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REVIEW

Understanding the genetics of sex determination in insects and its relevance to genetic pest management

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Abstract

Sex determination pathways regulate male and female-specific development and differentiation and offer potential targets for genetic pest management methods. Insect sex determination pathways are comprised of primary signals, relay genes and terminal genes. Primary signals of coleopteran, dipteran, hymenopteran and lepidopteran species are highly diverse and regulate the sex-specific splicing of relay genes based on the primary signal dosage, amino acid composition or the interaction with paternally inherited genes. In coleopterans, hymenopterans and some dipterans, relay genes are *Transformer* orthologs from the serine-arginine protein family that regulate sex-specific splicing of the terminal genes. Alternative genes regulate the splicing of the terminal genes in dipterans that lack *Transformer* orthologs and lepidopterans. *Doublesex* and *Fruitless* orthologs are the terminal genes. *Doublesex* and *Fruitless* orthologs are highly conserved zinc-finger proteins that regulate the expression of downstream proteins influencing physical traits and courtship behaviours in a sex-specific manner. Genetic pest management methods can use different mechanisms to exploit or disrupt female-specific regions of different sex determination genes. Female-specific regions of sex determination genes can be exploited to produce a lethal gene only in females or disrupted to impede female development or fertility. Reducing the number of fertile females in pest populations creates a male-biased sex ratio and eventually leads to the local elimination of the pest population. Knowledge on the genetic basis of sex determination is important to enable these sex determination pathways to be exploited for genetic pest management.

KEYWORDS
complementary sex determiner, femaleless, masculinizer, sex-lethal, wasp overruler of masculinization

INTRODUCTION

Insect sex determination pathways comprise three main components: primary signal genes or mRNA, relay genes and terminal genes (Sawanth et al., 2016). Primary signal genes regulate the alternative splicing of relay gene transcripts to produce functional or non-functional proteins and vary amongst insect orders (Graham et al., 2003; Sawanth et al., 2016). Relay genes are mediators of the insect sex determination pathway that regulate the splicing of the downstream terminal gene transcripts based on the primary signal present (Verhulst, van de Zande, & Beukeboom, 2010). Terminal genes regulate the expression of downstream genes responsible for

sexually dimorphic physical and reproductive traits (Verhulst & van de Zande, 2015). Knowledge of the primary signals, relay genes and terminal genes is still lacking for many insect groups. Detailed knowledge of insect sex determination pathways for model organisms can provide insights into these pathways for insects where this knowledge is limited and identify areas where more research is required.

Genetic pest management methods can exploit or disrupt sex determination genes to suppress insect pest populations (Compton & Tu, 2022; Siddall et al., 2022; Yan et al., 2023). Transgenic constructs facilitating genetic pest management can be introduced into insect pest populations through intraspecific mating between wild and gene-edited populations. To reduce female viability or fertility, transgenic constructs can exploit the female-specific splicing of sex determination genes to produce a lethal gene in females only (Ant et al., 2012; Jin et al., 2013; Spinner et al., 2022) or express an exogenous nuclease that disrupts the female-specific regions of sex determination genes (Apte et al., 2024; Kandul et al., 2022; Kyrou et al., 2018; Meccariello et al., 2024; Simoni et al., 2020; Smidler et al., 2023; Yadav et al., 2023). Reducing the number of viable, fertile female offspring creates a male-biased sex ratio and reduces the total number of offspring produced in pest populations (Burt, 2003; Hamilton, 1967). Pest populations can be transiently suppressed by transgenic constructs inherited in a Mendelian manner and eliminated by transgenic constructs that bias their transmission to achieve non-Mendelian inheritance (Alphey & Bonsall, 2018; Grilli et al., 2021; Karmakar et al., 2022; Siddall et al., 2022). To develop effective pest management methods, it is essential to understand the molecular mechanisms underlying sex determination pathways, as this knowledge allows for targeted suppression of pest populations by disrupting specific sex determination genes.

This review discusses the different primary signals, relay genes and terminal genes in the sex determination pathways of selected dipteran, coleopteran, hymenopteran and lepidopteran species. Characteristics of the sex determination pathway components described include the protein domains, protein–protein interactions and sex-specific alternative splicing. The review considers how genetic pest management methods that aim to disrupt or exploit sex determination genes can be used to suppress insect pest populations.

INSECT SEX DETERMINATION PATHWAYS

Sex determination in insects is governed by diverse molecular mechanisms (Blackman, 1995; Shukla & Nagaraju, 2010; Verhulst, van de Zande, & Beukeboom, 2010). In sexually dimorphic species, a zygote's sexual fate is determined by genes located on single or multiple autosomes or sex chromosomes (Blackman, 1995). Insect sex determination pathways are strictly regulated and centred around three components: the primary signal (e.g. maternally deposited *Transformer* mRNA), relay gene (e.g. *Transformer* and *Transformer* orthologs) and terminal gene (e.g. *Doublesex*) (Geuverink & Beukeboom, 2014; Verhulst & van de Zande, 2015; Verhulst, van de Zande, & Beukeboom, 2010).

Primary signals

Primary signals are the most diverse components of the insect sex determination pathway (Geuverink & Beukeboom, 2014). Primary signals induce sex-specific splicing of *Transformer* orthologs in insect orders with different mechanisms (Avila & Erickson, 2007; Beye et al., 2003; Dübendorfer & Hediger, 1998; Erickson & Quintero, 2007; Gempe et al., 2009; Sawanth et al., 2016) (Figure 1). The diversity of primary signals in the insect sex determination pathway is partially illustrated in *Drosophila melanogaster* Meigen (Diptera, Drosophilidae), *Musca domestica* Linnaeus (Diptera, Muscidae), *Anopheles gambiae* Giles (Diptera, Culicidae), *Tribolium castaneum* Herbst (Coleoptera, Tenebrionidae), *Apis mellifera* Linnaeus (Hymenoptera, Apidae), *Nasonia vitripennis* Walker (Hymenoptera, Pteromalidae) and *Bombyx mori* Linnaeus (Lepidoptera, Bombycidae) that are discussed below.

Primary signals directly influence the sex-specific splicing of *Transformer* orthologs in non-Drosophilidae and coleopteran species and indirectly influence the sex-specific splicing of *Sex-lethal* mRNA in Drosophilidae species (Avila & Erickson, 2007; Dübendorfer & Hediger, 1998; Erickson & Quintero, 2007; Hediger et al., 2010; Shukla & Palli, 2012b) (Figure 1). In Drosophilidae, such as *D. melanogaster*, X-linked signal elements induce the expression of female-specific *Sex-lethal* mRNA that produces a functional *Sex-lethal* protein, which subsequently regulates sex-specific splicing of the *Transformer* orthologs (Avila & Erickson, 2007; Erickson & Quintero, 2007). In non-Drosophilidae females, such as *M. domestica*, and coleopterans, such as *T. castaneum*, maternally deposited *Transformer* mRNA encodes a functional Transformer protein (Dübendorfer & Hediger, 1998; Hediger et al., 2010; Rohlfing et al., 2023; Shukla & Palli, 2012b). This functional Transformer protein regulates alternative splicing of embryonic *Transformer* pre-mRNA, resulting in female offspring (Dübendorfer & Hediger, 1998; Shukla & Palli, 2012b). In female embryos, functional female-specific Transformer proteins autoregulate sex-specific splicing of embryonic *Transformer* pre-mRNA (Dübendorfer & Hediger, 1998; Gabrieli et al., 2010; Morrow et al., 2014; Shukla & Palli, 2012b).

The dosage of the primary signals can influence the activity of the Sex-lethal protein in Drosophilidae species (Avila & Erickson, 2007; Erickson & Quintero, 2007) (Figure 1). In Drosophilidae females, such as *D. melanogaster*, the expression of *Sex-lethal* is regulated by an "early" and "late promoter" in the early and later stages of embryonic development (Avila & Erickson, 2007). During early development in Drosophilidae females, the dosage of X-linked signal elements induces expression of *Sex-lethal* from the "early promoter" and subsequent female-specific splicing of the *Sex-lethal* transcripts (Avila & Erickson, 2007; Erickson & Quintero, 2007). The functional "early promoter" Sex-lethal protein forces the use of a suboptimal nucleotide sequence regulating alternative splicing and prevents the incorporation of an exon section coding a premature stop codon into the *Sex-lethal* mRNA expressed from the "late promoter" in females (Bell et al., 1991;Boggs et al., 1987; Inoue et al., 1990). Females produce functional "late promoter" Sex-lethal proteins, which regulate

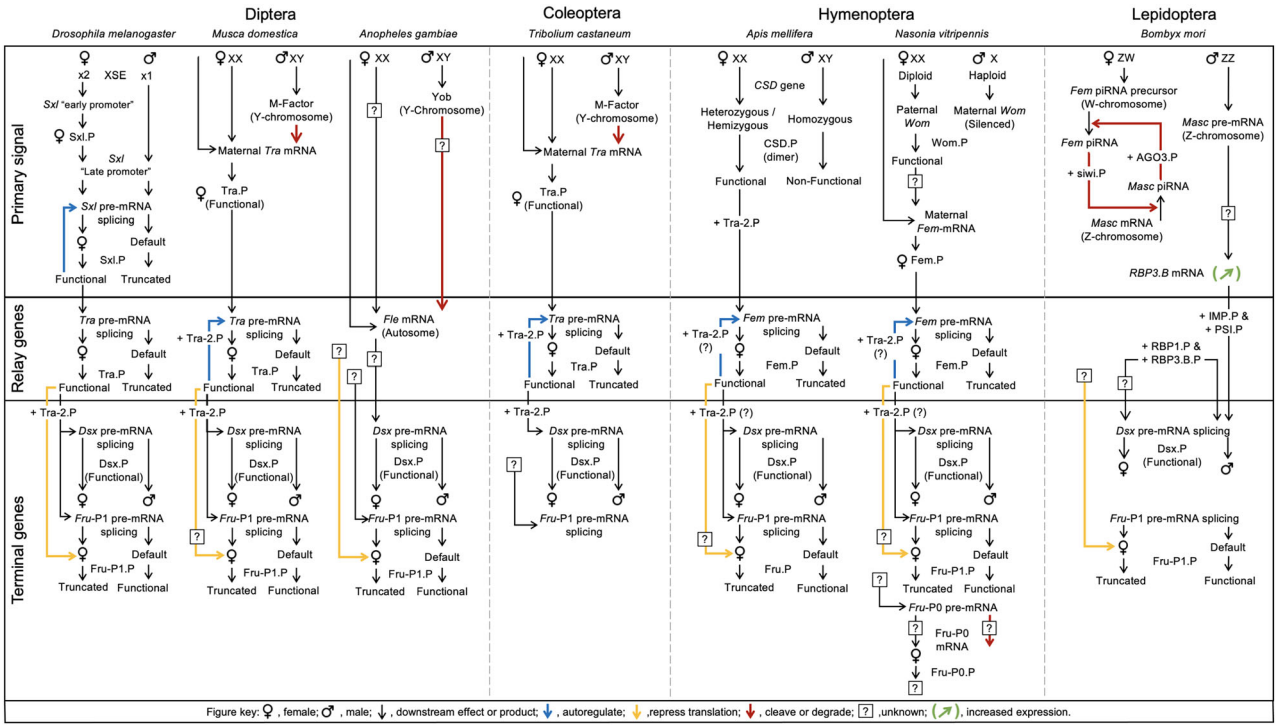

FIGURE 1 Sex determination pathway of the dipteran species *Drosophila melanogaster*, *Musca domestica and Anopheles gambiae*; the coleopteran species *Tribolium castaneum*, the hymenopteran species *Apis mellifera* and *Nasonia vitripennis*; and the lepidopteran species *Bombyx mori*. In dipteran, coleopteran and hymenopteran females, the primary signals, functional Sex-lethal ortholog, Transformer ortholog or Transformer paralog proteins, regulate female-specific splicing of *Transformer* ortholog pre-mRNA, the relay genes. Functional Transformer ortholog proteins in dipteran, coleopteran and hymenopteran females regulate sex-specific splicing of *Doublesex* pre-mRNA and *Fruitless*-P1 mRNA to produce a female-specific Doublesex isoform and a non-functional Fruitless-P1 protein. In dipteran, except for mosquitos, coleopteran and hymenopteran males, functional Transformer ortholog or Transformer paralog proteins are not produced. Resulting in the default splicing of *Transformer* ortholog pre-mRNA, *Doublesex* pre-mRNA and *Fruitless*-P1 pre-mRNA in dipteran, coleopteran and hymenopteran males. Males of dipteran, coleopteran and hymenopteran species produce non-functional Transformer ortholog proteins, male-specific Doublesex isoforms and functional Fruitless-P1 proteins. The sex determination pathway of *An. gambiae* is mostly unknown. The Yob male-determining factor and potential primary signal *Femaleless* mRNA were identified, but the exact interaction of the components remains to be determined. Unknown relay genes ensure male and female-specific Doublesex proteins are produced in *An. gambiae* males and females. Additionally, unknown factors regulate the production of functional and non-functional Fruitless-P1 proteins in *An. gambiae* males and females. Contrastingly, in the lepidopteran *B. mori*, a Masculinizer protein increases the expression of an RNA-binding protein that interacts with other RNA-binding proteins to regulate male-specific splicing of *Doublesex* pre-mRNA. In *B. mori* females, a female-specific piRNA inhibits the production of Masculinizer proteins through an RNA-silencing feedback loop. AGO3.P, Argonaute-3 protein; *CSD* gene, *Complementary sex determiner* gene; CSD.P, Complementary sex determiner protein; *Dsx*, *Doublesex*; *Fem*, *Feminizer*; Fem.P, Feminizer protein; *Fru*-P0, *Fruitless* derived from promotor 0; Fru-P0.P, Fruitless protein derived from the promotor 0; *Fru*-P1, *Fruitless* derived from promotor 1; Fru-P1.P, Fruitless protein derived from promotor 1; IMP.P, Insulin-like growth factor mRNA-binding protein; *Masc*, *Masculinizer*; PSI.P, P-element somatic inhibitor protein; RBP1.P, RNA-binding protein 1 protein; *RBP3.B*, *RNA-binding protein 3.B*; RBP3.B.P, RNA-binding protein 3.B protein; siwi.P, silkworm PIWI protein; *Sxl*, *Sex-lethal*; Sxl.P, Sex-lethal protein; *Tra*, *Transformer*; Tra.P, Transformer protein; Tra-2.P, Transformer-2 protein; *Wom*, *Wasp overruler of masculinization*; Wom.P, Wasp overruler of masculinization protein; XSE, X-linked signal elements.

sex-specific splicing of all subsequent *Sex-lethal* pre-mRNA. Functional "late promoter" Sex-lethal protein prevents the incorporation of an exon section with a premature stop codon into the *Transformer* mRNA during alternative splicing. Ensuring females produce functional Transformer proteins (Boggs et al., 1987; Inoue et al., 1990). In Drosophilidae males, *Sex-lethal* is only expressed from the "late promoter" as males contain one X-chromosomes and express one "dose" of X-linked signal elements (Avila & Erickson, 2007; Erickson & Quintero, 2007). *Sex-lethal* pre-mRNA in males is spliced in a default manner, and males produce a truncated, non-functional Sex-lethal protein.

The effect of the primary signal in the hymenopteran *A. mellifera* depends on the amino acid composition (Beye et al., 2003; Gempe et al., 2009) (Figure 1). In *A. mellifera*, the *Transformer* paralog, also known as the *Complementary sex determiner*, regulates alternative splicing of the *Transformer* ortholog, also known as *Feminizer* (Beye et al., 2003; Gempe et al., 2009). In *A. mellifera* females, two Complementary sex determiner proteins with different amino acid sequences bind together to produce a functional Complementary sex determiner protein complex (Beukeboom & Perrin, 2014; Beye et al., 2003; Gempe et al., 2009). Complementary sex determiner proteins of *A. mellifera* males contain the same sequence of amino acids and

TABLE 1 List of *Transformer* orthologs and paralogs involved in sex determination pathways of the dipterans *Drosophila melanogaster* Meigen (Drosophilidae), *Musca domestica* Linnaeus (Muscidae), *Anopheles gambiae* Giles (Culicidae); the coleopteran *Tribolium castaneum* Herbst (Tenebrionidae); the hymenopterans *Apis mellifera* Linnaeus (Apidae) and *Nasonia vitripennis* Walker (Pteromalidae); and the lepidopteran *Bombyx mori* Linnaeus (Bombycidae).

	Transformer	
Species	**Ortholog**	**Paralog**
Diptera		
D. melanogaster (Drosophilidae)	*Transformer* (*DmTra*)	-[b]
M. domestica (Muscidae)	*Transformer* (*MdTra*)	-
An. gambiae (Culicidae)	-	-
Coleoptera		
T. castaneum (Tenebrionidae)	*Transformer* (*TcTra*)	-
Hymenoptera[a]		
A. mellifera (Apidae)	*Feminizer* (*AmFem*)	*Complementary sex determiner* (*AmCSD*)
N. vitripennis (Pteromalidae)	*Feminizer* (*NvFem*)	-

[a]*Feminizer* in hymenopterans was recognised as a *Transformer* homologue based on the function and organisation of the protein domains. The low sequence similarity between dipteran *Transformer* orthologs and hymenopteran *Feminizer* orthologs was attributed to the quick evolution of the transformer gene.
[b]The absence of *Transformer* orthologs or paralogs involved in the sex determination pathway is indicated with "-".

produce a non-functional Complementary sex determiner protein complex (Beye et al., 2003; Gempe et al., 2009).

Products from paternal genes can suppress or enable the activity of maternally deposited *Transformer* mRNA in some dipteran, coleopteran and hymenopteran species (Geuverink et al., 2017; Hasselmann et al., 2008; Schmidt et al., 1997; Shukla & Palli, 2012b; Zou et al., 2020) (Figure 1). Maternally deposited *Transformer* mRNAs are primary signals in *M. domestica* (Dübendorfer & Hediger, 1998; Hediger et al., 2010) and *T. castaneum* (Shukla & Palli, 2012b). In *M. domestica* males, the product of a Y-linked gene inhibits the activity of the maternally deposited *Transformer* mRNA, and the resulting Transformer protein is non-functional (Hediger et al., 2010; Schmidt et al., 1997). The Y-chromosome of *T. castaneum* was predicted to contain a male-determining factor (Shukla & Palli, 2012b). In *N. vitripennis* females, maternally deposited *Feminizer* mRNAs and the paternally inherited *Wasp overruler of masculinization* genes are the primary signals (Verhulst, Beukeboom, & van de Zande, 2010; Zou et al., 2020) (Table 1). Maternally deposited *Feminizer* mRNA may be necessary to ensure female-specific splicing of embryonic *Feminizer* pre-mRNA (Verhulst, Beukeboom, & van de Zande, 2010). The maternally inherited *Wasp overruler of masculinization* gene is not transcribed due to the gene being silenced (Zou et al., 2020). This supports predicted models for *N. vitripennis* sex determination where proteins produced from the paternally inherited DNA induce the transcription of maternally inherited *Transformer* mRNA (Geuverink et al., 2017; van de Zande & Verhulst, 2014; Zou et al., 2020). The exact function of the Wasp overruler of masculinization protein produced from the paternally inherited DNA has not been determined (Geuverink et al., 2017; Zou et al., 2020).

In the lepidopteran *B. mori*, the PIWI interfering RNA (piRNA), known as *Feminizer* piRNA, is the primary signal (Kiuchi et al., 2014) (Figure 1). This contrasts with dipteran, coleopteran and hymenopteran females, where the primary signals encode functional proteins that bind to embryonic *Transformer* and *Feminizer* transcripts, respectively, and regulate sex-specific splicing (Geuverink & Beukeboom, 2014; Shukla & Palli, 2012b; Verhulst & van de Zande, 2015; Verhulst, van de Zande, & Beukeboom, 2010). Lepidopterans have a ZZ/WZ sex determination pathway where males are the homozygous sex (ZZ) and females are the heterozygous sex (WZ) (Beukeboom & Perrin, 2014; Ennis, 1979).

The primary signals of *B. mori* regulate the sex determination pathway through an RNA-silencing feedback loop (Kiuchi et al., 2014) (Figure 1). From the Z-chromosome of *B. mori*, a sex determination gene, known as *Masculinizer*, is expressed in females and males (Kiuchi et al., 2014). Masculinizer proteins are necessary to produce male-specific Doublesex proteins in *B. mori* (Kiuchi et al., 2014) and other lepidopterans (Bi et al., 2022; Lee et al., 2015; Visser et al., 2021). Whether Masculinizer proteins directly or indirectly affect the male-specific Doublesex proteins has not been determined (Bi et al., 2022; Visser et al., 2021). In *B. mori* females, the *Feminizer* piRNA is expressed from multiple copies of the *Feminizer* piRNA sequence on the W-chromosome (Kiuchi et al., 2014). A section of the *Feminizer* piRNA in *B. mori* is complementary to a section of the *Masculinizer* mRNA (Kiuchi et al., 2014). The *Feminizer* piRNA complexes with the silkworm PIWI protein, known as SIWI (Kawaoka et al., 2008; Kiuchi et al., 2014). The *Feminizer*-SIWI complex identifies and cleaves the *Masculinizer* mRNA in *B. mori* (Kiuchi et al., 2014). The cleaved *Masculinizer* mRNA is processed into *Masculinizer* piRNA (Kiuchi et al., 2014). The *Masculinizer* piRNA complexes with the Argonaute-3 (AGO3) protein (Kawaoka et al., 2008; Kiuchi et al., 2014). The *Masculinizer*-AGO3 complex identifies and cleaves unprocessed *Feminizer* transcripts (Kiuchi et al., 2014). The *Feminizer* piRNA sequences were sometimes located directly next to each other, which possibly caused multiple *Feminizer* piRNAs to be expressed in a single transcript (Kiuchi et al., 2014). The unprocessed transcripts with multiple *Feminizer* piRNAs would be processed into a single *Feminizer* piRNA, and the cycle begins again.

The *Masculinizer* gene is a possible relay gene in the sex determination pathway of lepidopteran species (Visser et al., 2021; Yang et al., 2021). Lepidopteran males lacking functional Masculinizer proteins produce female-specific *Doublesex* mRNA or are non-viable (Bi et al., 2022; Kiuchi et al., 2014; Lee et al., 2015; Visser et al., 2021). Masculinizer proteins commonly contain two zinc-finger motifs, two proline-rich domains, a masculinising domain and a nuclear localisation signal (Kiuchi et al., 2014; Visser et al., 2021). Zinc-finger domains are

involved in the DNA and RNA binding, and proline-rich domains are involved in protein–protein interaction (Kiuchi et al., 2014; Visser et al., 2021; Williamson, 1994). The presence of these domains has led to speculations that Masculinizer proteins are involved in regulating the lepidopteran sex determination genes at the transcriptional level. However, the expression of Masculinizer proteins lacking the zinc-finger domains does not affect the production of male-specific *Doublesex* mRNA or male viability (Kiuchi et al., 2019). The proline-rich and masculinizing domains of the Masculinizer proteins are highly conserved and may be responsible for influencing the alternative splicing of downstream genes (Visser et al., 2021). The exact mechanisms used by the Masculinizer proteins to influence the male and female-specific splicing of *Doublesex* mRNA are unknown, and the identity of the *Masculinizer* gene as the relay gene has not been confirmed (Yang et al., 2021).

Relay genes

Relay genes, also known as transducers, key genes and switch genes, regulate the splicing of the downstream terminal gene (Verhulst, van de Zande, & Beukeboom, 2010) (Table 1). *Transformer* and *Transformer* orthologs are relay genes that communicate the sexual fate of insects directed by primary signals to the downstream terminal gene of the sex determination pathway (Sawanth et al., 2016; Verhulst, van de Zande, & Beukeboom, 2010) (Figure 1). *Transformer* genes are less conserved than the terminal genes *Doublesex* and *Fruitless* (Geuverink & Beukeboom, 2014). Relay genes have been identified in most insect orders, with the exception of Lepidoptera (Sawanth et al., 2016; Verhulst, van de Zande, & Beukeboom, 2010; Visser et al., 2021; Yang et al., 2021). The *Transformer* ortholog, known as the *Feminizer*, was recognised as the relay gene in Hymenoptera based on the similar function and organisation of the protein domains (Hasselmann et al., 2008). In several dipteran and coleopteran species, sex-specific splicing of *Transformer* pre-mRNA results in males and females producing non-functional and functional Transformer proteins, respectively (Avila & Erickson, 2007; Dübendorfer & Hediger, 1998; Erickson & Quintero, 2007; Sawanth et al., 2016; Shukla & Palli, 2012b). In several hymenopteran species, males produce non-functional Feminizer proteins and females produce functional Feminizer proteins due to sex-specific splicing of *Feminizer* pre-mRNA (Beye et al., 2003; Gempe et al., 2009; Sawanth et al., 2016).

Transformer genes have a moderately conserved evolution compared to other components of the sex determination pathway (Geuverink & Beukeboom, 2014). DNA sequences of *Transformer* orthologs, including the functional domains, are poorly conserved between and within insect orders (Laslo et al., 2022). Amino acid sequences of Transformer proteins between *Anastrepha* species (Diptera, Tephritidae) indicated an amino acid similarity above 85% (Ruiz et al., 2007). Transformer proteins of *Anastrepha* species have 54%–56% similarity to the amino acid sequence of Transformer proteins in species from the same family; *Ceratitis capitata* Wiedemann (Diptera, Tephritidae) and *Bactrocera oleae* Rossi (Diptera, Tephritidae)

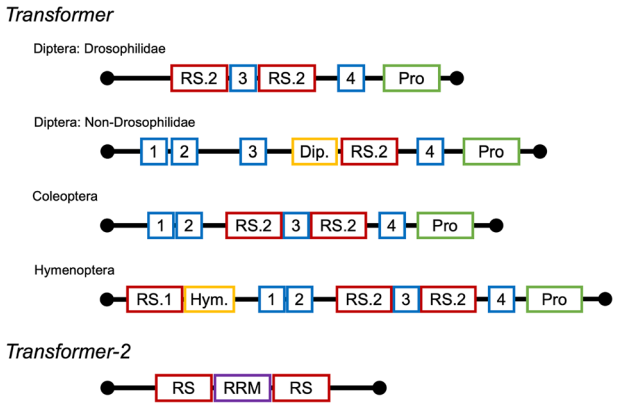

FIGURE 2 Genomic architecture of *Transformer* and *Transformer-2* genes. The number and position of autoregulation domains and arginine/serine-rich domains differ between dipteran, coleopteran and hymenopteran species (Adapted from Geuverink and Beukeboom (2014) and Nguantad et al. (2020)). A, autoregulation domain; Dip., dipteran specific domain; Hym., hymenopteran specific domain; Pro, proline-rich domain; RRM, RNA-recognition motif; RS.1/2, arginine/serine-rich domain.

(Ruiz et al., 2007). This has prevented the identification of *Transformer* orthologs from extensively researched lineages with excellent genomic resources (Laslo et al., 2022). Due to the high nucleotide sequence diversity, the *Feminizer* gene of Hymenoptera was characterised based on the organisation of protein functional domains and the protein function (Hasselmann et al., 2008).

Relay gene paralogs of the insect sex determination pathway evolved independently (Beye et al., 2003; Hasselmann et al., 2008; Traut et al., 2006). *Feminizer* paralogs, such as the *Complementary sex determiner* in Hymenoptera, have been co-opted into the sex determination pathway (Beye et al., 2003; Hasselmann et al., 2008) (Table 1). In Hymenoptera, the *Complementary sex determiner* gene arose from a recent duplication event and was co-opted as the primary signal of the sex determination pathway (Beye et al., 2003; Hasselmann et al., 2008). *Feminizer* duplications in Hymenoptera also occurred independently of the *Complementary sex determiner* gene (Jia et al., 2016). In Hymenoptera, *Feminizers* have been completely and partially duplicated several times independently in various extant species and ancestral species (Jia et al., 2016). DNA sequence analysis of partial *Feminizer* paralogs suggests the functional domains are under positive selection, indicating the genes are being co-opted for an unknown function (Jia et al., 2016).

Transformers are from the serine-arginine protein family, which regulates RNA-splicing (Fredericks et al., 2015; Howard & Sanford, 2015; Jeong, 2017) (Figure 2). Serine-arginine proteins influence RNA-splicing by promoting or stabilising the binding of general alternative splicing proteins (Fredericks et al., 2015; Howard & Sanford, 2015; Jeong, 2017). Serine-arginine proteins contain N-terminal domains that facilitate protein–protein interactions and the C-terminal RNA-recognition motifs that bind to specific RNA sequences (Crispino et al., 1994; Jeong, 2017; Wahl et al., 2009; Zahler et al., 1993). In dipterans and coleopterans, Transformers lack

RNA-recognition motifs and are unable to interact with RNA (Belote et al., 1989; Boggs et al., 1987; Gotoh, Zinna, et al., 2016; Hediger et al., 2010; Inoue et al., 1992; Nguantad et al., 2020; Shukla & Palli, 2012b). Transformer-2 is a serine-arginine protein that contains the RNA-binding motif and can bind to mRNA (Amrein et al., 1988; Burghardt et al., 2005; Goralski et al., 1989; Shukla & Palli, 2013) (Figure 2). Transformer and Transformer-2 promote the formation of a splicing complex (Burghardt et al., 2005; Hoshijima et al., 1991; Inoue et al., 1992; Shukla & Palli, 2013; Tian & Maniatis, 1993). Hymenopteran Feminizer proteins belong to the serine-arginine protein family and are suspected to complex with Transformer-2 proteins (Biewer et al., 2015; Geuverink et al., 2017; Nissen et al., 2012; Verhulst, Beukeboom, & van de Zande, 2010).

Sex-specific splicing of *Transformer* or *Feminizer* mRNA in males and females leads to the production of non-functional and functional Transformer or Feminizer proteins, respectively (Boggs et al., 1987; Inoue et al., 1990; Zou et al., 2020). In dipteran and coleopteran males, an exon containing a premature, in-frame stop codon is incorporated into *Transformer* mature mRNA during splicing (Inoue et al., 1990; Shukla & Palli, 2012b). Male-specific *Transformer* mRNA encodes a non-functional, truncated Transformer protein (Inoue et al., 1990; Shukla & Palli, 2012b). In females, the exon containing the premature, in-frame stop codon is excluded from *Transformer* pre-mRNA during alternative splicing (Gabrieli et al., 2010; Inoue et al., 1990; Morrow et al., 2014). Female-specific *Transformer* mRNA encodes a functional Transformer protein (Gabrieli et al., 2010; Inoue et al., 1990; Morrow et al., 2014). In hymenopterans, exons containing premature, in-frame stop codons are incorporated and excluded from *Feminizer* mature mRNA in males and females, respectively (Gempe et al., 2009; Hasselmann et al., 2008; Verhulst, Beukeboom, & van de Zande, 2010), resulting in the production of truncated, non-functional Feminizer proteins in males and functional Feminizer proteins in females.

Sex-specific splicing of *Transformer* orthologs or *Sex-lethal* is autoregulated to ensure the continued production of sex-specific Transformer proteins (Nguantad et al., 2020; Rohlfing et al., 2023; Sawanth et al., 2016; Tanaka et al., 2018). During autoregulation, a protein regulates the splicing of its mRNA (Calhoun & Hatfield, 1975). Excluding the family Drosophilidae, sex-specific splicing of *Transformer* ortholog mRNA is regulated by the sex-specific Transformer proteins in dipterans and coleopterans (Nguantad et al., 2020; Sawanth et al., 2016). Similarly, sex-specific Feminizer proteins were speculated to regulate the sex-specific splicing of *Feminizer* pre-mRNA in hymenopterans (Gempe et al., 2009; Verhulst, Beukeboom, & van de Zande, 2010). In most insect orders, except for the Drosophilidae family, autoregulation of sex-specific splicing of *Transformer* orthologs and *Feminizer* orthologs is facilitated by four small, scattered domains (Geuverink & Beukeboom, 2014; Nguantad et al., 2020; Sawanth et al., 2016; Tanaka et al., 2018; Verhulst, van de Zande, & Beukeboom, 2010). In Drosophilidae, *Transformer* orthologs lack two of the small, scattered domains that facilitate *Transformer* autoregulation (Tanaka et al., 2018; Verhulst, van de Zande, & Beukeboom, 2010). Sex-lethal regulates sex-specific splicing of the *Transformer* ortholog and sex-specific splicing of *Sex-lethal* transcripts (Bell et al., 1991; Inoue et al., 1990).

Transformer proteins regulate male or female-specific splicing of *Doublesex* pre-mRNA in *D. melanogaster* (Hediger et al., 2004; Hoshijima et al., 1991) (Figure 1) (Table 1). These serine-arginine proteins regulate sex-specific splicing by promoting or stabilising the binding of general alternative splicing proteins (Jeong, 2017; Ryner & Baker, 1991; Tacke & Manle, 1999; Verhulst, van de Zande, & Beukeboom, 2010). In females, nucleotide sequences of the male-specific or female-specific *Doublesex* exon and flanking introns that regulate alternative splicing are recognised by the Transformer and Transformer-2 (TRA/TRA2) complex (Hedley & Maniatis, 1991; Hoshijima et al., 1991; Ryner & Baker, 1991; Tacke & Manle, 1999; Verhulst, van de Zande, & Beukeboom, 2010). The TRA/TRA2 complex binds to the *Doublesex* transcripts and regulates alternative splicing to promote the incorporation of female-specific *Doublesex* exons or remove the male-specific *Doublesex* exons. This produces female-specific *Doublesex* transcripts and results in the production of female-specific Doublesex proteins. In males, the lack of functional Transformer proteins results in the default splicing of *Doublesex* transcripts and the production of male-specific Doublesex proteins (Hedley & Maniatis, 1991; Hoshijima et al., 1991; Ryner & Baker, 1991; Shukla & Nagaraju, 2010; Tacke & Manle, 1999; Tian & Maniatis, 1992).

The TRA/TRA2 complex and, potentially, the Feminizer and Transformer-2 complex regulate the sex-specific splicing of *Doublesex* pre-mRNA in other dipteran and coleopteran species (Hediger et al., 2004; Hoshijima et al., 1991; Shukla & Palli, 2012a), and hymenopteran species (Gempe et al., 2009; Oliveira et al., 2009; Verhulst, Beukeboom, & van de Zande, 2010) (Figure 1) (Table 1). In coleopterans and dipterans, except for mosquitos, the TRA/TRA2 complex regulates the sex-specific splicing of *Doublesex* pre-mRNA to ensure females produce female-specific Doublesex proteins (Concha et al., 2010; Hediger et al., 2004; Shukla & Nagaraju, 2010; Shukla & Palli, 2012a). In hymenopteran females, Feminizer and Transformer-2 are required to produce female-specific *Doublesex* mature mRNA; however, whether Feminizer forms a complex with Transformer-2 remains to be determined (Gempe et al., 2009; Geuverink et al., 2017; Oliveira et al., 2009; Verhulst, Beukeboom, & van de Zande, 2010). Default splicing of *Doublesex* pre-mRNA results in male-specific Doublesex proteins in dipteran, coleopteran and hymenopteran males (Concha et al., 2010; Gempe et al., 2009; Hediger et al., 2004; Oliveira et al., 2009; Shukla & Nagaraju, 2010; Shukla & Palli, 2012a; Verhulst, Beukeboom, & van de Zande, 2010).

Several factors involved in mosquito sex determination have been identified and characterised, but the complete pathway is unknown (Criscione et al., 2013; Hall et al., 2015; Krzywinska et al., 2016). Multiple, diverse male-determining genes have been identified in mosquitos, including *Yob* in *Anopheles gambiae* Giles (Diptera, Culicidae) (Krzywinska et al., 2016), *Guy1* in *Anopheles stephensi* Rothwell (Diptera, Culicidae) (Criscione et al., 2013), and *Nix* in *Aedes aegypti* Linnaeus (Diptera, Culicidae) (Hall et al., 2015), and *Aedes albopictus* Skuse (Diptera, Culicidae) (Liu et al., 2020). The male-determining

genes *Yob* and *Guy1* have unique nucleotide sequences and produce proteins with similar structures (Criscione et al., 2013; Krzywinska et al., 2016). Unlike *Yob* and *Guy1*, the male-determining gene *Nix* is a *Transformer-2* paralog (Hall et al., 2015).

Anopheles gambiae and other mosquito genomes lack the *Transformer* gene (Geuverink & Beukeboom, 2014; Krzywinska et al., 2021) (Table 1). Krzywinska et al. (2021) identified an *An. gambiae Transformer-2* paralog, termed *Femaleless*, that is highly conserved and present only in *Anopheles* species (Krzywinska et al., 2021). The Femaleless protein is necessary for female development and was predicted to bind directly to nucleotide sequences regulating the alternative splicing of *Doublesex* (Krzywinska et al., 2021). Unlike Transformer proteins, Femaleless proteins may not need to complex with Transformer-2 proteins to regulate alternative splicing (Krzywinska et al., 2021). A single splice variant of *Femaleless* is expressed by *An. gambiae* males and females (Krzywinska et al., 2021). In *An. gambiae* males, the Femaleless proteins may be inactivated by the male-determining factor Yob through an unknown mechanism (Krzywinska et al., 2016, 2021). Similarly, the *Transformer-2* paralog in *Ae. aegypti*, *Nix*, is continuously expressed and influences the splicing of the terminal gene, *Doublesex* and the development of the female reproductive organs (Liu et al., 2020).

Several proteins influencing the sex-specific splicing of *Doublesex* transcripts in the lepidopteran *B. mori* have been identified (Suzuki et al., 2008; Zheng et al., 2019). In contrast to dipteran, coleopteran and hymenopteran species, default splicing of the *Doublesex* pre-mRNA in lepidopteran species produces a female-specific *Doublesex* mRNA (Suzuki et al., 2001). Lepidopteran *Doublesex* pre-mRNA lacks TRA/TRA-2 complex binding sites (Ohbayashi et al., 2001; Suzuki et al., 2001). In *B. mori* males, the Insulin-like growth factor mRNA-binding protein binds the male-specific exon of *Doublesex* pre-mRNA, exon 4, and promotes the binding of the P-element somatic inhibitor (Suzuki et al., 2008, 2010). Additionally, RNA-binding protein 1 and RNA-binding protein 3B bind to the male-specific exons of *Doublesex* pre-mRNA, exons three and four, respectively (Zheng et al., 2019). The P-element somatic inhibitor directly interacts with RNA-binding protein 3B and the RNA-binding protein 1 to produce a male-specific *Doublesex* mRNA (Suzuki et al., 2008; Zheng et al., 2019). Masculinizer proteins directly or indirectly increase the production of RNA-binding protein 3B in *B. mori* males (Zheng et al., 2019). Additionally, the *B. mori* Zinc-finger-2 splicing factor may regulate male-specific splicing of *Doublesex* pre-mRNA directly or indirectly (Gopinath et al., 2016).

Sex-specific splicing of *Fruitless* pre-mRNA derived from the first *Fruitless* promotor is regulated by the TRA/TRA2 complex in several dipterans (Gailey et al., 2006; Heinrichs et al., 1998; Ito et al., 1996; Meier et al., 2013; Pane et al., 2005; Ryner et al., 1996). *Fruitless* genes contain multiple promoters and multiple 5′ and 3′ alternative splice sites, which enables the expression of numerous transcript variants (Heinrichs et al., 1998; Ryner et al., 1996). However, only *Fruitless* pre-mRNA derived from the first promoter (*Fruitless*-P1 pre-mRNA) is spliced in a sex-specific manner. In *D. melanogaster*, exon 2 of the *Fruitless*-P1 pre-mRNA has two nucleotide sequences that regulate alternative splicing and one nucleotide sequence that is recognised by the TRA/TRA2 complex. In females, the TRA/TRA2 complex binds to the *Fruitless*-P1 pre-mRNA near the nucleotide sequence regulating female-specific splicing and promotes the binding of the general alternative splicing proteins (Heinrichs et al., 1998; Ito et al., 1996; Ryner et al., 1996). The female-specific region of exon 2 is incorporated into the *Fruitless*-P1 mature mRNA. The female-specific *Fruitless*-P1 mature mRNA codes for two proteins with different translation start sites; however, the first protein-coding region contains an in-frame, premature stop codon (Heinrichs et al., 1998; Ryner et al., 1996). A truncated, non-functional female-specific Fruitless-P1 protein and a full-length, functional Fruitless-P1 protein are expected to be produced in females. Males lack functional Transformer proteins, and *Fruitless*-P1 pre-mRNA is spliced in a default manner. Sex-specific splicing prevents the female-specific region of exon 2 from being incorporated into the male-specific *Fruitless*-P1 mature mRNA, resulting in a functional Fruitless-P1 protein being produced in males. The TRA/TRA2 complex also regulates the alternative splicing of male-specific *Fruitless*-P1 pre-mRNA in *M. domestica* (Meier et al., 2013) and *C. capitata* (Pane et al., 2005).

Feminizer proteins may regulate the sex-specific splicing of *Fruitless*-P1 pre-mRNA in hymenopterans (Bertossa et al., 2009; Gempe et al., 2009; Hasselmann et al., 2008). Female-specific exons of *Fruitless* pre-mRNA in *N. vitripennis* and *A. mellifera* lack nucleotide sequences recognised by the TRA/TRA2 complex (Bertossa et al., 2009). Conserved repeat sequences have been identified close to or in the female-specific exons of *Doublesex* and *Fruitless* orthologs of *N. vitripennis* and *A. mellifera*. A conserved repeat sequence in the female-specific exon of *Fruitless* in *N. vitripennis* and *A. mellifera* is located at the same distance from the nucleotide sequence regulating female-specific splicing as the nucleotide sequence recognised TRA/TRA2 complex in *D. melanogaster*. Female-specific Feminizer protein in *A. mellifera* regulates sex-specific splicing of *Doublesex* pre-mRNA (Gempe et al., 2009; Hasselmann et al., 2008) (Table 1). The location of the conserved repeat sequences in the female-specific exons of *Doublesex* and *Fruitless* orthologs of *N. vitripennis* and *A. mellifera* suggests Feminizer protein orthologs may regulate female-specific splicing of *Fruitless* pre-mRNA (Bertossa et al., 2009). The alternative splicing of sex-specific exon or exon regions of *Fruitless*-P1 pre-mRNA in *N. vitripennis* and *A. mellifera* ensures males produce functional Fruitelss-P1 proteins and females produce truncated Fruitless-P1 proteins, similarly to *D. melanogaster* (Bertossa et al., 2009).

Splicing regulatory proteins specific to insect orders may regulate the sex-specific splicing of *Fruitless*-P1 pre-mRNA in insects lacking *Transformer* orthologs (Gailey et al., 2006; Krzywinska et al., 2021; Suzuki et al., 2010; Ueno et al., 2023). In *An. gambiae*, potential nucleotide sequences regulating sex-specific splicing have been identified on *Doublesex* and *Fruitless* pre-mRNA (Gailey et al., 2006; Krzywinska et al., 2021). Femaleless proteins were speculated to bind to the potential nucleotide sequences regulating sex-specific splicing. In *B. mori*, P-element somatic inhibitor complexes with RNA-binding protein 1 and RNA-binding protein 3B and the protein complexes directly bind to nucleotide sequences regulating sex-specific alternative splicing in the male-specific exons of *Doublesex* pre-mRNA (Suzuki

et al., 2010; Ueno et al., 2023). The nucleotide sequences regulating sex-specific alternative splicing of Doublesex pre-mRNA have been identified in the male-specific exon of *B. mori Fruitless*-P1 mRNA. However, direct binding of the potential, respective alternative splicing regulators to the potential splicing regulatory sequences remains to be investigated (Gailey et al., 2006; Krzywinska et al., 2021; Suzuki et al., 2010; Ueno et al., 2023). *Anopheles gambiae* and *B. mori* males and females are expected to produce functional and truncated Fruitless-P1 proteins, respectively, through the alternative-specific splicing of sex-specific exons in *Fruitless*-P1 pre-mRNA, similar to *D. melanogaster* (Gailey et al., 2006; Ueno et al., 2023).

Multiple mechanisms may act simultaneously to prevent the production of functional, female-specific Fruitless-P1 proteins (Lee et al., 2000; Usui-Aoki et al., 2000). In *D. melanogaster* females, the female-specific *Fruitless*-P1 mature mRNA was predicted to have two protein-coding regions; an upstream region coding a truncated, non-functional protein and a downstream region coding a potentially functional protein (Heinrichs et al., 1998; Ryner et al., 1996). However, previous studies found that functional, female-specific Fruitless-P1 proteins were absent from nerve tissue expressing *Fruitless*-P1 pre-mRNA in females (Lee et al., 2000; Usui-Aoki et al., 2000). In *D. melanogaster* males, *Sex-lethal* mature mRNA codes two protein-coding regions, but no functional, male-specific Sex-lethal proteins were produced (Bopp et al., 1996). The organisation of the protein-coding regions in *Fruitless* resembles that of *Sex-lethal* in *D. melanogaster* (Bopp et al., 1996; Usui-Aoki et al., 2000). Translation of the upstream protein-coding region of *Sex-lethal* mRNA inhibited the production of a functional Sex-lethal protein in males (Bopp et al., 1996). This suggests that translation of the truncated, non-functional female-specific Fruitless-P1 protein-coding region would inhibit the translation of the downstream Fruitless-P1 long protein-coding region in females (Usui-Aoki et al., 2000). Additionally, TRA/TRA2 complex bound to mRNA has been shown to inhibit translation and, as a result, may prevent the production of a protein from the long protein-coding region from the *Fruitless*-P1 mRNA in females (Usui-Aoki et al., 2000).

Terminal genes

Doublesex orthologs and *Fruitless* orthologs are terminal genes (Burtis et al., 1991; Hildreth, 1965; Panara et al., 2019; Shukla & Nagaraju, 2010) (Figure 1). These terminal genes regulate the expression of genes linked to male or female-specific phenotypic traits. *Doublesex* and *Fruitless* have been identified in many insect orders, including Diptera, Hymenoptera and Lepidoptera (Baral et al., 2019; Gailey et al., 2006; Geuverink & Beukeboom, 2014; Yang et al., 2021). Doublesex protein orthologs are highly conserved, and functional domains of Fruitless protein orthologs have a high degree of similarity in the amino acid sequence.

Doublesex orthologs

Doublesex orthologs regulate the expression of genes linked to physical traits that are unique to males or females (Burtis et al., 1991; Hildreth, 1965; Panara et al., 2019; Shukla & Nagaraju, 2010) (Figure 1). These genes are also known as the central nexus and executor and are the most conserved component of the insect sex determination pathways (Baral et al., 2019; Geuverink & Beukeboom, 2014; Graham et al., 2003). In several Diptera, Coleoptera, Hymenoptera and Lepidoptera species, *Doublesex* pre-mRNA is sex-specifically spliced to produce functional, sex-specific Doublesex proteins in males and females (Burtis & Baker, 1989; Shukla & Nagaraju, 2010).

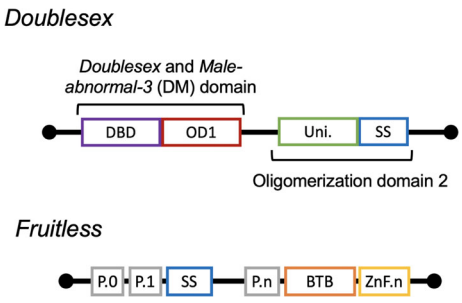

FIGURE 3 Genomic architecture of the *Doublesex* and *Fruitless* genes. The Doublesex and Male-abnormal-3 domain in the N-terminal of the *Doublesex* gene contains a DNA-binding domain (DBD) and an Oligomerization domain 1 (OD1). The Oligomerization domain 2 (OD2) in the C-terminal of the *Doublesex* gene contains a unisex and sex-specific region (Adapted from Geuverink and Beukeboom (2014)). Fruitless isoforms contain an N-terminal bric à brac, tramtrack and Broad-Complex (BTB) domain and a C-terminal zinc-finger motif that can differ between Fruitless isoforms. Male-specific Fruitless-P1 isoforms also contain a male-specific amino acid sequence upstream of the BTB domain. Similarly, female-specific Fruitless-P0 isoforms unique to *N. vitripennis* were predicted to contain amino acid sequence upstream of the BTB domain (Adapted from Bertossa et al. (2009), Salvemini et al. (2010) and Sato and Yamamoto (2020).

The functional domains of Doublesex proteins have different degrees of conservation (Baral et al., 2019; Beukeboom & Perrin, 2014; Geuverink & Beukeboom, 2014; Yang et al., 2021). In the orders Diptera, Coleoptera and Lepidoptera, the DNA-binding domain and the female-specific region of the Doublesex protein are conserved, whereas the male-specific region is evolving rapidly (Baral et al., 2019). The rapid evolution of the male-specific regions of Doublesex proteins may facilitate the development of male-specific adaptations (Baral et al., 2019). Contrastingly, in some eusocial hymenopteran species, such as *A. mellifera*, the DNA-binding domain and the male-specific region of the Doublesex protein are conserved, and the female-specific region is evolving rapidly (Baral et al., 2019). The rapid evolution of female-specific regions of Doublesex in these hymenopteran species may be attributed to the hierarchy systems of these social insects (Baral et al., 2019).

Doublesex encodes a zinc-finger protein that regulates the expression of downstream genes (Erdman & Burtis, 1993; Ohbayashi et al., 2001; Oliveira et al., 2009; Shukla & Palli, 2012a) (Figure 3). The transcription factor, Doublesex, belongs to a group of zinc-finger proteins known as Doublesex and Male-abnormal-3 Related Transcription (Dmrt) factors (Raymond et al., 1998). This group of transcription

factors is characterised by an N-terminal zinc-finger DNA-binding domain in male and female-specific Doublesex proteins (Burtis et al., 1991; Erdman & Burtis, 1993). This DNA-binding domain, known as the Doublesex and Male-abnormale-3 domain, was initially identified in the *Doublesex* and *Male-abnormal-3* genes from *D. melanogaster* and *Caenorhabditis elegans* (Chromadorea, Rhabditidae), respectively (Erdman & Burtis, 1993; Raymond et al., 1998; Shen & Hodgkin, 1988). Adjacent to Doublesex and Male-abnormale-3 domain is the Oligomerization domain 1, which facilitates the binding of two Doublesex proteins around the target sequence (An et al., 1996; Cho et al., 2007; Erdman et al., 1996; Gotoh, Ishiguro, et al., 2016; Gotoh, Zinna, et al., 2016; Hediger et al., 2004; Ito et al., 2013; Oliveira et al., 2009; Scali et al., 2005; Shukla & Palli, 2012a). Doublesex and Male-abnormal-3 Related Transcription factors contain different C-terminal domains that enable them to regulate the transcription of various genes involved in sex determination and differentiation (Burtis & Baker, 1989; Panara et al., 2019; Raymond et al., 1998).

The Oligomerization domain 2 enables Doublesex proteins to regulate the transcription of downstream genes in a sex-specific manner (Erdman et al., 1996; Panara et al., 2019) (Figure 3). The N-terminal region of the Oligomerization domain 2 is present in males and females and facilitates binding between two Doublesex proteins (Burtis et al., 1991; Erdman et al., 1996). The C-terminal region of the Oligomerization domain 2 differs between males and females (Burtis et al., 1991; Erdman et al., 1996). Sex-specific regions of the Oligomerization domain 2 are incorporated through alternative splicing of *Doublesex* pre-mRNA (Shukla & Nagaraju, 2010; Verhulst & van de Zande, 2015). Unisex and sex-specific regions of the Oligomerization domain 2 are necessary for the interaction of two Doublesex proteins and may facilitate sex-specific interaction with proteins regulating the transcription (An et al., 1996).

Doublesex proteins regulate the transcription of genes related to sex-specific traits in a sex, tissue and age-specific manner in *D. melanogaster* (Burtis et al., 1991). Male and female-specific Doublesex proteins bind to similar transcription regulatory sequences (An et al., 1996; Burtis et al., 1991; Erdman et al., 1996). Doublesex proteins may regulate the expression of downstream genes in opposite directions (Burtis et al., 1991; Verhulst & van de Zande, 2015). The expression of the yolk protein is repressed by the male-specific Doublesex isoform and is enhanced by the female-specific Doublesex isoform (Burtis et al., 1991; Hediger et al., 2004). The expression of genes linked to abdominal pigmentation is indirectly enhanced and repressed by male and female Doublesex isoforms (Kopp et al., 2000; Williams et al., 2008). The expression of downstream genes, such as the pheromone genes, may be regulated by the Doublesex isoforms of females, but do not appear to be regulated by male Doublesex isoforms (Shirangi et al., 2009).

Fruitless orthologs

Fruitless orthologs regulate the expression of genes linked to male-specific courtship behaviours and the development of nerve tissue (Billeter et al., 2006; Gailey et al., 2006; Song et al., 2002). Genes linked to different traits are regulated by different Fruitless isoforms (Anand et al., 2001; Billeter et al., 2006; Ryner et al., 1996; Song et al., 2002). Fruitless isoforms present in both males and females regulate genes linked to the development of the central nervous system (Anand et al., 2001; Dornan et al., 2005; Ryner et al., 1996; Song et al., 2002) and will not be discussed further. Sex-specific splicing only occurs in *Fruitless* pre-mRNA encoding Fruitless isoforms that regulate the expression of genes linked to male-specific courtship behaviour (Heinrichs et al., 1998; Ryner et al., 1996). Male-specific Fruitless proteins are functional (Bertossa et al., 2009; Gailey et al., 2006; Meier et al., 2013; Ryner et al., 1996; Ueno et al., 2023). In contrast, proteins coded on female-specific *Fruitless* mRNA may be truncated, functional or remain untranslated (Bertossa et al., 2009; Lee et al., 2000; Ryner et al., 1996; Usui-Aoki et al., 2000).

Fruitless encodes a highly conserved zinc-finger protein that regulates the expression of downstream genes (Heinrichs et al., 1998; Ito et al., 1996; Ryner et al., 1996) (Figure 3). The transcription regulator, Fruitless, belongs to a group of zinc-finger proteins known as the bric à brac, tramtrack and Broad-Complex (BTB) zinc-finger proteins (Zollman et al., 1994). The BTB zinc-finger proteins are characterised by an N-terminal protein–protein interaction domain that is present in sex-specific isoforms and isoforms present in males and females (Heinrichs et al., 1998; Ryner et al., 1996; Zollman et al., 1994). The protein–protein interaction domain, known as the BTB domain, was initially identified in the BTB transcription regulators in *D. melanogaster* (Zollman et al., 1994). The C-terminal of the individual BTB zinc-finger proteins may contain different DNA-binding zinc-finger domains that recognise different nucleotide sequences (Heinrichs et al., 1998; Ryner et al., 1996; Zollman et al., 1994). The BTB domains and shared zinc-finger domains in Fruitless proteins from dipteran, hymenopteran, lepidopteran and coleopteran species have very similar amino acid sequences (Bertossa et al., 2009; Gailey et al., 2006).

Multiple promoters and nucleotide sequences regulating alternative splicing in the *Fruitless* gene enable the production of different Fruitless isoforms (Heinrichs et al., 1998; Ryner et al., 1996) (Figure 1). *Fruitless* genes have four or more potential promotors that ensure different Fruitless isoforms are expressed in the correct developmental phase and tissue (Billeter et al., 2006; Heinrichs et al., 1998; Ryner et al., 1996; Song et al., 2002). Except for *Fruitless*-P1 pre-mRNA, Fruitless proteins derived from downstream promotors are present in males and females (Anand et al., 2001; Dornan et al., 2005; Ryner et al., 1996; Song et al., 2002). Only males produce functional Fruitless-P1 proteins as sex-specific splicing of *Fruitless*-P1 pre-mRNA in females results in truncated, non-functional Fruitless-P1 proteins or prevents translation of the Fruitless-P1 proteins (Heinrichs et al., 1998; Ryner et al., 1996). However, *N. vitripennis* females are the only known exception, as the females produce a female-specific Fruitless protein derived from the promotor 0 (P0) (Bertossa et al., 2009). The female-specific Fruitless-P0 isoforms were predicted to contain a BTB domain and different zinc-finger domains found in functional male-specific Fruitless-P1 proteins and a stretch of amino

acids on the N-terminal of the BTB domain. Fruitless proteins may have multiple isoforms due to the incorporation of different zinc-finger domains in the 3′ end during alternative splicing.

Male-specific Fruitless-P1 isoforms regulate the expression of genes linked to male-specific courtship behaviours (Demir & Dickson, 2005; Gailey & Hall, 1989; Ito et al., 1996; Kimura, Hachiya, Koganezawa, Tazawa, & Yamamoto, 2008; Ryner et al., 1996; Song et al., 2002). Courtship behaviours of male insects involve a sequence of species-specific behaviours in response to a courtship-stimulating source (Dauwalder, 2011; Gailey et al., 1991; Salvemini et al., 2010; Yamamoto & Koganezawa, 2013). In *D. melanogaster* and *M. domestica*, individual male-specific Fruitless-P1 isoforms regulate the male's ability to distinguish between males and females and perform distinct courtship steps (Anand et al., 2001; Billeter et al., 2006; Demir & Dickson, 2005; Gailey & Hall, 1989; Hall, 1994; Meier et al., 2013). Male-specific Fruitless-P1 isoforms contain different zinc-finger domains and regulate the expression of different genes. The development of the *muscle of Lawrence* and the *muscle of Lawrence-like* gene in *D. melanogaster* and *An. gambiae* was induced by the male-specific Fruitless-P1 isoform-C (Billeter et al., 2006; Demir & Dickson, 2005; Dornan et al., 2005; Gailey et al., 1991, 2006). In *B. mori*, male-specific Fruitless-P1 proteins regulate the production of the pheromone-binding proteins in male antennae, which enable the recognition of potential mates during pre-courtship behaviour (Ueno et al., 2023). The function of the female-specific Fruitless-P0 protein unique to *N. vitripennis* is unknown (Bertossa et al., 2009; Salvemini et al., 2010).

APPLICATION OF SEX DETERMINATION GENES IN GENETIC PEST MANAGEMENT

Genetic pest management can rely on intraspecific mating between transgenic and wild insect populations to introduce desired traits into pest populations (Compton & Tu, 2022; Siddall et al., 2022; Yan et al., 2023). Transgenic insects may introduce desired traits through exogenous nucleases encoded on transgenic constructs. To suppress pest populations, the exogenous nucleases may disrupt essential genes (Burt, 2003), or sex determination genes (Kandul et al., 2019; Simoni et al., 2020). The clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated (Cas) gene editing tool is commonly used as the exogenous nuclease (Compton & Tu, 2022; Siddall et al., 2022; Yan et al., 2023). The RNA-guided Cas endonuclease can target different genes and be used in various species (Burt, 2003; Galizi et al., 2016; Jinek et al., 2012; Sun et al., 2017; Windbichler et al., 2008). Transgenic constructs can be inherited in a Mendelian or non-Mendelian manner and provide self-limiting or self-sustaining genetic pest management, respectively (Alphey & Bonsall, 2018; Grilli et al., 2021; Karmakar et al., 2022; Siddall et al., 2022).

Sex determination genes can be disrupted or exploited by genetic pest management methods to suppress pest populations (Compton & Tu, 2022; Siddall et al., 2022; Yan et al., 2023). Disrupting female-specific exons of sex determination genes leads to the abnormal development of internal reproductive organs and male-specific traits in genetic females (XX) (Kyrou et al., 2018; Meccariello et al., 2024; Simoni et al., 2020; Yadav et al., 2023). Components of sex determination genes that regulate sex-specific splicing can be exploited to ensure exogenous lethal compounds are only produced in females (Ant et al., 2012; Jin et al., 2013; Spinner et al., 2022). Reducing the number of fertile females creates a male-biased sex ratio that can lead to the local extinction of pest populations (Burt, 2003; Hamilton, 1967).

Self-limiting genetic pest management

Self-limiting genetic pest management methods transiently suppress insect pest populations (Alphey et al., 2008; Alphey & Bonsall, 2018; Grilli et al., 2021; Karmakar et al., 2022; Siddall et al., 2022). Transgenic constructs providing self-limiting genetic pest management, hereafter referred to as self-limiting transgenic constructs, are inherited in a Mendelian manner. Due to the self-limiting transgenic constructs being inherited by a subset of the transgenic insect's progeny, the self-limiting transgenic constructs only propagate in populations for a limited number of generations. Different self-limiting transgenic constructs providing different pest management methods have been developed (Galizi et al., 2014; Heinrich & Scott, 2000; Kandul et al., 2019; Thomas et al., 2000; Windbichler et al., 2007, 2008). This section will discuss self-limiting genetic pest management methods that use sex determination genes; specifically the female-specific Release of Insects carrying a Dominant Lethal (fsRIDL) (Ant et al., 2012; Jin et al., 2013; Spinner et al., 2022), the precision-guided sterile insect technique (pgSIT) (Kandul et al., 2019) and the Y-chromosome-linked gene editor (Tolosana et al., 2024).

Female-specific Release of Insects carrying a Dominant Lethal traditionally uses female-specific promotors to induce mortality through the sex-specific expression of an exogenous cytotoxic compound (Heinrich & Scott, 2000; Thomas et al., 2000). During fsRIDL, regulating the expression of a tetracycline transactivator (tTA) with a female-specific promoter ensures that the tTA is only produced in females. In females, the tTA binds to a tetracycline operator (tetO) sequence, which regulates the transcription of a lethal gene. Tetracycline binds to and prevents the tTA binding to the tetO sequence (Heinrich & Scott, 2000; Thomas et al., 2000). Males and females containing a fsRIDL transgenic construct are viable when reared on a tetracycline diet; however, removing the tetracycline causes female-specific lethality.

The *tTA* gene and tetO sequence can be incorporated into the same or two different transgenic constructs to facilitate fsRIDL (Ant et al., 2012; Concha et al., 2020; Gong et al., 2005; Leftwich et al., 2014; Li et al., 2021; Ogaugwu et al., 2013; Schetelig et al., 2009, 2021; Schetelig & Handler, 2012; Spinner et al., 2022). In the single-component fsRIDL system, the *tTA* gene and tetO sequence are encoded on a single transgenic construct (Ant et al., 2012; Concha et al., 2020; Gong et al., 2005; Leftwich

et al., 2014; Li et al., 2021; Schetelig et al., 2021; Spinner et al., 2022). The tetO is located directly upstream of the promoter responsible for regulating the expression of the *tTA* gene. Overexpression of the tTA protein is thought to induce lethality by interfering with gene expression. In the two-component fsRIDL system, the *tTA* gene and the tetO sequence are encoded on different transgenic constructs (Concha et al., 2020; Ogaugwu et al., 2013; Schetelig et al., 2009; Schetelig & Handler, 2012). The *tTA* gene is regulated by a promoter that ensures continuous expression. The tetO sequence was placed upstream of a lethal gene that produces proteins which are cytotoxic or promote cell death.

Sex-specific splicing of sex determination genes has been exploited to facilitate single-component and two-component fsRIDL systems (Ant et al., 2012; Jin et al., 2013; Spinner et al., 2022). In the single-component fsRIDL system, female-specific lethality has been achieved by incorporating a female-specific *Transformer* intron downstream of the start codon for the *tTA* gene (Ant et al., 2012; Concha et al., 2020; Gong et al., 2005; Leftwich et al., 2014; Li et al., 2021; Schetelig et al., 2021; Spinner et al., 2022). The *Transformer* intron contains nucleotide sequences that regulate sex-specific splicing (Nguantad et al., 2020; Rohlfing et al., 2023; Sawanth et al., 2016; Tanaka et al., 2018). The TRA/TRA2 complex binds to and removes the female-specific *Transformer* intron in females, and females produce functional tTA proteins. Males lack functional Transformer proteins (Ant et al., 2012; Concha et al., 2020; Gong et al., 2005; Leftwich et al., 2014; Li et al., 2021; Schetelig et al., 2021; Spinner et al., 2022). As a result, the female-specific *Transformer* intron disrupts the protein-coding region of the *tTA* mature mRNA and males do not produce functional tTA proteins. Alternatively, minigenes of sex-specifically spliced sex determination genes can be incorporated into transgenic constructs to facilitate sex-specific expression of a lethal gene (Jin et al., 2013; Tan et al., 2013). Minigenes contain amplified exons and short regions of the flanking introns that are joined together to facilitate expression and alternative splicing of the gene (Cooper, 2005). The *tTA* gene is incorporated into a female-specific exon of the sex determination gene, and the tetO sequence is placed upstream. Only females will produce the tTA protein as the female-specific *Transformer* exon is not incorporated into male-specific *Transformer* mature mRNA. In the two-component fsRIDL system, a female-specific *Transformer* intron is integrated downstream of the start codon for the lethal gene to achieve female-specific lethality (Concha et al., 2020; Ogaugwu et al., 2013; Schetelig et al., 2009; Schetelig & Handler, 2012). Similar to the single-component fsRIDL system, the presence and absence of functional Transformer proteins will ensure that the products of the lethal gene are functional in females and non-functional in males. Cage and field trials, using the above mentioned self-limiting approaches have been conducted with different insects indicating varying degrees of success (Ant et al., 2012; Harvey-Samuel et al., 2014; Jin et al., 2013; Leftwich et al., 2014; Li et al., 2021; Shelton et al., 2020; Spinner et al., 2022).

The sex-specific lethality of fsRIDL removes the need to separate males and females for field releases (Alphey et al., 2008; Douglas & Turner, 2020; Lutrat et al., 2019). Removing females from transgenic populations is advantageous as females transmit pathogens and ensures transgenic males only mate with wild-type females (Knipling, 1959; Rendón et al., 2004). Transgenic insect populations for pest management methods are commonly produced in mass-rearing facilities (Lutrat et al., 2019). Initially, females and males were manually separated based on sex-specific phenotypic traits, which was time-consuming and labour-intensive (Lutrat et al., 2019; Papathanos et al., 2009). Genetic-sexing strains (GSS) and transgenic-sexing strains (TSS) use genetics and transgenics, respectively, to create visible sex-specific markers for male and female separation (Alphey et al., 2008; Douglas & Turner, 2020; Lutrat et al., 2019). In contrast, self-sexing strains contain a transgenic construct with components that induce sex-specific lethality.

During precision-guided sterile insect technique, sex determination genes are disrupted to impede female development (Apte et al., 2024; Kandul et al., 2022; Simoni et al., 2020; Smidler et al., 2023). The precision-guided sterile insect technique introduces sterile males into pest populations to create a transient male-biased sex ratio. In the above-referenced studies, sterile, male-only populations are produced by crossing transgenic populations homozygous for the Cas9 or single-guide RNA (sgRNA) transgenic constructs. Males were sterilised by disrupting genes linked to sperm development (Apte et al., 2024; Kandul et al., 2022; Simoni et al., 2020; Smidler et al., 2023). The sgRNAs targeted *Femaleless* or the female-specific exons of *Sex-lethal*, *Transformer* or *Doublesex* to impede the development of female zygotes.

Disrupting the different sex determination genes had unequal phenotypic effects (Apte et al., 2024; Kandul et al., 2022; Simoni et al., 2020; Smidler et al., 2023). Disrupting the female-specific exon of *Sex-lethal* in *D. melanogaster* and the *Femaleless* gene in *An. gambiae* caused female mortality (Kandul et al., 2022; Smidler et al., 2023). In *D. melanogaster*, *Transformer* or *Doublesex* knock-outs caused genetically female zygotes to become sterile and induced intersex phenotypes (Apte et al., 2024; Kandul et al., 2022). The sterility and intersex phenotypes resulted from abnormal development of reproductive organs and male-specific traits. The development of male-specific physical traits was greater in *Transformer* knock-outs than in *Doublesex* knock-outs.

Biasing the inheritance of the dominant female sterility mutation in the male germline is thought to reduce the reproductive capacity of female offspring (Tolosana et al., 2024). Previous research concluded that *An. gambiae* females required at least one functional allele of the female-specific exon of *Doublesex* to produce a fertile, wild-type female phenotype (Kyrou et al., 2018; Simoni et al., 2020). However, Tolosana et al. (2024) identified a mutation in the female-specific exon of *Doublesex* that caused females heterozygous for the mutation to produce a sterile, intersex phenotype. The resulting truncated Doublesex protein is hypothesised to have retained the DNA-binding and protein–protein binding activity, but, lacked the female-specific region of the Oligomerization domain 2. As a result, the truncated Doublesex protein would have interfered with the functional female-specific Doublesex protein and caused a mutation resulting in the production of a dominant female sterility phenotype.

The Y-chromosome-linked genome editor (YLE) may be used to favour the inheritance of a dominant female sterility mutation in the germline of *An. gambiae* males (Burt & Deredec, 2018; Tolosana et al., 2024). During YLE, the inheritance of a dominant female sterility mutation in the female-specific exon of *Doublesex* is favoured by a Cas9-sgRNA complex encoded on a Y-linked transgenic construct. The Cas9-sgRNA complex is active during early sperm development and creates a double-stranded break in the wild-type female-specific exon of *Doublesex*. The resulting double-stranded break is repaired via homology-directed repair using the female-specific exon of *Doublesex*, with the dominant female sterility mutation, as the repair template. As a result, all the progeny of the transgenic male inherit at least one copy of the dominant female sterility mutation in the female-specific exon of *Doublesex*. Integrating the transgenic construct, coding an exogenous nuclease that negatively effects the female fertility on the Y-chromosome, would prevent selection against the transgenic construct as the male carriers are unaffected (Burt & Deredec, 2018). Crossing of males, carrying the Y-linked transgenic construct for the Cas9-sgRNA complex and the dominant female sterility mutation, with wild-type females resulted in 70% to 95% of the progeny inheriting the dominant female sterility mutation. Empirical modelling indicated that number of transgenic males carrying the YLE and dominant female sterility mutation required to suppress pest populations was several times lower than other pest control strategies, including fsRIDL.

Self-sustaining genetic pest management

Self-sustaining genetic pest management methods aim to use synthetic gene drives to suppress pest populations for prolonged periods (Alphey & Bonsall, 2018; Grilli et al., 2021; Karmakar et al., 2022; Siddall et al., 2022). Gene drives are segments of DNA that manipulate genetic systems to distort their transmission to be inherited by more than 50% of the resulting daughter cells or offspring (Bier, 2022). Natural gene drives may bias their inheritance by copying themselves into different locations or preventing the inheritance of genes lacking the gene drive (Burt, 2014;McLaughlin Jr & Malik, 2017; Steinbrecher & Wells, 2019). Different synthetic gene drives express exogenous endonucleases, such as the Cas9-sgRNA complex, to mimic the inheritance biasing mechanisms of natural gene drives. Currently, the only self-sustaining genetic pest management methods developed to target sex determination genes are Homing gene drives (HGDs). Only HGDs targeting sex determination genes will be discussed further.

HGDs mimic the inheritance biasing mechanism of Homing endonuclease genes (McLaughlin Jr & Malik, 2017; Steinbrecher & Wells, 2019). Homing endonuclease genes achieve biased inheritance by cleaving and integrating themselves into homologous alleles that lack the homing endonuclease gene (Hafez & Hausner, 2012). This is known as "Homing". "Homing" in HGDs targeting sex determination genes has been done using Cas9-sgRNA ribonucleoproteins to disrupt female development genes (Kyrou et al., 2018; Meccariello et al., 2024; Simoni et al., 2020; Yadav et al., 2023). Hereafter, HGDs employing Cas9-sgRNA ribonucleoproteins to integrate into the female-specific exons of *Transformer* and *Doublesex* will be referred to as *Transformer* and *Doublesex*-integrating HGDs.

Disrupting the *Transformer* gene impeded female development, but *Transformer* genes have thus far not been shown to be suitable targets for population suppression HGDs (Meccariello et al., 2024). Functional Transformer proteins are required for sexual development and differentiation in females, but not males (Hediger et al., 2004; Hoshijima et al., 1991). *Ceratitis capitata* males carrying *Transformer*-integrating HGD construct were crossed with wild-type females. They produced genetic females with a sterile, intersex phenotype and genetic males (XY) with a fertile, male phenotype (Meccariello et al., 2024). The fertile male offspring carrying the *Transformer*-integrating HGD construct were crossed with wild-type females over multiple generations. However, these produced some genetic females with a fertile, male phenotype (XX-males) that carried or lacked the *Transformer*-integrating HGD construct, and genetic females with a sterile, intersex phenotype that lacked the *Transformer*-integrating HGD construct. The development of these offspring was attributed to the Cas9-sgRNA ribonucleoprotein being expressed in somatic cells and paternally deposited in gametes lacking the *Transformer*-integrating HGD construct. Crosses with XX-males and wild-type females only produced genetic female offspring with a fertile female phenotype. The initial male-biased sex ratio created by introducing males carrying the *Transformer*-integrating HGD construct was disrupted by the production of fertile female offspring and prevented the elimination of the pest population. Meccariello et al. (2024) proposed a "sterilising sex conversion drive" with an HGD transgenic construct that "Homes" into a female-specific fertility gene and carries sgRNA targeting the female-specific exon of the *Transformer* gene (Meccariello et al., 2024). Propagation of the "sterilising sex conversion drive" in a pest population would produce a male-biased sex ratio with XY-males and XX-males, and any resulting female progeny would be sterile. Disrupting the female-specific *Transformer* exon will ensure that all the progeny produced by fertile XX-males will be male. Mathematical modelling suggested that the "sterilising sex conversion drive" would be an efficient method to suppress *C. capitata* populations.

Homozygous disruption of the female-specific exon of *Doublesex* impeded female development in *An. gambiae* and might offer options for population suppression HGDs (Kyrou et al., 2018). Males and females heterozygous for the *Doublesex*-integrating HGD construct were crossed. The fertility and fitness of male progeny heterozygous and homozygous for the *Doublesex*-integrating HGD construct were similar to wild-type males. The fertility of the female progeny heterozygous for the *Doublesex*-integrating HGD construct was reduced compared to wild-type females. However, genetic females homozygous for the *Doublesex*-integrating HGD construct had sterile intersex phenotypes. Introducing males heterozygous for the *Doublesex*-integrating HGD construct into caged wild-type populations caused the number of offspring to be reduced with each successive generation and led to the complete suppression of the population after

several generations. A very small percentage of the offspring from the cage populations developed indels in the female-specific exon of *Doublesex* that were resistant to cleavage by Cas9-sgRNA ribonucleoprotein. The *Doublesex* gene is highly conserved, and the prevalence of the indels did not increase, suggesting that the indels would not greatly impede the spread of the *Doublesex*-integrating HGD construct.

Sex distorter gene drives (SDGDs) are similar to *Doublesex*-integrating HGDs but produce a homing endonuclease to create a male-biased sex ratio (Simoni et al., 2020). "Homing" of the SDGD construct into the female-specific *Doublesex* exon in the germline of males and females facilitated the biased inheritance of SDGD constructs. The homing endonuclease was expressed during sperm development to degrade the X-linked repeats and prevent the development of male gametes carrying X-chromosomes (Simoni et al., 2020; Windbichler et al., 2007, 2008). Introducing males heterozygous for the SDGD construct into caged wild-type populations resulted in male-biased sex ratios and the collapse of the population after several generations (Simoni et al., 2020). The homing endonuclease is not expressed in female gametes. However, if the homing endonuclease gene is lost or inactivated, the "Homing" of the SDGD construct in female gametes will result in the production of sterile intersex individuals and subsequent elimination of the pest populations (Kyrou et al., 2018; Simoni et al., 2020).

The efficiency of *Doublesex*-integrating HGD constructs was negatively affected when the HGD construct caused dominant female sterility in *Drosophila suzukii* Matsumura (Diptera, Drosophilidae) (Yadav et al., 2023). Females, heterozygous for the *Doublesex*-integrating HGD constructs, that induced the production of male-specific Doublesex proteins were sterile. Male-specific Doublesex proteins were produced due to the use of a weak nucleotide sequence regulating alternative splicing in the *Doublesex*-integrating HGD construct. Alternative splicing in the *Doublesex*-integrating HGD construct prevented the female-specific exon from being incorporated into the *Doublesex* mature mRNA during alternative splicing. Hereafter, *Doublesex*-integrating HGD constructs causing females to produce male-specific Doublesex proteins will be referred to as Dominant *Doublesex*-integrating HGD constructs. A stronger nucleotide sequence regulating alternative splicing was added to the *Doublesex*-integrating HGD construct to ensure the incorporation of the female-specific *Doublesex* exon in females. Females, heterozygous for the *Doublesex*-integrating HGD construct, which induced the production of male-specific Doublesex proteins, were fertile. Hereafter, *Doublesex*-integrating HGD constructs that prevent females from producing male-specific Doublesex proteins will be referred to as Recessive *Doublesex*-integrating HGD constructs. Individuals homozygous for the Dominant or the Recessive *Doublesex*-integrating HGD construct were sterile with female-like and male-like intersex phenotypes. Inheritance of the *Doublesex*-integrating HGD construct was greatest in the progeny from males heterozygous for the Recessive *Doublesex*-integrating HGD construct. The percentage of offspring inheriting the Dominant *Doublesex*-integrating HGD construct from heterozygous males was similar to the percentage of offspring inheriting the Recessive *Doublesex*-integrating HGD construct from heterozygous females. Modelling indicated that pest populations could be suppressed by repeatedly releasing males heterozygous for the Dominant or the Recessive *Doublesex*-integrating HGD construct, when the ratio of transgenic to wild-type males is low. However, single releases with a high ratio of transgenic to wild-type males would only suppress pest populations when releasing males heterozygous for the Recessive *Doublesex*-integrating HGD construct not the Dominant *Doublesex*-integrating HGD construct.

CONCLUSION

In the several dipteran, coleopteran, hymenopteran and lepidopteran species, some genes involved in the sex determination pathway are conserved while others are very diverse within and between insect orders. Very variable primary signals initiate male or female sex determination pathways based on the presence, absence or type of sex chromosome inherited paternally in the selected dipteran, coleopteran and hymenopteran species and maternally in the selected lepidopteran species. The downstream *Transformer*, *Fruitless* and *Doublesex* homologues are moderately and highly conserved, respectively, in insect species. As a result, identifying the primary signals may prove more challenging than identifying the relay and terminal genes in sex determination pathways in other species. This review on insect sex determination pathways describes known components of these pathways, thereby identifying gaps in the current knowledge and providing direction for future research.

The efficacy of different genetic pest management methods will vary depending on the sex determination gene targeted in the different insect species. Genetic pest management methods that induce female-specific mortality are more advantageous than others as they remove the need to separate males and females manually. Transgenic constructs facilitating genetic pest management methods can be supplemented with components, such as homing endonucleases, to induce female-specific mortality. Developed genetic pest management methods provide a base for developing genetic pest management methods in other species. However, it needs to be considered that disrupting orthologs of sex determination genes in other species may have a different effect than the one observed in the species where the genetic pest management method was developed. The efficacy of targeting sex determination genes in genetic pest management methods should, therefore, be assessed separately for each species. A comprehensive understanding of the molecular mechanisms underlying genetic pest management strategies is crucial for effectively targeting sex determination genes to achieve population suppression.

AUTHOR CONTRIBUTIONS

Conceptualization; investigation; writing—original draft; visualisation; writing—review and editing: Jade S. Ashmore. Writing—review and editing, supervision: Tuan A. Duong. Conceptualization, writing—review and editing, supervision: Bernard Slippers. Conceptualization;

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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