Natural and Engineered Sex Ratio Distortion in Insects

Austin Compton¹,²* and Zhijian Tu¹,²*

¹ Department of Biochemistry, Virginia Tech, Blacksburg, VA, United States, ² Fralin Life Sciences Institute, Virginia Tech, Blacksburg, VA, United States

Insects have evolved highly diverse genetic sex-determination mechanisms and a relatively balanced male to female sex ratio is generally expected. However, selection may shift the optimal sex ratio while meiotic drive and endosymbiont manipulation can result in sex ratio distortion (SRD). Recent advances in sex chromosome genomics and CRISPR/Cas9-mediated genome editing brought significant insights into the molecular regulators of sex determination in an increasing number of insects and provided new ways to engineer SRD. We review these advances and discuss both naturally occurring and engineered SRD in the context of the Anthropocene. We emphasize SRD-mediated biological control of insects to help improve One Health, sustain agriculture, and conserve endangered species.

Keywords: CRISPR/Cas9, sex chromosome, sex-determination, gene drive, endosymbiont bacteria, climate change, sex conversion, sex-specific lethality

INTRODUCTION

Sex of an insect is determined by the chromosome complement it inherits from its parents. The chromosome systems that underly genetic sex-determination are quite diverse among insect species (Bachtrog et al., 2014; Beukeboom and Perrin, 2014; Biedler and Tu, 2016). Flies and mosquitoes are among the many insects that evolved the XX/XY sex chromosome system, where the heterogametic (XY) individuals are males and the homogametic (XX) individuals are females. Lepidopterans such as the silkworm, Bombyx mori, evolved the ZZ/ZW sex chromosome system where ZZ males are the homogametic sex while heterogametic ZW individuals are females. Hemizygous sex chromosome systems are also found including XX/XO in several insect orders, where the males are hemizygous as they only have one X chromosome; and ZZ/ZO in some lepidopteran and tricopteran species, where the females are hemizygous (ZO). Although species within an insect order tend to share the same type of sex chromosome system, variations can occur within an order or even a family. In the aforementioned systems, the sex of an offspring is determined by the genotype of the gamete of the heterogametic or hemigametic parent. In Hymenopteran and Thysanopteran insects, however, sex is instead determined by the haplodiploidy of the individual, where fertilized diploid eggs (2n) develop to females while unfertilized haploid eggs (n) develop as males (reviewed in Beukeboom and Perrin, 2014; Biedler and Tu, 2016).

In contrast to the apparent plasticity and diversity of the sex-determining chromosome systems, two highly conserved transcription factors doublesex (dsx) and fruitless (fru) control the development of sexual dimorphism in all insects studied thus far (Herpin and Schartl, 2015; Biedler and Tu, 2016; Hopkins and Kopp, 2021). Sex-specific isoforms of the DSX and FRU proteins, which result from sex-specific splicing of their primary RNA transcripts, program sexual differentiation (Figure 1). Therefore, sex determination is a process in which the primary sex-specific signals, which reflect the sex-specific chromosome composition of the early embryo, are transduced in a signal cascade to modulate sex-specific splicing of the RNA transcripts of dsx and fru. Figure 1
presents two simplified models using \textit{dsx} as an example. In the vinegar fly \textit{Drosophila melanogaster}, the double dosage of the \textit{X} chromosome in the females (XX) initiates the transcription of the \textit{sex lethal (sxl)} gene in the early embryo, leading to the production of the primary signal which is the SXL protein (Figure 1A). The presence of SXL leads to the production of a functional protein isoform of \textit{transformer} (TRA), which in turn enables female-specific splicing of the primary RNA transcripts of \textit{dsx} and \textit{fru}. In the male (XY) embryo, the single \textit{X} chromosome fails to initiate the production of the primary signal SXL, leading to the default male-specific splicing of \textit{dsx} and \textit{fru} transcripts and hence the production of male-specific DSX and FRU protein isoforms. In the Mediterranean fruit fly \textit{Ceratitis capitata}, however, the default sex is female (Figure 1B). The primary signal, a male-determining factor (M factor) MoY (Meccariello et al., 2019), resides on the \textit{Y} chromosome. The presence of the M factor in the early male (XY) embryos results in male-specific splicing of the \textit{tra} pre-mRNA, leading to the production of a non-functional TRA protein and subsequently, male-specific splicing of \textit{dsx} and \textit{fru}. The lack of the \textit{Y} chromosome in females (XX) enables the TRA protein complex to catalyze the female-specific splicing of \textit{dsx} and \textit{fru}. The TRA intermediate evolved much faster than DSX and FRU and TRA is not found in all insects (Biedler and Tu, 2016). Recent advances have brought significant molecular insights into the diverse mechanisms of sex determination in an increasing number of insects (e.g., Kiuchi et al., 2014; Hall et al., 2015, 2016; Criscione et al., 2016; Krzywinska et al., 2016, 2021; Sharma et al., 2017; Meccariello et al., 2019; Qi et al., 2019; Wexler et al., 2019; Aryan et al., 2020; Liu et al., 2020; Zou et al., 2020; Lutrat et al., 2021; Zhuo et al., 2021).

A relatively balanced male to female sex ratio is expected for most insects. However, selection may shift the optimal sex ratios and meiotic drive and microbial manipulation can result in sex ratio distortion (SRD). In the following sections, we will review both naturally occurring and engineered SRD in the context of developing biological control of insects to help improve One Health, sustain agriculture, and conserve endangered species.

**Naturally Occurring Meiotic Drives Can Lead to Sex Ratio Distortion by Biased Production or Transmission of Gametes**

Gene drive refers to the phenomena in which one of the homologous chromosome pair, or an allele on one of the homologous chromosome pair, is transmitted to the next generation at a frequency greater than the expected 50% Mendelian segregation. If this bias results from a bias in the representation of gametes of a certain chromosome or genotype during meiosis (Figure 1C), then it is a meiotic drive. If this bias involves the sex chromosomes during meiosis in the heterogametic sex (e.g., during spermatogenesis in XY males), SRD will ensue. Meiotic and sex-linked drives have been discussed in a number of reviews including Jaenike (2001) and Courret et al. (2019). We will highlight a few examples emphasizing the concept and recent advances (Table 1). Meiotic drives typically involve two loci: a drive allele/locus and a drive-sensitive allele targeted by the drive locus (Lyttle, 1991). For example, the \textit{D. simulans} Winters drive is comprised of the \textit{Distorter on the X} (Dox) that targets \textit{Y}-chromosome repeats and kills the \textit{Y}-bearing sperm, leading to a female sex ratio bias (Tao et al., 2007a,b). Two autosomal suppressor alleles were found that counteract the drive (Tao et al., 2001, 2007a) and suppress \textit{Dox} via RNA interference (Lin et al., 2018). The intragenomic arms race between an SRD drive and its suppressor and resistance alleles may have resulted in cryptic SRDs that are derived from multiple waves of SRD invasion followed by suppression and/or resistance (e.g., Muirhead and Presgraves, 2021). In \textit{Aedes aegypti} mosquitoes a \textit{Y}-linked \textit{distorter} locus (D) is thought to disrupt the formation of the \textit{X}-bearing sperm by causing \textit{X}-chromosome breakage mainly at one of four sites during male meiosis I (Craig et al., 1960; Newton et al., 1976). Thus, D increases its own transmission and causes male-biased sex distortion (Figure 1C). This male bias is especially attractive in the context of controlling mosquito-borne infectious diseases as only female mosquitoes bite and transmit disease-causing pathogens. Suppressor alleles, resistant X chromosomes, and distortion enhancers associated with the D drive have been reported in various laboratory strains and wild populations (Wood, 1976; Suguna et al., 1977; Wood and Ouda, 1987; Owusu-Daaku et al., 1997). The \textit{Ae. aegypti} \textit{Y} chromosome is also called the M chromosome and it is similar to the \textit{X} chromosome (or the m chromosome) except for its male-determining locus M (Matthews et al., 2018). Strictly speaking, these M- and m-bearing chromosomes are homomorphic sex-determining chromosomes.

**Sex Ratio Can Be Skewed by Maternally Inherited Bacterial Endosymbionts**

Bacterial endosymbionts such as \textit{Wolbachia}, \textit{Rickettsia}, \textit{Cardinium} and \textit{Spiroplasma} can infect and manipulate the germline of insects that harbor them (reviewed in Werren et al., 2008; Ma et al., 2014; Hurst and Frost, 2015; Landmann, 2019). They are normally transmitted through the maternal germline. \textit{Wolbachia} is thought to infect more than half of all arthropod species (Weinert et al., 2015) and is well known for its induction of cytoplasmic incompatibility (CI) and CI-related applications in pest control (e.g., Laven, 1967; Dobson et al., 2002; Zheng et al., 2019). \textit{Wolbachia} can cause SRD by feminizing or killing males, inducing parthenogenesis, and regulating sex allocation. For example, \textit{Wolbachia} can cause damage in the dosage compensated \textit{X} chromosome that is only found in males to confer male-specific lethality in \textit{Drosophila} (Harumoto et al., 2018). \textit{Wolbachia} can also feminize and kill males by mis-regulating the factors in the sex determination pathway to shift \textit{dsx} splicing (Sugimoto and Ishikawa, 2012). \textit{Wolbachia} in female \textit{Eurema mandarina} butterflies (genotype ZW) prevents the production of \textit{Z}-bearing oocytes during oogenesis and initiates female development in ZO individuals, resulting in all-female offspring through a dual-acting meiotic drive (Kern et al., 2015; Kageyama et al., 2017). \textit{Wolbachia} and other intracellular symbionts can also mediate higher fertilization rates leading to a female bias in haplodiploid species (Wang et al., 2020; Bagheri et al., 2022).
FIGURE 1 | Simplified models of the sex-determination pathways in the vinegar fly *Drosophila melanogaster* (A) and the Mediterranean fruit fly *Ceratitis capitata* (B), and meiotic sex ratio distortion (C). (A) In *D. melanogaster*, embryos that inherit two X chromosomes (depicted as red DNA molecules) express the primary signal *Sex-lethal* (SXL) which effects the female-specific splicing of the *transformer* (*tra*) transcript, leading to the production of a functional TRA protein (TRA\(^F\)). The TRA\(^F\) protein complex enables the female-specific splicing of the *doublesex* pre-mRNA, leading to the production of the female DSX protein isoform (DSX\(^F\)) which programs female differentiation (left). In contrast, embryos with a single X chromosome do not express a functional SXL, resulting in a truncated non-functional TRA (TRA\(^M\)) and subsequently the default male-specific splicing of *dsx*. This leads to the production of DSX\(^M\), which programs male differentiation (right). The Y chromosome (depicted as a blue DNA molecule) does not directly participate in sex-determination. (B) In *C. capitata*, a dominant male-determining factor, *Maleness-on-the-Y* (MoY) resides on the Y chromosome (in blue). Expression of MoY somehow induces male-specific splicing of the *tra* pre-mRNA, leading to the production of truncated non-functional TRA (TRA\(^M\)) and subsequently the default male-specific splicing of *dsx*. This leads to the production of DSX\(^M\), which programs male differentiation (right). Embryos that do not inherit the Y chromosome (or MoY) produce a functional TRA protein (TRA\(^F\)) complex by default, which leads to the production of DSX\(^F\) and female differentiation (left). See Meccariello et al. (2019) and Primo et al. (2020) for details and for the concept of an autoregulatory loop. (C) Normal spermatogenesis of a heterogametic male with XY chromosomes will produce X- or Y-bearing sperms in equal proportion (left). In a hypothetical Y-linked X-shredder system (right), either natural or engineered, double-stranded DNA breaks along the X chromosome could lead to non-functional X-bearing sperms without affecting the Y chromosome-bearing sperms. As a result, sex ratio distortion will occur in the progeny of an affected heterogametic male (XY).
TABLE 1 | Examples of natural and engineered sex ratio distortion.

| Classification | Description | Species | References | Doi |
|----------------|-------------|---------|------------|-----|
| Meiotic        | Male-linked Distorter | Aedes aegypti | Craig et al., 1960; Newton et al., 1976 | doi: 10.1126/science.132.3443.188; doi: 10.1007/BF0005554 |
| Meiotic        | X-linked Distorter | Drosophila simulans | Muirhead and Presgraves, 2021 | doi: 10.1038/s41559-021-01543-8 |
| Meiotic        | X-shredder by I-Ppol | Anopheles gambiae | Galizi et al., 2014 | doi: 10.1038/ncomms4977 |
| Meiotic        | X-shredding by Cas9 | Anopheles gambiae | Galizi et al., 2016 | doi: 10.1038/srep3113 |
| Meiotic        | X-shredding by Cas9 | Drosophila melanogaster | Fasulo et al., 2020 | doi: 10.1371/journal.pgen.100864 |
| Meiotic        | X-shredding by Cas9 | Ceratitis capitata | Meccariello et al., 2021 | doi: 10.1186/s12915-021-01010-7 |
| Meiotic        | X-poisoning by Cas9 knockout of haploinsufficient genes | Drosophila melanogaster | Fasulo et al., 2020 | doi: 10.1371/journal.pgen.100864 |
| Sex determination | Female-to-male conversion by expression of Nix | Aedes aegypti | Hall et al., 2015; Aryan et al., 2020 | doi: 10.1126/science.aaa285; doi: 10.1073/pnas.20011321 |
| Sex determination | Female-to-male conversion by expression of Nix | Aedes albopictus | Lu et al., 2020; Lutrat et al., 2021 | doi: 10.1016/j.ibmb.2019.10331; doi: 10.1101/2021.07.28.003869 |
| Sex determination | Female-to-male conversion by disruption of Mdmd | Musca domestica | Sharma et al., 2017 | doi: 10.1126/science.aam549 |
| Sex determination | Maternal knockdown of tra results in all-male offspring | Blattella germanica | Wexler et al., 2019 | doi: 10.7554/eLife.4749 |
| Sex determination | Knockdown of Mosc results in male-specific lethality | Bombyx mori | Kuchi et al., 2014 | doi: 10.1038/nature1331 |
| Sex determination | Female-specific lethality by expression of Guyp | Anopheles stephensi | Criscone et al., 2016; Qi et al., 2019 | doi: 10.7554/eLife.192; doi: 10.7554/eLife.435 |
| Sex determination | Female-specific lethality by expression of Yob and fle | Anopheles gambiae | Krzywinska and Krzywinski, 2018; Krzywinska et al., 2021 | doi: 10.1186/s13071-018-3211-z; doi: 10.1016/j.cub.2020.12.0 |
| Sex determination | Female-specific lethality by depletion of Nlmd in embryos | Nilaparvata lugens | Zhuo et al., 2021 | doi: 10.1126/sciadv.abf923 |
| Sex determination | Gene drive targeting transformer | Ceratitis capitata | Carrami et al., 2018 | doi: 10.1073/pnas.171382511 |
| Sex determination | Knockdown of MoY feminizes males; ectopic expression masculinizes females | Ceratitis capitata | Meccariello et al., 2019 | doi: 10.1126/science.aax131 |
| Sex determination | Gene drive targeting female doublesex | Anopheles gambiae | Kryou et al., 2018; Simoni et al., 2020 | doi: 10.1038/s41587-019-0204-z; doi: 10.1038/s41587-018-0211-z |
| Sex determination | Female-specific lethality by Cas9 knockout of transformer | Bombyx mori | Zhang et al., 2018 | doi: 10.1038/s41006-018-0106-4 |
| Endosymbiont-induced | Meiotic drive and Feminization mediated by Wolbachia | Eurema mandarina | Kern et al., 2015; Kageyama et al., 2017 | doi: 10.1098/rsbl.2015.009; doi: 10.1002/evl3 |
| Endosymbiont-induced | Hamiltonella manipulation of fertilization | Bernisia tabaci | Shan et al., 2019 | doi: 10.1098/rsbl.2019.167 |
| Endosymbiont-induced | Sex ratio distortion by inhibition of fertilization | Trialeurodes vaporariorum | Wang et al., 2020 | doi: 10.1038/s41396-020-0717-0 |
| Endosymbiont-induced | Temperature-sensitive effects on sex allocation | Pezothrips kellyanus | Katlav et al., 2022 | doi: 10.1038/s41437-022-00505-5 |
| Endosymbiont-induced | Male killing Wolbachia | Drosophila bifasciata | Harumoto et al., 2015 | doi: 10.1098/rspb.2017.216 |
| Endosymbiont-induced | Male-killing Spiroplasma: mis-regulating dosage compensation/sex determination | Drosophila melanogaster | Cheng et al., 2016; Harumoto et al., 2016 | doi: 10.1016/j.cub.2016.03.05; doi: 10.1038/ncomms127 |
| Endosymbiont-induced | A male-killing Wolbachia: interacts with sex determination and dosage compensation | Ostrinia scapulative | Sugimoto and Ishikawa, 2012; Sugimoto et al., 2015; Fukui et al., 2015 | doi: 10.1098/rsbl.2011.111; doi: 10.1016/j.ibmb.2015.10.00; doi: 10.1371/journal.ppat.10050 |
| Endosymbiont-induced | Male-killing Wolbachia targeting the masculinizing gene | Ostrinia furnacalis | Fukui et al., 2015 | doi: 10.1371/journal.ppat.10050 |
| Endosymbiont-induced | Potential male-killing by Wolbachia | Anastrepha fraterculus | Conte et al., 2019 | doi: 10.1186/s12866-019-1652-y |

1 In these cases, sex-specific lethality is caused by mis-regulation of dosage compensation. Additional examples can be found in Hurst and Jiggins (2000), Jaenike (2001), and Kageyama et al. (2012). Some sex-determination factors listed are examples of good targets for SDR.
Engineering Sex Ratio Distortion Through Sex Conversion and Sex-Specific Lethality

Altering or perturbing factors involved in sex-determination (Figures 1A,B) could either result in sex conversion, or sex-specific lethality, or infertile intersex, depending on the relative position of the factor in the sex-determination pathway (i.e., top master switch such as SXL or MoY versus bottom effector such as DSX) and whether or not sex chromosome dosage compensation is required (reviewed in Biedler and Tu, 2016; Scott, 2021). Table 1 lists a number of candidates that can be manipulated to cause SRD in diverse insect species. We will only highlight a few recent examples in which SRDs were demonstrated over many generations when the factors were stably inherited as transgenes. Stable expression of a transgenic copy of Nix, a master switch for male determination in Ae. aegypti and Ae. albopictus (Hall et al., 2015), converted genetic females into fertile males and resulted in a clear SRD (Adelman and Tu, 2016; Aryan et al., 2020; Lutrat et al., 2021). On the other hand, stable germline transformation of a Y-linked primary signal Guy1 resulted in female-specific lethality in An. stephensi (Criscione et al., 2016). Unlike Ae. aegypti, X chromosome dosage compensation is needed in An. stephensi (Jiang et al., 2015) and Guy1 regulates dosage compensation by increasing the transcription of genes on the X chromosome in XY males (Qi et al., 2019). Transgenic expression of Guy1 in XX females result in abnormally high transcription of X-linked genes and hence lethality (Qi et al., 2019). Targeting the sex-specific exon (or its splicing signal) of dsx, a gene at the bottom of the sex-determination pathway, could also result in SRD. A gene drive was developed in An. gambiae that disrupts the formation of female dsxF transcript while leaving the male dsxM unaffected (Kyrou et al., 2018, see below for details), resulting in sterile intersex XX individuals without affecting the development or fertility of XY males. In addition to genetic manipulations mentioned above, SRD can also be achieved by silencing genes in the sex-determination pathway using interfering RNA (e.g., Pane et al., 2002; Whyard et al., 2015; Meccariello et al., 2019; Taracena et al., 2019). Other sex-specific phenomena can also be used to engineer SRD (e.g., Fu et al., 2010; Kandul et al., 2020; Li et al., 2021).

CRISPR/Cas9 Technology Expands Ways to Engineer Sex Ratio Distortion

An engineered X-shredder was developed in the malaria mosquito An. gambiae that uses an endonuclease I-Ppol to target ribosomal DNA repeats exclusive to the X chromosome to induce X chromosome breakage during spermatogenesis (Galizi et al., 2014). This results in the reduction of X-bearing sperm and > 95% male progeny. Releasing this X-shredder mosquito strain successfully suppressed a cage population, confirming its potential for genetic control. Unlike the D-locus in Ae. aegypti which is Y-linked and thus favors its own transmission at the expense of the X chromosome, the I-Ppol X-shredder is on an autosome and is transmitted at a 50% probability. Attempts to engineer a more powerful Y-linked sex ratio distorter have not been successful presumably due to inactivation of the Y-linked distorter transgene during meiosis (Turner, 2007; Taxarchi et al., 2019; Alcalay et al., 2021). As an alternative, the I-Ppol X-shredder in An. gambiae was integrated into a CRISPR/Cas9-based gene drive (Simoni et al., 2020) that targets the female doublesex (dsx) isoform as previously described (Kyrou et al., 2018). This new strain produces male-only progeny and eventually crashed cage populations in only 10–14 generations with a starting gene drive frequency as low as 2.5%, demonstrating the potential for large-scale applications (Simoni et al., 2020). Large cage trials that incorporated mosquito ecology showed further promise as the dsx gene drive suppressed the mosquito population within a year without selecting for resistance to the drive (Hammond et al., 2021).

An X-shredder was also developed using an RNA-guided CRISPR/Cas9 nuclease to target the X chromosome in An. gambiae (Galizi et al., 2016). The use of a programmable CRISPR/Cas9 nuclease has facilitated the development of X-shredders in other insect species including D. melanogaster (Fasulo et al., 2020) and C. capitata (Meccariello et al., 2021). All that are required are guide RNAs that target X-specific sequences/repeats and a male germline promoter that directs the expression of the Cas9 nuclease at the appropriate stage during meiosis. A bioinformatic pipeline was developed to identify X-specific sequences for shredding (Papathanos and Windbichler, 2018). Single cell RNA sequencing could enable discovery of appropriate promoters [reviewed in Compton et al. (2020)].

DISCUSSION: INSECT SEX RATIO DISTORTION IN THE ANTHROPOCENE

We discuss insect SRDs from three main perspectives in the context of the Anthropocene. First, we consider whether or not climate change may impact the sex ratio of wild insect populations. It is not yet clear whether climate change will introduce instability to the otherwise relatively stable sex-determination pathways in some insects. It is also not clear how climate change may affect the naturally occurring SRDs including those mediated by endosymbiotic bacteria. However, sex-biased heat-tolerance has been shown in diverse insects which may lead to shifting sex ratios in response to climate change (Edmands, 2021). Temperature can also affect female fertilization and alter the sex ratio in Hymenoptera parasitic wasps (Moiroux et al., 2014). One study demonstrated that longer drought periods caused by a delay in the timing of summer rainfall in the Mediterranean region led to a female sex ratio bias in the acorn weevil Bonal et al., 2015). Endosymbiotic manipulation of sex ratio in Pectothrips kellyanus Thrips is influenced by abiotic factors such as temperature (Katlav et al., 2022). It has also been suggested that temperature may differentially impact male and female body size, mortality, protandry, and population-level sex ratios of wild bees (Slominski and Burkle, 2019).

Second, engineered SRDs have shown great promise in controlling insect pests of agricultural and medical importance as discussed in previous sections. These efforts are critical to help sustain agriculture and improve human health. However, a
less discussed but perhaps equally important application of SRDs relates to conservation of wildlife or endangered species that are under the threat of insect-borne infectious diseases (National Academies of Sciences [NAS] et al., 2016). For example, only 20 of the 46 recorded forest bird species in Hawaii are still extant in the wild (Fortini et al., 2015). Many of these species are threatened by avian malaria, transmitted by the *Culex quinquefasciatus* mosquito. The presence of avian malaria constrains these birds to a narrow range of habitats that are unsuitable for the mosquito (Samuel et al., 2011). Climate change is expected to broaden the distribution of *C. quinquefasciatus*, thereby further shrink the habitat range for susceptible bird species (Benning et al., 2002; Ahumada et al., 2009; Samuel et al., 2011; Fortini et al., 2015). Integrating SRD-mediated mosquito population suppression strategies into the long-term conservation efforts would allow Hawaiian forest birds to reclaim lost habitat. New genomic resources1 and newly established genome-editing methods (Feng et al., 2021; Purusothaman et al., 2021) for *C. quinquefasciatus* provide the foundation for SRD development.

Finally, the potential environmental impacts of the engineered SRD control strategies should be critically evaluated in the context of current methods. Current insect control methods rely heavily on insecticides and increasing resistance has significantly reduced their effectiveness. Some widely used insecticides such as neonicotinoids, which were previously thought to be safe and highly target-specific (reviewed in Jeschke and Nauen, 2008), have been shown to have potential health risks (Thompson et al., 2020), can impact non-target invertebrates and insectivorous birds (Hallmann et al., 2014; Pisa et al., 2015), and potentially contribute to the decline of honeybees (Rundlöf et al., 2015). Genetic control strategies are species-specific as they all require mating and thus a direct impact on non-target species is not anticipated. However, some of the SRD-mediated methods, especially the ones involving gene drives, are very powerful and could potentially eliminate a pest species from a large region, which may have ecological consequences. There are ongoing efforts to fine-tune, confine or neutralize the power of some of the gene drive systems (Vella et al., 2017; Kandul et al., 2019; Noble et al., 2019; Li et al., 2020, 2021; Taxiarchi et al., 2021). It is unlikely that any one control measure will be the "silver bullet." Integration or selective implementation of a set of control measures most appropriate to the specific goals and the social and environmental context will likely be most effective. Therefore, continued development of a diverse range of genetic control methods for insect pests are important for promoting sustainable agriculture, improving One Health, and preserving wildlife.

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AC and ZT wrote and revised the manuscript. Both authors contributed to the article and approved the submitted version.

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1 https://www.ncbi.nlm.nih.gov/assembly/GCF_015732765.1

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