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A review on principles, applications and the current challenges to genome editing in insects

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Abstract
Genome editing is an important tool in synthetic biology and the past few decades has witnessed a tremendous pool of know-hows into the intricacies of the process. This technique can introduce altered traits in the organism's genome that can be extremely beneficial in controlling the spread of dangerous vector-borne diseases that torment our society today like dengue, malaria, chikungunya etc. In the past, random chemical and radiation mutagenesis were performed but now researchers rely on more site-specific mutations using CRISPR Cas, Zinc Finger endonucleases, TALENS, etc. Using gene drives for controlling the spread of the desired trait among the wild population and tackling public health issues are also discussed. The ultimate goal of this review paper is to highlight the major developments that have taken place in the field of genome editing in insects, to put into light the progress and challenges that have been overcome. Ultimately the current limitations and the future possibilities have also been pointed out.

Keywords: Genome editing, insects, mosquitoes, current scenario, challenges

1. Introduction
Class Insecta of Phylum Arthropoda is the largest class in the entire Animal Kingdom and includes a wide variety of species that are found in almost all the niches on this planet. It includes not just economically important insects like honey bee, silkworm but also notorious pests like cockroaches. They also act as vectors for diseases [1]. Their obvious demand as important organisms on this planet is thus unarguable coupled with their feasibility to act as model organisms. These have contributed to the vast database of genetic resources and research in this field is extremely beneficial for the betterment of human health, medicine, agriculture, and life sciences. Insects possess the unique ability to reduce organic wastes and turn them into their biomass. This is the reason why they are also called nature's greatest scavengers along with symbiotic microorganisms. They also play indispensable role in the food chain [2]. The bioconversion is said to be very efficient as it consumes very little natural resources whilst producing insignificant amounts of greenhouse gas. The model organism *Drosophila melanogaster* has played a pivotal role in biomedical science. In recent years, the progress in the field of genome editing has provided scientists and researchers with the capacity to induce gene knock-out (KO), knock-in (KI), and knock-down (KD) in non-model organisms as well [2].
Today, researchers can rapidly introduce sequence-specific modifications that are highly specific to a broad range of organisms and cells. The more precise a gene-editing tool is, the easier it becomes to control the experiment and predict the outcome [3]. The core techniques which are utilized include.

1. Zinc finger nucleases (ZFNs)
2. Transcription activator-like effector nucleases (TALENs)
3. Clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR associated protein 9 (Cas9)
4. Homing endonucleases or mega nucleases.

The ability of CRISPR-Cas9 and TALENs to recognize new genomic sequences has resulted in many important scientific discoveries. There have been breakthroughs in diverse fields including synthetic biology, human gene therapy, vector biology, genetics and drug development. The unique ability of genome editing to induce double-stranded breaks (DSBs) in target DNA sequences gives this technology the ability to make a wide array of genomic outcomes possible. These breaks are then repaired by cellular DNA repair pathways resulting in the introduction of site-specific genomic modifications [4–6] (4). Using this process NHEJ (non-homologous end joining) can cause genes to knock out via random base insertion and deletion. When a donor template homologous to the target chromosomal site is present, base insertion and integration of gene are done by Homology directed repair (HDR). The enzymes used for genome modifying can also serve as a foundation for artificial transcription factors, which are a class of tools with the ability to modulate any gene in the genome, hence they are broadly versatile. The possibility to introduce altered traits in insects in such a precise and refined manner, allows us to tackle public health issues and environmental issues in sustainable ways [7]. VBDs (vector-borne diseases are said to be responsible for 17% of all infectious diseases throughout the world) [8]. Numerous problems associated with VBDs that torment our society today can be solved by allowing the spread of these engineered traits throughout the wild population. Vulnerability to insecticides and pesticides can be restored in insect species by replacing the resistant alleles with sensitive ones. Besides that, sometimes new genes can also be inserted into organisms to make them sensitive to a particular molecule, thus allowing us to use that molecule as a novel biocide. Sometimes, genes that can lead to deleterious and fatal mutations and disturb the sex ratio of a population are spread to block the spread of these diseases and suppress invasive organisms in an environment-friendly manner.

2. History

Classical genetics banked on the discovery and subsequent analysis of spontaneous mutations investigated by stalwarts like Mendel, Morgan *et al*. Later, it was demonstrated by Muller and Auerbach that the mutagenesis rate can be further increased using radiation or chemical treatment [9,10]. Subsequent methods used transposon insertions for some organisms. However, these methods generated mutations at random sites in the genome. The first targeted genomic changes were produced in yeast and in mice in the 1970s and 1980s [11–14]. This gene targeting depended on the process of homologous recombination, which was remarkably precise but very inefficient, particularly in mouse cells. Recovery of the desired products required powerful selection and thorough characterization. Because of the low frequency and the absence of culturable embryonic stem cells in mammals other than mice, gene targeting was not readily adaptable to other species.

In the past, genome editing, and genetic engineering mainly involved the use of chemical and radiation mutagenesis. Over the years, research and development in this field led scientists to use transgenesis methods using transposable elements. Gloor *et al*. described the first use of targeted sequence modification or replacement where he used homologous recombination and gene conversion in double-stranded DNA for the creation of new alleles of selected endogenous genes in *Drosophila melanogaster* [15]. Despite being effective, this technology was quite inefficient and limited because transposon insertion and excision from the gene had to be edited for the creation of the double-stranded breaks in DNA and stimulate homology-directed repair. Later this original method was developed in such a way that almost every gene in *Drosophila* could be tagged with a transposon, this method was comparatively much more sophisticated. A more versatile method of genome editing was developed by Rong and his colleagues which could target any gene in the genome in *Drosophila*, hence allowing the desired editing [16]. One of the major drawbacks of this method was that the systems relied almost entirely upon a few transgenic lines of *Drosophila melanogaster*. For the recovery of recombinant varieties of insects, large screens were used. These techniques were fruitful for the fruit fly, but in other insects, the same techniques did not produce good results.

With the recent advancements in genetics, researchers today have a better understanding of how DNA-binding proteins like mega nucleases, zinc finger proteins and transcription activator-like effectors bind to other molecules. These DNA binding proteins have provided the possibility to create endonucleases having controllable site-specificity, thus allowing editing technologies with a broad range of applications to be developed.

Endonucleases that have a user-defined specificity were initially constructed with the help of many zinc finger binding domains that can easily recognize target DNA sequences and attach with DNA endonuclease like Fok1 thus resulting in the formation of Zinc finger nucleases (ZFNs). The efficacy of editing genes using this technology was demonstrated in the genome of *Drosophila* [17]. The challenges include difficulty in constructing the effective ZFNs and the high cost of their production. Most of the studies that involve ZFNs have mainly been technical.

The transcription activator-like effectors (TALEs) is a DNA binding protein system that has been derived from the plant pathogen *Xanthomonas*. It is highly programmable. compared to zinc finger-containing proteins [18]. Designing TALEs is much simpler but using the TALE system to create specific TALE-endonucleases (TALENs) can be quite tricky. A lot of reports are there that talk about the functionality of TALENs and the conditions under which they work best.

CRISPR/Cas (clustered regularly interspaced short palindromic repeats/ CRISPR associated proteins) is an adaptive immune system derived from bacteria that allows them to recognize and degrade foreign bacteriophage DNA. Cas9 is a DNA endonuclease protein that is derived from Streptococcus pyogenes. Its sequence can be determined by small associated RNAs (crRNA and tracrRNA) which are combined to form a guide RNA or gRNA. Compared to the previous techniques, this approach is different because in this case, Cas9 proteins do not have to be designed repeatedly. Instead, short specific gRNAs which possess the ability to guide Cas9 to the desired location have to be produced. Though it is a very recent discovery it is quickly being adopted by vector biologists all over the world because of its obvious benefits and advantages [19].

The CRISPR/Cas system is widely used in the field of vector biology as an important tool today because it can be used among a wide range of species with few optimization steps [20]. The main procedure consists of two parts- the Cas9

endonuclease that cuts the DNA and the synthetic guide RNA (sgRNA) that delivers the DNA endonuclease to the target site, programmed within its RNA sequence. These two elements can be delivered in insects either in the form of RNA, plasmid DNA, or encoded in the genome to increase efficiency. When combined, they efficiently disrupt the function of the target DNA sequence.

3. Programmable site-specific nucleases

3.1 Zinc finger nucleases

Zinc finger nucleases, popularly known as ZFNs were the first targeted nuclease to achieve widespread use. They are a fusion between custom-designed Cys2-His2 zinc-finger protein and the cleavage domain of the FokI restriction endonuclease [21]. In this structure, each finger comprises a 30 amino acid sequence that coordinates one Zinc atom using two cysteine and two histidine residues to contact a 3 base pair sequence of DNA [22]. Zinc fingers function as dimers, in which each monomer recognizes a nine to eighteen base pair 'half site' sequence with the help of the zinc finger binding domain. This dimerization is mediated with the help of the FokI cleavage domain, whose function is to cut DNA within five to seven base pair spacer sequences, separating the two flanking zinc finger binding sites as summarized in Figure 1 [17].

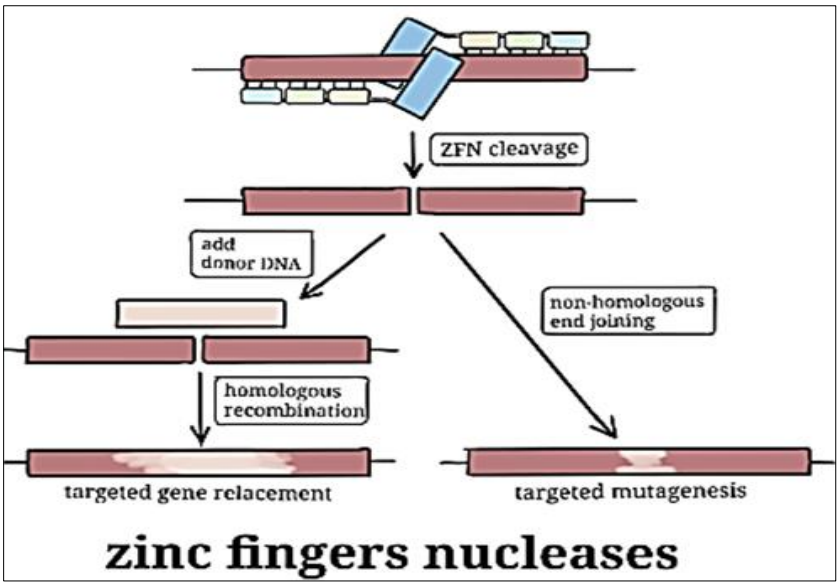

Fig 1: A diagrammatic representation of ZFNs

One of the problems with using zinc-finger nucleases in genome editing is that it can cause off-target mutations though many approaches have been used to enhance their specificity [23]. One of the best methods involves delivering the ZFNs to the cells in the form of protein. ZFNs possess an intrinsic property of cell penetration like other DNA-binding proteins [24]. The proteins are cell permeable. When delivered in their purified protein form, they show lesser off-target effects compared to when delivered in nucleic acid form. If double-stranded breaks are absent, then ZF Nickases can also facilitate gene correction by nicking or cleaving one strand of DNA and result in homology-directed repair (HDR). These are enzymes that consist of one catalytically inactivated ZFN monomer in combination with another ZFN monomer.

Compared to TALENs and CRISPR-Cas9, zinc finger arrays are much more difficult to construct. This is one of the reasons why their use is not widespread in laboratories. ZFNs also lack target flexibility.

3.2 TALE nucleases

Transcription activator-like effector proteins are bacterial effectors and their mechanism of recognizing DNA was discovered by Boch *et al*. in 2009 [25]. After the discovery, TALENs were created, and they could modify almost any gene. Like ZFNs, TALENs are modular in form and function and their structure comprises an amino-terminal TALE DNA-binding domain fused to a carboxy-terminal FolkI cleavage as shown in Figure 2.

TALENs have two major advantages over ZFNs. Firstly, assembly of the functional nuclease can be done easily within a less amount of time and experience and secondly TALENs have an improved specificity and reduced toxicity compared to ZFNs. TALENs have a large and highly repetitive structure, hence delivery to the destination is tricky. This problem has been overcome by the development of methods that deliver TALENs to cells in the form of mRNA or proteins. One of the concerns involved in the use of customized target protein is the possibility of mutagenesis in unintended sites [26].

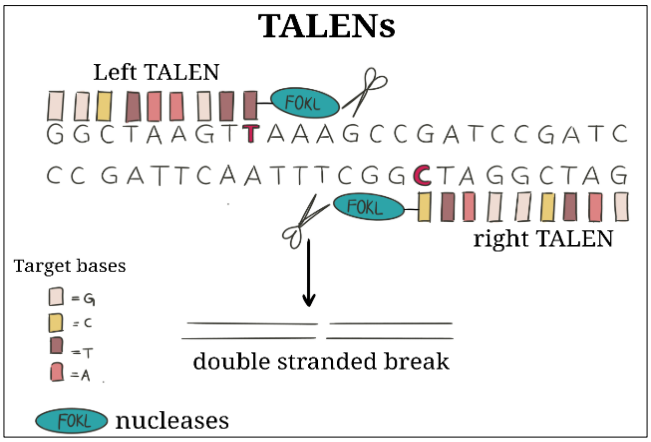

Fig 2: A diagrammatic representation of TALENs

3.3 CRISPR-Cas9

The most recent addition to genome editing is CRISPR-cas9 (Clustered regularly interspaced short palindromic repeats) which plays an important role in the bacterial adaptive immune system. The spacers represent an archive of former intruder encounters [27]. The type-II CRISPR system in bacteria is responsible for protecting against invading bacteriophages by RNA-guided DNA mediated cleavage with the help of Cas proteins. Short segments of foreign DNA when integrated within the CRISPR locus form the CRISPR RNA (crRNA) and they are annealed to trans-activating RNA (tracrRNA) to direct sequence-specific degradation of pathogenic DNA with the help of Cas protein. Mechanism- With the help of sequence-specific endonucleases, double-stranded breaks (nicks) can be created at the sites of interest which are then repaired using endogenous mechanisms (Figure 3). These repairs must be done very quickly because if broken chromosomes are present in the cells, they are unable to divide further and hence die. There are mainly two DNA repair pathways that are employed. The first one is non-homologous end joining (NHEJ) that allows small insertions and deletions at the sites of breakage and the second is homology-directed repair (HDR) that uses the information available on intact chromosomes to repair the broken one [28]. This feature is exploited by many scientists and researchers, and they trick the cells by giving them an artificial construct as a template, thus leading to desired insertions and deletions.

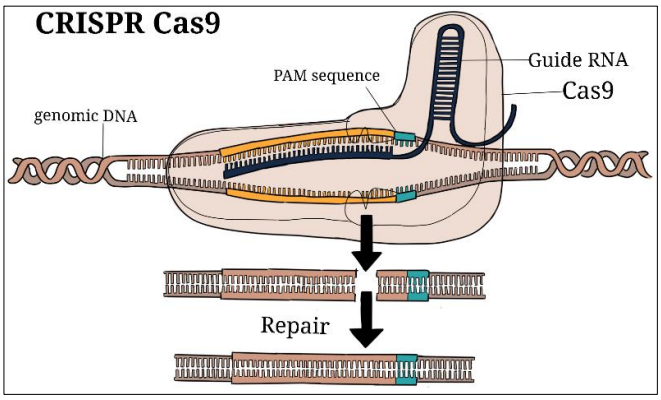

Fig 3: A simplified diagrammatic representation of CRISR Cas9

According to Doudna, Charpentier, and their co-workers in 2012, for target recognition, only a seed sequence within crRNA and a conserved protospacer adjacent motif (PAM) upstream of the crRNA binding site are required [29]. This entire system has been simplified for effective genome editing and now crRNA and tracrRNA together form a guide RNA (gRNA). This is responsible for directing the CAS9 to the target site [27].

CRISPR is extremely effective because it has eliminated the need for designing new proteins again and again for new target sites. It is flexible and user-friendly. This technique can easily transmit detrimental traits like infertility through the population of organisms. Cas9 is the easiest gene-editing tool to use but despite that, there are several reports of off-target mutations induced by Cas9. Guilinger *et al*. in 2014 fused the FokI cleavage domain with an inactivated Cas9 variant to generate hybrid nucleases to increase CRISPR-Cas9 specificity [30]. Kyrou *et al*. used CRISPR-Cas to target gene doublesex(dsx), involved in sex determination, and led to population suppression in *Anopheles gambiae* mosquitos [31]. Other studies indicate that protein engineering broadly enhances Cas9 specificity and alters the PAM requirements.

4. Homing endonucleases

These are also known as mega nucleases. There are different kinds of homing endonucleases but the members of the LAGLIDADG family of endonucleases are a collection of naturally occurring endonucleases that possess the ability to recognize and can cut long DNA sequences (14-40 bps). They show extreme specificity because of the ability to make extensive sequence contact [32].

Unlike ZFNs and TALENs, the binding domains of homing endonucleases are not modular. This is a drawback and limits their use in routine genome editing [33]. Recently a megaTALE has been developed by the fusion of a rare cleaving homing endonuclease with a TALE-binding domain. They cause highly specific gene modifications [34].

5. Progress in genome editing

Genome editing provides great convenience and reliability to gene function to *Drosophila*. Compared to RNA interference (RNAi), CRISPR-based screening has lower off-target effects [35]. CRISPR-based knock-in can induce precise site insertion and promotes tag-based protein studies for localization and interaction of both proteins and cell lineages. The Knock-in approach is employed to avoid side effects of transgenes hence demonstrating true transcription, translation of genes and proteins in a cell.

Novel economic insect strains can be generated much more rapidly compared to the traditional breeding methods [36]. Transgenic CRISPR/ Cas9 mediated gene knock out of nuclear polyhedrosis virus (NPV) genes was done for developing new silkworm strains with a high degree of resistance to the *Bombyx mori* nuclear polyhedron virus infection by Chen *et al* [37]. The silkworm fibroin heavy chain (FibH) was replaced with the major ampullate spidroin gene (MaSpa1) from the spider Nephila Clavicep using TALEN mediated Homology directed repair (HDR) by Xu *et al* [38]. As a result of this, only male populations were produced and their sex ratio could easily be regulated using W chromosome insertion. Female lethality can occur if an embryonic lethal gene is inserted into a fragment of the W chromosome. This method has been extremely useful for the silkworm industry because the males produce a better quality as well as a higher quantity of silk compared to the females as well as is a friendly but effective pest control strategy [39].

Gene drives are another big thing in the field of genome editing. They are a very good and successful vector control strategy, preventing the development of resistant variants. 'Active genetics', a new CRISPR/Cas9 based method established by Bier and his group can greatly bias the transmission of genetic traits to such an extent that it can bypass the traditional constraints of Mendelian inheritance [40]. This technology directs doublesex, thus resulting in complete population suppression of malaria vector *Anopheles gambiae* mosquito populations as well as the collapse of agricultural pest *Ceratitis capitata* [41, 42]. North *et al*. explored the potential of the driving Y chromosome for suppressing mosquito populations across regions of South Africa [43]. The main obstacle in this includes the formation of resistant alleles, which can lower the efficiency of gene drive systems. The mechanism of resistant allele formation was studied with the help of CRISPR gene and this study provided insights on

ways to overcome the issue [44]. Loss of function mutants for functional studies can be easily generated using the CRISPR/Cas9 method and has helped researchers know a lot about insects.

6. Applications of Genome editing

Genome editing has enormous potential in fighting insect-borne diseases because insects act as a vector for a variety of human, plant, and animal diseases. The control of insect vectors is a very important component in disease prevention but is quite expensive, requiring a lot of effort and money. Some methods include environmental modifications or the use of chemical insecticides but they have their side effects. Another strategy is the replacement of a wild disease-transmitting insect with the ones refractory to disease transmission. When compared to insecticide use, this strategy is much more beneficial as it is species-specific and self-sustaining.

Now, another major hurdle is that engineered traits do not provide a fitness benefit to an organism, but rather reduce fitness and hence are typically lost from the population because of the process of natural selection. That is why 'gene drives' are used [45, 46]. The Gene drive mechanism is used when we want to replace an important component of a wild population. It ensures that the engineered transgenes are spread throughout genotype or allele fixation in a modest number of generations. This creates the possibility to eliminate the spread of insect-borne diseases, ill effects of insect species, and biocide resistance [47]. Potential gene drive systems include naturally occurring selfish gene elements like transposons, meiotic drives, homing endonuclease genes (HEGs. In recent years a lot of progress has been made in CRISPR homing-based drive systems and they are incredibly efficient.

There are, however, various ethical issues associated with the development of gene drives because these systems are invasive and can easily spread beyond borders hence international agreements have to be made before they are released into the environment. HEGs, CRISPR homing endonuclease-based gene drive; meiotic drives have low release thresholds which means that even if a very small number of individuals are introduced in the population they can spread to a high frequency. Invasive gene drives are ideal in situations in which the goal is to spread genes over a very large area. What needs to be kept in mind is that once released, it is quite difficult to control and the pre-transgenic state cannot be restored easily.

Some other applications include,

6.1 Functional genomics

Gene editing technologies are very promising in the study of functional genomics because they are controllable mutagens. Insights into gene functions can be gained with the help of null alleles and mutant alleles in 'modal' insects. With the help of engineered endonucleases, insect biologists can create any allele by using DNA sequence-specific endonuclease activity to stimulate DNA repair mechanisms resulting in the formation of indels at the targeted locations or recombination or gene conversion at the target gene [48]. Leisch *et al*., DeGennaro *et al*. and McMeniman *et al*. illustrated the optimal use of gene editing technologies to answer important biological questions by creating null mutations. ZFNs are also used to create null mutations in *Aedes aegypti* genes implicated in host-finding and olfaction [49-51]. Merlin *et al*. showed the essential role in the functioning of the circadian clock in *Danaus plexippus* by creating null mutations in type 2 vertebrates like cryptochrome gene (CRY2) using ZFNs [52]. Daimon *et al*. and Enya *et al*. used TALENs for the creation of null mutation in *Bombyx mori* genes which were involved in the juvenile hormone synthesis and sterol metabolism, allowing researchers to make important insights into the development of these insects [53]. Zhang *et al*. and Daimon *et al*. demonstrated how gene editing systems can conduct somatic mosaic analysis besides heritable and vertically transmitted changes. By this research, they studied genes demonstrating pleiotropy [54, 55]. Insects can be injected with desired endonucleases during embryogenesis and this results in the development of insects with a huge number of cells with desired mutations. The cells in which both copies of their target gene are mutated, result in an insightful analysis of phenotypes without screening and establishing permanent lines of genetically modified insects.

ZFNs, TALENs, and CRISPR/Cas are not only good gene-editing tools, but they can also serve other functions because of their site-specific DNA binding capabilities. The endonuclease protein domain can be exchanged with other protein domains having different functions including activation or repression of transcription, chromatin modification, etc. This area has a lot of potentials and exploring this would allow insect biologists to control insect gene expression and insect phenotypes.

6.2 Insect control

Because of its simplicity, the CRISPR Cas9 system provides insects scientists a wide range of opportunities for managing insect pests. Sterile insect technique (SIT) is a sterility-based technique that is employed on specialized insect strains [56]. *Ceratitis capitata* is a line of medfly that is mass-reared and used in SIT programs around the world, contains a reciprocal translocation and temperature-sensitive lethal mutation which results in female-specific embryonic lethality followed by a brief heat shock at 34 degrees Celsius, which results in the production and release of only sterilized males [57]. Gene editing does not involve the use of exogenous foreign DNA or transgenes hence there are no such issues regarding releasing them into the environment.

Mosquito biologists have always been interested in eliminating the phenotypes that are responsible for their pest status by altering their genetic composition. Another interesting technology is the 'Gene drive' systems in which genes, genetic elements, and microbes skew the gene transmission patterns in such a way that the desired genotypes are over-represented in the progeny harboring these gene drives relative to what is expected if the gene transmission patterns strictly follow Mendelian expectations. When a certain genotype is overrepresented in a progeny, it will rapidly increase in frequency. Using gene drives results in the collapse of insect populations or the increase in the frequency of those alleles that make them less of a pest thus making them easier to control. Even though many gene drive systems are known till now, they haven't been very useful in pest control because the specific mechanisms behind gene drives are still unknown and hence cannot be subjected to manipulation. Moreover, they are species-specific.

Homing Endonuclease genes (HEGs) have the ability to easily spread and drive through populations and that is why they are

of great interest to insect biologists. They represent a natural case of gene drive [58, 59]. Their function is quite similar to ZFNs, TALENs, and Cas9 causing double-stranded breaks in DNA followed by homology-directed repair. After that gene conversion happens in which a copy of HEG is copied into the homologous chromosome lacking HEG. These are powerful drive systems when the rates of DNA cutting as well as gene conversion are high. A very good experiment performed on Anopheles gambiae indicates the powerful potential this technology has. I-PpoI is a homing endonuclease that cuts conserved sequence within a ribosomal RNA gene cluster on the X chromosome was inserted into an autosome of Anopheles gambiae using transposon-based technology and it was regulated in such a way that it would only be expressed during spermatogenesis [60]. During spermatogenesis, only Y chromosomes were produced and the X chromosomes were destroyed. When these male mosquitoes were made to mate with the wild-type females, only male progeny were produced. This created a disturbance in the sex ratio, ultimately leading to the collapse of the entire population. This lab-based experiment, when applied under natural conditions could allow vector biologists to eradicate the deadly vector of human malarial parasites. This strategy is widely applicable irrespective of the species of insect making this a very attractive tool for pest control.

7. Challenges to genome editing

Despite capturing the interest and imagination of insect scientists and vector biologists all over the globe, the widespread use of genome editing mainly depends upon two main factors: a. The technologies for delivering the systems at desired locations at the proper time and b. The amenability of target species to genetic analysis including controlled mating and screening.

Delivering the desired genetic technologies mainly depends upon the microinjection of preblastoderm embryos. The degree to which gene-editing technologies are adapted directly depends on this factor. For screening and identifying insects, we need to use molecular genotyping methods and that increases complexity.

Genome-edited organisms are different from transgenic organisms because these do not contain a foreign gene and do not involve gene vectors. As a result of which it is impossible to distinguish these organisms from the wild types found in nature.

Here, we attempt to summarize some of the major challenges faced by researchers today-

7.1 Delivery technology

One of the biggest challenges to genome editing is the difficulty is in the application of microinjection techniques in insect eggs. This is the main reason why genome editing has only shown optimal results when applied to a few selective species such as butterflies, mosquitos, and silkworms [61]. There are lots of parameters still left to be explored including proper penetration techniques, injection time and placement, egg collection time, and sealing them after incubation [62]. Most insects have eggs covered by a hard shell which causes difficulty in microinjection. In the case of silkworms; an interesting technique called the double needle system is used that involves piercing the eggshell with a tungsten needle and injects plasmids into them using capillary glass needles Cas9 ribonucleoprotein (RNP) can be delivered to arthropod germline by injection into adult female mosquitoes without injecting eggs using a technique called receptor-mediated ovary transduction [63].

7.2 Screening

One of the biggest obstacles is the problem in the selection of an edited insect from a brood population. Fluorescent protein genes and body-color genes are often used as selectable marker genes in transgenic-based genome editing. Another technique is using RGR (ribozyme-gRNA-ribozyme) structure with multiple sgRNAs some of which code for the body color and some for target genes in a single vector. This allows the selection of mutants based upon easily detectable characteristics such as fluorescence and body color [64].

7.3 KI efficiency

In silkworms, CRISPR-based Knock-ins have failed several times. NHEJ induces double-stranded breaks in DNA and hence has a lower probability of occurrence compared to HDR in a successful TALEN-based Knock-in. Inhibition of NHEJ pathway factors Ku70 and DNA ligase 4 improves the efficiency of gene knock-in. Cohesive ends after double-stranded breaks are induced with the help of cpf1, which is suitable for HDR repair [65].

7.4 On/Off target effects

The probability of off-target effects is lower in CRISPR/Cas9 compared to RNAi but still, the potential of occurrence can lead to various experimental errors. Chances of off-target effects can be reduced to some extent by designing a computer aided sgRNA design [66].

8. Future prospects

Future work must mainly focus on developing precise genome editing techniques that are highly efficient and have low probabilities of off-target effects for large fragment knock-in and single-base pair editing. There are a lot of RNA functions that are still left to be studied and new techniques are being developed to edit non-coding RNA, micro RNAs, and so on. Ultimately the two main motives are to eradicate a species and to hinder the vector's ability to transmit the parasite [67]. Last but not least, the transfer of gene editing mosquitoes from the laboratory to the field requires proper study, monitoring, and development of specific regulations [68].

9. Conclusion

TALENS are highly sensitive and specific towards modifications in the DNA. Hence, they are an indispensable tool in the field of genome editing. Being relatively cheaper they are ideal for wide range of applications. ZFNs are also used in both practical and mechanistic studies. Still, a lot of genetic analysis is still left to be done. CRISPR has provided scientists the ability to modify, delete and insert DNA almost anywhere in the insect genome. This has revolutionized the genomics of insects. Despite the great success of genome editing, there are still a lot of challenges that need to be overcome before the full potential of genome editing is realized. The need of the hour is to develop a tool that can introduce genomic modifications without causing DNA breaks. One of the options includes the development of targeted recombinases that can be reprogrammed to recognize specific DNA sequences. Recent research tells us Cas9 complex can lead to single base editing without DNA breaks,

even though it is still not properly known how this technology can be used therapeutically. By linking the genomic modifications induced by targeted nucleases with their self-inactivating vectors improves the specificity of genome editing to some extent because in such a case the frequency of off-target modification is directly proportional to the duration of cellular exposure to the nuclease.

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T.P.C.; writing, review and editing, A.R.; writing original draft preparation

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13. Conflicts of Interest

The authors declare no conflict of interest.

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