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Review

Genetic Technologies for Sustainable Management of Insect Pests and Disease Vectors

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Abstract: Recent advancements in genetic and genome editing research, augmented by the discovery of new molecular tools such as CRISPR, have revolutionised the field of genetic engineering by enabling precise site-specific genome modifications with unprecedented ease. These technologies have found a vast range of applications, including the development of novel methods for the control of vector and pest insects. According to their genetic makeup and engineering, these tools can be tuned to impose different grades of impact on the targeted populations. Here, we review some of the most recent genetic control innovations under development, describing their molecular mechanisms and performance, highlighting the sustainability potentials of such interventions.

Keywords: sustainability; genetic control; disease vectors; genome editing; gene drive

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1. Introduction

A few harmful insect species dramatically threaten humankind by directly or indirectly affecting numerous aspects of society, especially public health, the environment, and the economy. Vector-borne diseases (VBDs) including malaria, dengue, schistosomiasis, trypanosomiasis, leishmaniasis, yellow fever, Japanese encephalitis, and onchocerciasis, collectively claim around 700,000 lives per year [1]. In addition to this, it has been estimated that between 17 and 40% of essential food crops are destroyed annually by agricultural pests. Tropical and subtropical regions, where a great proportion of the population struggle with poverty and malnutri tion, are among the most affected by agricultural pests and vector-borne diseases [2]. Furthermore, globalization, population growth, and climate change, along with forest degradation and urbanization, have exacerbated the burden of insect vectors [3]. For instance, an increase in frequency and severity of extreme climate events has caused crop damages with consequent loss of food productivity, and has affected the ecosystems, causing a shift of species distribution and prolonged disease transmission seasons [4]. For instance, many species of exotic mosquitoes of the genus *Aedes*, which are vectors of several arboviruses, are now also established in Europe [4], where they caused outbreaks of Dengue in France in 2015 [5] and Chikungunya in Italy in 2007 and 2017 [6].

Historically, efforts to manage harmful insects have significantly reduced the impact of many infectious diseases. Nonetheless, global eradication of the major vector-borne diseases remains a major challenge [7]. Integrated pest and vector management (IPVM), which incorporates a panel of control strategies, active engagement of local communities, modelling of population dynamics and disease transmission, risk assessment, and monitoring, has been adopted worldwide to promote sustainable interventions for controlling insect vectors (Figure 1) [8,9]. Traditional control methods including cultural, biological, physical, and chemical approaches can be advantageous locally, but they are unsuitable for area-wide control programmes [4,10–13]. Although insecticides play a key role in the reduction of morbidity and mortality of various VBDs [11], concerns about their potential

hazard on ecosystems and human health exist among the public and scientific community [12]. The extensive use of insecticides has led to increasing genetic resistance in insects, which progressively reduces their effectiveness [14]. Similarly, while similarly, pathogens have developed resistance to drugs, affecting the efficacy of treatments [15]. Due to the above issues and the necessity to implement alternative and sustainable tools to combat pests and disease vectors, genetic control strategies have received growing interest over the last decades. The consistent progress of genome editing technologies and mathematical modelling has led to the improved performance of existing approaches and the development of new ones. In this review, we describe the status of genetic control of harmful insects and their capacity to exert a sustainable effect.

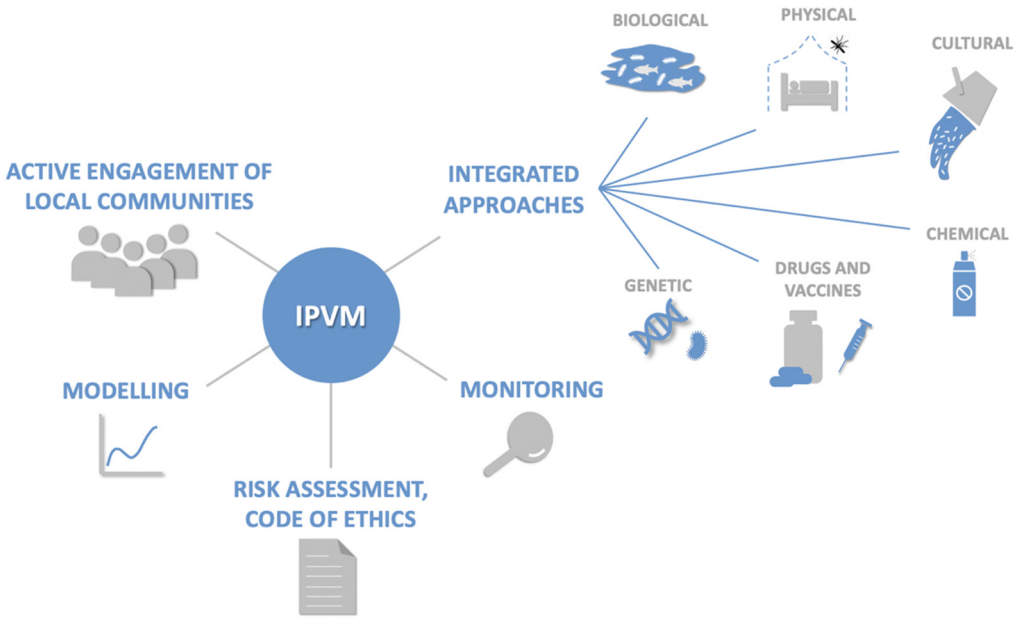

Figure 1. Integrated pest and vector management (IPVM). IPVM integrates biological, physical, cultural, chemical, and genetic vector control practices with active engagement of local communities risk assessment and code of ethical aspects, monitoring and modelling of population dynamics and disease transmission. Cultural practices are aimed at rendering the environment unfavourable for the spread of insects, for example, by removing larvae breeding sites or improving water supply. Biological control exploits the use of natural predators such as larvivorous fish against mosquito larvae [13]. Physical control creates a barrier between the insect and the host, for example, using bed-nets, whose efficacy can be improved by adding insecticides (insecticide-treated nets, ITNs) and indoor residual sprays (IRSs) [7].

2. Genetic Control

Genetic control methods rely on insect mating to transmit a genetic element or modification to the offspring to introduce a desirable trait in a wild population. Such strategies can be designed to either reduce insect population size or, in the case of pathogen transmitting insects, mitigate the vectorial capacity. The two approaches are, respectively, known as population suppression and population replacement (Figure 2). On the one hand, population suppression is pursued by imposing a fitness load to the target population, to reduce the number of vectors to an extent that disease transmission is not supported. This can be achieved either by affecting insect fertility [16], sex determination [17], or biasing the population sex ratio in favour of male, which is usually the non-damaging or non-disease transmitting sex [18–20]. On the other hand, population replacement relies on the engineering of genetic traits that interfere with the insect-hosted life cycle of the pathogen. Another type of classification of genetic control technologies considers the persistence of the modification in the population to distinguish self-limiting and self-sustaining strategies

(Figure 2). In the first case, the genetic modification is programmed to disappear from the population after a number of generations, according to several factors and parameters including fitness, inheritance, and mechanism of selection, which can be predetermined via mathematical modelling and laboratory testing. Therefore, repeated mass releases of transgenic insects are usually required to achieve the desired outcome. By contrast, in the case of self-sustaining systems, the genetic element is designed to increase in frequency over generations, even when associated with a fitness cost and seeded at low proportion in the target population [21].

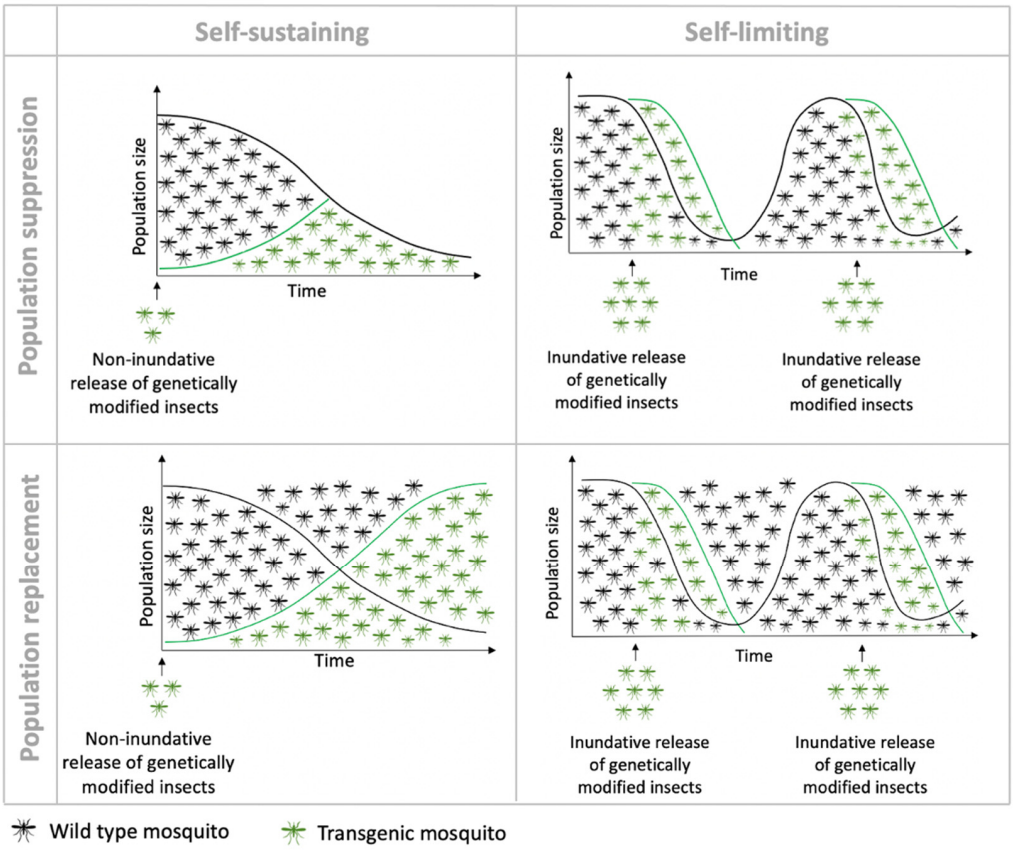

Figure 2. Self-sustaining vs. self-limiting technologies for population suppression or population replacement. Genetically modified insects engineered with self-sustaining traits either for population suppression (**top-left** panel) or population replacement (**bottom-left** panel) are expected to increase in frequency into the population over generations even when released at low initial frequency. Conversely, repetitive mass releases of mosquitoes engineered with self-limiting technologies are usually needed to maintain the desired effect over time (**right** panels).

Paratransgenesis

In addition to the manipulation of the insect genome, symbiotic microorganisms including bacteria [22–30], fungi [31–34], and viruses [35] can be engineered to develop alternative genetic control strategies. This approach, known as paratransgenesis, aims at expressing and delivering anti-pathogen effector molecules once the engineered symbiont is reintroduced into the vector [36]. Paratransgenesis is compatible with both traditional and other genetic control methods [37], while having the potential for self-sustained or self-limiting effect according to the stability of the recombinant DNA and transmission rate of the symbiont. Examples of well-characterized paratransgenic systems include: the bacteria *Rhodococcus rhodnii* engineered to express Cecropin A to interfere with *Trypanosoma cruzi* in the triatomine bug *Rhodnius prolixus*, vector of Chagas disease [23]; *Sodalis glossinidius*, a natural symbiont of Glossina spp., vector of African trypanosomiasis [22]; *Anopheles stephensi* and *Anopheles gambiae* recolonized by engineered strains of *Escherichia coli* [24,29],

Pantoea agglomerans [25,30], *Asaia* [26–28,38] and *Serratia* [39,40]. Confined semi-field studies employing *Asaia* in the two major malaria vectors *A. stephensi* and *A. gambiae* [41] have outlined key aspects regarding the behavioral ecology of paratransgenic mosquitoes and the transmission dynamics of the engineered symbiont in the vector population, through both horizontal (co-feeding and/or mating) and vertical (maternal and/or paternal inheritance) routes [42,43]. This implies that an engineered microorganism can be introduced into a mosquito population either by releasing non-biting paratransgenic males or through feeding stations, avoiding the temporary increase in mosquito density associated with release strategies [41], supporting the feasibility of paratransgenesis for future field applications. Introducing recombinant bacteria in the environment is subject to regulation and risk assessment, as required for any genetically modified organism. To mitigate the environmental impact (i.e., any undesired outcome such as the spread of recombinant microorganisms to non-targeted organisms or horizontal gene transfer), several technologies are being investigated. These include self-limiting paratransgenic systems in which the engineered symbiont reverts to a wild-type status at a predetermined rate [39] and microcapsule-based delivery strategies, which implies the release of the microorganism into the targeted organ of the insect [44]. An advantage of paratransgenesis over other control strategies is that the microorganisms used are relatively easy to isolate, engineer, and reintroduce into the vector and they can be produced at scale at an exceptionally low cost [36].

3. Self-Limiting Strategies

Self-limiting approaches require repeated releases of modified insects, which can be resource demanding, although they can be effective in geographically confined control efforts and potentially withdrawn by interrupting the releases. For this reason, self-limiting approaches can serve as an intermediate step before the release of a self-sustaining construct, to capture information on the behaviour of modified insects in the field or the interaction of the genetic construct with the environment.

3.1. Sterile Insects

Sterility in insects can be induced via radiation (sterile insect technique, SIT) (Figure 3), a long-established self-limiting strategy that has been practiced worldwide for the eradication, suppression, containment, and prevention of pests and vectors [45–49]. Once released, sterile males are expected to mate with wild females, producing no viable offspring, and hence reducing the population size, prticularly in the case of insect species that do not favour polyandry. Although the efficiency of SIT can be improved through male-only releases [50], radiation and mechanical sex sorting are usually time-consuming and resource-demanding. In addition, sterilisation and sorting steps often affect the fitness and competitiveness of the released insects besides being difficult to monitor and prevent accidental release of fertile insects [51]. Rather than using radiation to induce sterility, the incompatible insect technique (IIT) generates no viable progeny through a biological mechanism known as cytoplasmic incompatibility (CI), which is induced by the maternally inherited endosymbiotic bacteria *Wolbachia*. CI results in embryonic mortality when *Wolbachia*-infected males mate with wild-type females, whilst the progeny of the reciprocal mating and those having both parents infected, is fully viable. IIT involves male-only releases since accidental releases of *Wolbachia*-infected female would convert a population suppression strategy into population replacement, as mating between infected females and infected or wild-type males would be no longer incompatible [52]. To reduce the chance of this happening, IIT can be combined with SIT by exposing *Wolbachia*-infected insects to low doses of radiation to induce sterility without imposing detrimental fitness costs [53,54]. Technologies based on the release of insects carrying dominant lethal genetic elements (RIDL) [55] (Figure 4) can overcome some of these limitations by generating non-viable progeny from transgenic insects that are homozygous for an inducible, dominantlethal gene. It can be either a non-sex-specific (bi-sex RIDL) or a female-specific (fs-RIDL) gene,

which removes the difficulties related to the sex-sorting step [55]. Moreover, inducing lethality at an early developmental phase, such as at the embryonic stage [56–60], further reduces rearing costs, therefore enhancing sustainability of this approach. An example is the CRISPR-based precision guided SIT (pgSIT), initially developed in the *Drosophila* model, which envisages simultaneous sex sorting and sterilization of males without significant reduction in fitness [61,62].

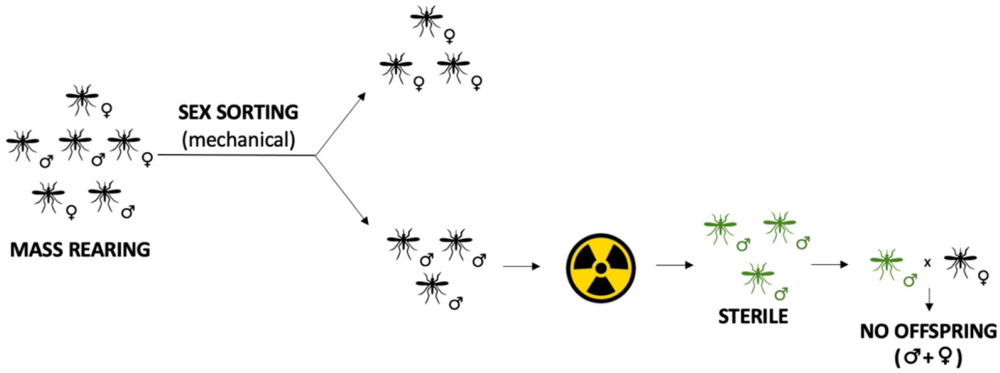

Figure 3. Sterile insect technique (SIT). Insects are mass reared and mechanically sexed. Males are exposed to radiation, which renders them sterile. Once released, males mate with wild-type females producing no viable offspring.

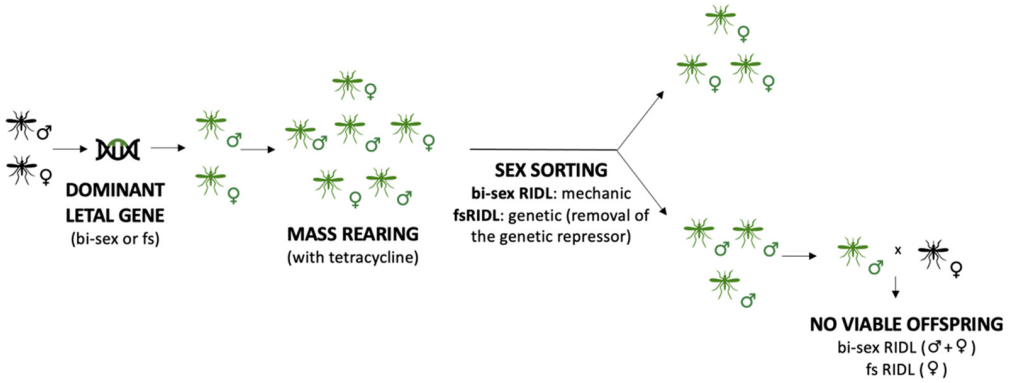

Figure 4. Bi-sex RIDL and fsRIDL. Insects are engineered with a dominant lethal (bi-sex RIDL) gene or a female-specific dominant lethal (fsRIDL) gene and mass reared in the presence of tetracycline, which represses the expression of the lethal genes during rearing stages. Males are sorted either mechanically (bi-sex RIDL) or genetically through removal of the antibiotic supplement from their diet (fsRIDL). Once males are released in the field, where tetracycline is not found, the RIDL system becomes active and the progeny resulting from the mating with wild females is not viable.

3.2. Autosomal Sex-Ratio Distorters

Another approach to control heterogametic pest species is to bias the sex ratio of the progeny by selectively interfering with the development of one of the two heterogametes during germline formation. The first synthetic sex-ratio distorter in mosquitoes was engineered in the malaria vector *A. gambiae* by expressing the I-*PpoI* endonuclease during the meiotic phase of spermatogenesis. This enzyme recognises and cleaves a specific sequence within the ribosomal DNA (rDNA) unit uniquely present in multiple copies on the centromeric region of the X chromosome in *A. gambiae*. Transgenic males carrying this construct generate over 95% male siblings as a result of the DNA double-strand breaks, which impairs the viability of X-bearing sperm (Figure 5) [18,63]. In contrast to female-killing systems, where lethality operates at the zygotic level, X shredding occurs meiotically (without apparent reduction in male fertility) therefore doubling the number of transgenic

male descendants and resulting in higher efficacy as compared with fs-RIDL [18]. Similar results were achieved by targeting the *A. gambiae* X chromosome with the CRISPR-Cas9 endonuclease [19], which paved the way for transferring synthetic sex distortion strategies for the suppression of other insect species, including the fruit fly *Drosophila melanogaster* [64] and the agricultural pest *Ceratitis capitata* [65]. However, the sex ratio distortion strategies developed so far in the laboratory carry the sex chromosome-targeting endonuclease in an autosomal locus. This results in only half of the progeny inheriting the transgene, according to Mendelian rules of inheritance, which is therefore gradually removed from the population (Figure 6) [66]. An alternative mechanism, named X poisoning, has been developed in *D. melanogaster* by inducing female lethality due to protein dose insufficiency generated by the disruption of X-linked haploinsufficient genes such as the *RpS6* ribosomal protein gene [64]. A different approach, known as Y-linked editor (YLE), involves the Y chromosome linkage of the endonuclease targeting X-linked haploinsufficient genes or required for female reproduction. According to modelling predictions, the invasiveness and related impact on the population is significantly higher when compared with other self-limiting strategies, primarily due to the reduced selection against the transgene, uniquely carried by unaffected males [67].

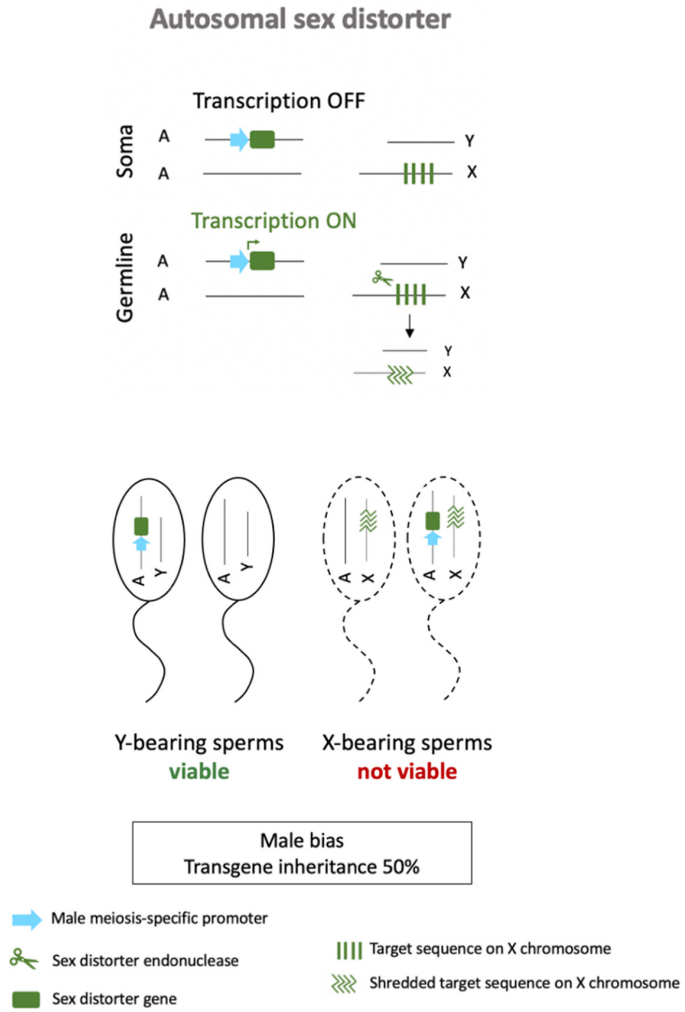

Figure 5. Autosomal sex-ratio distorters. Male bias can be achieved using an endonuclease, expressed under a male meiosis-specific promoter that cuts repetitive sequences exclusively located in one of the two sex chromosomes (the X chromosome in representation), such that only gametes of the other sex (Y-bearing sperms) would be able to fertilize the wild-type female oocytes. Autosomal sex distorters are considered self-limiting technologies because the transgene is located on autosomes, and thus inherited by only 50% of the progeny.

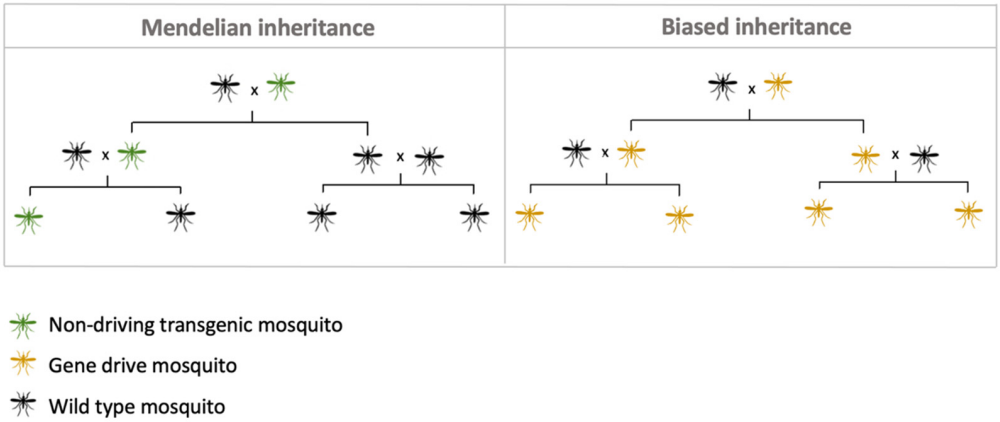

Figure 6. Mendelian vs. biased inheritance. Standard heterozygous genes (or transgenes) are inherited by 50% of the progeny following Mendelian rules of inheritance but would be eventually lost over generations if associated to a fitness cost. Conversely, gene drives bias Mendelian inheritance and can therefore spread in the population even if they confer fitness impairment.

4. Self-Sustaining Strategies

Self-sustaining technologies envision the introduction of genetic modifications that can persist and spread in the target population by biasing their inheritance to most of the progeny. These technologies, referred as "gene drives," were inspired by natural selfish genetic elements, which propagate in a population despite conferring no fitness advantage or even when posing additional costs (Figure 6) [68].

Some of the molecular mechanisms used by natural gene drives to spread into a population include toxin-antidote systems (such as the maternal effect dominant embryonic arrest (MEDEA) and underdominance (UD)), chromosomal translocations, and sequence-specific nucleases (meiotic drives and homing endonuclease genes (HEG)).

4.1. Toxin-Antidote-Based Gene Drives

Toxin-antidote-based gene drives (Figure 7) spread into the population by killing those individuals that do not co-inherit the antidote together with a genetically encoded lethal toxin. The toxin and the antidote can be designed either as *cis* acting (MEDEA) or *trans*-acting (underdominance, UD). Specifically, MEDEA elements, initially found in populations of the flour beetle *Tribolium castaneum* [69], have been synthetically engineered using maternally deposited microRNA (toxin) that mediates silencing of the maternally inherited *myd88* gene, involved in embryonic dorso-ventral pattern formation, in *D. melanogaster* [70] and in *Drosophila suzukii* [71]. The transgene also incorporates an embryonically expressed recoded copy of *myd88* (antidote), providing survival advantage only to the offspring that inherit it. Alternatively, MEDEA elements targeting either Notch signaling pathways or those involved in blastoderm formation have been shown to drive population replacement in caged fly populations [72]. UD elements consist of two constructs, one carrying a maternally expressed toxin and an embryonic antidote that rescues the lethal effect of the toxin located on the other construct, allowing only individuals carrying both constructs to be viable, and thus promoting the spread of the element. Synthetic UD have been proposed [73] and developed in *D. melanogaster* using miRNAs that target either *myd88* (named maternal-effect lethal underdominance (UD^{MEL})) [74] or the haploinsufficient ribosomal protein-coding gene *RpL14* [75]. Engineered underdominance has also been pursued through reciprocal chromosomal translocations [76,77] generating heterozygous individuals that are semi-sterile and less fit than homozygotes. A particular characteristic of many of these drives is that they are expected to behave in a frequency-dependent manner; transgenic insects are predicted to spread into a population and eventually reach fixation only if seeded above a critical threshold, which largely depends on the relative fitness of the engineered insects as well as the construct design [78]. Mathematical modelling suggests

that a combined MEDEA-underdominant system would result in a lower frequency-release threshold along with faster population modification and enhanced geographic stability [79].

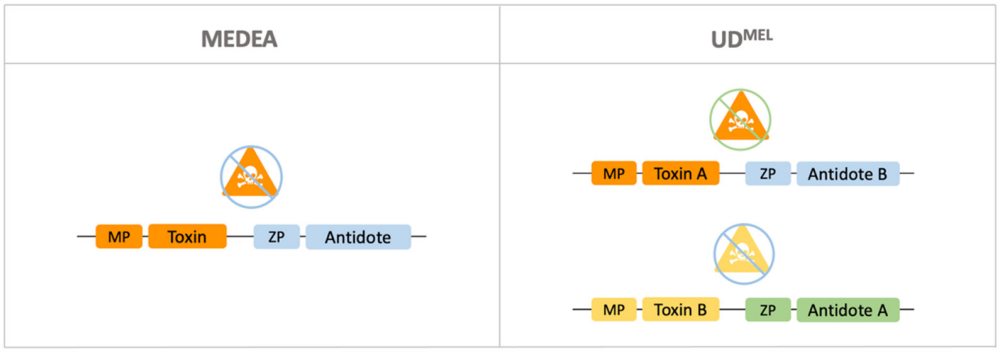

Figure 7. MEDEA and toxin-antidote-based underdominance (UD^{MEL}). Toxin-antidote-based gene drives express a toxin under the control of a maternal promoter that is deposited in the embryo and is lethal for the zygote unless rescued by an antidote. In MEDEA systems, the maternally expressed toxin and the zygotically-expressed antidote are linked on the same chromosome. UD^{MEL} consists of two constructs on different chromosomes, each bearing a maternally expressed toxin and a zygotically expressed antidote, which suppress the activity of the toxin expressed at the other locus. MEDEA and UD^{MEL} can both spread into a population by causing lethality of the offspring that do not inherit the element because the lethal activity of the maternally derived toxin. MP, maternal promoter and ZP, zygotic promoter.

4.2. Meiotic Drives

Meiotic drives elude the Mendelian law of allele segregation through several strategies including "gonotaxis" (in females), where they circumvent the polar bodies during oogenesis and move to the germline at the expense of the soma, and "gamete killing" (in males), which prevents the development of functional gametes that do not bear a copy of the driving factor [80]. Naturally occurring Y-drives have been found in populations of *Aedes aegypti* [81] and *Culex pipiens* [82] mosquitoes. Synthetic Y-linked sex distorters (Figure 8) have been proposed as self-sustaining tools for vector population suppression in species with XY male heterogametic systems [18,80,83,84]. Unlike autosomal sex distorters, the X-shredding endonuclease could be inserted on the Y chromosome and, if expressed during spermatogenesis, would result in progeny mainly composed of males that would inherit the transgene [63,83]. However, the transcriptional silencing of sex chromosomes during meiosis has so far impeded the implementation of synthetic Y-linked sex distorters [83], due to meiotic sex chromosome inactivation (MSCI) [84,85], an epigenetic process that is mediated by chromatin condensation of sex chromosomes at the meiotic stage of male gametogenesis [84]. Transcriptional signatures of MSCI were recently shown in the male germline of *A. gambiae*. The same study also highlighted genes and genomic locations that may be able to evade silencing, bringing a new wave of optimism for the successful engineering of Y-drive technologies to control the main malaria vector [85]. Mathematical models predict that synthetic Y drives would rapidly spread to fixation following relatively few inoculative releases of transgenic individuals, ultimately leading to suppression or even elimination of the population targeted [86]. The probability for an autosomal sex distorter to become a Y-drive seems extremely low as Y-linkage of the construct through remobilization of the genetic element is unlikely in *A. gambiae* in addition to meiotic inactivation [83]. This aspect reduces risk of unexpected invasiveness of autosomal sex distorters that may be deployed in the field.

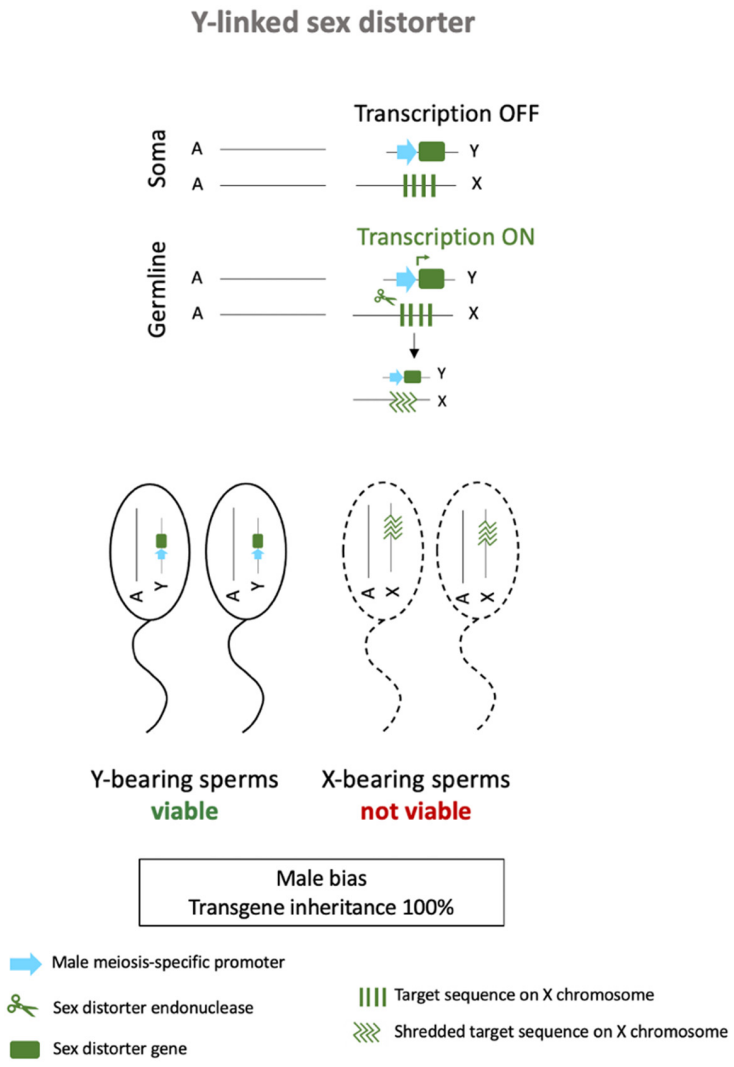

Figure 8. Y-linked sex-ratio distorters. Male bias can be achieved using the same mechanism of X shredding shown for autosomal sex distorters, although the endonuclease is placed on and expressed from the Y chromosome, rendering the Y-linked sex distorter self-sustaining, given that the transgene would be inherited by the whole male progeny.

4.3. Homing-Based Gene Drives

Homing-based gene drives (Figure 9) were inspired by natural selfish genetic elements such has homing endonuclease genes (HEGs) found in unicellular organisms. HEGs are able to cut specific DNA sequences and copy their coding sequence through homologous recombination (HR) using the HEG+ allele as template [87]. This process, known as "homing", results in the conversion of the HEG-bearing locus from heterozygosity to homozygosity, which is irreversible because the DNA recognition sequence of the endonuclease is lost after the insertion of the HEG genetic cassette. Using this mechanism, HEGs can spread in a population through super-Mendelian inheritance [87]. Synthetic HEGs can be designed to either interfere with the vectorial competence [88] or to target essential genes involved in survival or reproduction [87], finding applications in population replacement and suppression approaches, respectively. HEG-based gene drives to control vector and pest populations were proposed in the early 2000s [87], and few years later initial proof-of-principle was developed in *A. gambiae* [88,89] and *D. melanogaster* [90,91] using the I-SceI endonuclease. Alternatively, ZFNs and TALENs modular nucleases [92] have been explored to develop synthetic selfish elements in *D. melanogaster* [93], taking advantage of

their inbuilt programmability to target DNA sequences. However, limited stability, likely due to the genetic modularit has hampered their use in gene drive research [93].

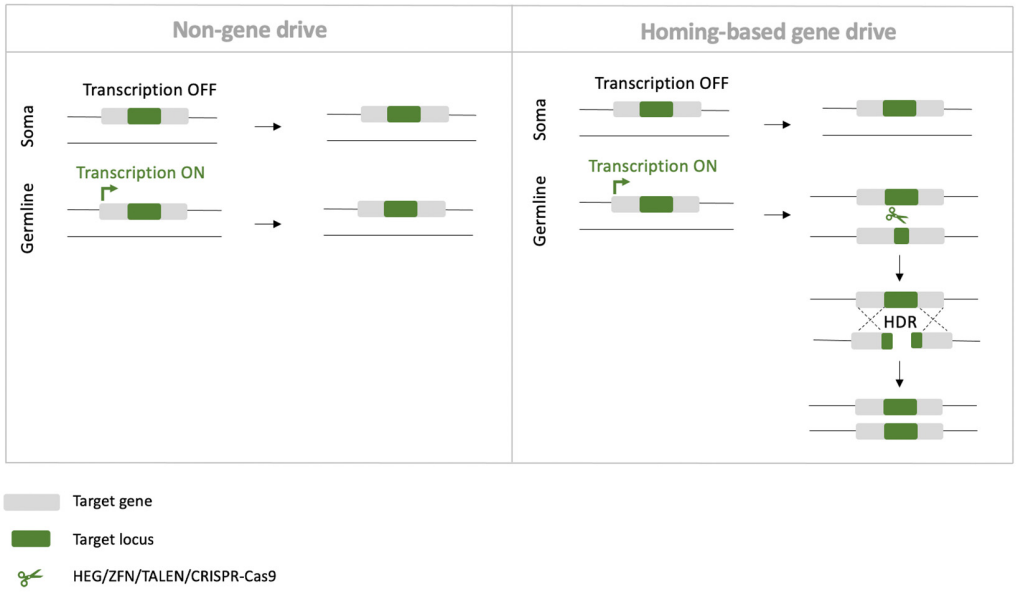

Figure 9. Homing-based gene drives. Homing-based gene drives bias Mendelian inheritance because the transgene is copied in both homologous chromosomes in the germline through a mechanism of double strand break (DSB), which can be induced by HEGs, ZFNs, TALENs, or CRISPR-Cas9 nucleases, followed by homology-directed repair (HDR).

The discovery of the bacterial CRISPR-Cas system [94,95] has clearly revolutionised the field of genome editing by means of RNA-programmable nucleases that perform site-specific modification in the genome (Figure 10) [96,97].

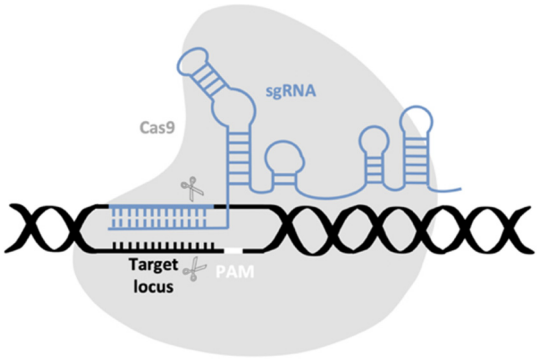

Figure 10. CRISPR-Cas9 system. The CRISPR-Cas9 system is composed of a Cas9 endonuclease that performs a DSB in the genome. The targeting specificity is provided by sgRNA, which directs the Cas9 onto the target locus on the DNA, which is flanked by a 2–6 bp long protospacer-adjacent motif (PAM).

CRISPR-Cas has been widely investigated as a versatile tool for site-specific mutagenesis and germline homing in the context of pest and vector control. Population suppressive CRISPR-Cas gene drives have been developed in the major African malaria vector *A. gambiae* by targeting either female fertility (*AGAP007280*) [16,98] or sex determination (*doublesex*) [17]. Despite a high transmission rate (up to 99.6%) of the gene drive element targeting gene *AGAP007280* [16], the spread of the drive into the caged mosquito population was halted by the generation of nuclease-resistant functional variants [16]. The development of resistant alleles has been mitigated by improving spatiotemporal expres-

sion of the Cas9 component [98], and by targeting the highly conserved genomic locus intron 4-exon 5 boundary in *doublesex* gene (*dsx*) [17]. This CRISPR-based homing drive has also been coupled with the X-shredding I-PpoI nuclease to generate a self-sustaining version of the autosomal sex-distorter (referred to as sex distorter gene drive, SDGD) in *A. gambiae* [20] (Figure 11).

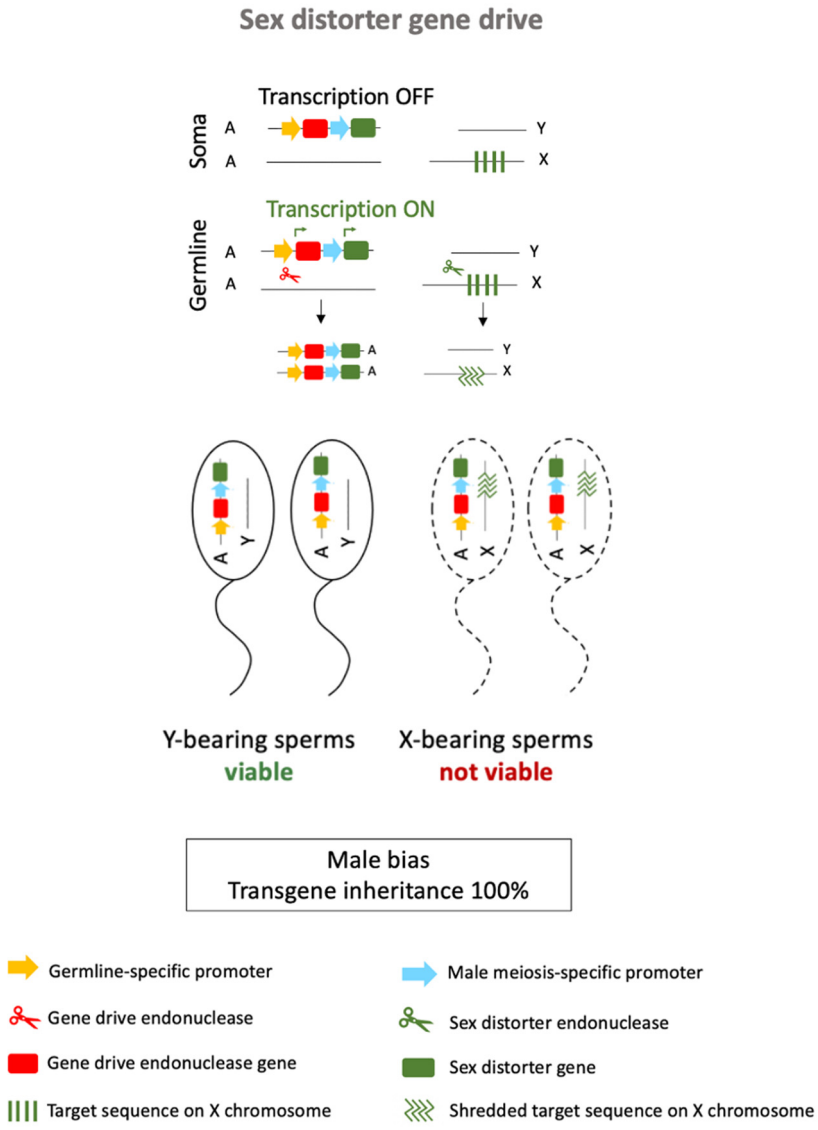

Figure 11. Sex-distorter gene drive. In SDGD (autosomal sex distorter coupled to a gene drive system) the gene drive endonuclease ensures the homing of the construct, while the sex-distorter endonuclease cuts and destroys the X chromosome, resulting in Y-bearing sperms only and male bias.

CRISPR-Cas gene drives aimed at population replacement have been developed in the Asian malaria vector *A. stephensi* [99] and in *Ae. aegypti* [100]. A wide variety of antimalarial effector genes used as "cargo", reviewed in [101], have shown effectiveness in reducing the vectorial ability of malaria-competent mosquitoes in laboratory studies, providing valuable tools for population modification [101]. Recently, a CRISPR-based gene drive strain has been developed in *A. gambiae* high homing rates (98 to 100%), no major fitness costs, and reduced resistance [102]. Additional parameters to be considered for successful implementation of these technologies are: the release threshold, epigenetic silencing, RNA interference, assortative mating, parasite and vector co-adaptation, and parental effect, impact the spread of the gene drive into the population [87,98,103–109]. Strategies to

mitigate the emergence of resistance at the target site include: tightening of spatiotemporal activity of the homing endonuclease to the germline [16,110], rational target site selection [87,111], combined releases of different gene drives [87], multiplexing [112–114] of sgRNAs to simultaneously target more than one site in the genome, and the use of nucleases that are more tolerant to mutations at the target site while maintaining on-target cleavage specificity. Alternatives to traditional CRISPR-based population replacement gene drives have been recently proposed to minimise the occurrence of resistance, such as integral gene drives (IGD) [115] and gene drive rescue systems [116]. IGDs envisage the drive and the anti-pathogen elements being integrated within endogenous loci while preserving their function. Such design should mitigate the emergence of resistant alleles as non-synonymous mutations would most likely perturb the normal gene expression and be selected against [115,117]. Alternatively, gene drive rescue systems use the Cas9-gRNA complex to target an essential gene while providing a recoded sequence that restores gene function. Evidence of the feasibility of this system has recently been shown in caged populations of *D. melanogaster* [116,118,119] and *A. stephensi* [120].

5. Split Drives

Split drives consist of distinct genetic elements, one containing the gRNA and the other the Cas9 endonuclease, which follow Mendelian patterns of transmission when inherited separately or biased when combined through mating [121]. This technology, which has been recently tested in *D. melanogaster* [122] and *Ae. aegypti* [100], achieves parameters of homing and emergence of resistance allele that are comparable to standard drives [102,122,123]. Recently, the population dynamics of a synthetic split drive that integrates the characteristics of the toxin-antidote systems (split drive killer rescue (SDKR)), have been explored [123]. SDKR consists of two unlinked constructs, one bearing the toxin with one or multiple gRNAs targeting the corresponding wild-type locus and the other construct carries the rescue gene and the Cas9 endonuclease. Therefore, homing can only occur when both constructs are present in the same organism, causing an increased frequency of the toxin in the population, which in turn creates a selective pressure on the rescue construct, leading to the diffusion of the SDKR in the population [123]. "Daisy drives", a more composite version of a simple two-element split drive, have been theoretically explored [124] with the aim to drive a transgene into a population for a limited number of generations before being halted by the loss of driving components. The elements of this system are arranged in series such that each is needed to drive the following, with the last element being the effector driven in higher frequencies. The gradual loss of the bottom elements, through mating with wild-type individuals from the population, leads the system to self-exhaust over time, eventually restoring the wild-type state of the population. Split drives and daisy-chain drives are considered to be "localised drives" because, assuming a limited gene flow between separate populations, even if the elements are introduced into a non-target population, they would not exceed the critical frequency to spread. Thus, they can be removed from the population, for example, by releasing wild-type individuals such as to lower the transgene frequency below the critical threshold [125].

6. Discussion and Conclusions

Genetic control engineering has prompted new solutions to control harmful insects with increased sustainability compared to chemical, physical, cultural, and biological control practices. Regardless of the design (i.e., population replacement/suppression or self-limiting/self-sustaining), it is important that the proposed genetic approach is compatible with other strategies employed in the target area. The species-specificity of mating-dependent genetic approaches, also minimises unwanted effects on non-targeted insects, which can be common with traditional approaches. However, genetic control methods could potentially also be effective within species complexes as a consequence of incomplete reproductive isolation and genetic flow between sibling species, which can nonetheless be controlled by the specificity of the technologies. The implementation

of efficient genetic control in the field may result in decreased use of insecticides and drugs, reducing the development of resistance, with potential benefits to the environment. Releasing genetically modified insects impacts the whole human community in the target area, irrespectively of the socio-economic status.

There have been numerous field releases of genetically modified insects, although they are only limited to sterile individuals that are not intended to introgress genetic material in the wild population. These include *Wolbachia*-based programmes for Dengue control in Australia, Asia, and South America [126,127]; sterile males in the malaria vector *A. gambiae* in Burkina Faso [128,129]; and RIDL systems engineered in the agricultural pests diamondback moth *Plutella xylostella* in New York [130] and the pink bollworm, *Pectinophora gossypiella* in Arizona [131], as well as in the *Ae. aegypti* mosquitoes as a measure against Zika virus outbreaks in the Cayman Island [132,133], Brazil [134], Malasya [135], and Florida [136].

Among the self-limiting strategies, YLEs are expected to have the greatest efficiency, although only explored theoretically so far. On the other hand, population dynamics of an autosomal sex distorter in *A. gambiae* have already been evaluated in semi-field conditions [66]. Other genetic strategies have shown the potential to impose a greater impact reducing the cost required for release, but envision the presence and relative persistence of a transgene in wild populations. Self-sustaining systems to control insects in geographical settings where large scale releases are impractical and not sustainable, have been explored. Among these, several homing-based gene drives have shown promising results for population suppression or replacement of caged populations of insects and, the latter, was recently evaluated in semi-field studies [137]. Although toxin-antidote-based systems are of interest because of their low frequency-release threshold, so far, technical difficulties have prevented the development of Medea and UD in many insects of medical and agricultural relevance. Nonetheless, RNA-guided nucleases-based toxins and genetic translocations may be used to improve the efficiency of these technologies [113]. Mathematical models of meiotic drives show great potential in suppressing insect populations. However, this technology was proved difficult to develop due to the meiotic silencing of transgenes located on the sex chromosomes. Split drives have been explored as an alternative to standard gene drives for local genetic control

The significant research progress in the field of gene drive has created the possibility of a future release in the environment. A transparent and responsible evaluation of efficacy and safety as well as careful assessment of risks and benefits of these technologies are of critical importance prior to field deployment [138]. A code of ethics for gene drive research has been proposed by incorporating established principles of responsible science, ecological stewardship, and public engagement to advocate for transparent research in the interest of society and humanity [139]. Acknowledging the potential outcome of the implementation of these technologies, frameworks indicating best practices in the field are fundamental for their deployment to eradicate vector-borne diseases and pests.

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