Applications of gene drive systems for population suppression of insect pests

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Abstract

Population suppression is an effective way for controlling insect pests and disease vectors, which cause significant damage to crop and spread contagious diseases to plants, animals and humans. Gene drive systems provide innovative opportunities for the insect pests population suppression by driving genes that impart fitness costs on populations of pests or disease vectors. Different gene-drive systems have been developed in insects and applied for their population suppression. Here, different categories of gene drives such as meiotic drive (MD), under-dominance (UD), homing endonuclease-based gene drive (HEGD) and especially the CRISPR/Cas9-based gene drive (CCGD) were reviewed, including the history, types, process and mechanisms. Furthermore, the advantages and limitations of applying different gene-drive systems to suppress the insect population were also summarized. This review provides a foundation for developing a specific gene-drive system for insect population suppression.

Introduction

According to the Mendelian segregation, a specific allele at a specific chromosomal locus has an equal chance (50:50) to exist in an individual gamete without any selective pressure. Gene-drive dynamics occur when a particular allele is segregated in the way to disrupt the Mendelian segregation. Gene drive systems have capabilities to provide innovative solutions to major environmental and public health problems (Hamilton, 1967; Curtis, 1968). The majority of developed gene drives are based on naturally occurring selfish genetic elements that work by rising in frequency with each generation without providing any fitness benefit to their hosts, causing non-Mendelian inheritance patterns (Burt, 2014). These characteristics of gene drive systems enable the scientist to overcome the genetic and evolutionary drawbacks of some desirable traits by disseminating them to wild-type populations or completely suppressing the population (Alphey *et al.*, 2013; Burt, 2014). As a result, gene drive systems facilitate the development of innovative strategies for reducing or eliminating the insect-born diseases and even overcome the development of pesticides resistances in a cost-effective and eco-friendly manner (Champer *et al.*, 2016). Furthermore, gene drives might also be used to spread desired traits to the population more quickly than natural selection to protect the endangered species by disseminating pathogen-resistant genes among targeted populations (Champer *et al.*, 2016).

The idea of utilization of gene drive to suppress the vector and pest population emerged in 1960 when naturally occurring male-producing factors were used to suppress the *Aedes aegypti* (Craig *et al.*, 1960; Curtis, 1968). Subsequently, a sex-determining chromosomal region in mosquitoes and its potential use for population suppression were defined (Hickey and Craig, 1966). Later on, Hamilton demonstrated the genes that distribute through the non-Mendelian process might cause population elimination (Hamilton, 1967) and Curtis explored the prospect of the transformation of malaria mosquito populations by employing self-spreading chromosomal variants (Curtis, 1968). The basic research in molecular biology and genetics continue until the possible, and advanced genetic engineering tools are available. In 1992, an idea and mechanism of utilizing transposable elements were proposed to drive a genetically engineered gene into the mosquito populations (Kidwell and Ribeiro, 1992). A substantial stimulus was the discovery of selfish genetic elements that originate in numerous single-cell organisms, called homing-endonuclease genes (HEGs) (Burt, 2003). Subsequently, homing endonuclease-based gene drive (HEGD) was developed with HEGs and used to suppress the population of malaria vectors (Stoddard, 2005). Population biologists and geneticists continued to study how to use different selfish genetic elements as the systematic source for the development of a gene-drive system in mosquitoes (James, 2005; Adelman *et al.*, 2007; Windbichler *et al.*, 2011). Then, the CRISPR/Cas9-based endonuclease system in

bacteria was discovered and modified to produce an artificial drive system (Jinek *et al.*, 2012; Rode *et al.*, 2019). CRISPR/Cas9-based gene drive (CCGD) was developed and used in *Drosophila melanogaster* (Gantz and Bier, 2015), *Anopheles stephensi* (Gantz *et al.*, 2015), *Anopheles gambiae* (Kyrou *et al.*, 2018), *Aedes aegypti* (Basu *et al.*, 2015; Williams *et al.*, 2020) and *Tribolium castaneum* (Drury *et al.*, 2017) to suppress and replace the wild-type population.

Nowadays, there is great interest in using of gene-drive technologies to suppress the pest populations (James, 2005). A systemic approach requires for designing effective and safe gene drive systems that involve in the establishment of this system by discovering naturally occurring or synthetic genetic mechanisms for gene spread (Letourneau and Burrows, 2001; James 2005). The most essential and critical criterion is that the gene-drive system must be strong enough to transfer the desirable effector genes to the next population (Boete and Koella, 2003). Four gene-drive systems, i.e. meiotic drive (MD), under-dominance (UD), HEGD and CCGD have been successfully developed and used to control the pest populations. The genetic alteration in gene-drive systems would continue in the future to manage the wild-type population of harmful pests (Godfray *et al.*, 2017; Asad *et al.*, 2020). The vital purpose of this review is to discuss different types of gene drives and their potential applications to control the pest and disease vector populations.

Gene-drive systems in insects

Meiotic drive (MD)

MD takes place when a specific heterozygous locus segregates at a higher rate than the typical Mendelian ratio by disabling or disturbing the homologous chromosome (Benedict, 2014). The sex chromosome-based MD states the inadequate transmission of X and Y chromosomes from individuals of heterogametic sex, resulting in a bias sex ratio between the offspring of the population (Helleu *et al.*, 2015). A lot of articles and reviews have discussed the development and detailed history of MD. We are focusing on X and Y MDs that have been successfully developed in insects during the past few years.

The two basic types, X and Y chromosome-based MD systems have been used in mosquito and *Drosophila*. In *Drosophila*, the X chromosome-based MD is associated with abnormal behavior of the Y chromosome during meiosis II, which can lead to failure of sister chromatid segregation (Keais *et al.*, 2017). The X chromosome-based MD can be very strong, often leading to 100% female progeny or near to it (Helleu *et al.*, 2015).

Y-drive is also linked with the breakage of X chromosome during anaphase I, which leads to the failure of crossover. The segregation distortion occurs during spermatogenesis and leads to Y chromatid non-disjunction at meiosis II or breakage of X chromosome for a failure of spermatogenesis. During meiosis, the non-random segregation of chromosomes occurs through the spermatogenesis of heterogametic male flies of *Trichosia pubescens,* although in a female-only single gamete is formed during meiosis. The development of only a single gamete is due to monocentric and monopolar spindles during the first meiotic division. In this case, non-random segregation of genetic material is due to the deactivation of paternal chromosomal kinetochores that are incapable of holding the microtubules of the spindle. (Rutkowska and Badyaev, 2008).

The potential for the MD is probably high in all sexual organisms, which can alter the natural populations by using the synthetic MD (Burt, 2003). The sex-chromosome drive should cause either local population collapse or the preference of suppressor alleles, and therefore, it is difficult to use. However, it will be self-limiting and continuous-release, similar to that required with the sterile insect technique (Alphey *et al.*, 2013).

Under-dominance (UD)

Underdominance (UD), also known as heterozygote inferiority, occurs when heterozygotes (or their progeny) have lower fitness than parental homozygotes (Hartl *et al.*, 1997). There are two basic types of engineer UD, two-locus UD and single-locus UD (fig. 1) (Dhole *et al.*, 2018).

In a two-allele UD system, one allele is generally fixed in large interbreeding insect populations, whereas the other allele is lost; this bi-stable switch, like the action of UD tends to make it a gene-drive mechanism (Dhole *et al.*, 2018). The initial frequency of the two alleles is the primary determinant for which one to lose. Hence, for two alleles, the heterozygote X/x (X and x are two alleles) has lower fitness than the parental homozygote X/X or x/x. These homozygous individuals can be reproductively separated from each other at extreme UD conditions when heterozygote individuals will be 100% lethal (Eppstein *et al.*, 2009). The model of two-locus UD represents two constructs that are positioned at independently segregating loci (fig. 1) (Dhole *et al.*, 2018). In single-locus UD, both constructs can insert a single locus (Marshall and Hay, 2012). The model of one-locus UD contains two engineered constructs, and each composes of four different linked elements of a coding gene for a lethal toxin, a suppressor specific to the promoter of another construct, a promoter to drive the toxin gene and a payload gene (the desired gene to be spread into population) (fig. 1) (Dhole *et al.*, 2018).

The mechanism to transform the insect population can be demonstrated by a transgenic construct based on UD. It presumes that wild-type (W^+/W^+) and under-dominant transgenic homozygotes (Ud/Ud) have an equal chance of fitness. By releasing (Ud)/(Ud) homozygotes into a target wild-type population, the (Ud) allele frequency might be increased above a 0.5 threshold frequency and then (Ud) alleles will probably eradicate the wild-type alleles for following generations. The eradication of these wild-type alleles continues even after the stopping release of transgenic under-dominant alleles (Davis *et al.*, 2001; Reeves *et al.*, 2014).

In the engineer UD system, it is presumed that even one copy of the heterozygous suppressor at one locus is enough to suppress all other copies of promoters on other constructs. So, only those individuals will survive who have one copy of the transgenic allele or no transgenic allele (Marshall and Akbari, 2016; Edgington and Alphey, 2017). The single-locus UD requires a high release of a population containing drive payload, making it difficult to engineer. In contrast, the two-locus UD system requires a low release of a population containing drive payload (Dhole *et al.*, 2018).

Homing endonuclease-based gene drive (HEGD)

The discovery of HEGD was initially stimulated by the finding of selfish genetic elements that originated in numerous single-cell organisms, named HEGs, and has been used to suppress the population of malaria vectors and other diseases related to human (Stoddard, 2005).

These HEG-encoded proteins can recognize and cut a 20- to 30-bp DNA sequence (Stoddard, 2005). However, once the
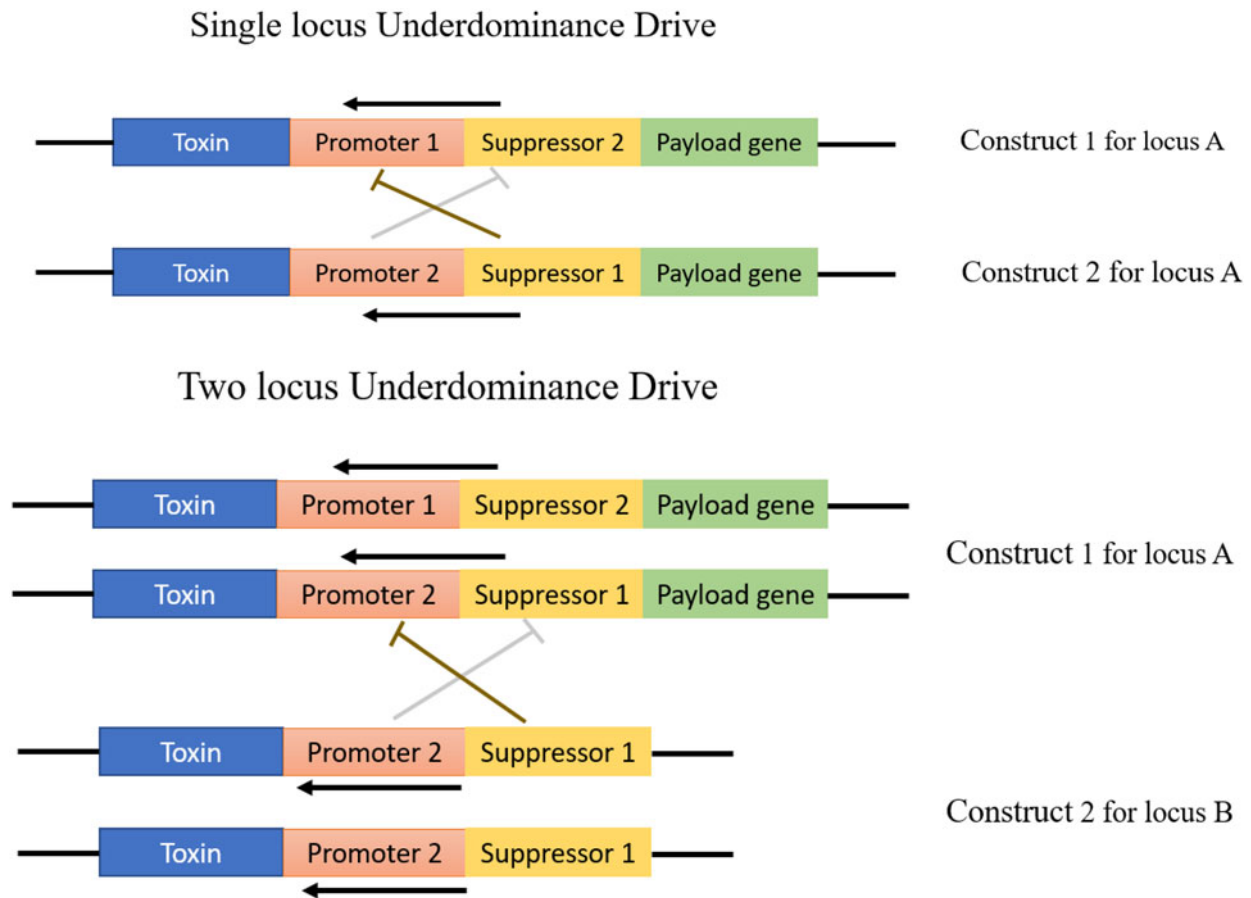
Single locus Underdominance Drive

Figure 1. Representation of under-dominance drive. The upper two constructs of under-dominance drive for single locus A and lower two constructs under-dominance for two locus of A and B. Each construct contains a promoter, a suppressor for each promoter, a toxin and payload gene.

HEGs come across into contact with a chromosome harboring the recognition sequence, then induce the double-strand DNA break (DSB), which is frequently repaired by homology-directed repair (HDR) and non-homologous end joining (NHEJ) (fig. 2). In the HDR, the HEG uses itself as a repairing template for this process, and as a consequence, the HEG element copies itself into its corresponding allele (Stoddard, 2011). Under these dynamics, the HEG frequency can be rapidly increased in a population (Windbichler *et al.*, 2011; Hammond *et al.*, 2016). The bias inheritance of HEG is through cutting the homologous chromosome and stimulating the copy of itself during the repair of the break. Various efforts have revolved about the prospect of using this gene-drive system to block the transmission of malaria by targeting mosquitoes (Galizi *et al.*, 2014). If this procedure takes place in the germline, the ratio of gametes that comprise the HEGD will be higher than 0.5 and it has been proved to be over 0.9 in a few cases (Burt, 2003).

Therefore, HEGD can be quickly spread to a population. Once a large number of individuals in the population are heterozygous, the population will collapse as heterozygote pairings will create non-viable or sterile offspring (Champer *et al.*, 2016). It is hard to redesign the HEG for a new target sequence (Chan *et al.*, 2013b).

CRISPR/Cas9-based gene drive (CCGD)

More recently, the development of the CRISPR/Cas9 system has simplified the engineering nucleases to cut the specific genomic sequence (Fu *et al.*, 2014). Cas9 was initially identified in the bacterial innate-immunity system and discovered as a novel class of sequence-specific endonuclease, whose target specificity was determined by its guide RNA (gRNA) (Kondo and Ueda, 2013; Mali *et al.*, 2013). Cas9 can be easily redesigned to target a new sequence merely by replacing the specificity of the gRNA, which is only a 20-bp sequence (fig. 3). The only limitation of this system is that the Cas9 will cleave the sequence, which is flanked by a protospacer-adjacent motif (PAM) at the 3′ end (Jinek *et al.*, 2012).

In CRISPR/Cas9-based gene-drive system, a CRISPR/Cas9 construct contains at least a coding sequence of the Cas9 endonuclease and a 20-bp gRNA that binds with the target site of the host genome. RNA-guided Cas9 causes a break of double-stranded DNA in the wild-type allele at heterozygous state (same as homing endonucleases), which might be repaired through homology-directed repair (HDR) (fig. 4). Developing this procedure in the germline will drive this Cas9 homologous element into the wild population (Drury *et al.*, 2017).

The first necessity for all kinds of endonuclease-based gene drives is to cleave the target sequence. Incomplete and improper cutting was a massive problem in transgenic mosquitoes and fruit flies, and HEG-based gene drive has 72% of cutting efficiency in mosquito and 86% in engineered fruit flies (Windbichler *et al.*, 2011; Chan *et al.*, 2013a). In contrast, target cutting rates of RNA-guided Cas9 exceeded up to 85–99% in the male of fruit flies (Kondo and Ueda, 2013). Hence, the highly efficient
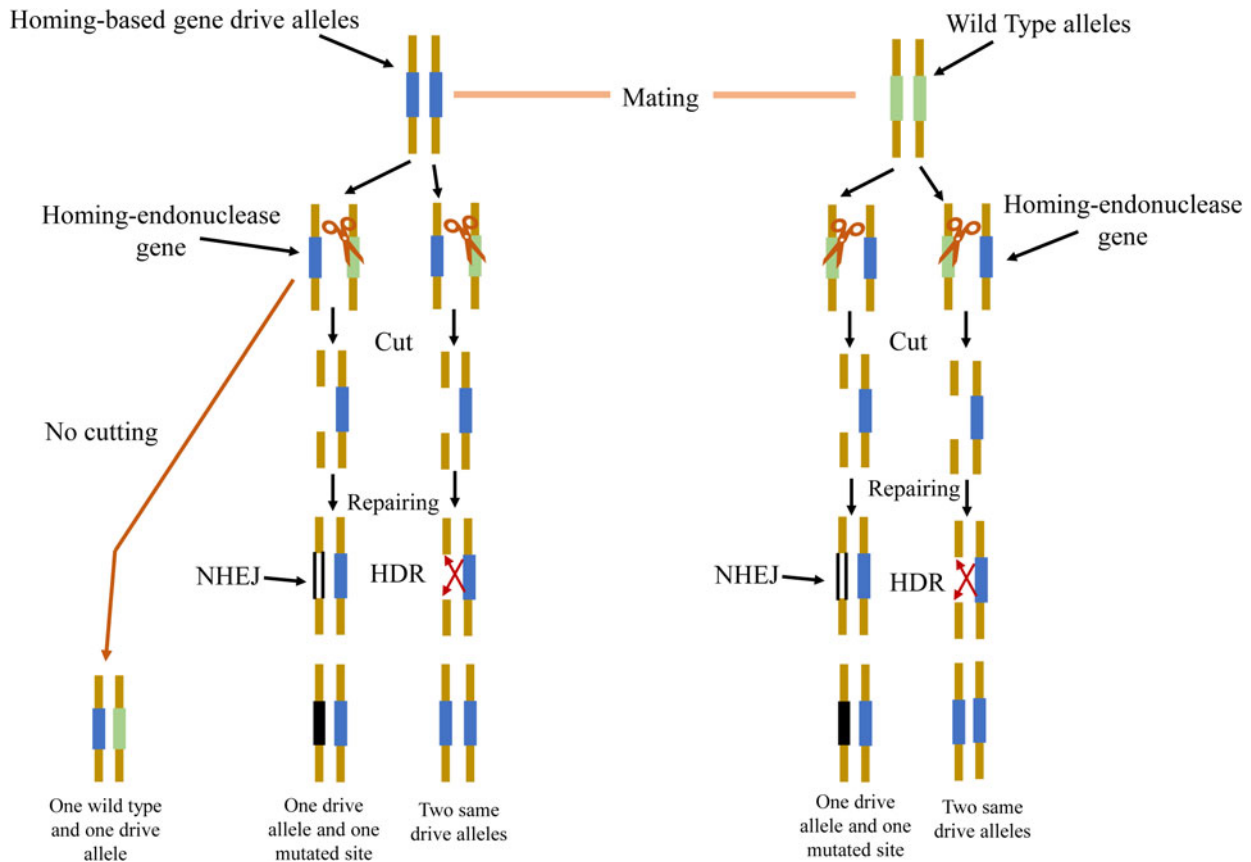
Figure 2. Representation of HEG-based gene drive. When the endonuclease-based gene drive organism mates with a wild-type organism, the endonuclease cutting system will be activated and cut the wild-type chromosome. If DNA break is repaired by using homologous recombination, then it will use drive chromosome containing the desired fragment as a template, copying drives into the wild-type chromosome (Bottom right). If endonuclease fails to cleave the sequence, no repairing occurs (bottom left). If homologous recombination fails to copy the template, the break will be repaired by non-homologous end-joining (bottom central).

CRISPR/Cas9 system allows researchers to construct a homologous CCGD to transfer any desired trait into the population (KaramiNejadRanjbar et al., 2018). The most exceptional challenging conditions require that the cleave sequence be repaired by HDR instead of non-homologous end joining (NHEJ). The rates of HDR are recognized to vary across the different species, developmental stages (Preston et al., 2006; Chan et al., 2013a; Asad et al., 2022), and different types of cells (Mali et al., 2013).

The major problem of CRISPR/Cas9 gene drive is the low-frequency rate of HDR. The HDR frequency rate can be increased when Cas9 protein is active at the early development stages, especially in germline tissues (Ren et al., 2013; KaramiNejadRanjbar et al., 2018). The HDR frequency rate increases in silkworms by knocking out the two genes (Ku70 and Ku80) involved in the NHEJ pathway (Ma et al., 2014; Xu et al., 2019).

Application of gene drive systems in insects

Application of meiotic drive for insect pests population suppression

The insect population can be suppressed by targeting female fertility, which has been achieved through MD. The X chromosome-based drive was constructed in the woodland fly; the male was driving X chromosome and produced 80–100% female offspring; most of the male offspring were sterile without Y chromosome

(Keais et al., 2017). The X-linked protein named heterochromatin protein 1 plays a vital role to develop sex-ratio MD in *Drosophila simulans*. The heterochromatin protein 1 driver (HP1D2) was constructed and this allelic driver prevents the Y chromatids from segregation during meiosis II to produce the female-biased progeny (Helleu et al., 2016). The natural fluctuation of Y-linked sex-ratio suppressors and chromosomal capability to regulate the gene expression was investigated in *D. simulans*. The Y-linked sex-ratio suppressors induce 63–93% female-based progeny (Branco et al., 2013). The natural occurrence of Y-chromosome-linked drive (Y-drive) has been identified and exposed to suppress insect populations by enhancing the male population (Lyttle, 1977; Wood and Newton, 1991). The Y-drive was observed in two mosquito species, *Culex pipiens* and *A. aegypti*, with a high ratio of sex bias (Helleu et al., 2015). The sex-linked MD system has been developed *A. aegypti*, which is associated with M (a male-determining gene) in chromosome Y, which leads to breakage of homologous chromosome X containing the m allele (Mori et al., 2004). In *D. affinis*, it has been confirmed that fathers siring all sons do not have a Y chromosome, and no morphological variations are linked with sex-ratio resistance to MD (Unckless et al., 2015). The persistence of X chromosome in the population of *D. neotestacea* differs between standard males and X chromosome-based males (Pieper and Dyer, 2016). Recently, the tethered gene-drive system has been developed for population suppression, in which a two-
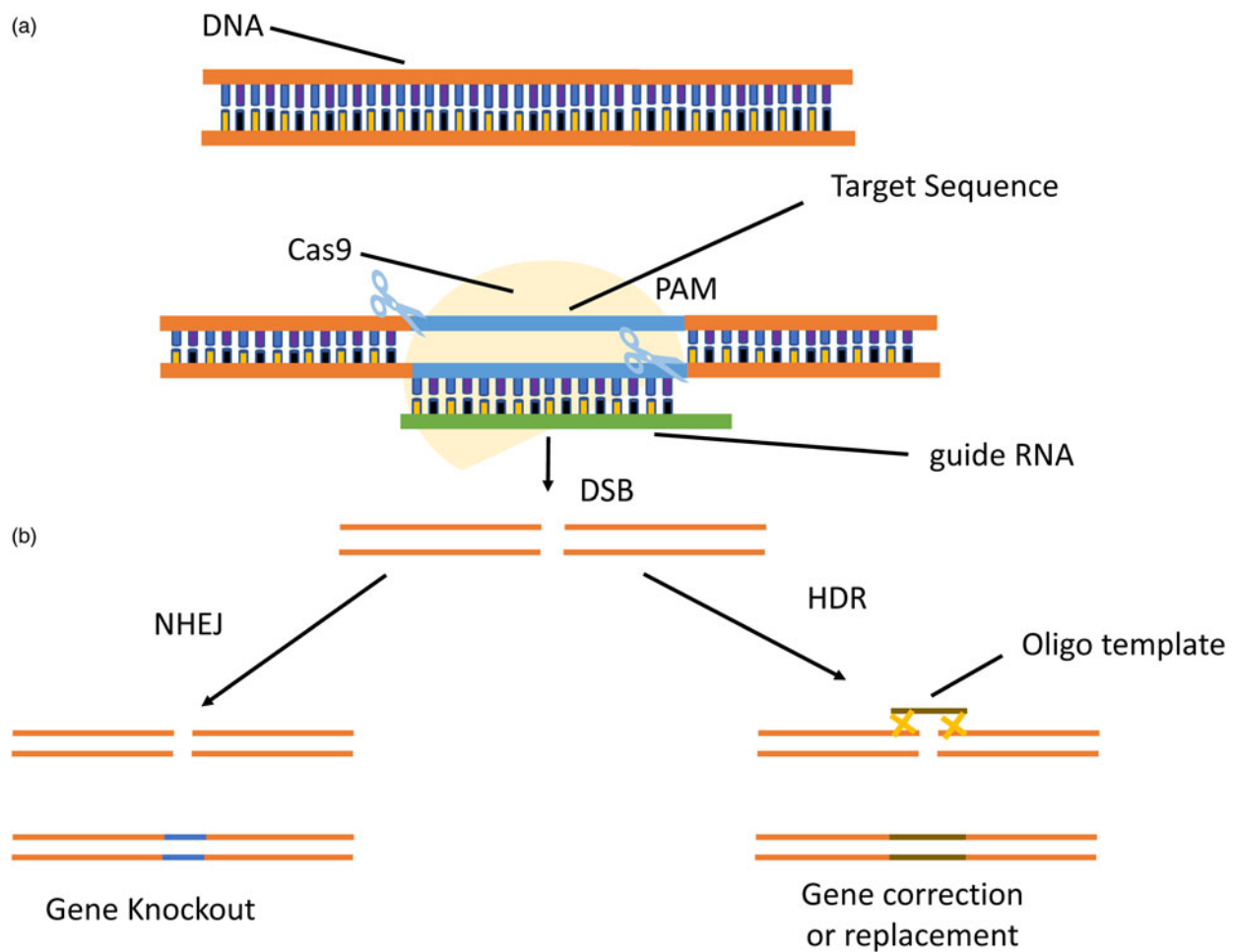
Figure 3. RNA guided CRISPR/Cas9 process. (a) The gRNA and Cas9 protein are delivered into the target cell. The gRNA directs Cas9 to bind target DNA. Cas9 will play a role as an endonuclease and cause a double-strand break (DSB), (b) DSB may be repaired by two processes: one is NHEJ resulting in gene knockout and the other is HDR resulting in gene correction and replacement.

locus UD and split homing drive were linked to one part of the split drive. This system shows better population-suppression results than the previous UD systems (Dhole *et al.*, 2019).

The sex-ratio meiotic-drive system plays a vital role in the process of speciation, sex separation, alteration of linkage pattern disequilibrium, interspecific competition and extinction (Unckless *et al.*, 2015).

Application of under-dominance for insect pests population suppression

An inherited character of UD causes a majority of males in the progeny of *A. aegypti* strain and the mass release of male-producing progeny was proposed for the control of *A. aegypti* (Craig *et al.*, 1960). A hypothetical model showed that an engineered UD-based gene drive system allows traits to be introduced into pest populations economically and efficiently (Davis *et al.*, 2001). The maternal-death under-dominance (UDMEL) gene-drive system was developed to modify and suppress local insect populations. The two maternal toxins individually settle on the different chromosomes. The zygotic antidotes connecting with two maternal toxins individually settling on the different chromosomes are capable of rescuing the maternal death of the other toxin. This model suggested that the spread of transgenic insects can be limited to local environments; a population

contained single-locus UDMEL may require the repeated release of wild-type males for population suppression (Akbari *et al.*, 2013). A UD transgenic construct is modified in *D. melanogaster* and introduced into the fly population to successfully make the flies refractory to spreading disease into the population (Reeves *et al.*, 2014).

UD gene drive has shown great potential for introducing refractory genes into the pest populations for controlling infective diseases, including malaria and dengue fever. Furthermore, the pest population has been controlled by UD knockdown/rescue system (Magori and Gould, 2006; Akbari *et al.*, 2013; Reeves *et al.*, 2014; Champer *et al.*, 2016). The UD-based gene-drive population models based on two-locus toxin and antitoxin show that UD could effectively change the neighboring populations (Champer *et al.*, 2020). The comparison between the two-locus and the one-locus UD systems exhibits that the one-locus UD system requires the massive release of the population compared to the two-locus UD (Dhole *et al.*, 2018).

UD requires the mass release of transgenic organisms into the population, which is not economically viable (table 1). Although UD can spread the desired mutation in the wild-type population, it is less stable than other potential gene-drive systems (table 1). In contrast, this system could be well suitable for the replacement of specified target populations, in which further spread is not required (Sinkins and Gould, 2006).
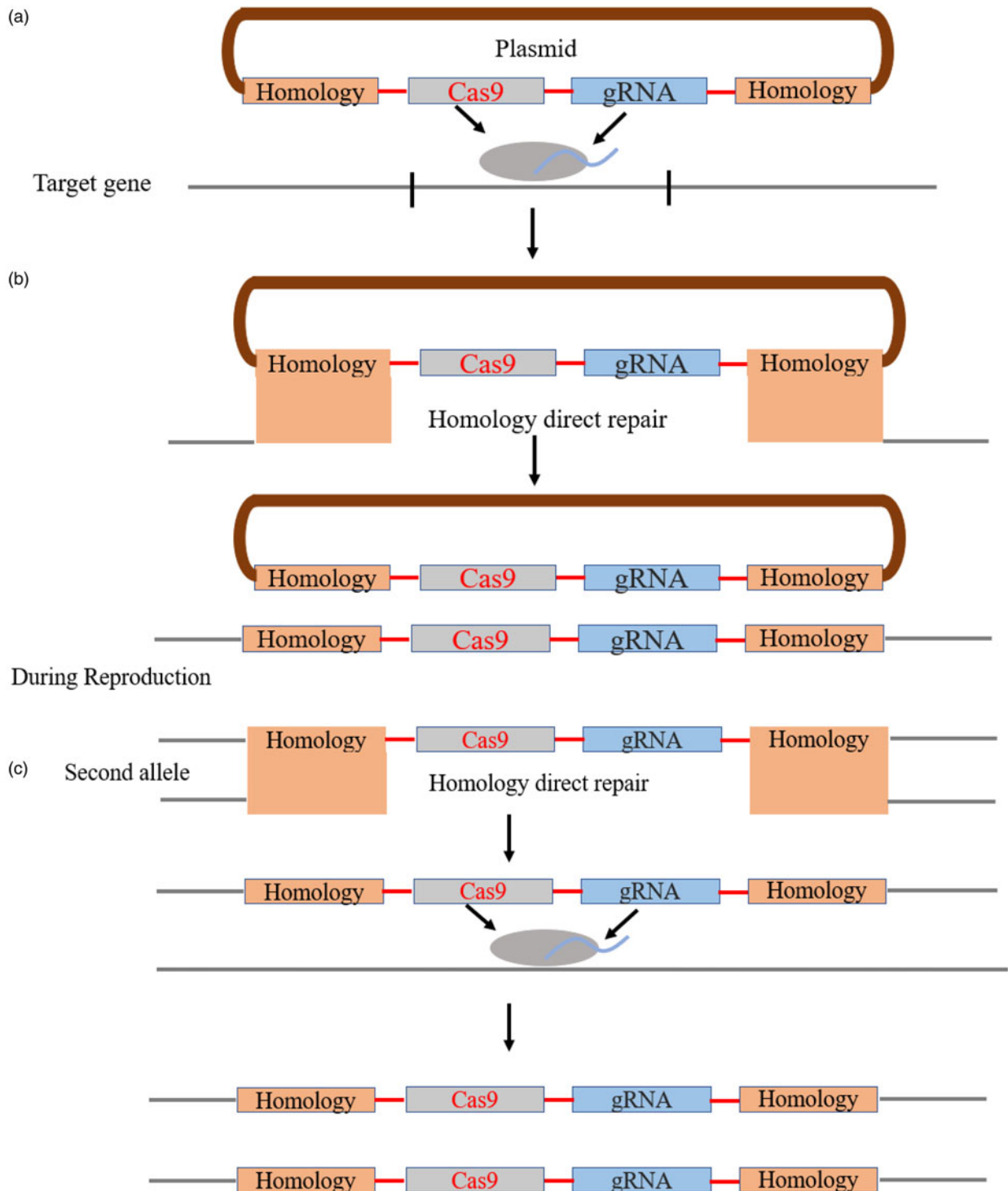
Figure 4. Representation of CRISPR/Cas9 based gene drive system. (a) The plasmid contains gRNA, Cas9 and other payload gene sequences. The gRNA will bind to a specific sequence and Cas9 will cut the double-strand DNA. (b) The gap will be repaired by HDR, and the targeted allele will be modified the same as plasmid containing sections. (c) During reproduction, this mutant allele in the heterozygous parent will be activated and cut the native wild-type allele, which is also repaired by HDR to generate two mutated gametes.

Application of homing endonuclease-based gene drive for insect population suppression

HEGD has been employed to suppress vector and pest populations by homing into a specific target locus or spreading a genetic payload, which leads to recessive lethality or sterility (Deredec

et al., 2008). HEGD using I-SceI gene elements has been developed in D. melanogaster with high rates of homing attained at both ectopic and homing sites, suggesting that the HEG-based gene-drive system may be feasible in other dipteran insects (Chan et al., 2011). The engineered homing endonucleases enhance the targeted mutagenesis and the homing in D.

Table 1. Comparison of gene-drive techniques

	Meiotic drive	Engineered underdominance	Homing endonuclease-based gene drive	CRISPR/Cas9-based gene drive
Mechanism	MD takes place (usually in males) when a specific heterozygous locus segregates at a rate of higher than the typical Mendelian ratio.	It is known as heterozygote inferiority and happens when heterozygotes (or their progeny) have lower fitness than parental homozygotes.	HEG recognizes and cleaves genomic DNA such that a gene cassette can be integrated into genome by HDR.	Cas9 protein cuts the specific genomic sequence guided by the sgRNA and the break is repaired by HDR.
Gene drive	Yes (low potential)	Yes (low potential)	Yes	Yes (high potential)
Drive efficiency	Unknown	Unknown	56%	90–100%
Mutation efficiency	Unknown	Unknown	1–9%	>90%
Rate of spread	Low	Very low	Fast	Very fast
The rate of resistance allele generation	Low	Moderate	High	Very high
Can the wild-type population be modified	Little	Little	Yes	Yes (with high rate)
Is there any risk of spread to non-target species	Close to zero	Close to zero	Low	No risk
Reference	(Alphey et al., 2008, Alphey et al., 2013, Lindholm et al., 2016).	(Magori and Gould, 2006, Akbari et al., 2013, Reeves et al., 2014, Champer et al., 2016)	(Windbichler et al., 2011, Chan et al., 2013b, Galizi et al., 2014)	(Li et al., 2015, Pavlovic et al., 2016)

melanogaster (Chan *et al.*, 2013*b*). To produce transgenic *A. gambiae*, HEG *I-SceI* was used as a gene-drive component to obtain high rates of site-specific chromosomal cleavage and gene conversion in the male germline (Windbichler *et al.*, 2011). The *A. gambiae* population might be eradicated by targeting the female-fertility gene with HEGs or driving by Y chromosome that transmitted to 75–96% of progeny (Deredec *et al.*, 2011). The combination of Y drive and fertility-targeting HEGD is more convenient to eradicate the disease by eradicating the vector (Deredec *et al.*, 2011). The HEG *I-PpoI* cleaves the gene responsible for disabling X-carrying spermatozoa on the X chromosome in *A. gambiae* to produce a male-biased sex ratio of *A. gambiae* (Windbichler *et al.*, 2007).

However, the HEGD system has not been tested in other insect species, such as lepidopterans, coleopterans, and orthopterans. Different kinds of homing genes are used in different species, and some of the homing genes work well in specific species (table 1). Therefore, this gene drive works differently in different species, and its efficacy mostly depends upon species and endonuclease genes.

Application of CRISPR/Cas9-based gene drive for insect pest population suppression

The application of a gene-drive system to modify the insect population has been improved by the CRISPR/Cas9 genome editing tool (McFarlane *et al.*, 2018). A CCGD targeting three genes (AGAP011377, AGAP007280, and AGAP005958) has been constructed in *A. gambiae* to cause phenotypic recessive female-sterility with a high transmission rate of 99.4% to the progeny (Hammond *et al.*, 2016). Another highly effective CCGD adopted from mutagenesis chain reaction (MCR) in *A. stephens* resulted in a high frequency to copy a 17-kb construct through HDR at target locus with site-specific integration manner, and the autonomous gene-drive components were introduced approximately 99% into progeny by outcrossing wild-type mosquitoes with transgenic lines (Gantz *et al.*, 2015). An MCR developed in *D. melanogaster* targeting yellow gene of *D. melanogaster* causes the conversion of heterozygous to homozygous flies and the cross of wild-type individuals with transgenic individuals resulted in 95–100% mutation in progeny (Gantz and Bier, 2015). A CCGD construct targeting the intron 4 and boundary of exon 5 of *dsx* gene resulted in blockage of female *dsx* function without disturbing the fertility and development of the male with the efficiency of 100% within 7–10 generations to cause the total population collapse of cage mosquito (Kyrou *et al.*, 2018). A CRISPR/Cas9 gene drive targeting the *transformer* (*tra*) gene exhibits the high efficiency in sex conversion of the females into males in *Ceratitis capitata* (KaramiNejadRanjbar *et al.*, 2018). This gene-drive system causes all-male population collapse and it may be used to control other harmful pests.

The CCGD can fulfill the basic requirements of a potential gene drive (table 1). Hence due to the successful construction of CRISPR/Cas9 gene drive in mosquitos and *D. melanogaster*, it may be constructed in other insect species such as major crop pests to modify or remove the pest population from the field. The major drawback in the application of CRISPR/Cas9 gene drive is a low rate of HDR because gene drive ultimately depends upon HDR (table 1).

Conclusions and future perspectives

Regardless of the possible benefits of various gene-drive systems, these systems may remain under the study, because it is unclear which type of gene-drive system is best suitable to overcome their engineering limitations and regulatory situation. The abovementioned gene-drive systems can potentially be used to suppress the insect pests population, but these systems have some limitations, which decreases the feasibility. We have discussed the four types of gene-drive systems, including the benefits and drawbacks in the application for insect pest population suppression, which will be helpful in the future to construct a potential gene-drive system to control the pest population. Recently, the CRISPR/Cas9-based gene-drive system in mosquitoes and *Drosphilla* has been successfully developed for population replacement, and it might be used for other crop pests in the future. The limitation is low HDR efficiency, which is partially due to the formation of resistant alleles. This limitation can be overcome by discovering an effective germline-specific promoter to drive the Cas9 expression effectively in germ cells. Considering the high drive activity of CRISPR/Cas9-based gene-drive system, future genetic and molecular work will be further proceeded to improve system efficiency and broaden their application to modify the wild-type population.

Gene drives show great promise as potentially new solutions to complex biological challenges, but they may also create risks. Payload genes may have unexpected effects, and certain gene drives may result in extinction. A gene drive might potentially have unintended consequences, such as spreading outside the specified geographical region or transferring over into another species. Concerns have also been expressed about the possibility of employing gene drives to cause economic damage. Considering that each of the systems discussed here is vulnerable to the development of drive resistance to variable degrees, more research into molecular designs will be required to prevent the creation of drive resistance. All these gene drive systems discussed above have been successfully constructed and evaluated under laboratory conditions to suppress the wild pest population. Due to certain limitations, these gene drive systems have not been tested under field conditions. It is necessary to conduct safe and controlled field experiments to achieve the numerous potential advantages of gene drive technology in a safe, ethical, and responsible manner. The genetic alteration in gene-drive systems will continue in the future to increase the feasibility of these gene-drive systems so that the wild-type population of harmful pests will be effectively managed under field conditions.

Conflict of interest. The authors declare that there is no conflict of interest.

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