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Forum

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Genetically Engineered Ricin Suppresses *Bactrocera dorsalis* (Diptera: Tephritidae) based on Demographic Analysis of Group-Reared Life Table

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Abstract

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), reduces the quantity and quality of many host fruits through the process of oviposition and larval feeding, and this insect has been considered a major insect pest in several Asian countries for decades. Using an earlier-developed, female-specific system that combines the toxicity of the ricin A chain (RTA) and the alternative RNA splicing property of *doublesex* (*Bddsx*), we show that transgenic male flies harboring the *RTA-Bddsx* transgene unevenly repress the pest population through inheritable effects. In age-stage, two-sex life-table analyses, high larval mortality and a delay in pupation were observed after introducing the transgene. The high male to female ratio in $DsRed^{+}$ flies demonstrates the lethal effect of ricin on females. The fitness of both the $DsRed^{+}$- and $DsRed^{-}$-transformed females was reduced as shown in the decrease of the net reproductive rate (R_0), intrinsic rate (r), and finite rate (λ) values compared with the wild-type populations. The integrity of the *RTA-Bddsx* transgene remained in more than 80% of $DsRed^{+}$ males after ten generations, supporting the stable inheritance of the transgene. All of the data from this study support the proposed *RTA-Bddsx* SIT approach, which provides a species-specific and environmentally friendly method of suppressing, rather than eradiating, *B. dorsalis*.

Key words: *doublesex* gene of *Bactrocera dorsalis*, ricin A chain, age-stage, two-sex life table, sterile insect technique.

In many Asian countries, the oriental fruit fly, *Bactrocera dorsalis* (Hendel), reduces the quantity and quality of a number of fruits, and for decades, it has been considered one of the major insect pests. Many traditional methods of *B. dorsalis* management, including methyl eugenic/protein baiting, insecticide application, the sterile insect technique (SIT), and orchard management, have been widely applied (Vargas et al. 2015), but due to high labor costs, the development of resistance, and ecological safety considerations, more advanced, environmentally friendly technologies are urgently needed to control this pest.

Although the development of new pesticides with different modes of action is necessary for successful pest management, traditional chemical screening is a time-consuming and expensive process. The 'release of insects carrying a dominant lethal' (RIDL) is an innovative approach to pest management that has been proposed as a genetic enhancement of the SIT (Heinrich and Scott 2000, Thomas et al. 2000, Alphey and Andreasen 2002). This approach utilizes the Tet-Off system (Gossen and Bujard 1992) to regulate the expression of sex-specific lethal gene(s), thus avoiding classical radiation treatments to generate males for the SIT program. Because female insects are usually responsible for agricultural and human health problems, they are the main targets of pest management projects, so the genes involved in sex determination are first to be proposed and screened as suitable targets for female-specific lethal genes (Raphael et al. 2004). To date, the 'transformer' and 'doublesex' genes of several insect species, which have alternative splicing properties, have been successfully applied for genetic sexing in various insect pests (Fu et al. 2007, 2010, Ant et al. 2012, Schetelig and Handler 2012, Jin et al. 2013, Ogaugwu et al. 2013, Leftwich et al. 2014).

Adding conditional modifications of natural toxins through well-known mechanisms can provide these substances with additional functions for practical pest control needs. Ricin is a natural plant toxin, and it has been used as a biological weapon. A number of recent studies have been conducted to determine methods of applying the potent toxic effects of ricin for cancer therapy (de Virgilio et al. 2010). The toxin belongs to the class II ribosome-inactivating protein family and has an A chain and B chain (RTA and RTB) linked by a disulfide bond. RTA has RNA N-glycosidase activity, which makes it a potent inhibitor of 28S rRNA functions in eukaryotic ribosomes and RTB is a nontoxic carbohydrate-binding

protein that facilitates the uptake of RTA into cells (Halling et al. 1985, Lamb et al. 1985, Endo and Tsurugi 1987, Lord et al. 1994, Spooner and Lord, 2015). In previous studies, we combined the female-specific alternative splicing property of *Bddsx* with the toxicity of RTA to develop a hybrid system for female-specific lethality *in vitro* and *in vivo* (Huang et al. 2016a). Subsequently, an *RTA-Bddsx* transgene driven by the *actin 5C* promoter of *B. dorsalis* in the *piggyBac* vector was used to establish transgenic flies (Huang et al. 2016b).

Recently, the population-level effects of fitness costs have been studied in two female-specific RIDL insects by measuring the time evolution of transgenes either with or without tetracycline. The persistent periods of an introduced transgene can be estimated through its competition with wild-type alleles under different rearing conditions (Harvey-Samuel et al. 2014). Indeed, determining the effects of a transgene in a pest population is critical for the success of the SIT program. Although the Tet-Off system is not applied here, the transformed male heterozygotes carrying *RTA-Bddsx* are available, and they are ideal for tracing the effects of the transgene in a population of *B. dorsalis*. A life-table study can be used to comprehensively summarize the effects of the transgene on the survival rate, development, and reproductive ability of flies, and this approach has been applied in many fields of population ecology, including invasive species population biology (Sakai et al. 2001), demographic ecotoxicology (Stark and Banks 2003), and the timing of pest control (Chi 1990). The traditional female, age-specific life table excludes the male population as well as the sex ratio, which renders these tables inapplicable to studies such as SIT and RIDL (Huang and Chi 2012). In this demographic study, a well-established age-stage, two-sex life-table experiment was adopted to analyze both sexes and their variable developmental rates (Chi and Liu 1985; Chi 1988).

All of the data presented here support the proposed SIT approach using the *RTA-Bddsx* system and indicate that this technique unevenly represses the pest population with inheritable effects through the release of transgenic male flies and provides a species-specific and environmentally friendly method of suppressing, rather than eradicating, *B. dorsalis*.

Materials and Methods

Fruit Fly Strains

In this study, a laboratory strain of *B. dorsalis*, which has been maintained in the laboratory for over 13 years, was used as a source of the wild-type flies. Eight transgenic F_3 males from LERQ-1-2(F2) M08, LERQ-1-7(F2) M10, and LERQ-1-8(F2) M02 revealed high male percentages (94.6–100%) and were randomly selected and mated with wild-type females (Huang et al. 2016b). The flies were maintained at 28°C under a photoperiod of 12:12 (L:D) h. Adult flies were housed in cages for mating and egg-laying; hatched larvae were cultured through periodic transfer to fresh food.

The Age-Stage, Two-Sex Life Table Study

For the age-stage, two-sex life table study, eight *RTA-Bddsx* transformed male flies (F_3) were backcrossed with 40 wild-type females to generate F_4 flies. The numbers of eggs, larvae, pupae, and adults were examined and recorded daily. After pupation, individuals with *DsRed* expression were recorded. The male and female numbers of $DsRed^+$ and $DsRed^-$ flies were recorded daily after eclosion for sex ratio calculation. Ten pairs of lab-reared wild-type flies were used in a parallel control experiment, and 50 eggs were randomly collected. For further study of the fecundity of female flies, three sets of experiments with 1) 25 pairs of wild-type flies, 2) 25 pairs of $DsRed^+$ flies, and 3) 25 pairs of $DsRed^-$ flies were conducted in a random manner after eclosion. The number of eggs laid by all female was recorded daily for their remaining life span. The numbers of hatched eggs in different groups were also examined during the three days after eggs were laid.

Life Table Analysis

According to the age-stage, two-sex life table theory (Chi and Liu 1985, Chi 1988), all of the raw data collected from different developmental stages and the sex information were analyzed using the computer program TWOSEX-MSChart (Chi 2016) for Windows which is available at http://140.120.197.173/Ecology/Download/Twosex-MSChart.rar. Because the insects were reared in groups, the number of individuals that survived to age x and in each stage were recorded. The survival rate (s_{xj}) to each age-stage unit can be calculated as

$$s_{xj} = \frac{n_{xj}}{n_{01}} \tag{1}$$

where n_{01} is the number of eggs used at the beginning of life table study and n_{xj} is the number of insects that survived to age x and stage j. Because we recorded the total number of eggs (E_x) laid by all female adults (the fourth life stage) at age x, the female age-specific fecundity f_{x4} can be calculated as

$$f_{x4} = \frac{E_x}{n_{x4}} \tag{2}$$

According to Chi and Liu (1985), the net reproductive rate is calculated as

$$R_0 = \sum_{x=0}^{\infty}\sum_{j=1}^{m} s_{xj} f_{xj} \tag{3}$$

where m is the number of life stages. The age-specific survival rate (l_x, the probability that a newly laid egg survives to age x) and the age-specific fecundity (m_x, the mean fecundity of individuals at age x) are calculated as follows:

$$l_x = \sum_{j=1}^{m} S_{xj} \tag{4}$$

$$m_x = \frac{\sum_{j=1}^{m} S_{xj} f_{xj}}{\sum_{j=1}^{m} S_{xj}} \tag{5}$$

The intrinsic rate can be estimated by using following equation

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1 \tag{6}$$

The finite rate of increase (λ) is calculated as $\lambda = e^r$ and the mean generation time is calculated as $T = (\ln R_0)/r$.

In order to estimate the standard errors of population parameters using the bootstrap technique, the longevity data for each individual and daily fecundity of each female adult are needed. Based on the age at death of each individual that was recorded during the experimental period, the longevity and sex of each individual can be calculated. In addition, the information from the daily number of eggs laid by each living female was used to assign the mean fecundity to each female. This procedure also will not affect the l_x and m_x because Chi (1988) proved the relationship between R_0 and F.

Table 1. Differential expression patterns of *DsRed* gene in pupal and adult stages of F_4 flies after eight transformed F_3 males back-crossing to wild-type females

F_4 lines	F_3 male lines	Eggs	Larvae	DsRed$^-$ Pupae	DsRed$^+$ Pupae	DsRed$^-$ Males	DsRed$^-$ Females	DsRed$^+$ Males	DsRed$^+$ Females
LERQ1-2(F4)M01	LERQ1-2(F3)M08-1	168	140	67	67	29	28	39	15
LERQ1-2(F4)M02	LERQ1-2(F3)M08-2	235	211	123	80	58	57	57	12
LERQ1-2(F4)M03	LERQ1-2(F3)M08-3	83	68	31	32	15	16	20	11
LERQ1-2(F4)M04	LERQ1-2(F3)M08-4	231	180	103	72	52	47	42	13
LERQ1-7(F4)M02	LERQ1-7(F3)M10-1	70	50	19	25	8	10	20	5
LERQ1-8(F4)M01	LERQ1-8(F3)M02-1	184	150	84	59	38	43	36	11
LERQ1-8(F4)M02	LERQ1-8(F3)M02-2	234	138	76	59	44	30	39	10
LERQ1-8(F4)M03	LERQ1-8(F3)M02-3	173	96	47	40	22	22	27	7
Total		1378	1018	550	434	266	253	280	84

DsRed$^+$ phenotype is underlined.

Therefore, this practice will not affect the population parameters. Due to the variable longevity of females, this practice can still reveal the variability of fecundity found in female adults. All data calculated for each individual were subjected to the bootstrap method with 100,000 resampling for estimating the standard errors of population parameters. Differences between treatments were then compared by using the paired bootstrap test (Efron and Tibshirani 1993, Polat-Akköprü et al. 2015).

Genomic DNA Isolation and Analysis of the Integrity of the *RTA-Bddsx* Transgene

Genomic DNA was isolated from the heads of the transformed fruit flies according to a previously reported method (Huang et al. 2016b). The primers PB-5587F (5′-CCCGTAATGCAGAAGAA GACCATG-3′) and PB-6522R (5′-CGCAGCGTGACCGCTACA CTTG-3′) were used to examine the integrity of the *actin 5C* promoter and the *RTA-Bddsx* gene, and PCR amplification was performed as follows: 94°C for 2 min; 35 cycles of 94°C for 45 s, 56°C for 1 min, and 72°C for 2.5 min; and 72°C for 10 min.

Results and Discussion

Life Table: High Larval Mortality, a Delay in Pupation, and a High Male/Female Ratio After Introduction of the *RTA-Bddsx* Transgene

Eight transgenic F_3 males from high-male-percentage (94.6–100%) F_2 flies were selected and mated with wild-type females, and a parallel wild-type experiment was simultaneously conducted as a control (Huang et al. 2016b). A total of 1,378 eggs were collected from the transformed population (Table 1), and the expression of the *DsRed* gene, a fluorescent marker, was used as an indicator of external DNA fragment insertion. To clarify our results, the integrant of the transgene in all eight of the transgenic males used in the study were confirmed to have the DsRed$^+$/RTA$^+$ genotype (Table 2). After backcrossing with the wild-type females, offspring were produced that included DsRed$^-$ males, DsRed$^-$ females, DsRed$^+$ males, and DsRed$^+$ females with percentages of 30.12, 28.65, 31.71, and 9.51%, respectively (Table 1). In the following age-stage-sex population structure analyses, the survival rates (s_{xj}) were recorded daily at different developmental periods throughout the entire lifespan, with sex determined at the adult stage (Fig. 1). In Figure 1A, the wild-type flies show high hatching and pupation rates, and the developmental periods were nearly consistent in the larval and pupal stages. The observed sex ratio of males to females was nearly the same after eclosion. However, the survival rates varied between the DsRed$^+$ and DsRed$^-$ offspring of the *RTA-Bddsx* transformed group, and they exhibited higher larval mortality (~30%) than their wild-type counterparts. These lethal events might have been partially caused by internal *RTA* expression driven by the *actin 5C* promoter. The pupation rate of the DsRed$^+$ flies was lower than that of the DsRed$^-$ flies, and the DsRed$^+$ group also exhibited a one-day delay in pupation (Fig. 1B). Moreover, the high ratio of males to females among the transformed flies (~3:1) is considered to result from the female-lethal effect of RTA. The number of male and female flies was nearly the same among the non-fluorescent individuals (Fig. 1B). The appearance of transformed females indicates that the *actin 5C* promoter-*RTA-Bddsx* transgene lost its function during chromosomal rearrangement (Huang et al. 2016b). The transformed females constituted 9.51% of the total transformed F_4 population. Interestingly, the transformed male flies showed a life span close to 118 days, which is longer than the 108 days for normal males (Fig. 1).

Table 2. Distribution of the integrant *RTA* gene in the DsRed$^-$ and DsRed$^+$ offspring of transformed *B. dorsalis*

		Sample size	Full RTA (%)
LERQ(F3)	DsRed$^+$ males	8	8 (100.0)
LERQ(F4)	DsRed$^-$ males	25	0 (0.0)
LERQ(F4)	DsRed$^-$ females	25	0 (0.0)
LERQ(F4)	DsRed$^+$ males	25	23 (92.0)
LERQ(F4)	DsRed$^+$ females	25	0 (0.0)

Reduced Fecundity of Transformed Females Compared With Wild-Type Flies

Before a practical application of the proposed RTA control approach can be developed, the fecundities of DsRed$^-$ and DsRed$^+$/RTA$^-$ females and their effects on the wild-type population must be determined. The age-specific survival rate (l_x), the female age-stage specific fecundity (f_{x4}), the age-specific fecundity (m_x), and the age-specific maternity (l_xm_x) of *B. dorsalis* were shown in Figure 2. When the data among the transformed and wild-type flies were compared, the age-specific survival rates of both the DsRed$^-$ and DsRed$^+$/RTA$^-$ females presented dramatic decreases during the pre-adult stages (Fig. 2B and C). In addition, the age-specific fecundity (m_x values) revealed similar behavior between the wild-type and DsRed$^-$ females; however, different patterns were observed between the wild-type and DsRed$^+$/RTA$^-$ females (Fig. 2). In the wild-type and DsRed$^-$ females, the maximum number of eggs were laid during the first 25 days following adult emergence and gradually declined throughout the aging process (Fig. 2A and B). However, the m_x

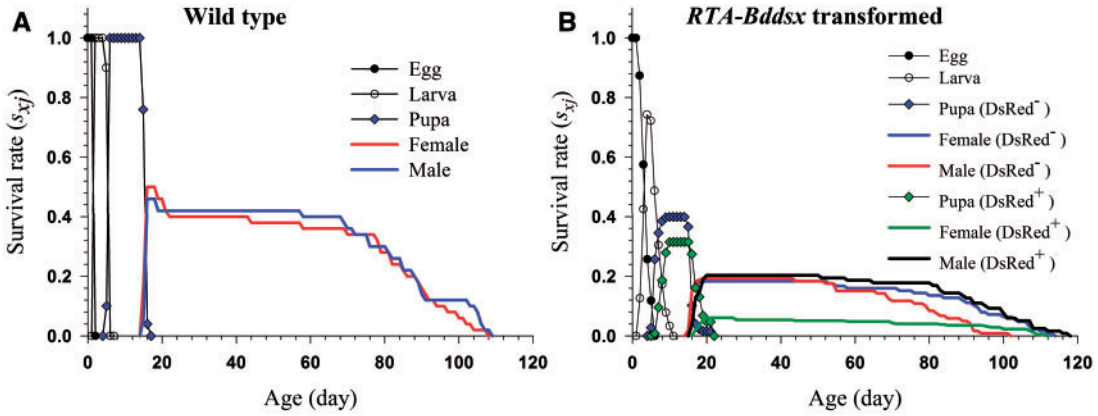

Fig. 1. Age-stage specific survival rate (s_{xj}) of wild-type and *RTA-Bddsx* transformed *B. dorsalis*. (A) Ten pairs of newly emerged wild-type flies were paired and 50 eggs were randomly collected to perform life table study. The numbers of hatched larvae, pupae, and adults were recorded daily until death. (B) Eight *RTA-Bddsx* transformed male flies (F_3) were backcrossed with 40 wild-type females to generate F_4 transgenic flies. Individuals within the transformed group were examined using fluorescent microscopy to distinguish DsRed$^+$ and DsRed$^-$ flies during the pupal stage. (Online figure in color.)

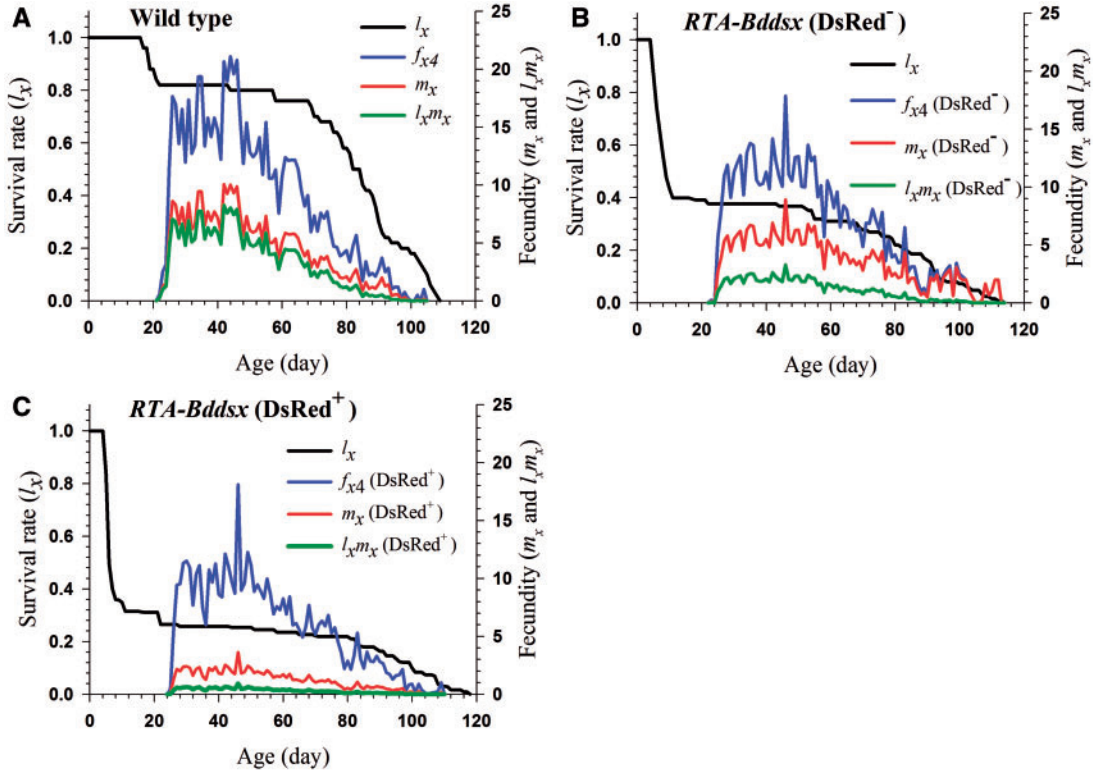

Fig. 2. Analyses and comparison of the reproductive abilities among wild-type, DsRed$^-$ and DsRed$^+$ transgenic female flies. (A) Twenty-five pairs of newly emerged wildtype flies were randomly selected and paired. (B) Twenty-five pairs of newly emerged DsRed flies after *RTA-Bddsx* male and wild-type female backcross were sampled randomly and self-crossed. (C) Twenty-five pairs of newly emerged flies of *RTA-Bddsx* transformed DsRed$^+$ flies were sampled randomly and self-crossed. The eggs laid by each female were collected daily for the rest of its life span to determine fecundity. (Online figure in color.)

values of the DsRed$^+$/RTA$^-$ females remained consistently low during the entire 70-day period after emergence (Fig. 2C). The wild-type females had the highest fecundity among the three groups tested as indicated by their l_xm_x values. Although the m_x value of the DsRed$^-$ flies was comparable to that of the wild-type females, the l_x values were far less. In the case of the DsRed$^+$/RTA$^-$ females, their fecundities were low, and only a limited number of offspring could be observed (Fig. 2C).

One of the most useful population parameters for fecundity measurements is the net reproductive rate (R_0), which is the total number of offspring that an individual can produce during its lifetime. Our data revealed a significant difference among the wild-type, DsRed$^-$ and DsRed$^+$/RTA$^-$ females, which presented R_0 values of 272.40, 98.54, and 24.32, respectively (Table 3). These results clearly indicate that the fecundity of the DsRed$^-$ and surviving DsRed$^+$/RTA$^-$ females was significantly reduced compared with that of the wild-type female flies. In addition, the intrinsic rate of increase (r) and the finite rate of increase (λ) were calculated; these values represent the theoretical rate of increase of a population at a stable age-stage distribution. The r values dropped from 0.1481 to

Table 3. Population parameters (mean ± SE) assessments among cohorts with wild-type, DsRed$^-$ and DsRed$^+$ transgenic female flies

Parameter	Cohort with wild-type females	Cohort with *RTA-Bddsx* females (DsRed$^-$)	Cohort with *RTA-Bddsx* females (DsRed$^+$/RTA$^-$)
Net reproductive rate (R_0)	272.4 ± 48.2	98.5 ± 5.7	24.3 ± 2.8
Intrinsic rate (r, day^{-1})	0.1481 ± 0.0057	0.1114 ± 0.0016	0.0725 ± 0.0030
Finite rate (λ, day^{-1})	1.1597 ± 0.0065	1.1178 ± 0.0018	1.0751 ± 0.0032
Mean generation time (T, day)	37.85 ± 0.32	41.19 ± 0.12	44.40 ± 0.30

Standard errors were estimated by using 100,000 bootstraps. Means followed by different letter are significantly different between two treatments using the paired bootstrap test at the 5% significance level.

Table 4. The patterns of DsRed expression and *RTA* gene in F_4–F_{10} progeny of *RTA-Bddsx* transformed flies

Generation	DsRed$^+$ pupae	DsRed$^+$ male	DsRed$^+$/*RTA*$^+$ male
F_4	434 (46.14%)	280 (76.90%)	10/10 (100.00%)
F_5	217 (40.49%)	121 (72.46%)	10/10 (100.00%)
F_6	272 (49.07%)	150 (60.00%)	8/10 (80.00%)
F_7	255 (45.42%)	144 (68.25%)	9/10 (90.00%)
F_8	465 (49.89%)	319 (69.03%)	10/10 (100.00%)
F_9	127 (51.42%)	74 (65.49%)	9/10 (90.00%)
F_{10}	232 (43.77%)	121 (71.18%)	9/10 (90.00%)

0.0725 day^{-1} in the wild-type control and DsRed$^+$/RTA$^-$, whereas the λ value was 1.0751 day^{-1} in the DsRed$^+$/RTA$^-$, which was lower than the λ value of the wild-type cohort (1.1597 day^{-1}) (Table 3). Decreasing values of r and λ in the DsRed$^-$ or DsRed$^+$/RTA$^-$-transformed females indicated their reduced ability to compete with the wild-type females in the population. Based on the genomic analysis, the randomly sampled DsRed$^-$ flies did not have a functional RTA gene (Table 2), so this phenomenon of fecundity reduction might be affected by the introduction of the external gene fragment(s) to its insertion position. In addition to the higher r and λ values, the mean generation time (T) of the wild-type flies was 37.85 days, which is shorter than the 41.19 and 44.4 days for both T values of the *RTA-Bddsx* transformed DsRed$^-$ and DsRed$^+$ flies, respectively (Table 3). The extended T values of the transformed flies might reflect fitness costs related to physiological adjustments. These results indicated that the fecundities of the DsRed$^-$ and DsRed$^+$/RTA$^-$ females decreased. The presence of DsRed$^+$/RTA$^-$ females suggested a natural protection mechanism to overcome the pressure from endogenous RTA toxins. Although the contribution of the DsRed$^+$/RTA$^-$ females to the rate of population increase can be ignored, it might be important to avoid eradicating the *B. dorsalis* population after repeated releases of transformed males. These results are consistent with the broader goal of SIT, which is to suppress insect pest populations rather than totally eradicating them (Enkerlin 2005).

The *RTA-Bddsx* Transgene Is Stably Inherited in Male Offspring After Backcross to a Wild-Type Female

Because the DsRed$^+$/RTA$^+$ females do not survive, the heterozygous *RTA-Bddsx* transgenic males must backcross to wild-type females for strain maintenance. During the period from F_4 to F_{10}, DsRed$^+$ pupae accounted for 40.49 to 51.42% of the population, and this result is consistent with Mendelian law. However, the male percentages of the progenies after backcrossing to the wild-type females were always above 50% in the DsRed$^+$ group (from 60.0 to 76.9%), and this observation suggests that the female-lethal function still remains (Table 4). In addition, data from genomic analyses showed that the *RTA-Bddsx* transgene was stable and present in >80% of the DsRed$^+$ male progeny (Table 4). The data clearly demonstrated that the transgene inserted in the transformed flies was inheritable and deliverable to their offspring. Moreover, the transformed males can be assigned as carriers with the ability to spread the *RTA-Bddsx* transgene into field populations of *B. dorsalis* and prolong the female-lethal effect from one generation to the next.

In previous RIDL studies, the Tet-Off system has been a useful conditional expression system because it provides a permissive-condition bypass of female lethality in the presence of tetracycline, which is helpful for establishing a homozygous transformation line. However, a recent report has indicated that the application of tetracycline might produce unexpected adverse effects that exert a fitness cost to the transformed organisms (Harvey-Samuel et al. 2014). Therefore, the concentrations of tetracycline must be applied carefully so that they repress transgene expression but are not harmful to normal female development. Based on our observations here, the Tet-Off step may not be necessary and can be skipped in the *RTA-Bddsx* system because heterozygous flies can be used for pest control directly with a red-fluorescent sorting process that can be applied during the pupae stage to distinguish the transformed and non-transformed flies before release. Because the surviving DsRed$^+$/RTA$^-$ females presented limited contributions to the population fecundity, their presence will be diluted quickly, and their effect can be ignored. The advantages of abandoning conditional expression include skipping several time-consuming steps for homozygote selection, excluding potentially adverse effects from the tetracycline treatment and inbreeding, and saving the executive costs spent on additional tetracycline applications.

After comprehensive age-stage two-sex life-table analyses, a number of population parameters related to the growth, survival, reproduction, and intrinsic rate of increase were recorded and compared among various groups of *B. dorsalis*. The percentage of F_4 DsRed$^-$ males significantly reduced from nearly 50 to 30.12% compared with that of the wild-type flies. The ratio of DsRed$^-$ females also declined, and these flies had a relatively low fecundity compared with that of the wild-type females. The percentage of F_4 DsRed$^+$ males remained high, and >90% retained the complete *actin 5C* promoter and *RTA-Bddsx* transgene; thus, these males are expected to act as carriers to spread the female-lethal effects in the population. In the case of the DsRed$^+$/RTA$^-$ females, they represented a small percentage of the population and had a low fecundity; therefore, their effects on the entire population could be ignored. Moreover, the competitive ability of the transformed males was also comparable with that of wild-type males from our preliminary observations. All of the data presented here support the *RTA-Bddsx* system as a permissive SIT approach for the control of *B. dorsalis* through environmentally friendly and target-specific features. This approach also indicates the potential for assigning new uses to available natural toxins with known functions.

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Erratum

Correction of "Chang C., Huang C. Y., Dai S. M., Atlihan R., Chi H. 2016. Genetically engineered ricin suppresses *Bactrocera dorsalis* (Diptera: Tephritidae) based on demographic analysis of group-reared life table. Journal of Economic Entomology."

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Following publication of the above article, it was found that the letters showing the significant difference in Table 3 were missing and the curve of l_x of Fig. 2c was wrong. Table 3 should read as the following:

Table 3. Population parameters (mean ± SE) assessments among cohorts with wild-type, DsRed$^-$ and DsRed$^+$ transgenic female flies

Parameter	Cohort with wild-type females	Cohort with *RTA-Bddsx* females (DsRed$^-$)	Cohort with *RTA-Bddsx* females (DsRed$^+$/RTA$^-$)
Net reproductive rate (R_0)	272.4 ± 48.2a	98.5 ± 5.7b	24.3 ± 2.8c
Intrinsic rate (r, day^{-1})	0.1481 ± 0.0057a	0.1114 ± 0.0016b	0.0725 ± 0.0030c
Finite rate (λ, day^{-1})	1.1597 ± 0.0065a	1.1178 ± 0.0018b	1.0751 ± 0.0032c
Mean generation time (T, day)	37.85 ± 0.32c	41.19 ± 0.12b	44.40 ± 0.30a

Fig. 2 should look as:

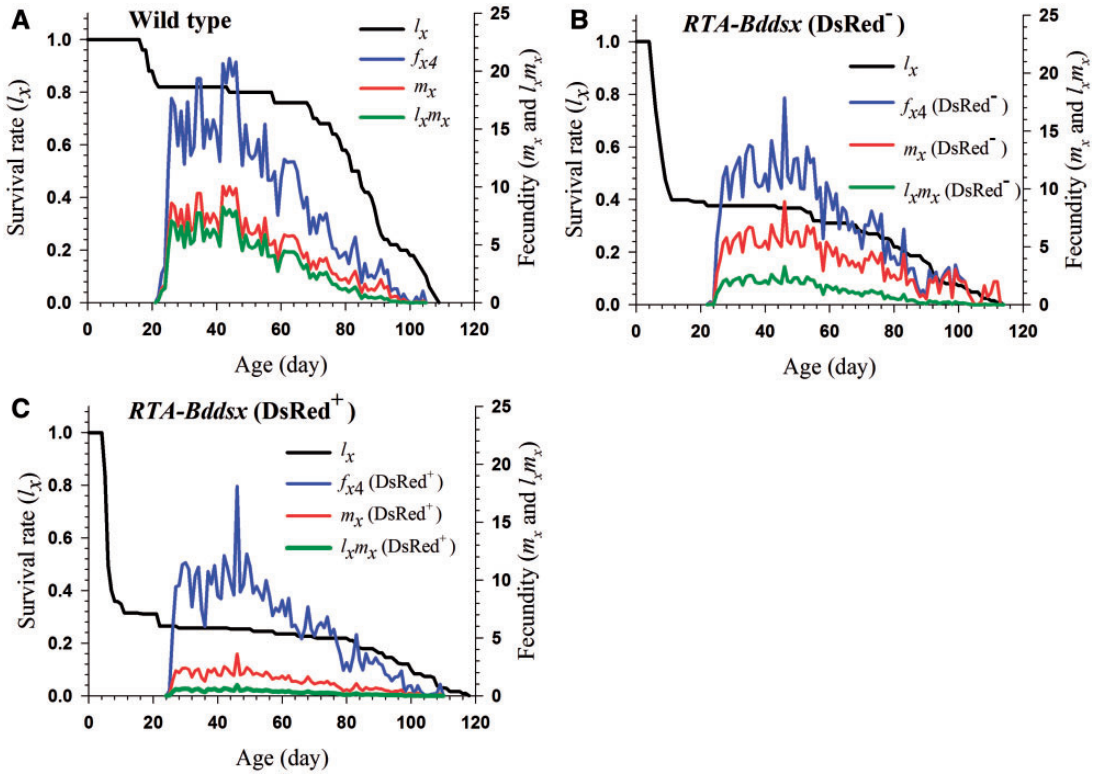

Fig. 2. Analyses and comparison of the reproductive abilities among wild-type, DsRed$^-$ and DsRed$^+$ transgenic female flies. (A) Twenty-five pairs of newly emerged wildtype flies were randomly selected and paired. (B) Twenty-five pairs of newly emerged DsRed$^-$ flies after *RTA-Bddsx* male and wild-type female back-cross were sampled randomly and self-crossed. (C) Twenty-five pairs of newly emerged flies of *RTA-Bddsx* transformed DsRed$^+$ flies were sampled randomly and self-crossed. The eggs laid by each female were collected daily for the rest of its life span to determine fecundity. (Online figure in color.)