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Generating and testing the efficacy of transgenic Cas9 in *Tribolium castaneum*

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Abstract

CRISPR/Cas9 genome editing has now expanded to many insect species, including *Tribolium castaneum*. However, compared to *Drosophila melanogaster*, the CRISPR toolkit of *T. castaneum* is limited. A particularly apparent gap is the lack of Cas9 transgenic animals, which generally offer higher editing efficiency. We address this by creating and testing transgenic beetles expressing Cas9. We generated two different constructs bearing basal heat shock promoter-driven Cas9, two distinct 3′ UTRs, and one containing Cas9 fused to EGFP by a T2A peptide. Analyses of Cas9 activity in each transgenic line demonstrated that both designs are capable of inducing CRISPR-mediated changes in the genome in the absence of heat induction. Overall, these resources enhance the accessibility of CRISPR/Cas9 genome editing for the *Tribolium* research community and provide a benchmark against which to compare future transgenic Cas9 lines.

KEYWORDS
Cas9, CRISPR, transgenic, Tribolium

INTRODUCTION

Tribolium castaneum is a continually evolving genetic model, and *Tribolium* research complements and offers several benefits over the standard and popular insect model of *Drosophila melanogaster*. Not only is *Tribolium* a model for evolutionary and developmental questions of biology (Adamski et al., 2019; Beeman et al., 1989; Brown et al., 1994, 2009; Pointer et al., 2021; Schroder et al., 2008), but also a model for regulating agricultural pests (Campbell et al., 2022; Kandul et al., 2019; Rosner et al., 2020). Moreover, it is a representative of the order Coleoptera, which comprises approximately 25% of animal species as well as 40% of known insect species (Hunt et al., 2007).

The genetic tractability of *Tribolium* has been substantially improved using several strategies. *Tribolium* can be genetically manipulated in the lab through insertional mutagenesis using multiple transposable element-based systems (ie, minos, piggybac, etc.), allowing for the creation of mutant lines and transgene incorporation (Berghammer et al., 1999; Lorenzen et al., 2003; Pavlopoulos et al., 2004). RNAi is effective and durable through injection of dsRNA into all life stages, and in the early embryo using parental RNAi (Brown et al., 1999; Bucher et al., 2002; Tomoyasu & Denell, 2004). The GAL4/UAS system is effective for controlling transgene expression (Rylee et al., 2018; Schinko et al., 2010). Finally, CRISPR has recently been used by multiple labs to create knock-in and knock-out lines (Adrianos et al., 2018; Gilles et al., 2015, 2019; He et al., 2019; Rylee et al., 2018).

Since the first demonstration of CRISPR/Cas9-mediated genome editing in *Tribolium* (Gilles et al., 2015), its use, in this species, has

been limited to a handful of reports. In most cases, Cas9 is supplied via injection of a plasmid (Gilles et al., 2015; He et al., 2019; Rylee et al., 2018) or purified protein (Adrianos et al., 2018) and leads to mutagenesis or homology-directed repair (HDR). Recently, ReMOT (Receptor-Mediated Ovary Transduction of Cargo) Control (Chaverra-Rodriguez et al., 2018) was used successfully to target the *Tribolium cardinal* gene (Shirai & Daimon, 2020). This method of delivery is promising for emerging models because it allows researchers to imprecisely inject materials into adults rather than embryos, thereby saving time and resources needed for training and optimization of embryo injections. However, a major drawback is that ReMOT Control only permits CRISPR/Cas9 mutagenesis; one cannot combine the technique with an exogenous DNA template for the induction of homology directed recombination (HDR). Additionally, while the authors were able to successfully mutate the *cardinal* gene, their reported mutagenesis rates were substantially below those reported in other species (Shirai & Daimon, 2020).

Whether it's transposon transposition, PhiC31 mediated insertion or recombination (Bateman et al., 2006; Groth et al., 2004), or CRISPR/Cas9 genome editing, the efficiency and heritability of each process is increased when the key enzyme is supplied endogenously. In addition, limiting enzyme expression to the germline not only increases the chances of germline transmission of the induced edits but reduces potential toxicity to the injected developing embryo. In *Drosophila* and mosquitos, germline sources of Cas9 are available (Gratz et al., 2014; Kondo & Ueda, 2013; Li et al., 2017; Port et al., 2014). A key limiting factor in generating an endogenous source of Cas9, and in particular a source limited to the germline, is the identification of enhancers and promoters for tissue specific expression, a problem that is exacerbated in emerging animal models lacking extensive characterization of such regulatory elements. Despite the presence of homologues to elements validated in other insects, there remain few well-characterized and tested promoters for tissue-specific expression in *Tribolium* (Khan et al., 2021; Lai et al., 2018). Alternatively, a proven methodology has been to utilize a basal promoter to drive low ubiquitous expression (eg, heat shock protein promoters). Indeed, on the available Cas9 plasmid (Gilles et al., 2015) for *Tribolium*, Cas9 is expressed from a core portion of the *Tribolium hsp68* promoter (Schinko et al., 2010) and does not require a heat pulse to induce editing (Gilles et al., 2015; Rylee et al., 2018).

Here, we report our creation of two in vivo endogenous sources of Cas9 facilitating general purpose genome modification in *Tribolium castaneum*. Utilizing the established expression of Cas9 from the *Tribolium hsp68* promoter (Gilles et al., 2015), we engineered a piggyBac transposable element carrying the previously described Cas9 cassette with a retinal GFP marker for screening (pB-hs-Cas9-hs). In the second piggyBac element, Cas9 is fused to EGFP via the viral T2A peptide, enabling spatiotemporal monitoring of expression, and the *hsp68* 3'UTR is exchanged for the *Tribolium nanos* 3' UTR, along with a retinal *vermilion* marker for screening (pB-hs-Cas9-GFP-nanos). For each cassette, we isolated at least one transgenic line that was capable of being homozygous viable. Subsequent injection of gRNAs resulted in specific phenotypic and molecular modifications of the host genome, demonstrating the functionality of endogenous Cas9. These transgenic lines will increase the accessibility and feasibility of genome modification in *Tribolium*.

RESULTS AND DISCUSSION

Generation of transgenic Cas9 *T. castaneum* lines

To streamline CRISPR genome editing in *T. castaneum*, we sought to generate lines in which the Cas9 coding sequence is stably integrated into the genome. To this end, we excised Cas9 under the control of the basal *hsp68* promoter from the original *T. castaneum* Cas9 vector bhsp68-Cas9 (Gilles et al., 2015) and inserted it into a piggyBac vector containing a 3XP3-GFP marker for random genomic integration (Horn & Wimmer, 2000). The uninduced basal *hsp68* promoter and associated 3' UTR have previously been demonstrated to provide sufficient Cas9 expression for genome editing in *Tribolium* (Gilles et al., 2015; Rylee et al., 2018). Subsequently, we sought to spatially restrict the activity of Cas9 to the posterior region of the embryo by exchanging the hsp68 3'UTR with the 3'UTR of *Tribolium nanos*. The 3'UTR of *Drosophila nanos* is critical for both localization and translation at the embryonic posterior pole (Gavis & Lehmann, 1992, 1994). Nonetheless, whereas the function of *Tribolium nanos* appears to be conserved (Schmitt-Engel et al., 2012), whether similar functions of the 3' UTR are conserved for *Tribolium nanos* remain unknown. As a means to monitor Cas9 expression from this construct, we also fused EGFP to Cas9 via the T2A peptide. The resulting construct was placed into a piggyBac vector containing a 3XP3-v^+ as a marker for transgenesis (Siebert et al., 2008).

Both constructs, pB-hs-Cas9-hs and pB-hs-Cas9-GFP-nanos, were injected into *m26* embryos (Lorenzen et al., 2007) and surviving individual males and females were outcrossed to v^w beetles. To identify pB-hs-Cas9-hs insertions, progeny were screened for GFP expression in the retina (Figure 1a) and to identify pB-hs-Cas9-GFP-nanos insertions, progeny were screened for the restoration of pigment in the eye (Figure 1b). We isolated three independent lines for pB-hs-Cas9-hs and two for pB-hs-Cas9-GFP-nanos; however, only one line of each was capable of existing in a homozygous viable state, 5556 and 3231, respectively. The generation of homozygous stocks in *Tribolium* is not straight forward (Strobl et al., 2018). Furthermore, with respect to the retinal markers for each transgenic line, 3XP3-GFP (line 5556) and 3XP3-v^+ (line 3231), one copy of either marker was phenotypically similar to two copies; we could not distinguish a homozygous from a heterozygous individual based on the transgenic marker. As such, to create a homozygous line we were dependent upon setting up crosses between siblings, one male and one female, and following the progeny of each cross for at least three generations before concluding the original sibling cross was between two homozygous individuals. Nevertheless, our scheme for testing Cas9 functionality was not dependent upon having a homozygous stock available immediately and the test presented below were performed in a mixture of heterozygotes and homozygous animals.
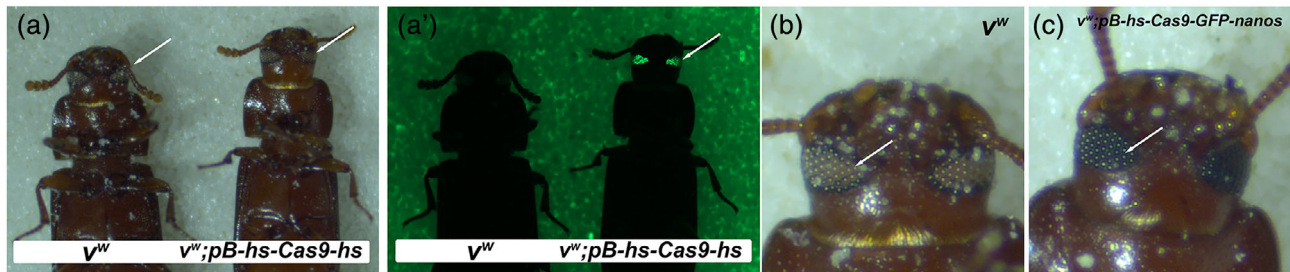
FIGURE 1 *Tribolium castaneum* genetic eye markers of Cas9 transgenic lines. (A, A') Comparison of v^w to pB-hs-Cas9-hs (*piggyBac 3XP3-GFP*; *hsp68-nls-Cas9-nls-hsp3'UTR*). Both contain the loss of eye pigment due to the presence of v^w, the loss of *vermillion* (see arrows in A), whereas pB-hs-Cas9-hs is distinguished by GFP (3XP3-GFP) expression in the retina. (b) Comparison of v^w to (c). pB-hs-Cas9-GFP-nanos (*piggyBac 3XP3-v^+*; *hsp68-nls-Cas9-nls-T2A-EGFP-nanos3'UTR*). Note the pigmented eyes in pB-hs-Cas9-GFP-nanos (arrows) due to the transgene rescue (3XP3-v^+) of *vermillion*
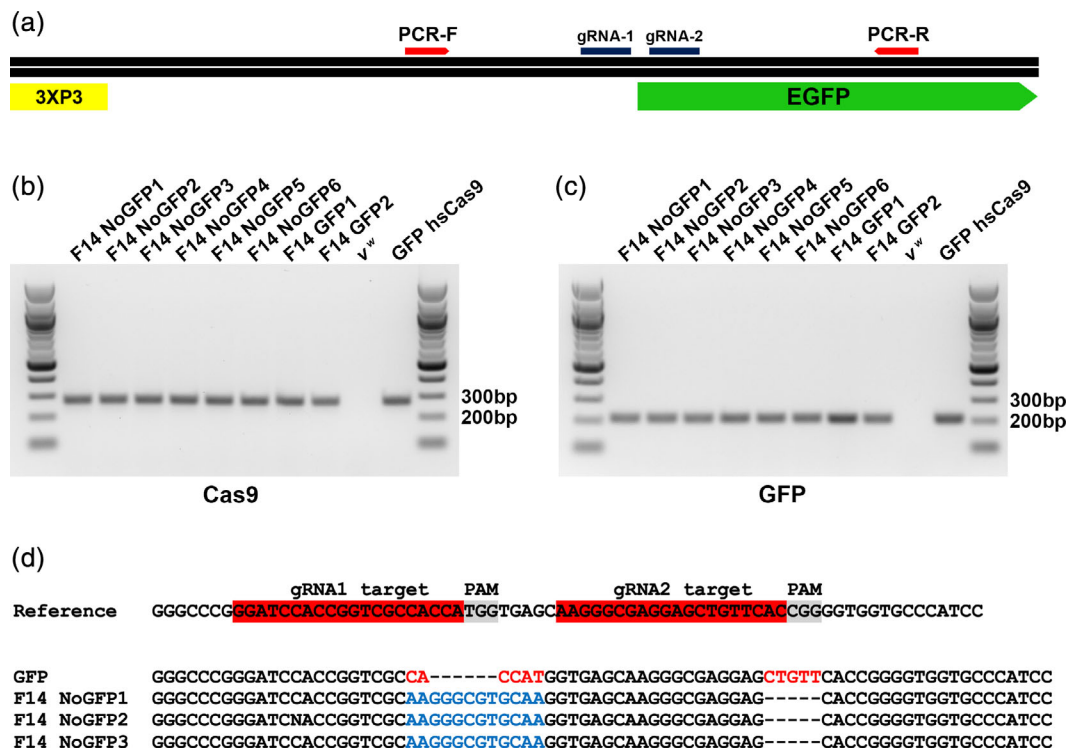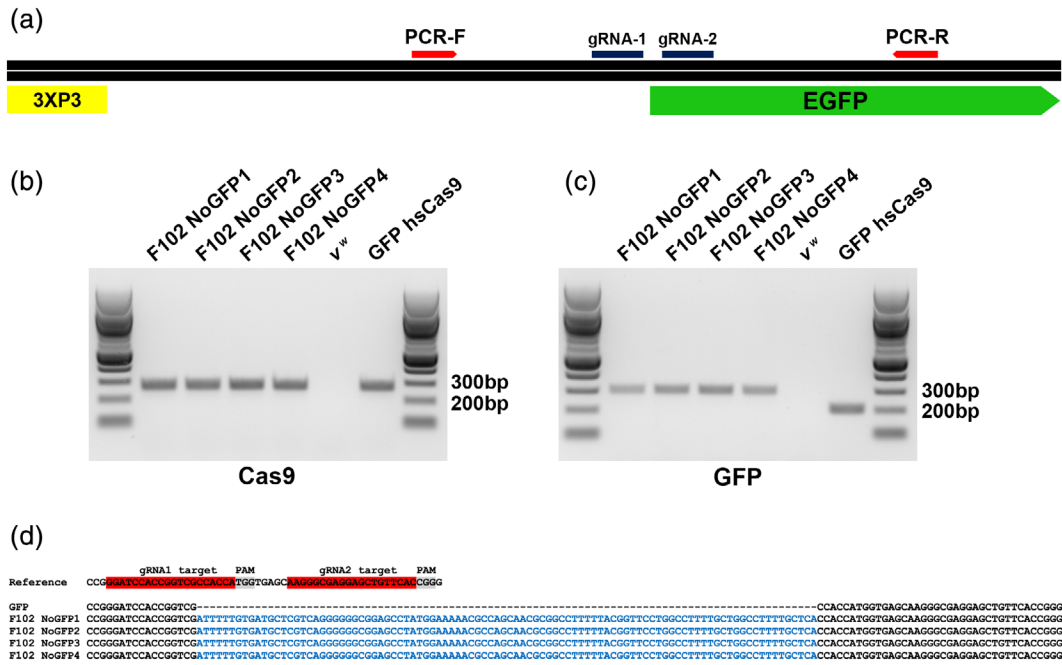

FIGURE 2 Characterization of CRISPR/Cas9 induced mutation in progeny of female-14. (a) Schematic showing the locations of gRNA targets, primers for PCR amplification, EGFP, and the 3XP3 promoter within the transgenic insertion. (b and c) gel images of PCR amplification products of Cas9 (b) or GFP (c) from genomic DNA extracted from individual progeny with or without GFP expression visible in the retina, v^w, and parental 5556 beetle containing the pB-hs-Cas9-hs construct which serve as negative and positive controls. (d) Alignment of sequences from GFP amplification products from progeny lacking GFP expression as compared to the parental pB-hs-Cas9-hs transgenic beetle; red bases indicate bases deleted and blue bases represent insertions as compared to the wild type

Transgenic Cas9 is functional

To test the inserted pB-hs-Cas9-hs, line 5556, for functionality, we made use of the 3XP3-GFP region of the integrated piggyBac transposon. We coinjected plasmids expressing two different sgRNAs flanking the start codon of EGFP, theoretically leading to indels that would disable GFP translation initiation (Figure 2a) into embryos that were heterozygous, homozygous, or completely lacked the transgenic insertion. 110 G0 embryos survived to adults that contained GFP expression in the retina,

the marker for the transgenic Cas9 construct, and thus genotypically could be either homozygous or heterozygous for the transgenic Cas9 construct. These adults were individually outcrossed to v^w beetles. The progeny of each cross was screened for loss of GFP expression in the eye. Genomic DNA was collected from beetles lacking GFP expression, and a region spanning the predicted mutation site, as well as a short span in the Cas9 transgene, were PCR amplified. Eleven crosses yielded progeny with no GFP expressed in the eye. Nine of these crosses generated an approximate phenotypic ratio of 1:1 with respect to the absence or

FIGURE 3 Characterization of CRISPR/Cas9 induced mutation in progeny of female-102. (a) Schematic showing the locations of gRNA targets, primers for PCR amplification, GFP, and the 3XP3 promoter within the transgenic insertion. (b and c) Gel images of PCR amplification products of Cas9 (b) or GFP (c) from gDNA extracted from individual progeny with or without GFP expression visible in the eyes, v^w, and parental 5556 beetle containing the pB-hs-Cas9-hs construct which serve as negative and positive controls. (d) Alignment of sequences from GFP amplification products from progeny lacking GFP expression as compared to the parental pB-hs-Cas9-hs transgenic beetle; blue bases represent insertions as compared to the wild type

presence of GFP; the expected ratio of a cross containing an individual that was heterozygous for the transgenic construct. In addition, amplification of both GFP and Cas9 failed using genomic DNA recovered from the progeny of these nine crosses, suggesting the loss of GFP was not due to Cas9 editing but rather the G0 individual was heterozygous for the insert. However, we recovered amplification products for both GFP and Cas9 in the two remaining lines that yielded GFP-negative progeny, F14 and F102. Sequencing of the GFP PCR product (Figures 2b,3b and S1a) revealed F14 progeny contained a complex indel within the gRNA-1 site, and a 5 bp deletion within the gRNA-2 site (Figure 1c), suggesting both sgRNAs were capable of mediating Cas9 directed editing. In contrast, the F102 progeny contained a 96 bp insertion within the gRNA-1 site (Figures 3c and S1b); the sequence of the 96 bp is identical to a region of pU6b-BsaI-gRNA vector.

Like the first construct, for pB-hs-Cas9-GFP-nanos, we targeted the wild-type *vermilion* gene within the *piggyBac 3XP3-v⁺* inserted cassette for editing. As previously reported, the v^w strain contains a partial deletion of the *vermilion* gene wherein only the 3′ end of the gene is present (Adrianos et al., 2018). Hence, our sgRNA targets the second exon of the *vermilion* transgene present in the piggyBac transposable element (Figure 4a). The sgRNA was injected into line #3231, embryos that were heterozygous, homozygous, or completely lacked the transgenic insertion. We recovered 87 G0 injected adults and upon subsequent mating to v^w demonstrated 40 of the surviving G0 adults were heterozygous for the transgenic construct upon the expected phenotypic Mendelian ratio of 1:1 of pigmented to non-pigmented eyes in the progeny. Therefore,

the progeny of these 40 crosses were not examined for any potential Cas9 editing. 4 G0 crosses resulted in no progeny and out of the 43 remaining crosses we recovered one G0 male cross that contained two progeny that lacked eye pigmentation among many pigmented siblings. PCR amplification (Figure 4b) and sequencing of these two progeny revealed and confirmed a single base pair deletion localized to the sgRNA target site (Figures 4c and S1c). The deletion results in a premature stop codon in the second exon of the transgene thus eliminating a functional protein (Figure 4c). These results indicate that both construct/transgenic lines are capable of inducing Cas9 mediated genome editing. For each construct we did not recover many mutagenesis events. This may be due to the basal expression levels provided by the *hsp68* core promoter used in both of our expression cassettes. It was reported that the *hsp68* core promoter gives only basal levels of expression, while expression is significantly higher when an approximately 450 bp region upstream of the core promoter was included (Schinko et al., 2010). In addition, our results do not permit a comparison between mutagenesis rates of constructs, given our experiments were designed only for testing functionality and different sgRNAs and target genes were utilized in each case.

However, increasing ubiquitous expression of Cas9 could result in toxicity. With respect to potential toxicity of expressing low levels of Cas9, we can only comment on the observed different rates of G0 crosses resulting in no progeny. In line 5556, pB-hs-Cas9-hs, the construct significantly affected male fecundity; 17 of the 54 GO males did not produce progeny whereas 5 of the 47 G0 females did not produce progeny (Table 1). In the case of line 3231, pB-hs-Cas9-GFP-
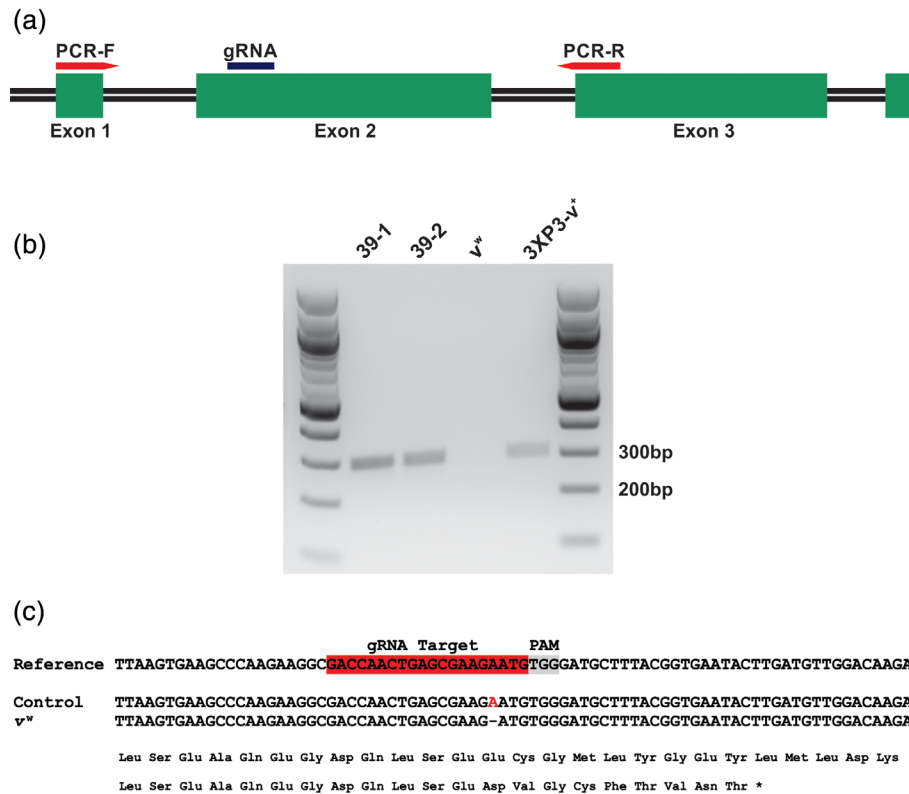
FIGURE 4 Characterization of CRISPR/Cas9 induced mutation in *vermillion* transgene. (a) Schematic showing the locations of the gRNA target and PCR primers within the first three exons of the *vermillion* transgene. (b) Gel image of the amplification products. (c) Alignment of sequences and protein translation from a pB-hs-Cas9-GFP-nanos, and from animals 39–1 and 39–2. Red bases indicate bases that are deleted in the mutated beetles

TABLE 1 Fecundity comparisons of males and females with respect to transgenic Cas9 construct

	Males	Females	Σ
piggyBac 3XP3-GFP; hsp68-nls-Cas9-nls-hsp3′UTR			
Yielded progeny	37	42	79
No progeny	17	5	22
Σ	54	47	101
			$p = .0152$[a]
piggyBac 3XP3-v^{w+}; hsp68-nls-Cas9-nls-T2A-EGFP-nanos3′UTR			
Yielded progeny	27	20	47
No progeny	0	4	4
Σ	27	24	51
			$p = .0272$[a]

[a]Fisher's exact test, significant difference between females and males.

nanos, we observed the opposite. Four of the 20 G0 female crosses did not produce progeny, whereas all 27 G0 male crosses produced progeny (Table 1), a significant reduction in female fecundity. Male reproduction varied significantly between the two constructs ($p < .004$) but female reproduction did not ($p > .25$). Although these data are consistent with the suggestion that the female-specific nanos 3′UTR is less harmful to male reproduction, our current results cannot

distinguish whether the differences observed are due to Cas9 activity, insertion location of transgenes, or differences in constructs and associated toxicity. For example, due to low expression from the *hsp68* core promoter we were not capable of utilizing GFP expression, via the T2A peptide (Kim et al., 2011), as a surrogate for Cas9 activity in the pB-hs-Cas9-GFP-nanos transgenic line 3231.

Altogether, we have generated the first *Tribolium* lines expressing Cas9 endogenously, adding to the ever-growing set of genome editing tools available to the community. Our work demonstrates the feasibility of these resources and also sets a baseline against which to compare other promoter-Cas9 combinations. As observed in other insect species, further work is needed to identify and characterize usable regulatory regions to drive robust germline Cas9 expression (Gratz et al., 2014; Kondo & Ueda, 2013; Li et al., 2017). Nonetheless, these transgenic Cas9 lines will increase the accessibility of CRISPR/Cas9 editing in *Tribolium* for the research community.

EXPERIMENTAL PROCEDURES

Tribolium husbandry and strains

All animals were raised at 28°C on a standard flour yeast mix. The following strains were utilized: *vermilion^{white}* (v^w), ([Lorenzen

et al., 2002]), m26, a v^w line with a X-linked insertion of the piggyBac transposase marked with 3XP3-DsRed (Lorenzen et al., 2007), piggyBac *3XP3-GFP; hsp68-nls-Cas9-nls-hsp3′UTR* (line #5556 [this study]), and *piggyBac 3XP3-v^+; hsp68-nls-Cas9-nls-T2A-EGFP-nanos3′UTR* (line #3231[this study]). Injections were performed at 25°C and embryos were then returned to 28°C for development and hatching.

Vectors

All vectors generated in this study are available through the Drosophila Genomics Resource Center at Indiana University.

pB-hs-Cas9-hs: piggyBac 3XP3-GFP; hsp68-nls-Cas9-nls-hsp3′UTR

The hsp68-nls-Cas9-nls-hsp3′UTR cassette was excised from p(bhsp68-Cas9) (Gilles et al., 2015) Addgene (#65959)) using flanking AscI sites and ligated into AscI-linearized pBac-3XP3-GFP (Horn & Wimmer, 2000).

pB-hs-Cas9-GFP-nanos: piggyBac 3XP3-v^+; hsp68-nls-Cas9-nls-T2A-EGFP-nanos3′UTR

T2A-EGFP was amplified from pX458 (a gift from Feng Zhang, Addgene #48138), nanos3′UTR was amplified from v^w genomic DNA, and the fragments were assembled into p(bhsp68-Cas9) using NEBuilder HiFi assembly (NEB, Ipswich, MA). The hsp68-nls-Cas9--nls-T2A-EGFP-nanos3′UTR cassette was excised using flanking AscI sites and ligated into AscI-linearized pBac-3XP3-v^+ (Siebert et al., 2008).

pU6b-BsaI-gRNA GFP1 and GFP2

The 20-mer protospacer sequences 5′-GGATCCACCGGTCGCCACCA-3′ and 5′-AAGGGCGAGGAGCTGTTCAC-3′ were cloned into BsaI-digested the pU6b-BsaI-gRNA ((Gilles et al., 2015), Addgene (#65956)) by NEBuilder HiFi-mediated ssDNA oligo bridging using an ssDNA oligo consisting of the protospacer flanked by 25 bp regions of homology.

pU6b-BsaI-gRNA Tc vermilion

The 20-mer protospacer sequence 5′-GACCAACTGAGCGAAGAATG-3′ was cloned into BsaI-digested pU6b-BsaI-gRNA (Gilles et al., 2015), Addgene (#65956)) by NEBuilder HiFi-mediated ssDNA oligo bridging as above using an ssDNA oligo consisting of the protospacer flanked by 25 bp regions of homology.

Tribolium transgenesis

pB-hs-Cas9-hs and pB-hs-Cas9-GFP-nanos were each resuspended in 1× injection buffer (0.5 mM KCl; 0.01 mM NaPO4 buffer pH 7.5) at a concentration of 1 μg/μl and injected into *m26* embryos. Individual surviving G0 adults were crossed to v^w and progeny were screened for expression of GFP in the retina or pigmented eyes, depending upon the injected construct. In total, three independent lines were isolated from pB-hs-Cas9-hs injections, but only one was capable of achieving homozygosity (Line # 5556). Two independent lines were isolated from pB-hs-Cas9-GFP-nanos and only one was capable of achieving homozygosity (Line # 3231).

Tribolium CRISPR injection and detection

A mixture consisting of both GFP sgRNA plasmids (500 ng/μl of each) in 1× injection buffer (0.5 mM KCl; 0.01 mM NaPO$_4$ buffer pH 7.5) was injected into embryos of line #5556. Individual surviving G0 adults were first screened to confirm the presence of 3XP3-GFP expression in the retina before individually crossing to v^w. Individual G0 males were mated to 2–3 v^w females and individual G0 females were mated to 2–3 v^w males. Progeny were then subsequently screened for the loss of GFP expression in the retina. The progeny from individual crosses that lost GFP expression were saved and subjected to PCR and sequence confirmation for CRISPR/Cas9 editing.

The sgRNA plasmid for *vermilion* (500 ng/μl) was resuspended in 1× injection buffer (0.5 mM KCl; 0.01 mM, NaPO$_4$ buffer pH 7.5) and injected into embryos of line #3231. Individual G0 males were mated to 2–3 v^w females and individual G0 females were mated to 2–3 v^w males. Progeny were then subsequently screened for the loss of pigment in the retina. The progeny from individual crosses that lost pigment were saved and subjected to PCR and sequence confirmation for CRISPR/Cas9 editing.

PCR and sequence confirmation of CRISPR/Cas9 editing

Genomic DNA was isolated from individual *Tribolium* by crushing each individual in 50 μl of extraction buffer (100 mM Tris–HCl, 50 mM EDTA, 1% SDS) with a pestle in an Eppendorf tube. The mixture was subjected to a five-minute incubation at 95°C, then chilled on ice. The mixture was then digested with Proteinase K (50 μg/ml) for 1 h at 55°C, followed by heat inactivation at 95°C for 5 min. 200 μl of 0.1× TE buffer was added to dilute the sample. Finally, 100 μl of the gDNA solution was purified using the Zymo Genomic DNA Clean and Concentrator −10 kit (Zymo Research #ZD4010) following the manufacturer's instructions.

Amplicons spanning the gRNA target sites were amplified from 1 μl of purified gDNA using HotStar PCR Master Mix (Qiagen). Half of each reaction was run on a 1.5% agarose gel, and the other half of the reaction was purified using the Qiaquick Gel Extraction Kit (Qiagen).

The purified fragments were submitted to Eurofins Genomics for Sanger sequencing, and the sequences were analysed using Sequencher (Gene Codes Corp.). Alignments were made using SnapGene. Additionally, a fragment of Cas9 was amplified to confirm the presence of the 3XP3-GFP; hsp68-nls-Cas9-nls-hsp3'UTR insertion.

The following PCR primers were used to amplify the GFP and Cas9 DNA fragments: GFP –5′-GCAAATAAACAAGCGCAGCTG-3′ and 5′-GTAGGTGGCATCGCCCTC-3′ and should amplify a 211 bp fragment. Cas9–5′-GGGATAAGCAATCTGGCAAA-3′ and 5′-CACGTGCCATTTCAATAACG-3′ and should amplify a 285 bp fragment. The GFP PCR products were sequenced with the following primer: 5′-CAAGCGCAGCTGAACAAGC-3′.

The following primers were used to amplify the *vermilion* DNA flanking the targeted gRNA site: 5′-ATGAGTTGCCCACTGAGACC CTCGTAAGTA-3′ and 5′-TGTTTGAACCATAACTCATACGCTGGAA TTAC-3′ and should amplify a 297 bp fragment. The vermillion PCR products were sequenced with the following primers: 5′-GAGACCCTCGTAAGTATGATTTAA-3′ and 5′-CGCTGGAATTACTTG TTATAG-3′.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Michael J. Wade, Andrew C. Zelhof, Gabriel E. Zentner conceived the idea of the work. All authors contributed to the research and Johnathan C. Rylee, Michael J. Wade, Andrew C. Zelhof, Gabriel E. Zentner wrote and edited the manuscript.

DATA AVAILABILITY STATEMENT

All data necessary for confirming the conclusions in this manuscript are included in this article and in supplemental figures and tables.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

FIGURE S1 Chromatography readouts of CRISPR/Cas9 editing. (a) Chromatograms from sequencing PCR products amplified from gDNA extracted from pB-hs-Cas9-hs control beetles, and the progeny from female 14 lacking GFP expression. (b) Chromatograms from sequencing PCR products amplified from gDNA extracted from pB-hs-Cas9-hs control beetles, and the progeny from female 109 lacking GFP expression. The site of the insertion is shown (c) chromatograms from sequencing PCR products amplified from gDNA extracted from pB-hs-Cas9-GFP-nanos control beetles, and the progeny from 39–1 lacking eye pigment. Red boxes indicate deletions, and blue boxes indicate insertions