when read from left to right, in the 5' to 3' direction. One of skill in the art would be aware that a given DNA sequence is understood to define a corresponding RNA sequence which is identical to the DNA sequence except for replacement of the thymine (T) nucleotides of the DNA with uracil (U) nucleotides. Thus, providing a specific DNA sequence is understood to define the exact RNA equivalent. A given first polynucleotide sequence, whether DNA or RNA, further defines the sequence of its exact complement (which can be DNA or RNA), which hybridizes perfectly to the first polynucleotide by forming Watson-Crick base-pairs. By “essentially identical” or “essentially complementary” to a target gene or a fragment of a target gene is meant that a polynucleotide strand (or at least one strand of a double-stranded polynucleotide) is designed to hybridize (generally under physiological conditions such as those found in a living plant or animal cell) to a target gene or to a fragment of a target gene or to the transcript of the target gene or the fragment of a target gene; one of skill in the art would understand that such hybridization does not necessarily require 100% sequence identity or complementarity. A first nucleic acid sequence is “operably” connected or “linked” with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For example, a promoter sequence is “operably linked” to DNA if the promoter provides for transcription or expression of the DNA. Generally, operably linked DNA sequences are contiguous.

[0014] The term “polynucleotide” commonly refers to a DNA or RNA molecule containing multiple nucleotides and generally refers both to “oligonucleotides” (a polynucleotide molecule of 18-25 nucleotides in length) and longer polynucleotides of 26 or more nucleotides. Polynucleotides also include molecules containing multiple nucleotides including non-canonical nucleotides or chemically modified nucleotides as commonly practiced in the art; see, *e. g.*, chemical modifications disclosed in the technical manual “RNA Interference (RNAi) and DsiRNAs”, 2011 (Integrated DNA Technologies Coralville, IA). Generally, polynucleotide as described herein, whether DNA or RNA or both, and whether single- or double-stranded, comprise at least one segment of 18 or more contiguous nucleotides (or, in the case of double-stranded polynucleotides, at least 18 contiguous base-pairs) that are essentially identical or complementary to a fragment of equivalent size of the DNA of a target gene or the target gene’s RNA transcript. Throughout this disclosure, “at least 18 contiguous” means “from about 18 to about 10,000, including every whole number point in between”. Thus, embodiments of this

invention include compositions including oligonucleotides having a length of 18-25 nucleotides (18-mers, 19-mers, 20-mers, 21-mers, 22-mers, 23-mers, 24-mers, or 25-mers), or medium-length polynucleotides having a length of 26 or more nucleotides (polynucleotides of 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 110, about 120, about 130, about 140, about 150, about 160, about 170, about 180, about 190, about 200, about 210, about 220, about 230, about 240, about 250, about 260, about 270, about 280, about 290, or about 300 nucleotides), or long polynucleotides having a length greater than about 300 nucleotides (*e. g.*, polynucleotides of between about 300 to about 400 nucleotides, between about 400 to about 500 nucleotides, between about 500 to about 600 nucleotides, between about 600 to about 700 nucleotides, between about 700 to about 800 nucleotides, between about 800 to about 900 nucleotides, between about 900 to about 1000 nucleotides, between about 300 to about 500 nucleotides, between about 300 to about 600 nucleotides, between about 300 to about 700 nucleotides, between about 300 to about 800 nucleotides, between about 300 to about 900 nucleotides, or about 1000 nucleotides in length, or even greater than about 1000 nucleotides in length, for example up to the entire length of a target gene including coding or non-coding or both coding and non-coding portions of the target gene). Where a polynucleotide is double-stranded, such as the dsRNAs described in the Examples, its length can be similarly described in terms of base pairs. Double-stranded polynucleotides, such as the dsRNAs described in the Examples, can further be described in terms of one or more of the single-stranded components.

[0015] The polynucleotides described herein can be single-stranded (ss) or double-stranded (ds). “Double-stranded” refers to the base-pairing that occurs between sufficiently complementary, anti-parallel nucleic acid strands to form a double-stranded nucleic acid structure, generally under physiologically relevant conditions. Embodiments include those wherein the polynucleotide is selected from the group consisting of sense single-stranded DNA (ssDNA), sense single-stranded RNA (ssRNA), double-stranded RNA (dsRNA), double-stranded DNA (dsDNA), a double-stranded DNA/RNA hybrid, anti-sense ssDNA, or anti-sense ssRNA; a mixture of polynucleotides of any of these types can be used. In some embodiments, the polynucleotide is double-stranded RNA of a length greater than that which is typical of naturally occurring regulatory small RNAs (such as endogenously produced siRNAs and mature miRNAs). In some embodiments, the polynucleotide is double-stranded RNA of at least about 30 contiguous base-pairs in length. In embodiments, the polynucleotide is double-stranded RNA with a length of between about 50 to about 500 base-pairs. In embodiments, the polynucleotide can include components other than standard ribonucleotides, *e. g.*, an embodiment is an RNA that includes terminal deoxyribonucleotides.

[0016] One or more effective polynucleotides of any size can be used, alone or in combination, in the various methods and compositions described herein. In some embodiments, a single polynucleotide is used in a mosquitocidal composition (*e. g.*, a composition to be provided to a mosquito or to be applied to an environment, or a recombinant DNA construct useful for making a transgenic cell). In

embodiments, a mixture or pool of different polynucleotides is used; in such cases the polynucleotides can be for a single target gene or for multiple target genes, or for a single or multiple mosquito species.

[0017] In various embodiments, the polynucleotides described herein include naturally occurring nucleotides, such as those which occur in DNA and RNA. In some embodiments, the polynucleotide is a combination of ribonucleotides and deoxyribonucleotides, for example, synthetic polynucleotides consisting mainly of ribonucleotides but with one or more terminal deoxyribonucleotides or one or more terminal dideoxyribonucleotides or synthetic polynucleotides consisting mainly of deoxyribonucleotides but with one or more terminal dideoxyribonucleotides. In embodiments, the polynucleotide includes non-canonical nucleotides such as inosine, thiouridine, or pseudouridine. In embodiments, the polynucleotide includes chemically modified nucleotides. Examples of chemically modified oligonucleotides or polynucleotides are well known in the art; see, for example, U.S. Patent Publication 2011/0171287, U.S. Patent Publication 2011/0171176, U.S. Patent Publication 2011/0152353, U.S. Patent Publication 2011/0152346, and U.S. Patent Publication 2011/0160082, which are herein incorporated by reference. Illustrative examples include, but are not limited to, the naturally occurring phosphodiester backbone of an oligonucleotide or polynucleotide which can be partially or completely modified with phosphorothioate, phosphorodithioate, or methylphosphonate internucleotide linkage modifications, modified nucleoside bases or modified sugars can be used in oligonucleotide or polynucleotide synthesis, and oligonucleotides or polynucleotides can be labeled with a fluorescent moiety (*e. g.*, fluorescein or rhodamine) or other label (*e. g.*, biotin).

[0018] The term “recombinant”, as used to refer to a polynucleotide (such as the recombinant RNA molecules or recombinant DNA constructs described herein), means that the polynucleotide is not a naturally occurring molecule, *i. e.*, that human intervention is required for the polynucleotide to exist. A recombinant polynucleotide is produced using recombinant nucleic acid techniques, or by chemical synthesis, and can include heterologous combinations of sequences (*e. g.*, heterologous combinations of a promoter and a DNA encoding an RNA to be expressed, or an RNA molecule that includes concatenated segments of a target gene that do not in nature occur adjacent to one another). A recombinant polynucleotide can be biologically produced in a cell (such as a bacterial or plant or animal cell), for example, when that cell is transfected or transformed with a vector encoding the recombinant polynucleotide. A recombinant polynucleotide can include sequences of nucleotides designed *in silico* using appropriate algorithms.

[0019] The polynucleotides described herein are generally designed to suppress or silence one or more genes (“target genes”). The term “gene” refers to any portion of a nucleic acid that provides for expression of a transcript or encodes a transcript. A “gene” can include, but is not limited to, a promoter region, 5’ untranslated regions, transcript encoding regions that can include intronic regions, 3’ untranslated regions, coding regions, or combinations of these regions. In embodiments, the target genes can include coding or non-coding sequence or both. In other embodiments, the target gene has a sequence identical to or complementary to a messenger RNA, *e. g.*, in embodiments the target gene is a cDNA.

[0020] As used herein, the term “isolated” refers to separating a molecule from other molecules normally associated with it in its native or natural state. The term “isolated” thus may refer to a DNA molecule that has been separated from other DNA molecule(s) which normally are associated with it in its native or natural state. Such a DNA molecule may be present in a recombined state, such as a recombinant DNA molecule. Thus, DNA molecules fused to regulatory or coding sequences with which they are not normally associated (*e.g.*, in a heterologous combination), for example as the result of recombinant techniques, are considered isolated, even when integrated as a transgene into the chromosome of a cell or present with other DNA molecules.

[0021] By “mosquitocidally effective” is meant effective in inducing a physiological or behavioural change in a mosquito (adult, larval, and/or egg) such as, but not limited to, growth stunting, increased mortality, decrease in reproductive capacity or decreased fecundity, decrease in or cessation of feeding behavior or movement, or decrease in or cessation of metamorphosis stage development. In embodiments, application of a mosquitocidally effective amount of the polynucleotide, such as a dsRNA molecule, to an area or surface decreases the viable mosquito population of that area or surface. While there is no upper limit on the concentrations and dosages of a polynucleotide as described herein that can be useful in the methods and compositions provided herein, lower effective concentrations and dosages will generally be sought for efficiency and economy. Non-limiting embodiments of mosquitocidally effective amounts of a polynucleotide include a range from about 10 nanograms per milliliter to about 100 micrograms per milliliter of a polynucleotide in a sprayable liquid form, or from about 10 milligrams per acre to about 100 grams per acre of polynucleotide applied in the form of a bait or sprayable liquid or dispersible particulates to an area or surface, or from about 0.001 to about 0.1 microgram per milliliter of polynucleotide in an artificial mosquito diet. Where compositions as described herein are topically applied to an area or surface (*e. g.*, sprayed or dusted on plants or other surfaces in an environment), the concentrations can be adjusted in consideration of the volume of spray or other treatment applied to the area or surface. In embodiments, a mosquitocidal composition contains about 0.5 to about 2.0 milligrams per milliliter, or about 0.14 milligrams per milliliter of a dsRNA (or a single-stranded 21-mer) such as the polynucleotides described herein. In embodiments, a mosquitocidal composition contains about 0.5 to about 1.5 milligrams per milliliter of a dsRNA polynucleotide as described herein of about 50 to about 200 or more nucleotides. In embodiments, the mosquitocidal composition contains at least one polynucleotide as described herein at a concentration of about 0.01 to about 10 milligrams per milliliter, or about 0.05 to about 2 milligrams per milliliter, or about 0.1 to about 2 milligrams per milliliter. When using long dsRNA molecules that can be processed into multiple oligonucleotides (*e. g.*, multiple mosquitocidal polynucleotides encoded by a single recombinant DNA molecule of this invention), lower concentrations can be used.

[0022] The present embodiments relate to methods and compositions for mosquito control, in particular mosquito genera and species that are vectors of disease. Disclosed herein are target genes identified as useful for designing polynucleotide agents for mosquito control. Also disclosed are sequences for suppressing one or more mosquito target genes. Several embodiments relate to

polynucleotide agents, for example, in the form of dsRNA, that suppress mosquito target genes. In some embodiments, polynucleotides and recombinant DNA molecules and constructs useful in mosquito control. Several embodiments relate to mosquitocidal compositions. Several embodiments relate to methods of identifying mosquitocidally efficacious polynucleotide agents, for example double-stranded RNA molecules, for mosquito control, and methods for identifying mosquito genes that are likely to represent essential functions, making these genes preferred targets for RNAi-mediated silencing and control of mosquitoes.

[0023] Several embodiments relate to methods and compositions for mosquito control by inhibiting in the mosquito the expression of one or more target genes selected from the group consisting of: (a) a gene identified in Tables 2 and 3; (b) a gene encoding a proteasome protein selected from the group consisting of: Proteasome Alpha 2, Proteasome Beta 5, Proteasome regulatory subunit 2, Proteasome regulatory subunit 3, Proteasome regulatory subunit 8, Proteasome regulatory subunit 9, Proteasome regulatory GTPase SAR1, 26S protease regulatory subunit 6; (c) a gene encoding an ATPase protein selected from the group consisting of: V-ATPase subunit A, V-ATPase subunit B, V-ATPase subunit D, V-ATPase subunit E, Actin 6; (d) a gene encoding a ribosomal protein selected from the group consisting of: Ribosomal protein Large subunit 3, Ribosomal protein Large subunit 7, Ribosomal protein Large subunit 19, Ribosomal protein Large subunit 40, Ribosomal protein Small subunit 21, Ribosomal protein Small subunit 4; (e) a gene encoding a vesicle protein selected from the group consisting of: Vesicle Sorting protein 2, Vesicle Sorting protein 4, Vesicle Sorting protein 16A, Vesicle Sorting protein 20, Vesicle Sorting protein 24, Vesicle Sorting protein 27, Vesicle Sorting protein 28, Vesicle Sorting protein Shrb (Snf7), Vesicle Sorting protein Chmp1, Secretory vesicle protein 6, Secretory vesicle protein 23, Coatamer Protein Alpha, Coatamer Protein Beta, Coatamer Protein Beta Prime, Coatamer Protein Delta, Coatamer Protein Epsilon, Coatamer Protein Zeta; (f) a gene encoding an actin; (g) a gene encoding a protein related to behaviour selected from the group consisting of: Bendless, scribbler, capa receptor, diapause hormone receptor, Ebl, Ether-a-go-go, flightless-I, foraging, juvenile hormone epoxide hydrolase, neuropeptide F, pheromone biosynthesis activating neuropeptide receptor, semaphorin 1a, shaker, slowmo, stress-sensitive B, turtle, twitchin, myosin heavy chain, nervana, N-synaptobrevin, nuclear hormone receptor 25, syntaxin, and bent; (h) a gene encoding a glycolysis and energy metabolism protein selected from the group consisting of: BiP, citrate synthase, glycogen synthase, hexokinase, isocitrate dehydrogenase, pyruvate dehydrogenase, phosphofructokinase, GTPase activator, GTPase Ran, sarcoplasmic Ca²⁺ ATPase, Ca²⁺ ATPase, hydrogen exporting ATPase, NADH dehydrogenase, F1F0 ATPase, spermatogenesis ATPase, beta-ketoacyl synthase, pyruvate kinase, and knickkopf; (i) a gene encoding a protein turnover and mitosis protein selected from the group consisting of: cyclins including cyclin A, cyclin B, cyclin B3, cyclin C, cyclin D, cyclin E, cyclin G, cyclin H, cyclin J, cyclin K, cyclin T, and cyclin Y, Ubiquitin conjugating enzyme E1, chromosome segregation protein, CDC5, COP9 signalosome, and ketel; (j) a gene encoding a gene expression protein selected from the group consisting of: cleavage polyadenylation specificity factor, E1A/CREB-binding protein, RNA helicases, RNA polymerases, Arf1, Bx42, and eft2; (k) a gene

encoding a protein-folding, stress, and heat shock response protein selected from the group consisting of: protein disulfide isomerase, heat shock cognate 70, chaperonin TCPI, chaperonins, carrier protein, and HSP70; and (l) a gene encoding a miscellaneous protein selected from the group consisting of: spermatogenesis ATPase, pitchoune, and insect proteins. Several embodiments relate to methods and compositions for mosquito control by inhibiting in the mosquito the expression of one or more target genes selected from the group consisting of **SEQ ID NOs.:1 – 105 and 897 - 1132**. In embodiments, inhibiting the expression of one or more target gene in the mosquito (adult, larva, and/or egg) results in stunting or mortality. In embodiments, contacting of the polynucleotide (e.g., dsRNA) to the mosquito is by oral delivery, or by non-oral contact, *e. g.*, by absorption through the cuticle, or through a combination of oral and non-oral delivery.

[0024] Several embodiments relate to polynucleotides of use in methods and compositions for mosquito control. In some embodiments, the mosquitocidally effective polynucleotide is a dsRNA. In embodiments, the mosquitocidally effective polynucleotide comprises an RNA strand comprising a sequence having about 95% to about 100% identity or complementarity with a mosquito target gene. In embodiments, the mosquitocidally effective polynucleotide comprises a sequence that is about 95% to about 100% identical or complementary to a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896**. In embodiments, the mosquitocidally effective polynucleotide is a dsRNA comprising an RNA strand comprising at least 21, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50 or more contiguous nucleotides that are identical or complementary to a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896**. In embodiments, the polynucleotide is a dsRNA comprising an RNA strand comprising a sequence that is identical or complementary to a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896**. In embodiments, the mosquitocidally effective polynucleotide is a dsRNA that suppresses a target gene in the mosquito and stunts growth, development or reproduction by the mosquito, or kills the mosquito. In embodiments, the mosquitocidally effective polynucleotide is dsRNA which is at least 21 base pairs in length. In embodiments, the mosquitocidally effective polynucleotide is dsRNA which is at least 50 base pairs in length. In embodiments, the mosquitocidally effective polynucleotide is dsRNA which is at least 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, or more base pairs in length. In embodiments, the polynucleotide is dsRNA which is (a) is blunt-ended, or (b) has an overhang at at least one terminus, or (c) includes at least one stem-loop. In embodiments the dsRNA is chemically modified. In embodiments, the polynucleotide is dsRNA which is produced by (a) chemical synthesis, or (b) expression in a microorganism, or (c) expression in a eukaryotic cell. Various embodiments include those in which the mosquitocidally effective polynucleotide is provided in an ingestible composition such as a bait, or in a sprayable or otherwise easily dispersible composition; such compositions can include components such as a carrier agent, a surfactant, an organosilicone, a lipid, a

sugar, a non-polynucleotide mosquitocide, an insecticidal bacterial toxin, a safener, a mosquito attractant, a pheromone, and an insect growth regulator.

[0025] Several embodiments relate to a mosquitocidally effective polynucleotide molecule, such as a dsRNA, which includes one or more segments including 18 or more contiguous nucleotides, for example, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides, having 95% to about 100% (*e. g.*, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) complementarity with a fragment of a mosquito target gene identified in Tables 2 or 3. Several embodiments relate to a mosquitocidally effective polynucleotide molecule, such as a dsRNA, which includes one or more segments including 18 or more contiguous nucleotides (for example 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides) having 100% complementarity with a fragment of a mosquito target gene selected from the group consisting of (a) a gene identified in Tables 2 and 3; (b) a gene encoding a proteasome protein selected from the group consisting of: Proteasome Alpha 2, Proteasome Beta 5, Proteasome regulatory subunit 2, Proteasome regulatory subunit 3, Proteasome regulatory subunit 8, Proteasome regulatory subunit 9, Proteasome regulatory GTPase SAR1, 26S protease regulatory subunit 6; (c) a gene encoding an ATPase protein selected from the group consisting of: V-ATPase subunit A, V-ATPase subunit B, V-ATPase subunit D, V-ATPase subunit E, Actin 6; (d) a gene encoding a ribosomal protein selected from the group consisting of: Ribosomal protein Large subunit 3, Ribosomal protein Large subunit 7, Ribosomal protein Large subunit 19, Ribosomal protein Large subunit 40, Ribosomal protein Small subunit 21, Ribosomal protein Small subunit 4; (e) a gene encoding a vesicle protein selected from the group consisting of: Vesicle Sorting protein 2, Vesicle Sorting protein 4, Vesicle Sorting protein 16A, Vesicle Sorting protein 20, Vesicle Sorting protein 24, Vesicle Sorting protein 27, Vesicle Sorting protein 28, Vesicle Sorting protein Shrb (Snf7), Vesicle Sorting protein Chmp1, Secretory vesicle protein 6, Secretory vesicle protein 23, Coatomer Protein Alpha, Coatomer Protein Beta, Coatomer Protein Beta Prime, Coatomer Protein Delta, Coatomer Protein Epsilon, Coatomer Protein Zeta; (f) a gene encoding an actin; (g) a gene encoding a protein related to behaviour selected from the group consisting of: Bendless, scribbler, capa receptor, diapause hormone receptor, Ebl, Ether-a-go-go, flightless-I, foraging, juvenile hormone epoxide hydrolase, neuropeptide F, pheromone biosynthesis activating neuropeptide receptor, semaphorin 1a, shaker, slowmo, stress-sensitive B, turtle, twitchin, myosin heavy chain, nervana, N-synaptobrevin, nuclear hormone receptor 25, syntaxin, and bent; (h) a gene encoding a glycolysis and energy metabolism protein selected from the group consisting of: BiP, citrate synthase, glycogen synthase, hexokinase, isocitrate dehydrogenase, pyruvate dehydrogenase, phosphofructokinase, GTPase activator, GTPase Ran, sarcoplasmic Ca²⁺ ATPase, Ca²⁺ ATPase, hydrogen exporting ATPase, NADH dehydrogenase, F1F0 ATPase, spermatogenesis ATPase, beta-ketoacyl synthase, pyruvate kinase, and knickkopf; (i) a gene encoding a protein turnover and mitosis protein selected from the group consisting of: cyclins including cyclin A, cyclin B, cyclin B3, cyclin C, cyclin D, cyclin E, cyclin G, cyclin H, cyclin J, cyclin K, cyclin T, and cyclin Y, Ubiquitin conjugating

enzyme E1, chromosome segregation protein, CDC5, COP9 signalosome, and ketel; (j) a gene encoding a gene expression protein selected from the group consisting of: cleavage polyadenylation specificity factor, E1A/CREB-binding protein, RNA helicases, RNA polymerases, Arf1, Bx42, and eft2; (k) a gene encoding a protein-folding, stress, and heat shock response protein selected from the group consisting of: protein disulfide isomerase, heat shock cognate 70, chaperonin TCP1, chaperonins, carrier protein, and HSP70; and (l) a gene encoding a miscellaneous protein selected from the group consisting of: spermatogenesis ATPase, pitchoune, and insect proteins. In embodiments, the mosquitocidally effective polynucleotide, such as a single-stranded RNA transcript, or one strand of a dsRNA, includes multiple segments each of 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides with a sequence of about 95% to about 100% (*e. g.*, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) complementarity with a fragment of one or more DNA sequences selected from the group consisting of **SEQ ID NOs.:1 – 105 and 897 - 1132**. In embodiments, the mosquitocidally effective polynucleotide, such as dsRNA, includes 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides having 100% complementarity with a fragment of a DNA sequence selected from the group consisting of **SEQ ID NOs.:1 – 105 and 897 - 1132**. In embodiments, the mosquitocidally effective polynucleotide, such as dsRNA, includes segments corresponding to different regions of the mosquito target gene, or can include multiple copies of a segment. In other embodiments, the mosquitocidally effective polynucleotide, such as dsRNA, includes multiple segments, each of 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides with a sequence of about 95% to about 100% (*e. g.*, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) complementarity with a fragment of a different mosquito target gene; in this way multiple mosquito target genes, or multiple mosquito species, can be suppressed.

[0026] Several embodiments relate to a mosquitocidally effective polynucleotide, such as dsRNA, which inhibits the expression of one or more mosquito target selected from the group consisting of (a) a gene identified in Tables 2 and 3; (b) a gene encoding a proteasome protein selected from the group consisting of: Proteasome Alpha 2, Proteasome Beta 5, Proteasome regulatory subunit 2, Proteasome regulatory subunit 3, Proteasome regulatory subunit 8, Proteasome regulatory subunit 9, Proteasome regulatory GTPase SAR1, 26S protease regulatory subunit 6; (c) a gene encoding an ATPase protein selected from the group consisting of: V-ATPase subunit A, V-ATPase subunit B, V-ATPase subunit D, V-ATPase subunit E, Actin 6; (d) a gene encoding a ribosomal protein selected from the group consisting of: Ribosomal protein Large subunit 3, Ribosomal protein Large subunit 7, Ribosomal protein Large subunit 19, Ribosomal protein Large subunit 40, Ribosomal protein Small subunit 21, Ribosomal protein Small subunit 4; (e) a gene encoding a vesicle protein selected from the group consisting of: Vesicle Sorting protein 2, Vesicle Sorting protein 4, Vesicle Sorting protein 16A, Vesicle Sorting protein 20, Vesicle Sorting protein 24, Vesicle Sorting protein 27, Vesicle Sorting

protein 28, Vesicle Sorting protein Shrb (Snf7), Vesicle Sorting protein Chmp1, Secretory vesicle protein 6, Secretory vesicle protein 23, Coatomer Protein Alpha, Coatomer Protein Beta, Coatomer Protein Beta Prime, Coatomer Protein Delta, Coatomer Protein Epsilon, Coatomer Protein Zeta; (f) a gene encoding an actin; (g) a gene encoding a protein related to behaviour selected from the group consisting of: Bendless, scribbler, capa receptor, diapause hormone receptor, Ebl1, Ether-a-go-go, flightless-I, foraging, juvenile hormone epoxide hydrolase, neuropeptide F, pheromone biosynthesis activating neuropeptide receptor, semaphorin 1a, shaker, slowmo, stress-sensitive B, turtle, twitchin, myosin heavy chain, nervana, N-synaptobrevin, nuclear hormone receptor 25, syntaxin, and bent; (h) a gene encoding a glycolysis and energy metabolism protein selected from the group consisting of: BiP, citrate synthase, glycogen synthase, hexokinase, isocitrate dehydrogenase, pyruvate dehydrogenase, phosphofructokinase, GTPase activator, GTPase Ran, sarcoplasmic Ca²⁺ ATPase, Ca²⁺ ATPase, hydrogen exporting ATPase, NADH dehydrogenase, F1F0 ATPase, spermatogenesis ATPase, beta-ketoacyl synthase, pyruvate kinase, and knickkopf; (i) a gene encoding a protein turnover and mitosis protein selected from the group consisting of: cyclins including cyclin A, cyclin B, cyclin B3, cyclin C, cyclin D, cyclin E, cyclin G, cyclin H, cyclin J, cyclin K, cyclin T, and cyclin Y, Ubiquitin conjugating enzyme E1, chromosome segregation protein, CDC5, COP9 signalosome, and ketel; (j) a gene encoding a gene expression protein selected from the group consisting of: cleavage polyadenylation specificity factor, E1A/CREB-binding protein, RNA helicases, RNA polymerases, Arf1, Bx42, and eft2; (k) a gene encoding a protein-folding, stress, and heat shock response protein selected from the group consisting of: protein disulfide isomerase, heat shock cognate 70, chaperonin TCP1, chaperonins, carrier protein, and HSP70; and (l) a gene encoding a miscellaneous protein selected from the group consisting of: spermatogenesis ATPase, pitchoune, and insect proteins. Several embodiments relate to a dsRNA having a length greater than that which is typical of naturally occurring regulatory small RNAs (such as endogenously produced siRNAs and mature miRNAs), *i. e.*, the dsRNA is at least about 30 contiguous base-pairs in length. In embodiments, the dsRNA has a length of between about 50 to about 500 base-pairs. In some embodiments, the dsRNA is at least 50 base pairs in length. In embodiments, the dsRNA is formed from two separate, essentially complementary strands (*e. g.*, where each strand is separately provided, or where each strand is encoded on a separate DNA molecule, or where the two strands are encoded on separate sections of a DNA and are separately transcribed or made separate, for example, by the action of a recombinase or nuclease), wherein at least one RNA strand includes a sequence of about 95% to about 100% (*e. g.*, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) identity or complementarity with at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides of a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896**. In embodiments, the dsRNA is blunt-ended, *e. g.*, two separate, equal-length strands of RNA which form the dsRNA through intermolecular hybridisation. In embodiments, the dsRNA has an overhang at one or both ends (termini), *e. g.*, two separate, unequal-length strands of RNA which form the dsRNA through intermolecular hybridisation; the overhang can be a single nucleotide or 2, 3, 4, 5, 6, or more

nucleotides, and can be located on the 5' end or on the 3' end of a strand. In embodiments, the dsRNA includes at least one stem-loop, *e. g.*, a single RNA molecule that forms a dsRNA with a "hairpin" secondary structure through intramolecular hybridization. In embodiments, the dsRNA is formed from a single self-hybridizing hairpin transcript, wherein one "arm" of the hairpin includes a sequence of about 95% to about 100% (*e. g.*, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) identity or complementarity with at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides of a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896**. In embodiments, the dsRNA includes multiple stem-loops, with or without spacer nucleotides between each stem-loop. The dsRNA can be chemically synthesized (*e. g.*, by *in vitro* transcription, such as transcription using a T7 polymerase or other polymerase), or can be produced by expression in a microorganism, by expression in a plant cell, or by microbial fermentation. The dsRNA can be chemically modified, *e. g.*, to improve stability, ease of formulation, or efficacy. In some embodiments, the dsRNA molecule is provided in a microbial or plant cell that expresses dsRNA, or in a microbial fermentation product.

[0027] In some embodiments, mosquitocidally effective polynucleotides are generally designed to induce regulation or suppression of mosquito target genes, which can be coding sequence or non-coding sequence. Examples of such mosquito target genes include those disclosed in **Tables 2 and 3**. In some embodiments, mosquitocidally effective polynucleotides are designed to have a nucleotide sequence essentially identical or essentially complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides of a sequence of a mosquito target gene or cDNA (*e. g.*, **SEQ ID NOs.:1 – 105 and 897 - 1132**) or to the sequence of RNA transcribed from a mosquito target gene.

Selection of Mosquitocidally Effective Polynucleotide by "Tiling"

[0028] Mosquitocidally effective polynucleotides need not be of the full length of a target gene, and in many embodiments are of much shorter length in comparison to the target gene. An example of a technique that is useful for selecting mosquitocidally effective polynucleotides is "tiling", or evaluation of polynucleotides corresponding to adjacent or partially overlapping segments of a target gene.

[0029] Mosquitocidally effective polynucleotides can be identified by "tiling" target genes in selected length fragments, for example, in fragments of 100 – 300 nucleotides in length, with partially overlapping regions (*e. g.*, of about 25 nucleotides) along the length of the target gene. In some embodiments, polynucleotide sequences are designed to correspond to (have a nucleotide identity or complementarity with) regions that are unique to the target gene; the selected region of the target gene can include coding sequence or non-coding sequence (*e. g.*, promoter regions, 3' untranslated regions, introns and the like) or a combination of both. In some embodiments, a set of polynucleotides is designed for each of the target genes listed in **Tables 2 and 3**. In some embodiments, a set of polynucleotides comprising 200 – 300 nucleotides with a sequence complementary to a fragment of a target gene having a sequence selected from **SEQ ID NOs.:1 – 105 and 897 - 1132** are designed so that

each polynucleotide's sequence overlaps about 25 nucleotides of the next adjacent polynucleotide's sequence, in such a way that the set of polynucleotides in combination cover the full length of the target gene. The tiling procedure can be repeated, if desired. In some embodiments, a polynucleotide found to provide desired mosquitocidal activity can itself be subjected to a tiling procedure. For example, multiple overlapping polynucleotides are designed, each of 50 – 60 nucleotides in length and with a sequence complementary to the fragment of a target gene having a sequence selected from SEQ ID NOs.:1 – 105 and 897 - 1132 for which a single polynucleotide of 300 nucleotides was found to be effective. Additional rounds of tiling analysis can be carried out, where polynucleotides as short as 18, 19, 20, or 21 nucleotides are tested.

[0030] In some embodiments where it is of interest to design a polynucleotide effective in suppressing multiple target genes, the multiple target gene sequences are aligned and polynucleotides designed to correspond to regions with high sequence homology in common among the multiple targets.

Conversely, where it is of interest to design a polynucleotide effective in selectively suppressing one among multiple target sequences, the multiple target gene sequences are aligned and polynucleotides are designed to correspond to regions with no or low sequence homology in common among the multiple targets.

[0031] The polynucleotides are tested by any convenient means for efficacy in silencing the target gene. Examples of a suitable tests include the bioassays described herein in the Examples. Another test involves the topical application of the polynucleotide either directly to individual mosquitoes or to an environment to be protected from a mosquito infestation. One desired result of treatment with a mosquitocidally effective polynucleotide is prevention or control of a mosquito infestation, *e. g.*, by inducing in a mosquito a physiological or behavioural change such as, but not limited to, growth stunting, increased mortality, decrease in reproductive capacity, decrease in or cessation of feeding behavior or movement, or decrease in or cessation of metamorphosis stage development.

[0032] Mosquitocidally effective polynucleotides of any size can be used, alone or in combination, in the various compositions and methods described herein. In some embodiments, a single mosquitocidally effective polynucleotide is used to make a composition (*e. g.*, a composition for topical application, or a recombinant DNA construct useful for making a transgenic plant). In other embodiments, a mixture or pool of different mosquitocidally effective polynucleotides is used; in such cases the mosquitocidally effective polynucleotides can be for a single target gene or for multiple target genes. In some embodiments, a mosquitocidally effective polynucleotide is designed to target different regions of a target gene. In some embodiments, a mosquitocidally effective polynucleotide includes multiple segments that correspond to different exon regions of a target gene, and “spacer” nucleotides which do not correspond to a target gene can optionally be used in between or adjacent to the segments.

Thermodynamic Considerations in Selecting Mosquitocidally Effective Polynucleotides

[0033] Mosquitocidally effective polynucleotides can be designed or their sequence optimised using thermodynamic considerations. For example, mosquitocidally effective polynucleotides can be selected

based on the thermodynamics controlling hybridization between one nucleic acid strand (*e. g.*, a polynucleotide or an individual siRNA) and another (*e. g.*, a target gene transcript). Methods and algorithms to predict nucleotide sequences that are likely to be effective at RNAi-mediated silencing of a target gene are known in the art. Non-limiting examples of such methods and algorithms include “i-score”, described by Ichihara *et al.* (2007) *Nucleic Acids Res.*, 35(18): 123e; “Oligowalk”, publicly available at rna.urmc.rochester.edu/servers/oligowalk and described by Lu *et al.* (2008) *Nucleic Acids Res.*, 36:W104-108; and “Reynolds score”, described by Khovorova *et al.* (2004) *Nature Biotechnol.*, 22:326-330.

Permitted mismatches

[0034] By “essentially identical” or “essentially complementary” is meant that the mosquitocidally effective polynucleotide (or at least one strand of a double-stranded polynucleotide) has sufficient identity or complementarity to the target gene or to the RNA transcribed from a target gene (*e. g.*, the transcript) to suppress expression of a target gene (*e. g.*, to effect a reduction in levels or activity of the target gene transcript and/or encoded protein). Mosquitocidally effective polynucleotides need not have 100 percent identity or complementarity to a target gene or to the RNA transcribed from a target gene to suppress expression of the target gene (*e. g.*, to effect a reduction in levels or activity of the target gene transcript or encoded protein, or to provide control of an insect species). In some embodiments, the mosquitocidally effective polynucleotide or a portion thereof is designed to be essentially identical to, or essentially complementary to, a sequence of at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides in either the target gene or the RNA transcribed from the target gene. In some embodiments, the mosquitocidally effective polynucleotide or a portion thereof is designed to be exactly identical to, or exactly complementary to, a sequence of at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides in either the target gene or the RNA transcribed from the target gene. In certain embodiments, an “essentially identical” polynucleotide has 100 percent sequence identity or at least about 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent sequence identity when compared to the sequence of 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides in either the endogenous target gene or to an RNA transcribed from the target gene. In certain embodiments, an “essentially complementary” polynucleotide has 100 percent sequence complementarity or at least about 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent sequence complementarity when compared to the sequence of at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides in either the target gene or RNA transcribed from the target gene.

[0035] Mosquitocidally effective polynucleotides containing mismatches to the target gene or transcript can be used in certain embodiments of the compositions and methods described herein. In

some embodiments, the mosquitocidally effective polynucleotide includes 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides that are essentially identical or essentially complementary to a segment of equivalent length in the target gene or target gene's transcript. In certain embodiments, a polynucleotide of 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides that is essentially identical or essentially complementary to a segment of equivalent length in the target gene or target gene's transcript can have 1 or 2 mismatches to the target gene or transcript (*i. e.*, 1 or 2 mismatches between the polynucleotide's contiguous nucleotides and the segment of equivalent length in the target gene or target gene's transcript). In certain embodiments, a polynucleotide of 20 or more nucleotides that contains a contiguous 19 nucleotide span of identity or complementarity to a segment of equivalent length in the target gene or target gene's transcript can have 1 or 2 mismatches to the target gene or transcript. In certain embodiments, a polynucleotide of 21 continuous nucleotides that is essentially identical or essentially complementary to a segment of equivalent length in the target gene or target gene's transcript can have 1, 2, or 3 mismatches to the target gene or transcript. In certain embodiments, a polynucleotide of 22 or more nucleotides that contains a contiguous 21 nucleotide span of identity or complementarity to a segment of equivalent length in the target gene or target gene's transcript can have 1, 2, or 3 mismatches to the target gene or transcript.

[0036] In designing mosquitocidally effective polynucleotides with mismatches to an endogenous target gene or to an RNA transcribed from the target gene, mismatches of certain types and at certain positions that are more likely to be tolerated can be used. In certain embodiments, mismatches formed between adenine and cytosine or guanosine and uracil residues are used as described by Du *et al.* (2005) *Nucleic Acids Res.*, 33:1671–1677. In some embodiments, mismatches in 19 base-pair overlap regions are located at the low tolerance positions 5, 7, 8 or 11 (from the 5' end of a 19-nucleotide target), at medium tolerance positions 3, 4, and 12-17 (from the 5' end of a 19-nucleotide target), and/or at the high tolerance positions at either end of the region of complementarity, positions 1, 2, 18, and 19 (from the 5' end of a 19-nucleotide target) as described by Du *et al.* (2005) *Nucleic Acids Res.*, 33:1671–1677. Tolerated mismatches can be empirically determined in routine assays such as those described herein in the Examples.

[0037] In some embodiments, the mosquitocidally effective polynucleotides comprise additional nucleotides for reasons of stability or for convenience in cloning or synthesis. In one embodiment, the polynucleotide is a dsRNA including an RNA strand with a segment of at least 21 contiguous nucleotides of a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087** and further including an additional 5' G or an additional 3' C or both, adjacent to the segment. In another embodiment, the mosquitocidally effective polynucleotide is a double-stranded RNA including additional nucleotides to form an overhang, for example, a dsRNA including 2 deoxyribonucleotides to form a 3' overhang.

Embedding Active Triggers in Neutral Sequence

[0038] In some embodiments, a sequence corresponding to the target gene and which is responsible for an observed suppression of the target gene is embedded in “neutral” sequence, *e.g.*, inserted into additional nucleotides that have no sequence identity or complementarity to the target gene. Neutral sequence can be desirable, *e.g.*, to increase the overall length of a polynucleotide. For example, it can be desirable for a mosquitocidally effective polynucleotide to be of a particular size for reasons of stability, cost-effectiveness in manufacturing, or biological activity.

[0039] In some insects, the size of a polynucleotide may affect efficacy of dietary uptake. For example, it has been reported that in the coleopteran species, *Diabrotica virgifera*, dsRNAs greater than or equal to approximately 60 base-pairs (bp) are required for biological activity in artificial diet bioassays; see Bolognesi *et al.* (2012) PLoS ONE 7(10): e47534. doi:10.1371/journal.pone.0047534.

Thus, in one embodiment, a 21-base-pair sequence corresponding to a mosquito target gene in **Tables 2** or **3** is embedded in neutral sequence of an additional 39 base pairs, thus forming a mosquitocidally effective polynucleotide of about 60 base pairs. In another embodiment, a single 21-base-pair sequence is found to be efficacious when embedded in larger sections of neutral sequence, *e.g.*, where the total polynucleotide length is from about 60 to about 300 base pairs. In another embodiment, at least one segment of at least 21 contiguous nucleotides of a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087** is embedded in larger sections of neutral sequence to provide a mosquitocidally effective polynucleotide. In another embodiment, segments from multiple sequences selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087** are embedded in larger sections of neutral sequence to provide a mosquitocidally effective polynucleotide.

[0040] It is anticipated that the combination of certain mosquitocidally effective polynucleotides (*e.g.*, dsRNA comprising a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087**, or active fragments thereof) with one or more non-polynucleotide pesticidal agents will result in an improvement in prevention or control of mosquito infestations, when compared to the effect obtained with the mosquitocidally effective polynucleotide alone or the non-polynucleotide pesticidal agent alone. Routine bioassays such as the bioassays described herein in the Examples are useful for defining dose-responses for mosquito larval or adult mortality or growth inhibition using combinations of the mosquitocidally effective polynucleotide and one or more non-polynucleotide mosquitocidal agents (*e.g.*, an insecticidal protein or insect growth inhibitor). One of skill in the art can test combinations of mosquitocidally effective polynucleotide and non-polynucleotide mosquitocidal agents in routine bioassays to identify combinations of bioactives that are desirable for use in mosquito control.

METHODS OF MOSQUITO CONTROL

[0041] Provided herein are methods of mosquito control comprising contacting a mosquito with a mosquitocidally effective polynucleotide, such as a dsRNA, designed to suppress a target gene in the mosquito.

[0042] An embodiment is a method of mosquito control including contacting a mosquito with a

mosquitocidally effective polynucleotide including a nucleotide sequence that is complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides with a sequence of about 95% to about 100% (e. g., about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) complementarity or identity with a target gene, or an RNA transcribed from the target gene. In embodiments, the method includes contacting a mosquito with a mosquitocidally effective polynucleotide including a nucleotide sequence that is complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides of multiple target genes, or multiple alleles of a target gene, or an RNA transcribed from the target gene. In embodiments of these methods, the target gene is a gene essential to normal metabolic function or to normal reproduction in a mosquito. In embodiments, the target gene is one or more genes selected from the group consisting of: (a) a gene identified in Tables 2 and 3; (b) a gene encoding a proteasome protein selected from the group consisting of: Proteasome Alpha 2, Proteasome Beta 5, Proteasome regulatory subunit 2, Proteasome regulatory subunit 3, Proteasome regulatory subunit 8, Proteasome regulatory subunit 9, Proteasome regulatory GTPase SAR1, 26S protease regulatory subunit 6; (c) a gene encoding an ATPase protein selected from the group consisting of: V-ATPase subunit A, V-ATPase subunit B, V-ATPase subunit D, V-ATPase subunit E, Actin 6; (d) a gene encoding a ribosomal protein selected from the group consisting of: Ribosomal protein Large subunit 3, Ribosomal protein Large subunit 7, Ribosomal protein Large subunit 19, Ribosomal protein Large subunit 40, Ribosomal protein Small subunit 21, Ribosomal protein Small subunit 4; (e) a gene encoding a vesicle protein selected from the group consisting of: Vesicle Sorting protein 2, Vesicle Sorting protein 4, Vesicle Sorting protein 16A, Vesicle Sorting protein 20, Vesicle Sorting protein 24, Vesicle Sorting protein 27, Vesicle Sorting protein 28, Vesicle Sorting protein Shrb (Snf7), Vesicle Sorting protein Chmp1, Secretory vesicle protein 6, Secretory vesicle protein 23, Coatomer Protein Alpha, Coatomer Protein Beta, Coatomer Protein Beta Prime, Coatomer Protein Delta, Coatomer Protein Epsilon, Coatomer Protein Zeta; (f) a gene encoding an actin; (g) a gene encoding a protein related to behaviour selected from the group consisting of: Bendless, scribbler, capa receptor, diapause hormone receptor, Eb1, Ether-a-go-go, flightless-I, foraging, juvenile hormone epoxide hydrolase, neuropeptide F, pheromone biosynthesis activating neuropeptide receptor, semaphorin 1a, shaker, slowmo, stress-sensitive B, turtle, twitchin, myosin heavy chain, nervana, N-synaptobrevin, nuclear hormone receptor 25, syntaxin, and bent; (h) a gene encoding a glycolysis and energy metabolism protein selected from the group consisting of: BiP, citrate synthase, glycogen synthase, hexokinase, isocitrate dehydrogenase, pyruvate dehydrogenase, phosphofructokinase, GTPase activator, GTPase Ran, sarcoplasmic Ca²⁺ ATPase, Ca²⁺ ATPase, hydrogen exporting ATPase, NADH dehydrogenase,

F1F0 ATPase, spermatogenesis ATPase, beta-ketoacyl synthase, pyruvate kinase, and knickkopf; (i) a gene encoding a protein turnover and mitosis protein selected from the group consisting of: cyclins including cyclin A, cyclin B, cyclin B3, cyclin C, cyclin D, cyclin E, cyclin G, cyclin H, cyclin J, cyclin K, cyclin T, and cyclin Y, Ubiquitin conjugating enzyme E1, chromosome segregation protein, CDC5, COP9 signalosome, and ketel; (j) a gene encoding a gene expression protein selected from the group consisting of: cleavage polyadenylation specificity factor, E1A/CREB-binding protein, RNA helicases, RNA polymerases, Arf1, Bx42, and eft2; (k) a gene encoding a protein-folding, stress, and heat shock response protein selected from the group consisting of: protein disulfide isomerase, heat shock cognate 70, chaperonin TCP1, chaperonins, carrier protein, and HSP70; and (l) a gene encoding a miscellaneous protein selected from the group consisting of: spermatogenesis ATPase, pitchoune, and insect proteins.

[0043] Another embodiment is a method of mosquito control including contacting a mosquito with an effective amount of a mosquitocidally effective polynucleotide, one strand of which includes a nucleotide sequence that is complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides with a sequence of about 95% to about 100% (e. g., about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) complementarity or identity with a target gene having a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene. In embodiments, the method includes contacting a mosquito with a mosquitocidally effective amount of a polynucleotide, one strand of which is complementary to at least 21 contiguous nucleotides of a target gene having a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene. In embodiments, the method results in inducing RNA interference, and mosquito mortality or failure to reproduce occurs.

[0044] Another embodiment is a method of mosquito control including providing in the diet of a mosquito a mosquitocidally effective polynucleotide including a nucleotide sequence that is complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides with a sequence of about 95% to about 100% (e. g., about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) complementarity or identity with a target gene having a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene. In embodiments, the method includes providing in the diet of a mosquito a mosquitocidally effective polynucleotide comprising a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene having a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene.

[0045] Another embodiment is a method of mosquito control including causing mortality or stunting in larvae of a mosquito by providing in the diet of the larvae at least one mosquitocidally effective polynucleotide including at least one silencing element, wherein the silencing element includes at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44,

45, 46, 47, 48, 49, 50 or more contiguous nucleotides of a sequence of about 95% to about 100% (e. g., about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) complementarity or identity with a target gene having a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene. In embodiments, the method

includes causing mortality or stunting in larvae of a mosquito by providing in the diet of the larvae at least one mosquitocidally effective polynucleotide including at least one silencing element, wherein the silencing element includes at least 21 contiguous nucleotides that are complementary to a target gene having a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene. In embodiments, the mosquitocidally effective polynucleotide comprises multiple silencing elements; each silencing element can comprise sequence that is complementary to a single target gene (e. g., multiple copies of a single silencing element sequence, or a combination of silencing elements that are complementary to different regions of a target gene) or to different target genes or different alleles of a target gene.

[0046] Another embodiment is a method of mosquito control including contacting a mosquito with a mosquitocidally effective polynucleotide including at least one segment that is identical or complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides of a target gene selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene. In embodiments, the method includes contacting a mosquito with a mosquitocidally effective polynucleotide including at least one segment that is identical or complementary to at least 21 contiguous nucleotides of a target gene selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene. In embodiments, the mosquitocidally effective polynucleotide comprises multiple segments; each segment can include sequence that is complementary to a single target gene (e. g., multiple copies of a single segment, or a combination of segments that are complementary to different regions of a target gene) or to different target genes or different alleles of a target gene.

[0047] Another embodiment is a method of mosquito control including contacting an environment infested by the mosquito with a composition including a mosquitocidally effective polynucleotide including a nucleotide sequence that is complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides of a target gene having a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene, thereby preventing or decreasing infestation by the mosquito. In embodiments, the method includes contacting an environment infested by the mosquito with a composition including a mosquitocidally effective polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene having a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene.

[0048] In embodiments of these methods, the mosquito is an *Aedes* sp., a *Culex* sp., or an *Anopheles* sp. In embodiments of these methods, (a) the mosquitoes are *Aedes aegypti* and the target gene has a DNA sequence selected from the group consisting of SEQ ID NOs.:1 – 36 and 897 – 975; or (b) the mosquitoes are *Culex quinquefasciatus* and the target gene has a DNA sequence selected from the group consisting of SEQ ID NOs.:37 – 71 and 1014 and 1060 – 1132; or (c) the mosquitoes are *Anopheles gambiae* or *Anopheles gambiae* species complex and the target gene has a DNA sequence selected from the group consisting of SEQ ID NOs.:72 – 105 and 976 – 1013 and 1015 – 1059.

[0049] In embodiments of these methods, the mosquito is an adult, pupa, larva, or egg. In embodiments the method is effective on multiple life-stages of mosquitoes. In embodiments of these methods, contacting or ingesting the mosquitocidally effective polynucleotide, such as a dsRNA, results in mortality or stunting of the mosquito, or failure of the mosquito to reproduce, or failure of the mosquito to progress through metamorphosis. In embodiments of these methods, the mosquito is in a population of mosquitoes and the population size or reproductive fitness is reduced.

[0050] In embodiments of these methods, the mosquitocidally effective polynucleotide is provided in an ingestible composition, a sprayable composition, a particulate or powder, a solid bait, or a liquid bait. In embodiments of these methods, contacting includes oral delivery to the mosquito, or includes non-oral delivery to the mosquito, or includes a combination of oral and non-oral delivery to the mosquito. In embodiments of these methods, the contacting includes providing the mosquitocidally effective polynucleotide in a composition that is ingested or contacted by the mosquito. In embodiments of these methods, the composition includes a solid, liquid, powder, suspension, emulsion, colloid, spray, encapsulation, microbeads, carrier particulates, granules, film, gel, or matrix. In embodiments of these methods, the composition includes a bacterial or eukaryotic cell expressing the mosquitocidally effective polynucleotide. In embodiments of these methods, the composition is provided in the form of a bait (such as a sugar bait), a trap, a sprayable liquid, a concentrate, dunks, dispersible granules, or ingestible particulates or cells. In embodiments of these methods, the composition further includes one or more components selected from the group consisting of a carrier agent, a surfactant, an organosilicone, a lipid (such as cationic lipids, dendrimers, neutral lipids, fatty acids, or compositions comprising lipid components such as liposomes), a sugar, a non-polynucleotide mosquitocide, a dipterocidal *Bacillus thuringiensis* toxin (or other insecticidal bacterially produced toxin), a safener, a mosquito attractant, a pheromone, and an insect growth regulator such as a ecdysis inhibitor. Embodiments of the mosquito control methods include providing mosquitocidally effective polynucleotides in the form of attractive toxic sugar baits, or as larvicidal formulations (*e. g.*, dispersible powders or granules or dunks, or sprayable liquids). In some embodiments the composition further comprises a cationic carbohydrate. In some embodiments the composition further comprises a cationic starch. In some embodiments the composition further comprises a cationic guar. In some embodiments the composition further comprises a branched Polyethylenimine. In embodiments, methods utilizing a combination of a mosquitocidally effective polynucleotide designed to suppress a mosquito target gene and an insecticidal protein or an insect growth regulator provides a level of

mosquito control that is greater than the effects of the polynucleotide and the insecticidal protein or an insect growth regulator components if tested separately.

[0051] In embodiments of these methods, the mosquitocidally effective polynucleotide is a double-stranded RNA (dsRNA). In embodiments, the dsRNA (a) is blunt-ended, or (b) has an overhang (e. g., an overhang of 1, 2, 3, or 4 nucleotides) at one terminus or both termini, and/or (c) includes at least one stem-loop. In embodiments, the dsRNA includes at least one stem-loop with additional secondary structure, such as mismatches or additional nucleotides within the “stem” of the stem-loop structure. In embodiments, the dsRNA is produced by (a) chemical synthesis, or (b) expression in a microorganism, or (c) expression in a eukaryotic cell. In embodiments, the dsRNA is chemically modified.

[0052] In embodiments of these methods, the mosquitocidally effective polynucleotide is a double-stranded RNA that includes a strand including at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides that are identical or complementary to a sequence selected from the group consisting of: **SEQ ID NOs.:106 – 896**. In specific embodiments of these methods, (a) the mosquitoes are *Aedes aegypti* and the double-stranded RNA includes one strand which is complementary to at least 21 contiguous nucleotides of a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:106 – 382 and 1133 – 1440**; or (b) the mosquitoes are *Culex quinquefasciatus* and the double-stranded RNA includes one strand which is complementary to at least 21 contiguous nucleotides of a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:383 – 647 and 1613 – 1614 and 1762 – 2087**; or (c) the mosquitoes are *Anopheles gambiae* or *Anopheles gambiae* species complex and the double-stranded RNA includes one strand which is complementary to at least 21 contiguous nucleotides of a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:648 – 896 and 1441 – 1612 and 1615 – 1761**.

MOSQUITOCIDAL COMPOSITIONS AND POLYNUCLEOTIDES

[0053] Provided herein are mosquitocidal compositions including a mosquitocidally effective amount of a recombinant polynucleotide, such as a dsRNA, designed to suppress a target gene in a mosquito.

[0054] An embodiment is a mosquitocidal composition including a mosquitocidally effective amount of a recombinant polynucleotide including at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides with a sequence of about 95% to about 100% (e. g., about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) complementarity with a fragment of a mosquito target gene, or an RNA transcribed from the mosquito target gene. In embodiments, the mosquitocidal composition includes a recombinant polynucleotide including a nucleotide sequence that is complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides of a mosquito target gene, or an RNA transcribed from the mosquito target gene. In embodiments, the mosquitocidal composition includes a recombinant polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous

nucleotides of a mosquito target gene, or an RNA transcribed from the mosquito target gene. In embodiments of these mosquitocidal compositions, the target gene is a gene essential to normal metabolic function or to normal reproduction in a mosquito. In embodiments, the target gene is selected from the group consisting of: (a) a gene identified by name in Tables 2 and 3; (b) a gene encoding a proteasome protein selected from the group consisting of: Proteasome Alpha 2, Proteasome Beta 5, Proteasome regulatory subunit 2, Proteasome regulatory subunit 3, Proteasome regulatory subunit 8, Proteasome regulatory subunit 9, Proteasome regulatory GTPase SAR1, 26S protease regulatory subunit 6; (c) a gene encoding an ATPase protein selected from the group consisting of: V-ATPase subunit A, V-ATPase subunit B, V-ATPase subunit D, V-ATPase subunit E, Actin 6; (d) a gene encoding a ribosomal protein selected from the group consisting of: Ribosomal protein Large subunit 3, Ribosomal protein Large subunit 7, Ribosomal protein Large subunit 19, Ribosomal protein Large subunit 40, Ribosomal protein Small subunit 21, Ribosomal protein Small subunit 4; (e) a gene encoding a vesicle protein selected from the group consisting of: Vesicle Sorting protein 2, Vesicle Sorting protein 4, Vesicle Sorting protein 16A, Vesicle Sorting protein 20, Vesicle Sorting protein 24, Vesicle Sorting protein 27, Vesicle Sorting protein 28, Vesicle Sorting protein Shrb (Snf7), Vesicle Sorting protein Chmp1, Secretory vesicle protein 6, Secretory vesicle protein 23, Coatamer Protein Alpha, Coatamer Protein Beta, Coatamer Protein Beta Prime, Coatamer Protein Delta, Coatamer Protein Epsilon, Coatamer Protein Zeta; (f) a gene encoding an actin; (g) a gene encoding a protein related to behaviour selected from the group consisting of: Bendless, scribbler, capa receptor, diapause hormone receptor, Eb1, Ether-a-go-go, flightless-I, foraging, juvenile hormone epoxide hydrolase, neuropeptide F, pheromone biosynthesis activating neuropeptide receptor, semaphorin 1a, shaker, slowmo, stress-sensitive B, turtle, twitchin, myosin heavy chain, nervana, N-synaptobrevin, nuclear hormone receptor 25, syntaxin, and bent; (h) a gene encoding a glycolysis and energy metabolism protein selected from the group consisting of: BiP, citrate synthase, glycogen synthase, hexokinase, isocitrate dehydrogenase, pyruvate dehydrogenase, phosphofructokinase, GTPase activator, GTPase Ran, sarcoplasmic Ca²⁺ ATPase, Ca²⁺ ATPase, hydrogen exporting ATPase, NADH dehydrogenase, F1F0 ATPase, spermatogenesis ATPase, beta-ketoacyl synthase, pyruvate kinase, and knickkopf; (i) a gene encoding a protein turnover and mitosis protein selected from the group consisting of: cyclins including cyclin A, cyclin B, cyclin B3, cyclin C, cyclin D, cyclin E, cyclin G, cyclin H, cyclin J, cyclin K, cyclin T, and cyclin Y, Ubiquitin conjugating enzyme E1, chromosome segregation protein, CDC5, COP9 signalosome, and ketel; (j) a gene encoding a gene expression protein selected from the group consisting of: cleavage polyadenylation specificity factor, E1A/CREB-binding protein, RNA helicases, RNA polymerases, Arf1, Bx42, and eft2; (k) a gene encoding a protein-folding, stress, and heat shock response protein selected from the group consisting of: protein disulfide isomerase, heat shock cognate 70, chaperonin TCP1, chaperonins, carrier protein, and HSP70; and (l) a gene encoding a miscellaneous protein selected from the group consisting of: spermatogenesis ATPase, pitchoune, and insect proteins. In embodiments, the mosquitocidal composition includes a recombinant polynucleotide including a nucleotide sequence that is complementary to at least 18 contiguous nucleotides of multiple target

genes (or their transcripts), or multiple alleles of a target gene (or its transcript), wherein the target gene is selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**.

[0055] Another embodiment is a mosquitocidal composition including a mosquitocidally effective amount of a recombinant polynucleotide including a nucleotide sequence that is complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides of a target gene having a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene. In embodiments, the mosquitocidal composition includes a mosquitocidally effective amount of a recombinant polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene having a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene. In embodiments, the mosquitocidal composition includes a mosquitocidally effective amount of a recombinant polynucleotide including a nucleotide sequence that is complementary to at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides of multiple target genes (or their transcripts), or multiple alleles of a target gene (or its transcript), wherein the target gene is selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**.

[0056] In embodiments of these mosquitocidal compositions, mosquitocidally effective polynucleotide is provided in a microbial or eukaryotic cell that expresses the recombinant polynucleotide, or in a microbial fermentation product. In embodiments of these mosquitocidal compositions, the composition is provided as a solid, liquid, powder, suspension, emulsion, colloid, spray, encapsulation, microbeads, carrier particulates, granules, film, gel, or matrix. In embodiments of these mosquitocidal compositions, the composition is provided in the form of a bait (such as a sugar bait), a trap, a sprayable liquid, a concentrate, dunks, dispersible granules, or ingestible particulates or cells. In embodiments of these mosquitocidal compositions, the composition further includes one or more components selected from the group consisting of a carrier agent, a surfactant, an organosilicone, a lipid (such as cationic lipids, dendrimers, neutral lipids, fatty acids, or compositions comprising lipid components such as liposomes), a sugar, a non-polynucleotide mosquitocide, a dipterocidal *Bacillus thuringiensis* toxin (or other insecticidal bacterially produced toxin), a safener, a mosquito attractant or pheromone, and an insect growth regulator such as a ecdysis inhibitor. Embodiments of attractive toxic sugar baits formulated with dsRNAs are described in the Examples. Other embodiments are larvicidal formulations such as dispersible powders or granules or dunks, or sprayable liquids, which are applied to mosquito breeding areas. In some embodiments the composition further comprises a cationic carbohydrate. In some embodiments the composition further comprises a cationic starch. In some embodiments the composition further comprises a cationic guar. In some embodiments the composition further comprises a branched Polyethylenimine. In embodiments, a mosquitocidal composition including a polynucleotide designed to suppress a mosquito target gene and at least one of a insecticidal bacterially produced toxin or an insect growth regulator provides a level of insect control that is greater

than the sum of the effects of the polynucleotide and the insecticidal bacterially produced toxin or an insect growth regulator components if tested separately.

[0057] In embodiments of these mosquitocidal compositions, the recombinant polynucleotide is a dsRNA. In embodiments, the dsRNA includes a strand including at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous ribonucleotides of a sequence selected from the group consisting of SEQ ID NOs.:106 – 896 and 1133 - 2087. In embodiments, the dsRNA includes a strand including at least 21 contiguous ribonucleotides of an RNA sequence selected from the group consisting of SEQ ID NOs.:106 – 896 and 1133 - 2087. In embodiments, the dsRNA includes a strand including multiple segments each at least 21 contiguous ribonucleotides of at least one RNA sequence selected from the group consisting of SEQ ID NOs.:106 – 896 and 1133 - 2087. In embodiments, the dsRNA (a) is blunt-ended, or (b) has an overhang (*e. g.*, an overhang of 1, 2, 3, or 4 nucleotides) at one terminus or both termini, and/or (c) includes at least one stem-loop. In embodiments, the dsRNA includes at least one stem-loop with additional secondary structure, such as mismatches or additional nucleotides within the “stem” of the stem-loop structure. In embodiments, the dsRNA is produced by (a) chemical synthesis, or (b) expression in a microorganism, or (c) expression in a eukaryotic cell. In embodiments, the dsRNA is chemically modified.

[0058] Embodiments of the mosquitocidal compositions intended for dispersal by spraying onto plants or in an outdoor environment optionally include the appropriate stickers and wetters for efficient surface coverage as well as UV protectants to protect polynucleotides, such as dsRNAs, from UV damage. Such additives are commonly used in the bioinsecticide industry and are known to those skilled in the art. Embodiments of the compositions include solid granular formulations that can be dispersed on the surface of standing water or other environments that contain mosquito larvae or where adult mosquitoes breed or feed. Embodiments of the compositions include solid baits, such as the attractive toxic sugar baits described in the Examples. In some embodiments the composition further comprises a cationic carbohydrate. In some embodiments the composition further comprises a cationic starch. In some embodiments the composition further comprises a cationic guar. In some embodiments the composition further comprises a branched Polyethylenimine.

[0059] Embodiments of mosquitocidal compositions include a “transfer agent” that, when combined with a composition including a mosquitocidally effective polynucleotide described herein that is topically applied to or comes into contact with the surface of a mosquito, enables the polynucleotide to enter the cells of the mosquito. Such transfer agents can be incorporated as part of the composition including a mosquitocidally effective polynucleotide, or can be applied prior to, contemporaneously with, or following application of the composition including a mosquitocidally effective polynucleotide as described herein. Suitable transfer agents include agents that increase permeability of the exterior of the mosquito or that increase permeability of cells of the mosquito to mosquitocidally effective polynucleotides. Examples of transfer agents include (a) surfactants, (b) an organic solvent or an aqueous solution or aqueous mixtures of organic solvents, (c) oxidizing agents, (d) acids, (e) bases, (f)

oils, (g) enzymes, or combinations thereof. Suitable transfer agents can cause the polynucleotide composition to take the form of an emulsion, a reverse emulsion, a liposome, or other micellar-like composition. Examples of transfer agents include counter-ions or other molecules that are known to associate with nucleic acid molecules, *e. g.*, cationic lipids, inorganic ammonium ions, alkyl ammonium ions, lithium ions, polyamines such as spermine, spermidine, or putrescine, cationic starches and other cations. Embodiments of transfer agents include organic solvents such as DMSO, DMF, pyridine, *N*-pyrrolidine, hexamethylphosphoramide, acetonitrile, dioxane, polypropylene glycol, or other solvents miscible with water or that dissolve phosphonucleotides in non-aqueous systems (such as is used in synthetic reactions). Embodiments of transfer agents include naturally derived or synthetic oils with or without surfactants or emulsifiers, *e. g.*, plant-sourced oils, crop oils (such as those listed in the 9th Compendium of Herbicide Adjuvants, publicly available on-line at herbicide.adjuvants.com), paraffinic oils, polyol fatty acid esters, or oils with short-chain molecules modified with amides or polyamines such as polyethyleneimine or *N*-pyrrolidine. Embodiments of transfer agents include organosilicone preparations. For example, a suitable transfer agent is an organosilicone preparation that is commercially available as SILWET L-77® brand surfactant having CAS Number 27306-78-1 and EPA Number: CAL.REG.NO. 5905-50073-AA, and currently available from Momentive Performance Materials, Albany, New York. Organosilicone compounds useful as transfer agents include, but are not limited to, compounds that include: (a) a trisiloxane head group that is covalently linked to, (b) an alkyl linker including, but not limited to, an *n*-propyl linker, that is covalently linked to, (c) a polyglycol chain, that is covalently linked to, (d) a terminal group.

RECOMBINANT DNA CONSTRUCTS

[0060] Provided herein are recombinant DNA constructs including a heterologous promoter operably linked to DNA encoding an RNA transcript designed to suppress a target gene in a mosquito.

[0061] An embodiment is a recombinant DNA construct including a heterologous promoter operably linked to DNA encoding an RNA transcript including a sequence of about 95% to about 100% identity or complementarity with a sequence selected from the group consisting of SEQ ID NOs.:1 – 105 and 897 - 1132.

[0062] An embodiment is a recombinant DNA construct including a heterologous promoter operably linked to DNA encoding an RNA transcript including at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides that are complementary to a target gene having a nucleotide sequence selected from the group consisting of: SEQ ID NOs.:1 – 105 and 897 - 1132, or an RNA transcribed from the target gene. In embodiments, the recombinant DNA construct includes a heterologous promoter operably linked to DNA encoding an RNA transcript including at least 21 contiguous nucleotides that are complementary to a target gene having a nucleotide sequence selected from the group consisting of: SEQ ID NOs.:1 – 105 and 897 - 1132, or an RNA transcribed from the target gene. In embodiments, the recombinant DNA construct includes a heterologous promoter operably linked to DNA encoding an

RNA transcript including multiple segments each of at least 21 contiguous nucleotides that are complementary to a target gene comprising a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene; each segment can include sequence that is complementary to a single target gene (*e. g.*, multiple copies of a single segment, or a combination of segments that are complementary to different regions of a target gene) or to different target genes or different alleles of a target gene; the segments need not be immediately contiguous in the RNA transcript.

[0063] An embodiment is a recombinant DNA construct including a heterologous promoter operably linked to DNA encoding an RNA transcript including at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous ribonucleotides of an RNA sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087**. In embodiments, the recombinant DNA construct includes a heterologous promoter operably linked to DNA encoding an RNA transcript including at least 21 contiguous ribonucleotides of an RNA sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087**. In embodiments, the recombinant DNA construct includes a heterologous promoter operably linked to DNA encoding an RNA transcript including multiple segments each of at least 21 contiguous nucleotides of an RNA sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087**; in embodiments the RNA transcript includes multiple copies of a single segment, or a combination of segments that are complementary to different regions of an RNA sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087**, or to different RNA sequences selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087**, or different alleles of an RNA sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087**; the segments need not be immediately contiguous in the RNA transcript.

[0064] An embodiment is a recombinant DNA construct including a heterologous promoter operably linked to DNA encoding an RNA transcript including a strand including a sequence selected from the group consisting of: **SEQ ID NOs.:106 – 896 and 1133 - 2087**. In embodiments, the recombinant DNA construct includes a heterologous promoter operably linked to DNA encoding an RNA transcript including a strand consisting of a sequence selected from the group consisting of: **SEQ ID NOs.:106 – 896 and 1133 - 2087**.

[0065] In embodiments of these recombinant DNA constructs, the RNA transcript includes dsRNA. In embodiments, the RNA transcript is essentially completely double-stranded (*e. g.*, an RNA transcript that self-hybridizes to form a hairpin structure with little or no single-stranded RNA outside of the loop, or two separate, hybridized strands of RNA). In embodiments, the dsRNA is blunt-ended. In embodiments, the dsRNA has an overhang at one or both ends (termini), *e. g.*, two separate, unequal-length strands of RNA which form the dsRNA through intermolecular hybridisation; the overhang can be a single nucleotide or 2, 3, 4, 5, 6, or more nucleotides, and can be located on the 5' end or on the 3' end of a strand. In embodiments, the dsRNA includes at least one stem-loop, *e. g.*, a single RNA molecule that forms a dsRNA with a “hairpin” secondary structure through intramolecular

hybridization. In embodiments, the dsRNA includes at least one stem-loop with additional secondary structure, such as mismatches or additional nucleotides within the “stem” of the stem-loop structure. In embodiments, the dsRNA is formed from a single self-hybridizing hairpin transcript, wherein one “arm” of the hairpin includes a sequence of about 95% to about 100% (*e. g.*, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%) identity or complementarity with at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more contiguous nucleotides of a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087**. In embodiments, the dsRNA includes multiple stem-loops, with or without spacer nucleotides between each stem-loop.

[0066] In embodiments, the RNA transcript includes both single-stranded and double-stranded regions. In embodiments, the recombinant DNA construct including a heterologous promoter operably linked to DNA encoding an RNA transcript which includes dsRNA encoded on a single RNA strand (*i. e.*, where at least part of the RNA transcript self-hybridizes). In embodiments, the recombinant DNA construct including a heterologous promoter operably linked to DNA encoding an RNA transcript which includes dsRNA encoded on multiple RNA strands, *e. g.*, where the RNA transcript is further processed (for example by endonucleases) into multiple RNAs, or wherein the recombinant DNA construct includes two heterologous promoters, each operably linked to DNA encoding a RNA transcript that forms part of a dsRNA.

[0067] In embodiments, the heterologous promoter used in the recombinant DNA construct is a promoter functional for expression of the RNA transcript in a bacterium, for example, in a bacterium selected from the group consisting of *Escherichia coli*, *Bacillus* species, *Pseudomonas* species, *Xenorhabdus* species, or *Photorhabdus* species. In embodiments, the heterologous promoter used in the recombinant DNA construct is a promoter functional in an eukaryotic cell, for example a promoter functional in yeast cells, insect cells, or plant cells. The recombinant DNA constructs are useful for the production of mosquitocidally effective polynucleotides described in the Examples, for use in compositions and methods of controlling mosquitoes. For example, the recombinant DNA constructs are useful in *in vitro* transcription, or for expression of the desired RNA molecule through microbial fermentation (*e. g.*, by expression in *E. coli* or other bacterium or in a yeast culture) or in a eukaryotic cell (*e. g.*, a plant cell or an insect cell).

[0068] In embodiments, the recombinant DNA construct also encodes additional elements, which can be operably linked to the promoter that drives expression of the DNA encoding an RNA transcript designed to suppress a target gene in a mosquito, or can be linked to a separate promoter. Examples of such elements include DNA encoding an insecticidal protein (*e. g.*, a *Bacillus thuringiensis* insecticidal protein) or DNA encoding an ecdysis inhibitor.

EXAMPLES

Example 1

[0069] This example illustrates non-limiting embodiments of coding DNA sequences useful as target genes for controlling insect species and for making mosquitocidal compositions, and identifies mosquitocidally effective polynucleotides. More specifically, target genes identified by name (annotation) and sequence identifier (SEQ ID NO.) for controlling mosquitoes are provided in **SEQ ID NOs.:1 – 105**, and mosquitocidally effective polynucleotide sequences ranging in size from 218 to 276 bp and designed to suppress these target genes are provided in **SEQ ID NOs.:106 – 896**.

[0070] Target gene sequences with the following sequence identifiers were identified from cDNAs sourced from transcriptomes of three mosquito species: **SEQ ID NOs.:1 – 36**, *Aedes aegypti* (yellow fever mosquito), **SEQ ID NOs.:37 – 71**, *Culex quinquefasciatus* (southern house mosquito), and **SEQ ID NOs.:72 – 105**, *Anopheles gambiae* (or *Anopheles gambiae* species complex). The mosquito target genes included genes encoding: proteasome proteins (Proteasome Alpha 2, Proteasome Beta 5, Proteasome regulatory subunit 2, Proteasome regulatory subunit 3, Proteasome regulatory subunit 8, Proteasome regulatory subunit 9, Proteasome regulatory GTPase SAR1, 26S protease regulatory subunit 6), ATPase proteins (V-ATPase subunit A, V-ATPase subunit B, V-ATPase subunit D, V-ATPase subunit E, Actin 6), ribosomal proteins (Ribosomal protein Large subunit 3, Ribosomal protein Large subunit 7, Ribosomal protein Large subunit 19, Ribosomal protein Large subunit 40, Ribosomal protein Small subunit 21, Ribosomal protein Small subunit 4), vesicle proteins (Vesicle Sorting protein 2, Vesicle Sorting protein 4, Vesicle Sorting protein 16A, Vesicle Sorting protein 20, Vesicle Sorting protein 24, Vesicle Sorting protein 27, Vesicle Sorting protein 28, Vesicle Sorting protein Shrb (Snf7), Vesicle Sorting protein Chmp1, Secretory vesicle protein 6, Secretory vesicle protein 23, Coatomer Protein Alpha, Coatomer Protein Beta, Coatomer Protein Beta Prime, Coatomer Protein Delta, Coatomer Protein Epsilon, Coatomer Protein Zeta), and actin (Actin 6). For each target gene, individual siRNAs were selected using an algorithm publicly available in the software RNAstructure (Reuter and Mathews (2010) *BMC Bioinformatics*, 11:129, doi: 10.1186/1471-2105-11-129) and the longer dsRNA or suppression sequences of a user-defined length and incorporating the selected siRNAs (**Table 2**) were designed using a proprietary algorithm and defined criteria; in general, multiple mosquitocidally effective polynucleotides were designed for a given target gene. The dsRNA or suppression sequences include **SEQ ID NOs.:106 – 382**, designed to target *Aedes aegypti* (yellow fever mosquito), **SEQ ID NOs.:383 – 647**, designed to target *Culex quinquefasciatus* (southern house mosquito), and **SEQ ID NOs.:648 – 896**, designed to target *Anopheles gambiae* (or *Anopheles gambiae* species complex).

Table 2

Target Gene Name	<i>Aedes aegypti</i>		<i>Culex quinquefasciatus</i>		<i>Anopheles gambiae</i>	
	TARGET GENE SEQ ID NO:	Polynucleotide SEQ ID NO: *	TARGET GENE SEQ ID NO:	Polynucleotide SEQ ID NO: *	TARGET GENE SEQ ID NO:	Polynucleotide SEQ ID NO: *
Proteasome Beta 5	1	106 – 110	37	383 – 393	72	648 – 658
V-ATPase subunit E	2	111, 112	38	394 – 396	73	659 – 663

Ribosomal protein Large subunit 3	3	113 – 122	39	397 – 406	74	664 – 671
Vesicle Sorting protein 16A	4	123 – 132	40	407 – 417	75	672 – 682
Ribosomal protein Large subunit 19	5	133 – 138	41	418 – 423	76	683 – 687
26S protease regulatory subunit 6	6	139 – 148	42	424 – 434	77	688 – 696
Coatomer Protein Beta	7	149 – 157	43	435 – 445	78	697 - 701
Vesicle sorting protein Shrb (Snf7)	8	158 – 163	44	446 – 451	79	702 – 707
Coatomer Protein Beta Prime	9	164 – 174	45	452 – 460	80	708 - 717
Ribosomal protein Small subunit 21	10	175	46	461	81	718
Ribosomal protein Small subunit 4	11	176 – 185	47	462 – 468	82	719 – 725
V-ATPase subunit A	12	186 – 195	48	469 – 478	83	726 - 736
Ribosomal protein Large subunit 40	13	196 – 200	49	479 – 484	84	737 – 743
Proteasome Regulatory Subunit 2	14	201 – 210	50	485 – 495	85	744 – 753
Secretory vesicle protein 23	15	211 – 221	51	496 – 506	86	754 – 761
Proteasome Alpha 2	16	222 – 228	52	507 – 511	87	762 – 764
Actin 6	17	229 – 239	53	512 – 522	88	765 – 773
Secretory vesicle protein 6	18	240 – 250	54	523 – 531	89	774 - 781
Coatomer Protein Alpha	19	251 – 260	55	532 - 542	90	782 – 792
Coatomer Protein Delta	20	261 – 271	56	543 – 552	91	793 - 801
Coatomer Protein Epsilon	21	272	57	553 – 558	92	802 - 812
Coatomer Protein Zeta	22	273 – 275	--	--	--	--
Ribosomal Protein Large Subunit 7	23	276 - 282	58	559 – 566	93	813 – 822
Proteasome Regulatory Subunit 3	24	283 – 293	59	567 – 575	94	823 - 833
Proteasome Regulatory Subunit 8	25	294 – 301	60	576 – 583	95	834 – 837
Proteasome Regulatory Subunit 9	26	302 – 311	61	584 – 590	96	838 – 845
Proteasome regulatory GTPase SAR1	27	312 – 317	62	591 – 595	97	846 – 848
V-ATPase subunit B	28	318 – 327	63	596 – 606	98	849 – 858
V-ATPase subunit D	29	328 – 333	64	607 – 614	99	859 – 866
Vesicle Sorting protein 2	30	334 – 342	65	615 – 618	100	867 – 873
Vesicle Sorting protein 4	31	343 – 352	66	619 – 627	101	874 – 878
Vesicle Sorting protein 20	32	353 – 359	67	628 – 630	102	879 – 884
Vesicle Sorting protein 24	33	360	68	631	103	885
Vesicle Sorting protein 27	34	361 – 368	69	632 – 640	104	886 – 893
Vesicle Sorting protein 28	35	369 – 376	70	641 – 644	105	894 – 896
Vesicle Sorting Protein Chmp1	36	377 – 382	71	645 – 647	--	--

* anti-sense nucleotide sequence complementary to the Target Gene (e.g., nucleotide sequence of the anti-sense strand of a dsRNA)

[0071] The mosquito target genes include genes encoding the following protein groups and subgroups:

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- (1) **proteasome proteins** including Proteasome Alpha 2 (e. g., proteins encoded by **SEQ ID NOs.:16, 52, 87**), Proteasome Beta 5 (e. g., proteins encoded by **SEQ ID NOs.:1, 37, 72**), Proteasome regulatory subunit 2 (e. g., proteins encoded by **SEQ ID NOs.:14, 50, 85**), Proteasome regulatory subunit 3 (e. g., proteins encoded by **SEQ ID NOs.:24, 59, 94**), Proteasome regulatory subunit 8 (e. g., proteins encoded by **SEQ ID NOs.:25, 60, 95**),

Proteasome regulatory subunit 9 (*e. g.*, proteins encoded by **SEQ ID NOs.:26, 61, 96**),
 Proteasome regulatory GTPase SAR1 (*e. g.*, proteins encoded by **SEQ ID NOs.:27, 62, 97**),
 and 26S protease regulatory subunit 6 (*e. g.*, proteins encoded by **SEQ ID NOs.:6, 42, 77**);

(2) **ATPase proteins** including V-ATPase subunit A (*e. g.*, proteins encoded by **SEQ ID NOs.:12, 48, 83**), V-ATPase subunit B (*e. g.*, proteins encoded by **SEQ ID NOs.:28, 63, 98**), V-ATPase subunit D (*e. g.*, proteins encoded by **SEQ ID NOs.:29, 64, 99**), V-ATPase subunit E (*e. g.*, proteins encoded by **SEQ ID NOs.:2, 38, 73**), Actin 6 (*e. g.*, proteins encoded by **SEQ ID NOs.:17, 53, 88**);

(3) **ribosomal proteins** including (a) **large ribosomal subunit proteins**, *e. g.*, Ribosomal protein Large subunit 3 (*e. g.*, proteins encoded by **SEQ ID NOs.:3, 39, 74**), Ribosomal protein Large subunit 7 (*e. g.*, proteins encoded by **SEQ ID NOs.:23, 58, 93**), Ribosomal protein Large subunit 19 (*e. g.*, proteins encoded by **SEQ ID NOs.:5, 41, 76**), Ribosomal protein Large subunit 40 (*e. g.*, proteins encoded by **SEQ ID NOs.:13, 49, 84**); and (b) **small ribosomal subunit proteins**, *e. g.*, Ribosomal protein Small subunit 21 (*e. g.*, proteins encoded by **SEQ ID NOs.:10, 46, 81**), Ribosomal protein Small subunit 4 (*e. g.*, proteins encoded by **SEQ ID NOs.:11, 47, 82**);

(4) **vesicle proteins** including (a) **vesicle sorting proteins**, *e. g.*, Vesicle Sorting protein 2 (*e. g.*, proteins encoded by **SEQ ID NOs.:30, 65, 100**), Vesicle Sorting protein 4 (*e. g.*, proteins encoded by **SEQ ID NOs.:31, 66, 101**), Vesicle Sorting protein 16A (*e. g.*, proteins encoded by **SEQ ID NOs.:4, 40, 75**), Vesicle Sorting protein 20 (*e. g.*, proteins encoded by **SEQ ID NOs.:32, 67, 102**), Vesicle Sorting protein 24 (*e. g.*, proteins encoded by **SEQ ID NOs.:33, 68, 103**), Vesicle Sorting protein 27 (*e. g.*, proteins encoded by **SEQ ID NOs.:34, 69, 104**), Vesicle Sorting protein 28 (*e. g.*, proteins encoded by **SEQ ID NOs.:35, 70, 105**), Vesicle Sorting protein Shrb (Snf7) (*e. g.*, proteins encoded by **SEQ ID NOs.:8, 44, 79**), Vesicle Sorting protein Chmp1 (*e. g.*, proteins encoded by **SEQ ID NOs.:36, 71**); (b) **secretory vesicle proteins**, *e. g.*, Secretory vesicle protein 6 (*e. g.*, proteins encoded by **SEQ ID NOs.:18, 54, 89**), Secretory vesicle protein 23 (*e. g.*, proteins encoded by **SEQ ID NOs.:15, 51, 86**); and (c) **coatamer proteins**, *e. g.*, Coatamer Protein Alpha (*e. g.*, proteins encoded by **SEQ ID NOs.:19, 55, 90**), Coatamer Protein Beta (*e. g.*, proteins encoded by **SEQ ID NOs.:7, 43, 78**), Coatamer Protein Beta Prime (*e. g.*, proteins encoded by **SEQ ID NOs.:9, 45, 80**), Coatamer Protein Delta (*e. g.*, proteins encoded by **SEQ ID NOs.:20, 56, 91**), Coatamer Protein Epsilon (*e. g.*, proteins encoded by **SEQ ID NOs.:21, 57, 92**), Coatamer Protein Zeta (*e. g.*, proteins encoded by **SEQ ID NO:22**); and

(5) **actin** including Actin 6 (*e. g.*, proteins encoded by **SEQ ID NOs.:17, 53, 88**).

[0072] The polynucleotide sequences include nucleotide sequences encoding double-stranded RNA for silencing target genes that encode:

- (1) **proteasome proteins** including Proteasome Alpha 2 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:222 – 228, 507 – 511, 762 – 764**), Proteasome Beta 5 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:106 – 110, 383 – 393, 648 – 658**), Proteasome regulatory subunit 2 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:201 – 210, 485 – 495, 744 – 753**), Proteasome regulatory subunit 3 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:283 – 293, 567 – 575, 823 – 833**), Proteasome regulatory subunit 8 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:294 – 301, 576 – 583, 834 – 837**), Proteasome regulatory subunit 9 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:302 – 311, 584 – 590, 838 – 845**), Proteasome regulatory GTPase SAR1 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:312 – 317, 591 – 595, 846 – 848**), and 26S protease regulatory subunit 6 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:139 – 148, 424 – 434, 688 – 696**);
- (2) **ATPase proteins** including V-ATPase subunit A (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:186 – 195, 469 – 478, 726 – 736**), V-ATPase subunit B (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:318 – 327, 596 – 606, 849 – 858**), V-ATPase subunit D (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:328 – 333, 607 – 614, 859 – 866**), V-ATPase subunit E (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:111, 112, 394 – 396, 659 – 663**), Actin 6 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:229 – 239, 512 – 522, 765 – 773**);
- (3) **ribosomal proteins** including (a) **large ribosomal subunit proteins**, *e. g.*, Ribosomal protein Large subunit 3 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:113 – 112, 397 – 406, 664 – 671**), Ribosomal protein Large subunit 7 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:276 – 282, 559 – 566, 813 – 822**), Ribosomal protein Large subunit 19 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:133 – 138, 418 – 423, 683 – 687**), Ribosomal protein Large subunit 40 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:196 – 200, 479 – 484, 737 – 743**); and (b) **small ribosomal subunit proteins**, *e. g.*, Ribosomal protein Small subunit 21 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:175, 461, 718**), Ribosomal protein Small subunit 4 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:176 – 185, 462 – 468, 719 – 725**);
- (4) **vesicle proteins** including (a) **vesicle sorting proteins**, *e. g.*, Vesicle Sorting protein 2 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:334 – 342, 615 – 618, 867 – 873**), Vesicle Sorting protein 4 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:343 – 352, 619 – 627, 874 – 878**), Vesicle Sorting protein 16A (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:123 – 132, 407 – 417, 672 – 682**), Vesicle Sorting protein 20 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID**

NOs.:353 – 359, 628 – 630, 879 – 884), Vesicle Sorting protein 24 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:360, 631, 885**), Vesicle Sorting protein 27 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:361 – 368, 632 – 640, 886 – 893**), Vesicle Sorting protein 28 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:369 – 376, 641 – 644, 894 – 896**), Vesicle Sorting protein Shrb (Snf7) (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:158 – 163, 446 – 451, 702 – 707**), Vesicle Sorting protein Chmp1 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:377 – 382, 645 – 647**); (b) **secretory vesicle proteins**, *e. g.*, Secretory vesicle protein 6 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:240 – 250, 523 – 531, 774 – 781**), Secretory vesicle protein 23 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:211 – 221, 496 – 506, 754 – 761**); and (c) **coatomer proteins**, *e. g.*, Coatomer Protein Alpha (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:251 – 260, 532 – 542, 782 – 792**), Coatomer Protein Beta (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:149 – 157, 435 – 445, 697 – 701**), Coatomer Protein Beta Prime (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:164 – 174, 452 – 460, 708 – 717**), Coatomer Protein Delta (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:261 – 271, 543 – 552, 793 – 801**), Coatomer Protein Epsilon (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:272, 553 – 558, 802 – 812**), Coatomer Protein Zeta (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:273 – 275**); and

(5) **actin** including Actin 6 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:229 – 239, 512 – 522, 765 – 773**).

[0073] The embodiments of polynucleotide sequences provided in **Table 2** are generally useful for RNA-mediated suppression of the corresponding target gene identified in **Table 2**. These polynucleotides are useful for controlling insects, especially mosquitoes, including the source species from which the target genes in **Table 2** were identified. RNA-mediated suppression of one or more of the target genes provided in **Table 2**, or use of one or more of the polynucleotides provided in **Table 2**, is useful for causing mortality or stunting, or otherwise controlling, target mosquitoes, especially mosquito species in the following genera: *Aedes*, *Culex*, and *Anopheles*.

[0074] In embodiments, compositions including a mosquitocidally effective polynucleotide for suppression of one or more of the target genes provided in **Table 2** (*e. g.*, a composition including an insecticidally effective amount of one or more of the polynucleotides provided in **Table 2**) are useful for controlling mosquito species, including mosquito species in the following genera: *Aedes*, *Culex*, and *Anopheles*. For example, a composition including an insecticidally effective amount of one or more of the polynucleotides provided in **Table 2** is useful for inducing growth stunting, increased mortality, decrease in reproductive capacity or decreased fecundity, decrease in or cessation of feeding behavior or movement, or decrease in or cessation of metamorphosis stage development, in mosquito

species, including mosquito species in the following genera: *Aedes*, *Culex*, and *Anopheles*.

Compositions include embodiments insecticidally effective on adult mosquitoes, larval mosquitoes, or mosquito eggs, or on combinations thereof.

[0075] In embodiments, RNA-mediated suppression of one or more of the target genes provided in

Table 2, or use of one or more of the polynucleotides provided in **Table 2**, is useful for inducing growth stunting, increased mortality, decrease in reproductive capacity or decreased fecundity, decrease in or cessation of feeding behavior or movement, or decrease in or cessation of metamorphosis stage development, in mosquito species, including mosquito species in the following genera: *Aedes*, *Culex*, and *Anopheles*. In embodiments, RNA-mediated suppression of one or more of the target genes having a sequence selected from the group consisting of **SEQ ID NOs.:1 – 36** is used to cause mortality or stunting in *Aedes* spp., such as *Aedes aegypti* adults or larvae, for example, by contacting *Aedes aegypti* adults, larvae, or eggs with an effective amount of a dsRNA comprising a sequence selected from the group consisting of **SEQ ID NOs.:106 – 382**. In embodiments, RNA-mediated suppression of one or more of the target genes comprising a sequence selected from the group consisting of **SEQ ID NOs.:37 – 71** is used to cause mortality or stunting in *Culex* spp., such as *Culex quinquefasciatus* adults or larvae, for example, by contacting *Culex quinquefasciatus* adults, larvae, or eggs with an effective amount of a dsRNA comprising a sequence selected from the group consisting of **SEQ ID NOs.:383 – 647**. In embodiments, RNA-mediated suppression of one or more of the target genes having a sequence selected from the group consisting of **SEQ ID NOs.:72 – 105** is used to cause mortality or stunting in *Anopheles* spp., such as *Anopheles gambiae* adults or larvae, for example, by contacting *Anopheles gambiae* adults, larvae, or eggs with an effective amount of a dsRNA comprising a sequence selected from the group consisting of **SEQ ID NOs.:648 – 896**.

[0076] Adult mosquitoes feed on naturally occurring plant sugars, and are attracted to sugar-containing compositions. Thus, an aspect of this invention includes mosquito bait compositions, such as attractive toxic sugar baits (ATSBs), including an insecticidally effective amount of one or more of the polynucleotides provided in **Table 2**. Typically, ATSBs include an aqueous solution of a sugar (such as sucrose) as a phagostimulant, an attractant such as fruit or flower scents, and an orally available toxin; other ingredients can include fruit juice or pulp, yeast, preservatives, stabilizers, and visual dyes. ATSBs can be sprayed (*e. g.*, on vegetation) or provided in indoor or outdoor bait stations. Examples of ATSBs which use non-polynucleotide toxins such as Spinosad, pyrethroids, boric acid, or eugenol include those described by Müller *et al.* (2010) *Malaria J.*, 9:210 – 216; Beier *et al.* (2012) *Malaria J.*, 11:31 – 37; Stewart *et al.* (2013) *PLoS ONE*, 8(12):e84168; and Qualls *et al.* (2014) *Acta Tropica*, 131:104 – 110. The disadvantage of ATSBs containing non-polynucleotide toxins is that these are non-selective and are toxic to non-target species including beneficial insects such as honey bees and other pollinators and potentially toxic to non-target species that might ingest a mosquito that has fed on the ATSB. Use of a polynucleotide such as the mosquitocidally effective polynucleotides provided herein permits a species-specific approach to mosquito control.

[0077] Other embodiments include larvicidal compositions including a polynucleotide such as the mosquitocidally effective polynucleotides provided herein. Larvicidal compositions formulated as a sprayable liquid or dispersible powder or granules can be deployed in and around mosquito breeding grounds such as stagnant water, cisterns, ponds, lakes, streams, and in vegetated areas.

Example 2

[0078] This example illustrates additional non-limiting embodiments of coding DNA sequences useful as target genes for controlling mosquitoes and for making mosquitocidally effective compositions, and identifies polynucleotide sequences useful for controlling mosquitoes. More specifically, embodiments of target genes identified by name (annotation) and sequence identifier (SEQ ID NO.) (Table 3) for controlling mosquitoes are provided in SEQ ID NOs.:897 – 1132, and embodiments of polynucleotide sequences ranging in size from 98 to 305 bp and designed to suppress these target genes are provided in SEQ ID NOs.:1133 – 2087.

[0079] Target gene sequences (Table 3) with the following sequence identifiers were identified from cDNAs sourced from transcriptomes of three mosquito species: SEQ ID NOs.:897 – 975, *Aedes aegypti* (yellow fever mosquito), SEQ ID NOs.:1014 and 1060 – 1132, *Culex quinquefasciatus* (southern house mosquito), and SEQ ID NOs.:976 – 1013 and 1015 – 1059, *Anopheles gambiae* (or *Anopheles gambiae* species complex). For each target gene, individual siRNAs were selected using an algorithm publicly available in the software RNAstructure (Reuter and Mathews (2010) *BMC Bioinformatics*, 11:129, doi: 10.1186/1471-2105-11-129) and the longer polynucleotide or suppression sequences of a user-defined length and incorporating the selected siRNAs (Table 3) were designed using a proprietary algorithm and defined criteria; in general, multiple polynucleotides were designed for a given target gene. The polynucleotide or suppression sequences include SEQ ID NOs.:1133 – 1440, designed to target *Aedes aegypti* (yellow fever mosquito), SEQ ID NOs.:1613 – 1614 and 1762 – 2087, designed to target *Culex quinquefasciatus* (southern house mosquito), and SEQ ID NOs.:1441 – 1612 and 1615 – 1761, designed to target *Anopheles gambiae* (or *Anopheles gambiae* species complex).

Table 3

Target Gene Name	<i>Aedes aegypti</i>		<i>Culex quinquefasciatus</i>		<i>Anopheles gambiae</i>	
	TARGET GENE SEQ ID NO:	Polynucleotide SEQ ID NO: *	TARGET GENE SEQ ID NO:	Polynucleotide SEQ ID NO: *	TARGET GENE SEQ ID NO:	Polynucleotide SEQ ID NO: *
Bendless	897	1133-1135	1110	1993-1995	989	1499-1503
BiP	898	1136-1139	1106	1975-1978	985	1481-1484
Scribbler	899	1140-1143	1107	1979-1983	986	1485-1488
calnexin	900	1144-1147	1105	1970-1974	984	1476-1480
Capa receptor	901	1148-1151	1112	2000-2003	990	1504-1510
citrate synthase	902	1152-1154	1101	1950-1954	980	1457-1461
COP9 signalosome	903	1155-1158	1104	1965-1969	983	1471-1475
Diapause hormone	904	1159-1161	--	--	1001	1555-1558

receptor						
Eb1	905	1162-1164	1115	2014-2017	993	1521-1523
Ether-a-go-go	906	1165-1168	1117	2021-2025	995	1529-1533
Flightless-I	907	1169-1172	1113	2004-2008	991	1511-1515
Foraging	908	1173-1176	1109	1989-1992	988	1494-1498
glycogen synthase	909	1177-1180	1102	1955-1959	981	1462-1466
hexokinase	910	1181-1184	1098	1936-1939	977	1446-1448
isocitrate dehydrogenase	911	1185-1188	1099	1940-1944	978	1449-1453
Juvenile hormone epoxide hydrolase	912	1189-1191	1118	2026-2029	997	1539-1542
Neuropeptide F	913	1192-1194	--	--	1000	1550-1554
Pheromone biosynthesis activating neuropeptide receptor	914	1195-1197	1120	2035-2037	999	1548-1549
protein disulfide isomerase	915	1198-1201	1103	1960-1964	982	1467-1470
pyruvate dehydrogenase	916	1202-1206	1100	1945-1949	979	1454-1456
semaphorin 2a	917	1207-1209	1116	2018-2020	994	1524-1528
shaker	918	1210-1213	1108	1984-1988	987	1489-1493
slowmo	919	1214-1215	1014	1613-1614	996	1534-1538
stress-sensitive B	920	1216-1217	1111	1996-1999	--	--
turtle	921	1218-1221	1114	2009-2013	992	1516-1520
twitchin	922	1222-1225	1119	2030-2034	998	1543-1547
phosphofructokinase	923	1226-1227	1121	2038-2040	1002	1559-1562
cyclin A	924	1228-1229	1122	2041-2044	1003	1563-1567
cyclin B	925	1230-1232	1123	2045-2049	1004	1568-1571
cyclin B3	926	1233-1234	1124	2050-2054	1005	1572-1576
cyclin C	927	1236	1125	2055-2058	1006	1577-1579
cyclin D	928	1237-1238	1126	2059-2063	--	--
cyclin E	929	1239-1241	--	--	1007	1580-1583
cyclin G	930	1242-1244	1127	2064-2068	1008	1584-1588
cyclin H	931	1245-1247	1128	2069-2071	1009	1589-1593
cyclin J	932	1248-1250	1129	2072-2074	1010	1594-1598
cyclin K	933	1251-1253	1130	2075-2078	1011	1599-1603
cyclin T	934	1254-1256	1131	2079-2082	1012	1604-1608
cyclin Y	935	1257-1259	1132	2083-2087	1013	1609-1612
cleavage polyadenylation specificity factor	936	1260-1264	1060	1762-1767	1015	1615-1618
RNA helicase	937	1265-1270	1061	1768-1773	1016	1619-1622
RNA polymerase	938	1271-1273	1062	1774-1777	1017	1623-1626
daf21	939	1274-1279	1063	1778-1782	1018	1627-1630
nucleic acid binding protein	940	1280-1284	1064	1783-1787	1019	1631-1634
GTPase activator	---	---	--	--	1020	1635-1638
RNA polymerase	---	---	--	--	1021	1639
RNA polymerase II	941	1285-1288	1065	1788-1789	1033	1677-1680
GTPase Ran	942	1289-1291	1066	1790-1794	1022	1640-1643
sarcoplasmic Ca ²⁺ -ATPase	943	1292-1294	1067	1796-1798	1023	1644-1647
E1A/CREB-binding protein	--	--	1068	1799-1804	1024	1648-1651
N-synaptobrevin	944	1295-1296	1069	1805-1807	1025	1652
ubiquitin conjugating enzyme E1	945	1297-1301	1070	1808-1812	1026	1653-1656

myosin heavy chain	946	1302-1305	1071	1813-1815	1027	1657-1658
eft2	947	1306-1311	1072	1816-1821	1028	1659-1662
spermatogenesis ATPase	948	1312-1317	1073	1822-1827	1029	1663-1666
ketel	949	1318-1323	1074	1828-1833	1030	1667-1670
F1F0 ATPase	950	1324-1325	1075	1834	1031	1671-1672
ABC transporter	951	1326-1330	1076	1835-1840	1032	1673-1676
bent	--	--	--	--	1034	1681-1684
beta-ketoacyl synthase	952	1331-1336	1077	1841-1846	1035	1685-1688
Ca2+ ATPase	953	1337-1339	1078	1847-1851	1036	1689-1692
nuclear hormone receptor 25	954	1340-1345	1079	1852-1856	1037	1693-1696
syntaxin	955	1346-1349	1080	1857-1861	1038	1697-1698
heat shock cognate 70	956	1350-1355	1081	1862-1867	1039	1699-1702
chromosome segregation protein	957	1356-1357	1082	1868	1040	1703-1704
RNA helicase	958	1358-1361	1083	1869-1873	1041	1705-1708
chaperonin TCP1	959	1362-1367	1084	1874-1878	1042	1709-1710
Rab11	960	1368-1370	1085	1879-1880	1043	1711-1712
RNA helicase	961	1371-1376	--	--	1044	1713-1714
knickkopf	962	1377-1381	1086	1881-1886	1045	1715-1718
pitchoune	963	1382-1385	--	--	1046	1719-1722
CDC5	964	1386-1391	1087	1887-1892	1047	1723-1726
chaperonin	965	1392-1396	1088	1893-1894	1048	1727-1730
RNA helicase	966	1397-1400	1089	1895-1900	1049	1731-1732
carrier protein	967	1401-1402	--	--	1050	1733-1734
Bx42	968	1403-1408	1090	1901-1905	1051	1735-1738
Arf1	--	--	1091	1906	1052	1739-1740
Insect protein	969	1409-1413	1092	1907-1913	1053	1741-1742
HSP70	970	1414-1419	1093	1914-1919	1054	1743-1746
Insect protein	971	1420-1425	--	--	1055	1747-1750
chaperonin	972	1426-1431	--	--	1056	1751-1754
hydrogen exporting ATPase	973	1432	1094	1920	1057	1755
NADH dehydrogenase	974	1433-1435	1095	1921-1926	1058	1756-1757
nervana	975	1436-1440	1096	1927-1932	1059	1758-1761
pyruvate kinase	--	--	1097	1933-1935	976	1441-1445

* anti-sense nucleotide sequence complementary to the Target Gene (e.g., nucleotide sequence of the anti-sense strand of a dsRNA)

[0080] The mosquito target genes include genes encoding the following protein groups and subgroups:

- 5 (1) **genes encoding proteins related to behaviour** including Bendless (*e. g.*, proteins encoded by SEQ ID NOs.:897, 1110, and 989), scribbler (*e. g.*, proteins encoded by SEQ ID NOs.:899, 1107, and 986), capa receptor (*e. g.*, proteins encoded by SEQ ID NOs.:901, 1112, and 990), diapause hormone receptor (*e. g.*, proteins encoded by SEQ ID NOs.:904 and 1001), Eb1 (*e. g.*, proteins encoded by SEQ ID NOs.:905, 1115, and 993), Ether-a-go-go (*e. g.*, proteins encoded by SEQ ID NOs.:906, 1117, and 995), flightless-I (*e. g.*, proteins encoded by SEQ ID NOs.:907, 1113, and 991), foraging (*e. g.*, proteins encoded by SEQ ID NOs.:908, 1109, and 988), juvenile hormone epoxide hydrolase (*e. g.*, proteins encoded by SEQ ID NOs.:912, 1118, and 997), neuropeptide F (*e. g.*, proteins encoded by SEQ ID NOs.:913 and 1000), pheromone
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biosynthesis activating neuropeptide receptor (*e. g.*, proteins encoded by SEQ ID NOs.:914, 1120, and 999), semaphorin 1a (*e. g.*, proteins encoded by SEQ ID NOs.:917, 1116, and 994), shaker (*e. g.*, proteins encoded by SEQ ID NOs.:918, 1108, and 987), slowmo (*e. g.*, proteins encoded by SEQ ID NOs.:919, 1014, and 996), stress-sensitive B (*e. g.*, proteins encoded by SEQ ID NOs.:920 and 1111), turtle (*e. g.*, proteins encoded by SEQ ID NOs.:921, 1114, and 992), twitchin (*e. g.*, proteins encoded by SEQ ID NOs.:922, 1119 and 998), myosin heavy chain (*e. g.*, proteins encoded by SEQ ID NOs.:946, 1071, and 1027), nervana (*e. g.*, proteins encoded by SEQ ID NOs.:975, 1096, and 1059), N-synaptobrevin (*e. g.*, proteins encoded by SEQ ID NOs.:944, 1069, and 1025), nuclear hormone receptor 25 (*e. g.*, proteins encoded by SEQ ID NOs.:954, 1079, and 1037), syntaxin (*e. g.*, proteins encoded by SEQ ID NOs.:955, 1080, and 1038), and bent (*e. g.*, proteins encoded by SEQ ID NO:1034);

(2) **glycolysis and energy metabolism proteins** including BiP (*e. g.*, proteins encoded by SEQ ID NOs.:898, 1106, and 985), citrate synthase (*e. g.*, proteins encoded by SEQ ID NOs.:902, 1101, and 980), glycogen synthase (*e. g.*, proteins encoded by SEQ ID NOs.:909, 1102, and 981), hexokinase (*e. g.*, proteins encoded by SEQ ID NOs.:910, 1098, and 977), isocitrate dehydrogenase (*e. g.*, proteins encoded by SEQ ID NOs.:911, 1099, and 978), pyruvate dehydrogenase (*e. g.*, proteins encoded by SEQ ID NOs.:916, 1100, and 979), phosphofructokinase (*e. g.*, proteins encoded by SEQ ID NOs.:923, 1121, and 1002), GTPase activator (*e. g.*, proteins encoded by SEQ ID NO:1020), GTPase Ran (*e. g.*, proteins encoded by SEQ ID NOs.:942, 1066, and 1022), sarcoplasmic Ca²⁺ ATPase (*e. g.*, proteins encoded by SEQ ID NOs.:943, 1067, and 1023), Ca²⁺ ATPase (*e. g.*, proteins encoded by SEQ ID NOs.:953, 1078, and 1036), hydrogen exporting ATPase (*e. g.*, proteins encoded by SEQ ID NOs.:973, 1094, and 1057), NADH dehydrogenase (*e. g.*, proteins encoded by SEQ ID NOs.:974, 1095, and 1058), F1F0 ATPase (*e. g.*, proteins encoded by SEQ ID NOs.:950, 1075, and 1031), spermatogenesis ATPase (*e. g.*, proteins encoded by SEQ ID NOs.:948, 1073, and 1029), beta-ketoacyl synthase (*e. g.*, proteins encoded by SEQ ID NOs.:952, 1077, and 1035), pyruvate kinase (*e. g.*, proteins encoded by SEQ ID NOs.:1097 and 976), and knickkopf (*e. g.*, proteins encoded by SEQ ID NOs.:962, 1086, and 1045);

(3) **protein turnover and mitosis proteins** including cyclins—such as cyclin A (*e. g.*, proteins encoded by SEQ ID NOs.:924, 1122, and 1003), cyclin B (*e. g.*, proteins encoded by SEQ ID NOs.:925, 1123, and 1004), cyclin B3 (*e. g.*, proteins encoded by SEQ ID NOs.:926, 1124, and 1005), cyclin C (*e. g.*, proteins encoded by SEQ ID NOs.:927, 1125, and 1996), cyclin D (*e. g.*, proteins encoded by SEQ ID NOs.:928 and 1126), cyclin E (*e. g.*, proteins encoded by SEQ ID NOs.:929 and 1007), cyclin G (*e. g.*, proteins encoded by SEQ ID NOs.:930, 1127, and 1008), cyclin H (*e. g.*, proteins encoded by SEQ ID NOs.:931, 1128, and 1009), cyclin J (*e. g.*, proteins encoded by SEQ ID NOs.:932, 1129, and 1010), cyclin K (*e. g.*, proteins encoded by SEQ ID NOs.:933, 1130, and 1011), cyclin T (*e. g.*, proteins encoded by SEQ ID NOs.:934, 1131, and 1012), and cyclin Y (*e. g.*, proteins encoded by SEQ ID NOs.:935, 1131,

and 1013), Ubiquitin conjugating enzyme E1 (*e. g.*, proteins encoded by **SEQ ID NOs.:945, 1070, and 1026**), chromosome segregation protein (*e. g.*, proteins encoded by **SEQ ID NOs.:957, 1082, and 1040**), CDC5 (*e. g.*, proteins encoded by **SEQ ID NOs.:964, 1087, and 1047**), COP9 signalosome (*e. g.*, proteins encoded by **SEQ ID NOs.:903, 1104, and 983**), and ketel (*e. g.*, proteins encoded by **SEQ ID NOs.:949, 1074, and 1030**);

(4) **gene expression proteins** including cleavage polyadenylation specificity factor (*e. g.*, proteins encoded by **SEQ ID NOs.:936, 1060, and 1015**), EIA/CREB-binding protein (*e. g.*, proteins encoded by **SEQ ID NOs.:1068 and 1024**), RNA helicases (*e. g.*, proteins encoded by **SEQ ID NOs.:937, 1061, and 1016; 958, 1083, and 1041; 961 and 1044; and 966, 1089, and 1049**), RNA polymerases (*e. g.*, proteins encoded by **SEQ ID NOs.:938, 1062, and 1017; 1021; and 941, 1065, and 1033**), Arf1 (*e. g.*, proteins encoded by **SEQ ID NOs.:1091 and 1052**), Bx42 (*e. g.*, proteins encoded by **SEQ ID NOs.:968, 1090, and 1051**), and eft2 (*e. g.*, proteins encoded by **SEQ ID NOs.:947, 1072, and 1028**);

(5) **protein-folding, stress, and heat shock response proteins** including protein disulfide isomerase (*e. g.*, proteins encoded by **SEQ ID NOs.:915, 1103, and 982**), heat shock cognate 70 (*e. g.*, proteins encoded by **SEQ ID NOs.:956, 1081, and 1039**), chaperonin TCP1 (*e. g.*, proteins encoded by **SEQ ID NOs.:959, 1084, and 1042**), chaperonins (*e. g.*, proteins encoded by **SEQ ID NOs.:965, 1088, and 1048; 972 and 1056**), carrier protein (*e. g.*, proteins encoded by **SEQ ID NOs.:967 and 1050**), and HSP70 (*e. g.*, proteins encoded by **SEQ ID NOs.:970, 1093, and 1054**); and

(6) **miscellaneous proteins** including spermatogenesis ATPase (*e. g.*, proteins encoded by **SEQ ID NOs.:948, 1073, and 1029**), pitchoune (*e. g.*, proteins encoded by **SEQ ID NOs.:963 and 1046**), and insect proteins (*e. g.*, proteins encoded by **SEQ ID NOs.:969, 1092, and 1053; 971 and 1055**).

[0081] The polynucleotide sequences include nucleotide sequences encoding double-stranded RNA for silencing target genes that encode:

(1) **genes encoding proteins related to behaviour** including Bendless (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1133 – 1135, 1993 – 1995, and 1499 – 1503**), scribbler (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1140 – 1143, 1979 – 1983, and 1485 – 1488**), capa receptor (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1140 – 1143, 1979 – 1983, and 1485 – 1488**), diapause hormone receptor (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:2259 – 2261 and 1555 – 1558**), Ebl (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1162 – 1164, 2014 – 2017, and 1521 – 1523**), Ether-a-go-go (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1165 – 1168, 2021 – 2025, and 1529 – 1533**), flightless-I (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1169 – 1172, 2004 – 2008, and 1511 – 1515**), foraging (*e. g.*, dsRNA with an anti-

sense strand encoded by any of SEQ ID NOs.:1173 – 1176, 1989 – 1992, and 1494 - 1498), juvenile hormone epoxide hydrolase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1189 – 1191, 2026 – 2029, and 1539 – 1542), neuropeptide F (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1192 – 1194 and 1150 – 1554), pheromone biosynthesis activating neuropeptide receptor (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1195 – 1197, 2035 – 2037, and 1548 – 1549), semaphorin 1a (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1207 – 1209, 2018 – 2020, and 1524 – 1528), shaker (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1210 – 1213, 1984 – 1988, and 1489 – 1493), slowmo (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1214 – 1215, 1613 – 1614, and 1534 – 1538), stress-sensitive B (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1216 – 1217 and 1996 – 1999), turtle (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1218 – 1221, 2009 – 2013, and 1516 – 1520), twitchin (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1222 – 1225, 2030 – 2034, and 1543 – 1547), myosin heavy chain (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1302 – 1305, 1813 – 1815, and 1657 - 1658), nervana (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1436 – 1440, 1927 – 1932, and 1758 – 1761), N-synaptobrevin (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1295 – 1296, 1805 – 1807, and 1652), nuclear hormone receptor 25 (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1340 – 1345, 1852 – 1856, and 1693 – 1696), syntaxin (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1346 – 1349, 1857 – 1861, and 1697 - 1698), and bent (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1681 – 1684);

- (2) **glycolysis and energy metabolism proteins** including BiP (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1136 – 1139, 1975 – 1978, and 1481 – 1484), citrate synthase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1152 – 1154, 1950 – 1954, and 1457 – 1461), glycogen synthase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1177 – 1180, 1955 -1959, and 1462 – 1466), hexokinase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1181 – 1184, 1936 – 1939, and 1446 – 1448), isocitrate dehydrogenase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1185 – 1188, 1940 – 1944, and 1449 - 1453), pyruvate dehydrogenase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1202 – 1206, 1945 – 1949, and 1454 – 1456), phosphofructokinase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1226 – 1227, 2038 – 2040, and 1559 – 1562), GTPase activator (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1635 – 1638), GTPase Ran (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1289 – 1291, 1790 – 1794, and 1640 – 1643), sarcoplasmic Ca²⁺ ATPase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1292 – 1294, 1796 – 1798,

and 1644 – 1647), Ca²⁺ ATPase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1337 – 1339, 1847 – 1851, and 1689 – 1692), hydrogen exporting ATPase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1432, 1920, and 1755), NADH dehydrogenase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1433 – 1435, 1921 – 1926, and 1756 – 1757), F1F0 ATPase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1324 – 1325, 1834, and 1671 – 1672), spermatogenesis ATPase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1312 – 1317, 1822 – 1827, and 1663 – 1666), beta-ketoacyl synthase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1331 – 1336, 1841 – 1846, and 1685 – 1688), pyruvate kinase (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1933 – 1935 and 1441 – 1445), and knickkopf (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1377 – 1381, 1881 – 1886, and 1715 – 1718);

- (3) **protein turnover and mitosis proteins** including cyclins—such as cyclin A (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1228 – 1229, 2041 – 2044, and 1563 – 1567), cyclin B (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1230 – 1232, 2045 – 2049, and 1568 – 1571), cyclin B3 (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1233 – 1234, 2050 – 2054, and 1572 – 1576), cyclin C (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1236, 2055 – 2058, and 1577 – 1579), cyclin D (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1237 – 1238 and 2059 – 2063), cyclin E (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1239 – 1241 and 1580 – 1583), cyclin G (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1242 – 1244, 2064 – 2068, and 1584 – 1588), cyclin H (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1245 – 1247, 2069 – 2071, and 1589 – 1593), cyclin J (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1248 – 1250, 2072 – 2074, and 1594 – 1598), cyclin K (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1251 – 1253, 2075 – 2078, and 1599 – 1603), cyclin T (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1254 – 1256, 2079 – 2082, and 1604 – 1608), and cyclin Y (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1257 – 1259, 2083 – 2087, and 1609 – 1612), Ubiquitin conjugating enzyme E1 (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1297 – 1301, 1808 – 1812, and 1653 – 1656), chromosome segregation protein (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1356 – 1357, 1868, and 1703 – 1704), CDC5 (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1386 – 1391, 1887 – 1892, and 1723 – 1726), COP9 signalosome (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1155 – 1158, 1965 – 1969, and 1471 – 1475), and ketel (*e. g.*, dsRNA with an anti-sense strand encoded by any of SEQ ID NOs.:1318 – 1323, 1828 – 1833, and 1667 – 1670);

- (4) **gene expression proteins** including cleavage polyadenylation specificity factor (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1260 – 1264, 1762 – 1767, and 1615 – 1618**), E1A/CREB-binding protein (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1799 – 1804 and 1648 – 1651**), RNA helicases (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1265 – 1270, 1768 – 1773, and 1619 – 1622; 1371 – 1376 and 1713 – 1714; and 1397 – 1400, 1895 – 1900, and 1731 – 1732**), RNA polymerases (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1271 – 1273, 1774 – 1777, and 1623 – 1626; 1639; and 1285 – 1288, 1788 – 1789, and 1677 – 1680**), Arf1 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1906 and 1739 – 1740**), Bx42 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1403 – 1408, 1901 – 1905, and 1735 – 1738**), and eft2 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1306 – 1311, 1816 – 1821, and 1659 – 1662**);
- (5) **protein-folding, stress, and heat shock response proteins** including protein disulfide isomerase (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1198 – 1201, 1960 – 1964, and 1467 – 1470**), heat shock cognate 70 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1350 – 1355, 1862 – 1867, and 1699 - 1702**), chaperonin TCP1 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1362 – 1367, 1874 – 1878, and 1709 – 1710**), chaperonins (*e. g.*, proteins encoded by dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1392 – 1396, 1893 – 1894, and 1727 – 1730; and 1426 – 1431 and 1751 – 1754**), carrier protein (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1401 – 1402 and 1733 – 1734**), and HSP70 (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1414 – 1419, 1914 – 1919, and 1743 – 1746**); and
- (6) **miscellaneous proteins** including spermatogenesis ATPase (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1312 – 1317, 1822 – 1827, and 1663 - 1666**), pitchoune (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1382 – 1385 and 1719 – 1722**), and insect proteins (*e. g.*, dsRNA with an anti-sense strand encoded by any of **SEQ ID NOs.:1409 – 1413, 1907 – 1913, and 1741 – 1742; and 1420 – 1425 and 1747 – 1750**).

[0082] The embodiments of polynucleotide sequences provided in **Table 3** are generally useful for RNA-mediated suppression of the corresponding target gene identified in **Table 3**. These polynucleotides are useful for controlling mosquitoes, including the source species from which the target genes in **Table 3** were identified. RNA-mediated suppression of one or more of the target genes provided in **Table 3**, or use of one or more of the polynucleotides provided in **Table 3**, is useful for causing mortality or stunting, or otherwise controlling, target mosquitoes, especially mosquito species in the following genera: *Aedes*, *Culex*, and *Anopheles*.

[0083] In embodiments, compositions including a polynucleotide for suppression of one or more of the target genes provided in **Table 3** (e. g., a composition including an insecticidally effective amount of one or more of the polynucleotides provided in **Table 3**) are useful for controlling mosquito species, including mosquito species in the following genera: *Aedes*, *Culex*, and *Anopheles*. For example, a composition including an insecticidally effective amount of one or more of the polynucleotides provided in **Table 3** is useful for inducing growth stunting, increased mortality, decrease in reproductive capacity or decreased fecundity, decrease in or cessation of feeding behavior or movement, or decrease in or cessation of metamorphosis stage development, in mosquito species, including mosquito species in the following genera: *Aedes*, *Culex*, and *Anopheles*. Compositions include embodiments insecticidally effective on adult mosquitoes, larval mosquitoes, or mosquito eggs, or on combinations thereof.

[0084] In embodiments, RNA-mediated suppression of one or more of the target genes provided in **Table 3**, or use of one or more of the polynucleotides provided in **Table 3**, is useful for inducing growth stunting, increased mortality, decrease in reproductive capacity or decreased fecundity, decrease in or cessation of feeding behavior or movement, or decrease in or cessation of metamorphosis stage development, in mosquito species, including mosquito species in the following genera: *Aedes*, *Culex*, and *Anopheles*. In embodiments, RNA-mediated suppression of one or more of the target genes having a sequence selected from the group consisting of **SEQ ID NOs.:897 – 975** is used to cause mortality or stunting in *Aedes* spp., such as *Aedes aegypti* adults or larvae, for example, by contacting *Aedes aegypti* adults, larvae, or eggs with an effective amount of a dsRNA comprising a sequence selected from the group consisting of **SEQ ID NOs.:1133 – 1440**. In embodiments, RNA-mediated suppression of one or more of the target genes having a sequence selected from the group consisting of **SEQ ID NOs.:1014 and 1060 – 1132** is used to cause mortality or stunting in *Culex* spp., such as *Culex quinquefasciatus* adults or larvae, for example, by contacting *Culex quinquefasciatus* adults, larvae, or eggs with an effective amount of a dsRNA comprising a sequence selected from the group consisting of **SEQ ID NOs.:1613 – 1614 and 1762 – 2087**. In embodiments, RNA-mediated suppression of one or more of the target genes having a sequence selected from the group consisting of **SEQ ID NOs.:976 – 1013 and 1015 – 1059** is used to cause mortality or stunting in *Anopheles* spp., such as *Anopheles gambiae* adults or larvae, for example, by contacting *Anopheles gambiae* adults, larvae, or eggs with an effective amount of a dsRNA comprising a sequence selected from the group consisting of **SEQ ID NOs.:1441 – 1612 and 1615 – 1761**.

[0085] Adult mosquitoes feed on naturally occurring plant sugars, and are attracted to sugar-containing compositions. Thus, an aspect of this invention includes mosquito bait compositions, such as attractive toxic sugar baits (ATSBs), including an insecticidally effective amount of one or more of the polynucleotides provided in **Table 3**. Typically, ATSBs include an aqueous solution of a sugar (such as sucrose) as a phagostimulant, an attractant such as fruit or flower scents, and an orally available toxin; other ingredients can include fruit juice or pulp, yeast, preservatives, stabilizers, and visual dyes. ATSBs can be sprayed (e. g., on vegetation) or provided in indoor or outdoor bait stations. Examples

of ATSBs which use non-polynucleotide toxins such as Spinosad, pyrethroids, boric acid, or eugenol include those described by Müller *et al.* (2010) *Malaria J.*, 9:210 – 216; Beier *et al.* (2012) *Malaria J.*, 11:31 – 37; Stewart *et al.* (2013) *PLoS ONE*, 8(12):e84168; and Qualls *et al.* (2014) *Acta Tropica*, 131:104 – 110. The disadvantage of ATSBs containing non-polynucleotide toxins is that these are non-selective and are toxic to non-target species including beneficial insects such as honey bees and other pollinators and potentially toxic to non-target species that might ingest a mosquito that has fed on the ATSB. Use of a mosquitocidally effective polynucleotide provided herein permits a species-specific approach to mosquito control.

[0086] Other embodiments include larvicidal compositions including a polynucleotide such as a dsRNA provided herein. Larvicidal compositions formulated as a sprayable liquid or dispersible powder or granules can be deployed in and around mosquito breeding grounds such as stagnant water, cisterns, ponds, lakes, streams, and in vegetated areas.

Example 3

[0087] This example illustrates non-limiting embodiments of assaying the effectiveness of polynucleotides designed to suppress mosquito target genes such as those disclosed in **Tables 2 and 3**. More specifically, this example describes assays of dsRNA with an anti-sense strand encoded by a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 – 2087**.

[0088] Blunt-ended, double-stranded RNAs with an anti-sense strand encoded by a sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 – 2087** are produced. In embodiments, assays are carried out using species-specific dsRNAs. Efficacy of each dsRNA is evaluated by one or more approaches: microinjection studies and evaluation of phenotypic effects in adult mosquitoes, feeding assays in mosquito larvae, sugar bait feeding studies in adult mosquitoes, and routine molecular analyses to measure target gene mRNA or protein suppression (*e. g.*, northern or Western blots). A dsRNA designed to silence a non-relevant gene, such as a dsRNA targeting green fluorescent protein (GFP), is used as a negative control. Mosquitocidal efficacy is validated with multiple or iterative assays. For example, dsRNAs found effective in larval feeding assays are advanced to adult feeding assays using sugar bait preparations. In addition, dsRNA sequences can be modified by tiling procedures to improve efficacy.

[0089] A non-limiting example of a larval feeding assay using *Aedes aegypti* (yellow fever mosquito, YFM) follows. Similar assays are designed for other mosquito species, *e. g.*, *Culex quinquefasciatus* or *Anopheles gambiae*. *Aedes aegypti* eggs are obtained from a commercial source. YFM larval diet is prepared by adding 250 microliters of stock diet solution (0.5 gram of 3:1 mixture of liver powder and brewers yeast mixed into 40 milliliters of sterile deionized water) to 100 milliliters of sterile deionized water; YFM larval diet is de-oxygenated prior to use by degassing overnight under vacuum. Approximately 2000 YFM eggs are hatched in ~100 milliliters de-oxygenated YFM larval diet. The larvae are ready for infestation after overnight incubation. Larvae are collected onto a #40 mesh sieve,

gently rinsed with sterile deionized water, and transferred to a fresh container with 250 milliliters sterile deionized water. The larvae are now ready for loading into a large particle flow cytometer such as a COPAS™ instrument (Union Biometrica, Holliston, MA, USA); larvae should be used within 24 hours. Larvae are distributed into assay containers (*e. g.*, flat-bottom 96-well plates pre-loaded with 200 microliters YFM larval diet), followed by addition of the test or control material. Typically wells contain a single larva each; 8 – 16 wells per test sample and 16 wells for untreated controls and a positive control are used. Plates are manually sealed, with a ventilation pinhole in the sealing film for each well. Plates are maintained at 27 degrees Celsius, 0:24, light:dark cycle, at 70% relative humidity for the required period before scoring. Mortality response can be seen in less than 24 hours, and assay quality remains good for up to 5 days; therefore assay duration may be modified, *e. g.*, scored at less than 1 day, or at 1, 2, 3, 4, or 5 days. To score mortality, wells are checked visually for larval movement; live larvae are very active and therefore inactive larvae are scored as dead.

[0090] Sugar bait feeding studies are carried out in adult mosquitoes. In embodiments, assays are carried out using species-specific polynucleotides. Sugar baits containing one or more of dsRNAs with an anti-sense strand encoded by a sequence selected from the group consisting of SEQ ID NOs.:106 – 896 and 1133 – 2087 are prepared using methods similar to those known in the art (see, *e. g.*, the ATSB compositions described by Müller *et al.* (2010) *Malaria J.*, 9:210 – 216; Beier *et al.* (2012) *Malaria J.*, 11:31 – 37; Stewart *et al.* (2013) *PLoS ONE*, 8(12):e84168; and Qualls *et al.* (2014) *Acta Tropica*, 131:104 – 110). In one embodiment, the sugar bait is an aqueous solution of sucrose (10% w/v) with an added attractant and the test dsRNA added at the desired concentration. The sugar bait can be provided as a liquid, *e. g.*, in a container or in droplets, or via a wick or other feeding device. In another embodiment, the dsRNA is provided in a sugar-containing solid or gel. The sugar bait is offered to adult mosquitoes, and mortality or other changes in phenotype are monitored. Controls include sugar bait with no dsRNA added; optionally, starved controls (no water or bait offered) are included in the event that the dsRNA reduced feeding behaviour.

Example 4

[0091] This example illustrates non-limiting embodiments of assaying the effectiveness of dsRNAs designed to suppress mosquito target genes such as those disclosed in Tables 2 and 3. More specifically, this example describes methods useful for assaying target gene transcript suppression by dsRNA with an anti-sense strand encoded by a sequence selected from the group consisting of SEQ ID NOs.:106 – 896 and SEQ ID NOs.:1133 – 2087.

[0092] Various dsRNAs were tested for their ability to suppress levels of target gene transcripts in adult *Aedes aegypti* mosquitoes. In embodiments, similar assays are designed for other mosquito species, *e. g.*, *Culex quinquefasciatus* or *Anopheles gambiae*. The representative *Aedes aegypti* target genes selected were *RpL19*, *Copi beta*, *Copi beta prime*, *RpS4*, *VATPaseA*, *actin 6*, and *vesicle sorting protein Chmp1*. *Aedes aegypti* adults were anesthetized on a -10 degrees Celsius chill plate. 100 nanoliters of a solution containing 1X PBS and dsRNA (10 milligrams/milliliter) were injected into the

thorax of each insect using a Nanoject II instrument (Drummond Scientific Co., Broomall, PA); 1 microgram dsRNA was administered per insect. After 48 hours, 12 individuals were collected from each treatment, frozen on dry ice, and levels of target gene transcripts were analyzed. As a control, dsRNA designed to target green fluorescent protein (GFP) was administered in the same way.

- 5 Transcript expression data were normalized to the geometric mean of control genes Ef1A and RPS13.

Table 4

Target Gene	Target Gene SEQ ID NO:	dsRNA SEQ ID NO:	% decrease in target gene mRNA level
actin 6	17	229	91%
coatomer protein (COPI) beta prime	9	164	52%
coatomer protein (COPI) beta	7	149	68%
ribosomal protein large subunit 19 (RPL19)	5	133	85%
ribosomal protein small subunit 4 (RPS4)	11	176	84%
vesicle sorting protein Chmp1	36	377	74%
vATPase subunit A	12	186	76%

10 [0093] The results demonstrated that all target genes tested in these experiments showed specific and statistically significant suppression of transcript levels at 48 hours after treatment; target gene transcript levels were reduced between 51 - 90%, compared to expression levels in the control GFP dsRNA injection (**Table 4**). These data indicated that each of the target-gene-specific dsRNAs effectively
15 generated a systemic and gene-specific RNAi response in adult *Aedes aegypti* mosquitoes.

[0094] In a separate series of experiments, various dsRNAs were tested for their ability to cause species-specific mortality in a larval feeding assay using *Aedes aegypti* larvae. The representative *Aedes aegypti* target genes selected were ribosomal protein large subunit 19, vesicle sorting protein Chmp1, and coatomer protein (COPI) beta prime. The larval feeding assay was carried out on *Aedes*
20 *aegypti* larvae using a procedure similar to that described in Example 3 above, with multiple doses of species-specific dsRNAs comprising **SEQ ID NO:133** (targeting ribosomal protein large subunit 19, “RPL19”, **SEQ ID NO:5**), **SEQ ID NO:377** (targeting vesicle sorting protein Chmp1, “Chmp1”, **SEQ ID NO:36**), and **SEQ ID NO:164** (targeting coatomer protein (COPI) beta prime, “COPI beta prime”, **SEQ ID NO:9**). dsRNAs (either a ~100 base-pair “GFP-100” dsRNA or ~300 base-pair “GFP-300”
25 dsRNA) targeting green fluorescent protein (“GFP”) were used as controls. Results for a first set of dsRNA formulations are shown in **Table 5**. These feeding assay data demonstrate that nucleic acids targeting species-specific genes in mosquito larvae cause larval mortality at a statistically significant level.

Table 5

Test Set	Target gene name	Target gene SEQ ID NO:	dsRNA SEQ ID NO:	dsRNA mg/mL	Mean % mortality	Standard error of the mean	Significant vs. control
YFM-2080	GFP (control)	--	-- (GFP-300)	0.25	0.0	0.00	NO
YFM-2080	RPL19	5	133	0.25	79	4.2	YES
YFM-2087	GFP (control)	--	--	1.0	17	11	NO
YFM-2087	Chmp1	36	377	0.25	25	7.2	NO
YFM-2087	Chmp1	36	377	0.5	29	4.2	NO
YFM-2087	Chmp1	36	377	1.0	83	8.3	YES
YFM-2090	GFP (control)	--	-- (GFP-300)	0.5	8.3	8.3	NO
YFM-2090	RPL19	5	133	0.5	42	8.3	NO
YFM-2090	GFP (control)	--	-- (GFP-300)	0.5	8.3	4.2	NO
YFM-2090	RPL19	5	133	0.5	54	11	YES
YFM-2090	Chmp1	36	377	0.5	42	15	NO
YFM-2090	GFP (control)	--	-- (GFP-300)	1.0	29	4.2	NO
YFM-2090	Chmp1	36	377	1.0	50	7.2	YES
YFM-2084	GFP (control)	--	-- (GFP-300)	0.1	0	0	NO
YFM-2084	RPL19	5	133	0.1	79	8.3	YES
YFM-2088	GFP (control)	--	-- (GFP-300)	0.1	0	0	NO
YFM-2088	GFP (control)	--	-- (GFP-300)	0.15	0	0	NO
YFM-2088	GFP (control)	--	-- (GFP-300)	0.2	0	0	NO
YFM-2088	GFP (control)	--	-- (GFP-300)	0.3	0	0	NO
YFM-2088	GFP (control)	--	-- (GFP-300)	0.4	42	4.2	YES
YFM-2088	RPL19	5	133	0.1	50	0	YES
YFM-2088	RPL19	5	133	0.15	88	0	YES
YFM-2088	RPL19	5	133	0.2	75	0	YES
YFM-2088	RPL19	5	133	0.3	46	8.36	YES
YFM-2088	RPL19	5	133	0.4	79	4.2	YES
YFM-2098	GFP (control)	--	-- (GFP-300)	0.01	8.3	4.2	NO
YFM-2098	GFP (control)	--	-- (GFP-300)	0.1	29	17	NO
YFM-2098	GFP (control)	--	-- (GFP-300)	0.2	12	7.2	NO
YFM-2098	RPL19	5	133	0.01	71	8.3	YES
YFM-2098	RPL19	5	133	0.1	62	7.2	YES
YFM-2098	RPL19	5	133	0.2	79	4.2	YES
YFM-2098	<i>Aedes aegypti</i> Chmp1	36	377	0.01	25	12	NO
YFM-2098	<i>Aedes aegypti</i> Chmp1	36	377	0.1	38	7.2	NO
YFM-2098	<i>Aedes aegypti</i> Chmp1	36	377	0.2	62	12	YES
YFM-2100	GFP (control)	--	-- (GFP-100)	0.1	4.2	4.2	NO
YFM-2100	GFP (control)	--	-- (GFP-100)	0.2	0	0	NO
YFM-2100	GFP (control)	--	--	0.3	0	0	NO

			(GFP-100)				
YFM-2100	GFP (control)	--	-- (GFP-100)	0.4	0	0	NO
YFM-2100	COPI beta prime	9	164	0.1	29	15	YES
YFM-2100	COPI beta prime	9	164	0.2	25	7.2	YES
YFM-2100	COPI beta prime	9	164	0.3	38	12	YES
YFM-2100	COPI beta prime	9	164	0.4	42	11	YES

[0095] In another series of experiments, various dsRNAs were tested for their ability to cause species-specific mortality in adult *Aedes aegypti* by injection. The representative *Aedes aegypti* target genes selected were ribosomal protein large subunit 19, vesicle sorting protein Chmp1, coatomer protein (COPI) beta, coatomer protein (COPI prime) beta prime, Ribosomal protein small Subunit 4, and Vacuolar ATPase A. The insects were evaluated for target gene knock-down (KD) at 48 hr post-injection. Insects (12 insects per treatment) were maintained on a -10°C chill plate and injected in the thorax with 100nL of solution containing 1xPBS and dsRNA (10mg/mL) using a Nanoject II instrument (Drummond Scientific Co., Broomall, PA). After 48 hr all individuals were collected from each treatment, frozen on dry ice and analyzed for target gene KD. Expression data was normalized to the geometric mean of EF1A and RPS13. All target genes showed specific transcript KD at 48 hrs ranging from 51% to 90% reduction compared to expression levels in the control GFP saRNA injection. These data indicated a potent systemic RNAi response is functional in adult *Aedes aegypti*. However, no significant mortality compared to the GFP control was observed.

Table 6. Percent target gene knockdown (KD) in dsRNA-injected *Aedes aegypti* adults.

Species	Target Gene SEQ ID NO	dsRNA SEQ ID NO	Target Gene	% Target gene mRNA knockdown	Significant versus GFP control	Significant Mortality versus GFP control
<i>Aedes aegypti</i>	17	229	ACTIN 6	90.70%	YES	NO
<i>Aedes aegypti</i>	9	164	COPI BETA PRIME	51.71%	YES	NO
<i>Aedes aegypti</i>	7	149	COPI BETA	68.16%	YES	NO
<i>Aedes aegypti</i>	5	133	RPL19	84.70%	YES	NO
<i>Aedes aegypti</i>	11	176	RPS4	84.37%	YES	NO

<i>Aedes aegypti</i>	36	377	CHMP 1	73.63%	YES	NO
<i>Aedes aegypti</i>	12	186	vATPase A	75.99%	YES	NO

[0096] In another series of experiments the retention of target gene knockdown over a period of several days for three gene targets was assessed. The gene targets tested were: Chmp1, RPL19 and vATPA. Target gene knockdown appeared to be retained over two to eight days with some slight variability (Table 7.) indicating that the selection of target gene may be important for inducing prolonged knock down.

Table 7. Time course retention of target gene knock down (KD) in dsRNA-injected *Aedes aegypti* adults.

ds RNA SEQ ID NO:	Amount injected	Target Gene	Days Post-injection	% Target gene mRNA knockdown	Significant versus GFP control
377	1 ug/adult	CHMP 1	2D	43.03%	YES
377	1 ug/adult	CHMP 1	4D	49.87%	YES
377	1 ug/adult	CHMP 1	7D	54.81%	YES
186	1 ug/adult	vATPA	2D	83.30%	YES
186	1 ug/adult	vATPA	7D	65.91%	YES
133	1 ug/adult	RPL19	2D	80.56%	YES
133	1 ug/adult	RPL19	5D	73.07%	YES
133	1 ug/adult	RPL19	8D	91.51%	YES
T55708	1 ug/adult	SNF7	2D	47.55%	YES
T55708	1 ug/adult	SNF7	5D	2.28%	NO
T55708	1 ug/adult	SNF7	8D	13.64%	NO

[0097] In order to rule out whether protein stability is a factor in preventing adult specific mortality in *Aedes aegypti*, Western blot analysis of two proteins (RPL7 and SNF7) was undertaken. The protein levels were monitored over several days (day 2, 5 and 7) post-injection of SEQ ID NO 133 (RPL 19) and SEQ ID NO 158 (SNF7). Very limited to no protein suppression was seen by Western blot.

Example 5.

[0098] The following example outlines a screen to identify the excipients capable of enhanced delivery and RNAi phenotypes post-feeding in *Aedes aegypti* larvae. A total of 1052 dsRNA formulations

utilizing forty nine unique excipient mixtures were tested in feeding bioassays for dsRNA-induced mortality in *Aedes aegypti* larvae. Formulation compositions including cationic guar or Polyethylenimine (PEI, branched, 10-25 kDa) and *Aedes aegypti* –specific dsRNAs targeting RPL19, vesicle sorting protein Chmp1, and COPI beta prime were tested for specific activity against larvae in feeding bioassays with multiple doses of dsRNA. **Table 8** illustrates bioassay results against *Aedes aegypti* utilizing dsRNA formulations with a cationic guar and **Table 9** illustrates bioassay results against *Aedes aegypti* larvae utilizing dsRNA formulations with branched PEI (10-25 kDa).

Formulation of dsRNA

[0099] Materials utilized in formulations included: dsRNA (10 mg/ml), PEI (10 mg/ml) in sodium acetate buffer pH 5.3 (0.05 M), Sodium acetate buffer pH 5.3 (0.05M), or cationic guar (in which case sodium acetate buffer was replaced with nuclease-free water). For the preparation of the formulations, dsRNA was added to a 1.5mL Eppendorf tube and the appropriate buffer (sodium acetate for PEI or water for cationic guar) was added to the tube followed by a brief vortexing period. Either PEI or cationic guar were then added and vortexing continued for 1 minute. Formulations were kept for a period of 10 minutes at room temperature before use. As Tables 8 and 9 illustrate, some of the formulations containing cationic guar or PEI had efficacious effects in *Aedes aegypti* larvae versus the GFP control. However, no obvious trend in dsRNA concentration was discernible.

Table 8. Summary of cationic guar formulation bioassay hits against *Aedes aegypti*. Treatments targeting Green Fluorescent Protein are negative control for the individual test sets.

Test Set	dsRNA SEQ ID NO	Target	dsRNA Concentration	Formulation	Mean % Mortality	SEM	Significant vs control
YFM-2080		GFP	0.25 mg/mL	dsRNA{TRUE}; dH2O{TRUE}; Adjuvant{cationic guar, 1 mg/ml}	0.00	0.00	NO
YFM-2080	133	RPL19	0.25 mg/mL	dsRNA{TRUE}; dH2O{TRUE}; Adjuvant{ cationic guar, 1 mg/ml}	79.17	4.17	YES
YFM-2087		GFP	1.0 mg/mL	dsRNA{TRUE}; dH2O{TRUE}; Adjuvant{DV 10496, 1 mg/ml}	16.67	11.02	NO
YFM-2087	377	<i>Aedes aegypti</i> Chmp1	0.25 mg/mL	dsRNA{TRUE}; dH2O{TRUE}; Adjuvant{	25.00	7.22	NO

				cationic guar, 1 mg/ml}			
YFM-2087	377	<i>Aedes aegypti</i> Chmp1	0.5 mg/mL	dsRNA{TRUE}; dH2O{TRUE}; Adjuvant{ cationic guar, 1 mg/ml}	29.17	4.17	NO
YFM-2087	377	<i>Aedes aegypti</i> Chmp1	1.0 mg/mL	dsRNA{TRUE}; dH2O{TRUE}; Adjuvant{ cationic guar, 1 mg/ml}	83.33	8.33	YES
YFM-2090		GFP	0.5 mg/mL	dsRNA{TRUE}; NaOAc{50 mM}; pH{5.3}; Adjuvant{cationic guar, 1.0 mg/ml}	8.33	8.33	NO
YFM-2090	133	<i>Aedes aegypti</i> RPL19	0.5 mg/mL	dsRNA{TRUE}; NaOAc{50 mM}; pH{5.3}; Adjuvant{cationic guar, 1.0 mg/ml}	41.67	8.33	NO
YFM-2090		GFP	0.5 mg/mL	dsRNA{TRUE}; NaOAc{50 mM}; pH{5.3}; Adjuvant {cationic guar, 1.5 mg/ml}	8.33	4.17	NO
YFM-2090	133	<i>Aedes aegypti</i> RPL19	0.5 mg/mL	dsRNA{TRUE}; NaOAc{50 mM}; pH{5.3}; Adjuvant {cationic guar, 1.5 mg/ml}	54.17	11.02	YES
YFM-2090	377	<i>Aedes aegypti</i> Chmp1	0.5 mg/mL	dsRNA{TRUE}; NaOAc{50 mM}; pH{5.3}; Adjuvant{cationic guar, 1.5 mg/ml}	41.67	15.02	NO
YFM-		GFP	1.0 mg/mL	dsRNA{TRUE}; NaOAc{50	29.17	4.17	NO

2090				mMolar}; pH{5.3}; Adjuvant{cationic guar, 1.5 mg/ml}			
YFM-2090	377	<i>Aedes aegypti</i> Chmp1	1.0 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant{cationic guar, 1.5 mg/ml}	50.00	7.22	YES

Table 9. Summary of PEI 10-25 kDa (branched) formulation bioassay hits against *Aedes aegypti*.
Treatments targeting Green Fluorescent Protein are negative control for the individual test sets.

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Testset ID	dsRNA SEQ ID NO	Target	dsRNA Concentration	Formulation	Mean % Mortality	SEM	Significant vs control
YFM-2084		GFP	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	0.00	0.00	NO
YFM-2084	133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	79.17	8.33	YES
YFM-2088		GFP	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	0.00	0.00	NO
YFM-2088		GFP	0.15 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI	0.00	0.00	NO

				10-25 kDa, branched 1:1 w/w}			
YFM-2088		GFP	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	0.00	0.00	NO
YFM-2088		GFP	0.3 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	0.00	0.00	NO
YFM-2088		GFP	0.4 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	41.67	4.17	YES
YFM-2088	133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	50.00	0.00	YES
YFM-2088	133	<i>Aedes aegypti</i> RPL19	0.15 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	87.50	0.00	YES
YFM-2088	133	<i>Aedes aegypti</i>	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50	75.00	0.00	YES

		RPL19		mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}			
YFM-2088	133	<i>Aedes aegypti</i> RPL19	0.3 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	45.83	8.33	YES
YFM-2088	133	<i>Aedes aegypti</i> RPL19	0.4 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	79.17	4.17	YES
YFM-2098		GFP	0.01 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	8.33	4.17	NO
YFM-2098		GFP	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	29.17	16.67	NO
YFM-2098		GFP	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	12.5	7.22	NO
YFM-	133	<i>Aedes aegypti</i>	0.01 mg/mL	dsRNA{TRUE}; NaOAc{50	70.83	8.33	YES

2098		RPL19		mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}			
YFM-2098	133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	62.5	7.22	YES
YFM-2098	133	<i>Aedes aegypti</i> RPL19	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	79.17	4.17	YES
YFM-2098	377	<i>Aedes aegypti</i> Chmp1	0.01 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	25.00	12.5	NO
YFM-2098	377	<i>Aedes aegypti</i> Chmp1	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	37.50	7.22	NO
YFM-2098	377	<i>Aedes aegypti</i> Chmp1	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	62.5	12.5	YES
YFM-2100		GFP	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3};	4.17	4.17	NO

				Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}			
YFM-2100		GFP	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	0.00	0.00	NO
YFM-2100		GFP	0.3 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	0.00	0.00	NO
YFM-2100		GFP	0.4 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	0.00	0.00	NO
YFM-2100	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	29.17	15.02	YES
YFM-2100	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	25.00	7.22	YES
YFM-2100	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.3 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa,	37.50	12.5	YES

				branched 1:1 w/w}			
YFM-2100	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.4 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	41.67	11.02	YES

[00100] Further to the data presented in the Tables above, target gene knockdown was evaluated in larval *Aedes aegypti* at 5 days post-feeding on those formulations that appeared active through induced mortality in bioassays. **Table 10** illustrates the significant target gene suppression observed for a select number of formulations tested in the feeding bioassays.

Table 10. Evaluation of target gene knockdown in *Aedes aegypti* larvae post feeding on PEI 10-25 kDa (branched) or cationic guar -based formulation. Treatments targeting green fluorescent protein are negative control dsRNA treatments for the individual testsets.

Testset ID	dsRNA SEQ ID NO.	Target	dsRNA Concentration	Formulation	% Target gene mRNA knockdown	Significant knockdown vs GFP control
BDX-01234	133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	5.57%	NO
BDX-01234	133	<i>Aedes aegypti</i> RPL19	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	-1.2%	NO
BDX-01234	377	<i>Aedes aegypti</i> Chmp1	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI	40.94%	YES

				10-25 kDa, branched 1:1 w/w}		
BDX-01234	377	<i>Aedes aegypti</i> Chmp1	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	12.85%	NO
BDX-01234	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	22.65%	NO
BDX-01234	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	3.3%	NO
BDX-01293	133	<i>Aedes aegypti</i> RPL19	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	-32.76%	NO
BDX-01293	133	<i>Aedes aegypti</i> RPL19	0.2 mg/mL	dsRNA{TRUE}; dH2O{TRUE}; Adjuvant{DV 10496, 1 mg/ml}	7.97%	NO
BDX-	377	<i>Aedes aegypti</i>	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50	-12.86%	NO

01293		Chmp1		mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}		
BDX-01293	377	<i>Aedes aegypti</i> Chmp1	0.2 mg/mL	dsRNA{TRUE}; dH2O{TRUE}; Adjuvant{DV 10496, 1 mg/ml}	14.02%	NO
BDX-01293	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.2 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	-11.65%	NO
BDX-01293	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.2 mg/mL	dsRNA{TRUE}; dH2O{TRUE}; Adjuvant{DV 10496, 1 mg/ml}	7.39%	NO
BDX-01365	133	<i>Aedes aegypti</i> RPL19	0.02 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	-10.57%	NO
BDX-01365	133	<i>Aedes aegypti</i> RPL19	0.04 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	-4.17%	NO
BDX-01365	133	<i>Aedes aegypti</i> RPL19	0.06 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI	0.99%	NO

				10-25 kDa, branched 1:1 w/w}		
BDX-01365	133	<i>Aedes aegypti</i> RPL19	0.08 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	0.81%	NO
BDX-01365	133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 1:1 w/w}	-13.68%	NO
BDX-01365	133	<i>Aedes aegypti</i> RPL19	0.01 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	-33.93%	NO
BDX-01365	133	<i>Aedes aegypti</i> RPL19	0.02 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	-8.78%	NO
BDX-01365	133	<i>Aedes aegypti</i> RPL19	0.04 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	6.68%	NO
BDX-01365	133	<i>Aedes aegypti</i>	0.06 mg/mL	dsRNA{TRUE}; NaOAc{50	21.95%	YES

		RPL19		mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}		
BDX-01365	133	<i>Aedes aegypti</i> RPL19	0.08 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	19.07%	YES
BDX-01365	133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	15.61%	YES
BDX-01405	133	<i>Aedes aegypti</i> RPL19	0.05 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	14.00%	NO
BDX-01405	133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	-28.00%	NO
BDX-01405	133	<i>Aedes aegypti</i> RPL19	0.15 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	-14.41%	NO

BDX-01405	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.05 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	-0.26%	NO
BDX-01405	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	6.89%	NO
BDX-01405	164	<i>Aedes aegypti</i> COPI BETA PRIME	0.15 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	15.98%	NO
BDX-01405	377	<i>Aedes aegypti</i> Chmp1	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	20.72%	NO
BDX-01405	377	<i>Aedes aegypti</i> Chmp1	0.15 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {PEI 10-25 kDa, branched 2:1 w/w}	-6.67%	NO

Specificity of formulation activity to larval stage:

[00101] Target gene suppression was further assessed in adult *Aedes aegypti* mosquitoes 3 days post-feeding on dsRNA formulations adjusted for delivery in 10% fructose or 10% sucrose. Weak to no

gene suppression was observed post sugar feeding in adult mosquitoes suggesting formulation-based efficacy was more potent in the aquatic larval stage of the mosquito (Table 11. below).

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Table 11. Results of sugar-based dsRNA formulation feeding in adult *Aedes aegypti*.

dsRNA SEQ ID NO	Target	dsRNA Concentration	Formulation	% Target gene mRNA knockdown	Significant knockdown vs GFP control
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE }; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Fructo se; PEI 10-25 kDa, branched 2:1 w/w}	0.68%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE }; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Fructo se; BSA_EDAC_P EI 10-25 kDa, branched 1:1 w/w}	-5.98%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE }; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Fructo se; BSA_EDAC_P EI 10-25 kDa, branched 2:1 w/w }	3.44%	NO

133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Fructose; AGA_EDAC_PEI 10-25 kDa, branched 1:1 w/w }	-7.24%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Fructose; AGA_EDAC_PEI 10-25 kDa, branched 2:1 w/w }	-0.48%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Sucrose; PEI 10-25 kDa, branched 2:1 w/w }	-0.59%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Sucrose; BSA_EDAC_PEI 10-25 kDa, branched 1:1 w/w }	-1.5%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3};	3.58%	NO

			Adjuvant {10_%_Sucrose; BSA_EDAC_P EI 10-25 kDa, branched 2:1 w/w }		
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE }; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Sucrose; AGA_EDAC_ PEI 10-25 kDa, branched 1:1 w/w }	-2.5%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE }; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Sucrose; AGA_EDAC_ PEI 10-25 kDa, branched 2:1 w/w }	11.58%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE }; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Fructose_P EI 10-25 kDa, branched 1:1 w/w }	13.48%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE }; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Fructose_DOTAP/D OPE_2:1 w/w }	-4.17%	NO

133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Fructose}	-14.00%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Sucrose_PEI_10-25 kDa, branched 1:1 w/w}	16.55%	YES
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Sucrose_DOTAP/DOPE_2:1 w/w}	-9.37%	NO
133	<i>Aedes aegypti</i> RPL19	0.1 mg/mL	dsRNA{TRUE}; NaOAc{50 mMolar}; pH{5.3}; Adjuvant {10_%_Sucrose}	6.14%	NO

[00102] All of the materials and methods disclosed and claimed herein can be made and used without undue experimentation as instructed by the above disclosure. Although the materials and methods of this invention have been described in terms of preferred embodiments and illustrative examples, it will be apparent to those of skill in the art that variations can be applied to the materials and methods described herein without departing from the concept, spirit and scope of this invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of this invention as defined by the appended claims.

What is claimed is:

1. A method of mosquito control, including:

- (a) contacting a mosquito with a polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene, or an RNA transcribed from the target gene, wherein the target gene is at least one gene selected from the group consisting of: (i) a gene identified by name in Tables 2 and 3; (ii) a gene encoding a proteasome protein selected from the group consisting of: Proteasome Alpha 2, Proteasome Beta 5, Proteasome regulatory subunit 2, Proteasome regulatory subunit 3, Proteasome regulatory subunit 8, Proteasome regulatory subunit 9, Proteasome regulatory GTPase SAR1, 26S protease regulatory subunit 6; (iii) a gene encoding an ATPase protein selected from the group consisting of: V-ATPase subunit A, V-ATPase subunit B, V-ATPase subunit D, V-ATPase subunit E, Actin 6; (iv) a gene encoding a ribosomal protein selected from the group consisting of: Ribosomal protein Large subunit 3, Ribosomal protein Large subunit 7, Ribosomal protein Large subunit 19, Ribosomal protein Large subunit 40, Ribosomal protein Small subunit 21, Ribosomal protein Small subunit 4; (v) a gene encoding a vesicle protein selected from the group consisting of: Vesicle Sorting protein 2, Vesicle Sorting protein 4, Vesicle Sorting protein 16A, Vesicle Sorting protein 20, Vesicle Sorting protein 24, Vesicle Sorting protein 27, Vesicle Sorting protein 28, Vesicle Sorting protein Shrb (Snf7), Vesicle Sorting protein Chmp1, Secretory vesicle protein 6, Secretory vesicle protein 23, Coatomer Protein Alpha, Coatomer Protein Beta, Coatomer Protein Beta Prime, Coatomer Protein Delta, Coatomer Protein Epsilon, Coatomer Protein Zeta; (vi) a gene encoding an actin; (vii) a gene encoding a protein related to behaviour selected from the group consisting of: Bendless, scribbler, capa receptor, diapause hormone receptor, Eb1, Ether-a-go-go, flightless-I, foraging, juvenile hormone epoxide hydrolase, neuropeptide F, pheromone biosynthesis activating neuropeptide receptor, semaphorin 1a, shaker, slowmo, stress-sensitive B, turtle, twitchin, myosin heavy chain, nervana, N-synaptobrevin, nuclear hormone receptor 25, syntaxin, and bent; (viii) a gene encoding a glycolysis and energy metabolism protein selected from the group consisting of: BiP, citrate synthase, glycogen synthase, hexokinase, isocitrate dehydrogenase, pyruvate dehydrogenase, phosphofructokinase, GTPase activator, GTPase Ran, sarcoplasmic Ca²⁺ ATPase, Ca²⁺ ATPase, hydrogen exporting ATPase, NADH dehydrogenase, F1F0 ATPase, spermatogenesis ATPase, beta-ketoacyl synthase, pyruvate kinase, and knickkopf; (ix) a gene encoding a protein turnover and mitosis protein selected from the group consisting of: cyclins including cyclin A, cyclin B, cyclin B3, cyclin C, cyclin D, cyclin E, cyclin G, cyclin H, cyclin J, cyclin K, cyclin T, and cyclin Y, Ubiquitin conjugating enzyme E1, chromosome segregation protein, CDC5, COP9 signalosome, and ketel; (x) a gene encoding a gene expression protein selected from the group consisting of: cleavage polyadenylation specificity factor, E1A/CREB-binding protein, RNA helicases, RNA polymerases, Arf1, Bx42, and eft2; (xi) a gene encoding a protein-folding, stress, and heat shock response protein selected from the

group consisting of: protein disulfide isomerase, heat shock cognate 70, chaperonin TCP1, chaperonins, carrier protein, and HSP70; and (xii) a gene encoding a miscellaneous protein selected from the group consisting of: spermatogenesis ATPase, pitchoune, and insect proteins;

(b) contacting a mosquito with an effective amount of a polynucleotide, one strand of which is complementary to at least 21 contiguous nucleotides of a target gene comprising a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene;

(c) providing in the diet of a mosquito a polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene comprising a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene;

(d) causing mortality or stunting in larvae of a mosquito by providing in the diet of the larvae at least one polynucleotide including at least one silencing element, wherein the silencing element includes at least 21 contiguous nucleotides that are complementary to a target gene comprising a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene;

(e) contacting a mosquito with a polynucleotide including at least one segment that is identical or complementary to at least 21 contiguous nucleotides of a target gene selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene; or

(f) contacting an environment infested by the mosquito with a composition including a polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene comprising a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene.

2. The method of claim 1, wherein the polynucleotide is provided in an ingestible composition, a sprayable composition, a particulate or powder, a solid bait, or a liquid bait.

3. The method of claim 1, wherein the contacting includes oral delivery to the mosquito, or includes non-oral delivery to the mosquito, or includes a combination of oral and non-oral delivery to the mosquito.

4. The method of claim 1, wherein the mosquito is an adult, pupa, larva, or egg.

5. The method of claim 1, wherein the contacting results in mortality or stunting of the mosquito.

6. The method of claim 1, wherein the mosquito is in a population of mosquitoes and the population is reduced.

7. The method of claim 1, wherein the mosquito is an *Aedes* sp., a *Culex* sp., or an *Anopheles* sp.

8. The method of claim 1, wherein:

(a) said mosquitoes are *Aedes aegypti* and the target gene has a DNA sequence selected from the group consisting of **SEQ ID NOs.:1 – 36 and 897 – 975**; or

(b) said mosquitoes are *Culex quinquefasciatus* and the target gene has a DNA sequence selected from the group consisting of **SEQ ID NOs.:37 – 71 and 1014 and 1060 – 1132**; or

(c) said mosquitoes are *Anopheles gambiae* or *Anopheles gambiae* species complex and the target gene has a DNA sequence selected from the group consisting of **SEQ ID NOs.:72 – 105 and 976 – 1013 and 1015 – 1059**.

9. The method of claim 1, wherein the contacting includes providing the polynucleotide in a composition that is ingested or contacted by the mosquito.

10. The method of claim 9, wherein the composition includes a solid, liquid, powder, suspension, emulsion, colloid, spray, encapsulation, microbeads, carrier particulates, granules, film, gel, or matrix.

11. The method of claim 9, wherein the composition includes a bacterial or eukaryotic cell expressing the dsRNA.

12. The method of claim 9, wherein the composition is provided in the form of a bait, a trap, a sprayable liquid, a concentrate, dunks, dispersible granules, or ingestible particulates or cells.

13. The method of claim 9, wherein the composition further includes one or more components selected from the group consisting of a carrier agent, a surfactant, an organosilicone, a lipid, a sugar, a non-polynucleotide mosquitocide, a dipterocidal *Bacillus thuringiensis* toxin, a safener, a mosquito attractant, a pheromone, and an insect growth regulator.

14. The method of claim 1, wherein the polynucleotide is a double-stranded RNA (dsRNA).

15. The method of claim 14, wherein the dsRNA (a) is blunt-ended, or (b) has an overhang at at least one terminus, or (c) includes at least one stem-loop.

16. The method of claim 14, wherein the dsRNA is produced by (a) chemical synthesis, or (b) expression in a microorganism, or (c) expression in a eukaryotic cell.

17. The method of claim 14, wherein the dsRNA is chemically modified.

18. The method of claim 14, wherein the double-stranded RNA includes a strand including a sequence selected from the group consisting of: **SEQ ID NOs.:106 – 896 and 1133 – 2087**.

19. The method of claim 14, wherein:

(a) said mosquitoes are *Aedes aegypti* and the double-stranded RNA includes one strand which is complementary to at least 21 contiguous nucleotides of a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:106 – 382 and 1133 – 1440**;

(b) said mosquitoes are *Culex quinquefasciatus* and the double-stranded RNA includes one strand which is complementary to at least 21 contiguous nucleotides of a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:383 – 647 and 1613 – 1614 and 1762 – 2087**; or

(c) said mosquitoes are *Anopheles gambiae* or *Anopheles gambiae* species complex and the double-stranded RNA includes one strand which is complementary to at least 21 contiguous nucleotides of a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:648 – 896 and 1441 – 1612 and 1615 – 1761**.

20. A mosquitocidal composition including a mosquitocidally effective amount of:

- (a) a recombinant polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a mosquito target gene, or an RNA transcribed from the mosquito target gene, wherein the target gene is at least one gene selected from the group consisting of:
- (i) a gene identified by name in Tables 2 and 3; (ii) a gene encoding a proteasome protein selected from the group consisting of: Proteasome Alpha 2, Proteasome Beta 5, Proteasome regulatory subunit 2, Proteasome regulatory subunit 3, Proteasome regulatory subunit 8, Proteasome regulatory subunit 9, Proteasome regulatory GTPase SAR1, 26S protease regulatory subunit 6; (iii) a gene encoding an ATPase protein selected from the group consisting of: V-ATPase subunit A, V-ATPase subunit B, V-ATPase subunit D, V-ATPase subunit E, Actin 6; (iv) a gene encoding a ribosomal protein selected from the group consisting of: Ribosomal protein Large subunit 3, Ribosomal protein Large subunit 7, Ribosomal protein Large subunit 19, Ribosomal protein Large subunit 40, Ribosomal protein Small subunit 21, Ribosomal protein Small subunit 4; (v) a gene encoding a vesicle protein selected from the group consisting of: Vesicle Sorting protein 2, Vesicle Sorting protein 4, Vesicle Sorting protein 16A, Vesicle Sorting protein 20, Vesicle Sorting protein 24, Vesicle Sorting protein 27, Vesicle Sorting protein 28, Vesicle Sorting protein Shrb (Snf7), Vesicle Sorting protein Chmp1, Secretory vesicle protein 6, Secretory vesicle protein 23, Coatomer Protein Alpha, Coatomer Protein Beta, Coatomer Protein Beta Prime, Coatomer Protein Delta, Coatomer Protein Epsilon, Coatomer Protein Zeta; (vi) a gene encoding an actin; (vii) a gene encoding a protein related to behaviour selected from the group consisting of: Bendless, scribbler, capa receptor, diapause hormone receptor, Eb1, Ether-a-go-go, flightless-I, foraging, juvenile hormone epoxide hydrolase, neuropeptide F, pheromone biosynthesis activating neuropeptide receptor, semaphorin 1a, shaker, slowmo, stress-sensitive B, turtle, twitchin, myosin heavy chain, nervana, N-synaptobrevin, nuclear hormone receptor 25, syntaxin, and bent; (viii) a gene encoding a glycolysis and energy metabolism protein selected from the group consisting of: BiP, citrate synthase, glycogen synthase, hexokinase, isocitrate dehydrogenase, pyruvate dehydrogenase, phosphofructokinase, GTPase activator, GTPase Ran, sarcoplasmic Ca²⁺ ATPase, Ca²⁺ ATPase, hydrogen exporting ATPase, NADH dehydrogenase, F1F0 ATPase, spermatogenesis ATPase, beta-ketoacyl synthase, pyruvate kinase, and knickkopf; (ix) a gene encoding a protein turnover and mitosis protein selected from the group consisting of: cyclins including cyclin A, cyclin B, cyclin B3, cyclin C, cyclin D, cyclin E, cyclin G, cyclin H, cyclin J, cyclin K, cyclin T, and cyclin Y, Ubiquitin conjugating enzyme E1, chromosome segregation protein, CDC5, COP9 signalosome, and ketel; (x) a gene encoding a gene expression protein selected from the group consisting of: cleavage polyadenylation specificity factor, E1A/CREB-binding protein, RNA helicases, RNA polymerases, Arf1, Bx42, and eft2; (xi) a gene encoding a protein-folding, stress, and heat shock response protein selected from the group consisting of: protein disulfide isomerase, heat shock cognate 70, chaperonin TCPI1, chaperonins, carrier

protein, and HSP70; and (xii) a gene encoding a miscellaneous protein selected from the group consisting of: spermatogenesis ATPase, pitchoune, and insect proteins; or

(b) a recombinant polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene comprising a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene.

21. The mosquitocidal composition of claim 20, wherein the recombinant RNA molecule is provided in a microbial or eukaryotic cell that expresses the recombinant RNA, or in a microbial fermentation product.

22. The mosquitocidal composition of claim 20, wherein the composition includes a solid, liquid, powder, suspension, emulsion, colloid, spray, encapsulation, microbeads, carrier particulates, granules, film, gel, or matrix.

23. The mosquitocidal composition of claim 20, wherein the composition is provided in the form of a bait, a trap, a sprayable liquid, a concentrate, dunks, dispersible granules, or ingestible particulates or cells.

24. The mosquitocidal composition of claim 20, wherein the composition further includes one or more components selected from the group consisting of a carrier agent, a surfactant, an organosilicone, a lipid, a sugar, a non-polynucleotide mosquitocide, a dipterocidal *Bacillus thuringiensis* toxin, a safener, a mosquito attractant or pheromone, and an insect growth regulator.

25. The mosquitocidal composition of claim 20, wherein the recombinant polynucleotide is a dsRNA.

26. The mosquitocidal composition of claim 25, wherein the dsRNA includes a strand including at least 21 contiguous ribonucleotides of an RNA sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087**.

27. The mosquitocidal composition of claim 25, wherein the dsRNA is at least 50 base pairs in length.

28. The mosquitocidal composition of claim 25, wherein the dsRNA (a) is blunt-ended, or (b) has an overhang at at least one terminus, or (c) includes at least one stem-loop.

29. The mosquitocidal composition of claim 25, wherein the dsRNA is produced by (a) chemical synthesis, or (b) expression in a microorganism, or (c) expression in a eukaryotic cell.

30. The mosquitocidal composition of claim 25, wherein the dsRNA is chemically modified.

31. A recombinant DNA construct including a heterologous promoter operably linked to DNA encoding an RNA transcript including:

(a) a sequence of about 95% to about 100% identity or complementarity with a sequence selected from the group consisting of **SEQ ID NOs.:1 – 105 and 897 - 1132**;

(b) at least 21 contiguous nucleotides that are complementary to a target gene comprising a nucleotide sequence selected from the group consisting of: **SEQ ID NOs.:1 – 105 and 897 - 1132**, or an RNA transcribed from the target gene;

(c) at least 21 contiguous ribonucleotides of an RNA sequence selected from the group consisting of **SEQ ID NOs.:106 – 896 and 1133 - 2087**; or

(d) a strand including a sequence selected from the group consisting of: **SEQ ID NOs.:106 – 896** and **1133 - 2087**.

32. The recombinant DNA construct of claim 31, wherein the RNA transcript includes dsRNA.

33. The recombinant DNA construct of claim 32, wherein the dsRNA is encoded on: (a) a single RNA strand, or (b) multiple RNA strands.

34. The recombinant DNA construct of claim 32, wherein the dsRNA (a) is blunt-ended, or (b) has an overhang at at least one terminus, or (c) includes at least one stem-loop.

35. The recombinant DNA construct of claim 31, wherein the heterologous promoter is functional for expression of the RNA transcript in a bacterium.

36. The recombinant DNA construct of claim 35, wherein the bacterium is selected from the group consisting of *Escherichia coli*, *Bacillus* species, *Pseudomonas* species, *Xenorhabdus* species, or *Photorhabdus* species.

37. The recombinant DNA construct of claim 31, wherein the heterologous promoter is functional in an eukaryotic cell.

38. A recombinant vector including the recombinant DNA construct of claim 31.

39. The recombinant vector of claim 31, wherein the recombinant vector is a recombinant plant virus vector or a recombinant baculovirus vector.

40. A chromosome or plastid including the recombinant DNA construct of claim 31.

41. A transgenic cell comprising in its genome the recombinant DNA construct of claim 31.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/45227

Box No. 1 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/US17/45227

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please see supplemental page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Groups I+, Claims 1-41; SEQ ID NO:1 (Target gene); SEQ ID NO: 106 (Polynucleotide)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/45227

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A01N 63/00; C07H 21/02; C12N 15/113; C12P 19/34 (2017.01)

CPC - A01N 63/00; C07H 21/02; C12N 15/113; C12P 19/34

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.
A	(NENE, V et al.) Genome sequence of <i>Aedes aegypti</i> , a major arbovirus vector. <i>Science</i> . 22 June 2007, Vol. 316, No. 5832; pages 1718-1723; Genbank Supplement, pages 1-2; DOI: 10.1126/science.1138878	1-41
A	US 2013/137747 A1 (ZHU, KY et al.) 30 May 2013; paragraphs [0035]-[0036], [0040]-[0041]	1-41
A	(EKERT, EV et al.) Control of larval and egg development in <i>Aedes aegypti</i> with RNA interference against juvenile hormone acid methyl transferase. <i>Journal of Insect Physiology</i> . November 2014, Epub 9 August 2014, Vol. 70; pages 143-150; abstract; page 4, 2nd column, 3rd paragraph; page 2, 2nd column, 3rd paragraph; page 5, 2nd column, 1st paragraph; DOI: 10.1016/j.jinsphys.2014.08.001	1-41
A	(ZHANG, X et al.) Chitosan/double-stranded RNA nanoparticle-mediated RNA interference to silence chitin synthase genes through larval feeding in the African malaria mosquito (<i>Anopheles gambiae</i>). <i>Insect Molecular Biology</i> . October 2010, Epub 8 July 2010, Vol. 19, No. 5; pages 683-693; abstract; Figure 4; page 684, 1st column, 4th paragraph; page 686, 2nd column, 2nd paragraph; page 690, 1st column, 2nd paragraph and 2nd column, 2nd paragraph; DOI: 10.1111/j.1365-2583.2010.01029.x	1-41

☐ 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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 November 2017 (17.11.2017)

Date of mailing of the international search report

28 DEC 2017

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US17/45227

----Continued from Box No. III: Observations where unity of invention is lacking----

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-41; SEQ ID NO:1 (Target gene); SEQ ID NO: 106 (Polynucleotide) are directed toward polynucleotides, compositions, and methods for mosquito control.

The polynucleotides, compositions, and methods for mosquito control will be searched to the extent that they encompass SEQ ID NO:1 (first exemplary Target gene); SEQ ID NO: 106 (first exemplary polynucleotide). Applicant is invited to elect additional target gene sequence(s), and associated polynucleotide sequence(s), with specified SEQ ID NO: for each, to be searched. Additional target gene(s) and associated polynucleotide sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 4 (in-part), 5 (in-part), 6 (in-part), 7 (in-part), 8 (in-part), 9 (in-part), 10 (in-part), 11 (in-part), 12 (in-part), 13 (in-part), 14 (in-part), 15 (in-part), 16 (in-part), 17 (in-part), 18 (in-part), 19 (in-part), 20 (in-part), 21 (in-part), 22 (in-part), 23 (in-part), 24 (in-part), 25 (in-part), 26 (in-part), 27 (in-part), 28 (in-part), 29 (in-part), 30 (in-part), 31 (in-part), 32 (in-part), 33 (in-part), 34 (in-part), 35 (in-part), 36 (in-part), 37 (in-part), 38 (in-part), 39 (in-part), 40 (in-part), and 41 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (target gene) and SEQ ID NO: 106 (polynucleotide). Applicants must specify the claims that encompass any additionally elected target gene sequence(s), and associated polynucleotide sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 2 (first exemplary elected Target gene); SEQ ID NO: 111 (first exemplary elected polynucleotide).

No technical features are shared between the sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a method of mosquito control, including: (a) contacting a mosquito with a polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene, or an RNA transcribed from the target gene; (b) contacting a mosquito with an effective amount of a polynucleotide, one strand of which is complementary to at least 21 contiguous nucleotides of a target gene, or an RNA transcribed from the target gene; (c) providing in the diet of a mosquito a polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene, or an RNA transcribed from the target gene; (d) causing mortality or stunting in larvae of a mosquito by providing in the diet of the larvae at least one polynucleotide including at least one silencing element, wherein the silencing element includes at least 21 contiguous nucleotides that are complementary to a target gene, or an RNA transcribed from the target gene; (e) contacting a mosquito with a polynucleotide including at least one segment that is identical or complementary to at least 21 contiguous nucleotides of a target gene, or an RNA transcribed from the target gene; or (f) contacting an environment infested by the mosquito with a composition including a polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene, or an RNA transcribed from the target gene; a mosquitocidal composition including a mosquitocidally effective amount of: (a) a recombinant polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a mosquito target gene, or an RNA transcribed from the mosquito target gene; or (b) a recombinant polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene, or an RNA transcribed from the target gene; a recombinant DNA construct including a heterologous promoter operably linked to DNA encoding an RNA transcript including: (a) a sequence of about 95% to about 100% identity or complementarity with a target sequence; (b) at least 21 contiguous nucleotides that are complementary to a target, or an RNA transcribed from the target gene; (c) at least 21 contiguous ribonucleotides of an RNA sequence; or (d) a strand; a recombinant vector including the recombinant DNA construct; a chromosome or plasmid including the recombinant DNA construct; a transgenic cell comprising in its genome the recombinant DNA construct.

However, these shared technical features are previously shared by US 2014/0013471 A1 to Monsanto Technology LLC (hereinafter 'Monsanto').

A method of mosquito control (method of pest control including mosquitoes; abstract; paragraph [0122]), including: (a) contacting a mosquito with a polynucleotide including a nucleotide sequence that is complementary to at least 21 contiguous nucleotides of a target gene (paragraphs [0099], [0110], [0122]; claim 1), (b) contacting a mosquito with an effective amount of an RNA transcribed from the target gene (paragraphs [0099], [0110], [0122]; claims 5, 7); (c) providing in the diet of a mosquito an RNA transcribed from the target gene (paragraphs [0099], [0110], [0122]; claims 5, 7); (e) contacting a mosquito with an RNA transcribed from the target gene (paragraphs [0099], [0110], [0122]; claims 5, 7); or (f) contacting an environment infested by the mosquito with an RNA transcribed from the target gene (paragraphs [0099], [0110], [0122]; claims 1, 5, 7); a mosquitocidal composition (insecticidal composition against mosquitoes; abstract; paragraphs [0025], [0099], [0122], [0135]) including a mosquitocidally effective amount (insecticidally effective amount; paragraph [0135]) of: (b) an RNA transcribed from the target gene (paragraphs [0014], [0122]; claim 5); a recombinant DNA construct (paragraph [0123]) including a heterologous promoter operably linked to DNA encoding an RNA transcript (a heterologous promoter operably linked to DNA encoding an RNA transcript; paragraph [0020], [0022]) including: (b) an RNA transcribed from the target gene; (c) at least 21 contiguous ribonucleotides of an RNA sequence (paragraphs [0021]-[0022]); a recombinant vector including the recombinant DNA construct (paragraph [0075]; claim 4); a chromosome including the recombinant DNA construct (a chromosome or plasmid including the recombinant DNA construct; paragraphs [0079], [0095]); a transgenic cell comprising in its genome the recombinant DNA construct (a transgenic cell comprising in its genome the recombinant DNA construct; paragraph [0093]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Monsanto reference, unity of invention is lacking.