

The *Drosophila Suzukii* Mating Rate Decrease for the Reproductive Interference of *Drosophila Melanogaster*

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Abstract

Background: *Drosophila suzukii* has been widely distributed all over the world since 2008, and it is a harmful pest causing great economic loss in many countries. Previous research has found that the presence of *Drosophila melanogaster* could reduce the emergence and egg laying of *Drosophila suzukii*. In order to figure out the potential mechanism of this phenomenon, we studied three potential factors including lifetime, larval interspecific competition, and reproductive interference.

Results: The results show that the *Drosophila suzukii* offspring number was significantly decreased when reared with *Drosophila melanogaster*. The lifetime and larval interspecific competition have no significant effect on the *Drosophila suzukii* population. Surprisingly, *Drosophila melanogaster* can cause reproductive interference with male *Drosophila suzukii*, which leads to a significant decline in the successful mating rate of the latter fruit fly.

Conclusions: The presence of *Drosophila melanogaster* causes the *Drosophila suzukii* population to decrease through the effect of reproductive interference, and the *Drosophila suzukii* successful mating rate is significantly decreased for the existence of *Drosophila melanogaster*.

Background

Drosophila suzukii (Diptera: Drosophilidae), which is also called spotted-wing drosophila (SWD), is originally from Asia, and has become a severe invasive pest in South America and Europe since 2008 [1, 2], causing huge economic losses in the fruit production industry [3]. Usually, *Drosophila suzukii* lay eggs within ripe and ripening fruit by using a sclerotized and serrated ovipositor that can penetrate and lead to physical damage of fruit skins [4, 5]. According to the literature, *D. suzukii* can lay up to 600 eggs (around 400 eggs on average) during their life span, and the minimum development time from oviposition to adult under optimal temperature is 8 days [6]. As for environmental adaptability, *D. suzukii* has a wide range of tolerance to climatic conditions, with a reproduction temperature from 10 °C up to 32 °C [7]. In addition, the activity and development temperature range are usually between 20 to 25 °C [8]. This pest also has wide host range, and up to now, there have been more than 150 species reported as hosts for *D. suzukii* [9, 10]. Due to this, it is almost impossible to find an effective way to eliminate this pest in a short time, for different hosts have non-identical ripening times, and wild hosts as well as ornamental plants can serve as refuges, so that *D. suzukii* can persist through survival challenges such as the low temperature in winter [11].

Currently, *Drosophila suzukii* has successfully invaded many areas and countries. The use of ovipositors can cause physical damage to intact fruit, and the larvae can speed up the process of decay, which provides an ideal food source to other phytophagous insects, so competition is almost inevitable with other *Drosophila* species, such as *Drosophila melanogaster*. Usually, competition includes intraspecific competition and interspecific competition. The outcome of competition depends on the relative strength of interspecific competition and intraspecific competition. Both types of competition are a struggle for

survival for every individual because of limited environmental resources [12]. To plant-eating insects, the importance of interspecific competition to community structure has been discussed by many ecologists [13, 14]. Interspecific competition widely exists among insects [15, 16]. The research by Denno analyzed 193 pairs of competitor species, and concluded that the interspecific competition affects the abundance and distribution of plant-eating insects [17]. The interspecific competition between two species is an interspecific interaction that could lead one species population to be reduced as the other species increases, which has great effect on species coexistence, habitat partitioning, food resource partitioning, and species replacement [18].

According to the niche theory, closely related species overlap in resource needs, so the competition can be very strong when two species first meet [19]. A previous study concluded that, in a lab environment, the presence of *D. melanogaster* can significantly reduce emergence and egg laying of *D. suzukii* through interspecific competition [20]. This is because most male *Drosophila* can produce a pheromone called *cis*-vaccenyl acetate (*cVA*) that used in courtship, aggression, and aggregation signaling, which has a disruptive effect on *D. suzukii* [20]. The number of successful matings is reduced when male *D. suzukii* encounter this pheromone. Similarly, in previous work, we found that when adults of *D. melanogaster* and *D. suzukii* were reared together for three generations, the whole population of *D. suzukii* would eventually die. Thus, in this study, we attempted to explore more alternative potential factors.

The current study shows that both *D. suzukii* and *D. melanogaster* have strong appetites for rotten fruit, and have identical feeding niches. For acquiring resource, two species might show competition, which might cost time, energy and increase risk for injury. When interspecific competition of two flies occurs, more time and energy might be used for competition, resulting in decreased in egg laying, and individuals might suffer physical damage, affecting the longevity of adults. Decreases in both egg laying and adult longevity could be important factors leading to the whole population of *D. suzukii* would be finally dying. Therefore, in our experiments, we investigated the effect of interspecific competition on the longevity and fecundity of the two flies. In addition to adult competition, larval-larval competition between *D. melanogaster* and *D. suzukii* was also studied. Besides, that, reproductive interference is also a kind of interspecific sexual interaction that the female reproductive success of one species is reduced because of the interference by another species [21]. The frequency of conspecific courtship and mating, fecundity of females, and fertility of eggs can be influenced by reproductive interference, and time, energy, nutrients, or gametes can be wasted in heterospecific sexual interactions, causing fitness loss for the individuals involved [22]. Reproductive interference might be a potential factor resulting in the population reduction of *D. suzukii*, and would be examined in this study.

Results

The offspring number and longevity comparison of two flies in two rearing conditions

The *D. suzukii* offspring numbers were significantly decreased when rearing with *D. melanogaster* (Fig. 1a). Under independent rearing condition, the offspring number of *D. suzukii* (117.37 ± 24.28) was significantly higher than in mixed rearing condition (40.47 ± 8.23 , $t_{17} = 3.13$, $P = 0.006$). The same result also found in *D. melanogaster* that the offspring number in independent condition (494.50 ± 36.92) was extremely higher than in mixed rearing condition (269.53 ± 47.05 , $t_{18} = 3.76$, $P < 0.01$).

As for longevity, two flies were affected differently under two conditions (Fig. 1b). Our result showed that the lifetime of *D. suzukii* in the independent condition (54 ± 2.70 days) had no apparent difference comparing with the mixed condition (61.6 ± 3.04 days, $t_{36} = -1.85$, $P = 0.07$). However, as for *D. melanogaster*, the lifetime in independent rearing condition (70.1 ± 1.05 days) was significantly higher than in the mixed rearing condition (79.25 ± 3.05 days, $t_{38} = -2.838$, $P = 0.007$).

The larval interspecific competition of two flies

We calculated the number of pupation and eclosion of two flies in larval interspecific competition experiments (Table 1). The results showed that for *D. suzukii*, eclosion had no significant difference between independent and mixed rearing conditions ($\chi^2 = 3.52$, $df = 1$, $P = 0.061$), which was similar as the result of pupation ($\chi^2 = 3.14$, $df = 1$, $P = 0.077$). As for *D. melanogaster*, the eclosion number still did not have much difference between independent and mixed rearing conditions ($\chi^2 = 0.515$, $df = 1$, $P = 0.473$), the pupation number had little difference between the two rearing conditions ($\chi^2 = 0.002$, $df = 1$, $P = 0.905$).

Table 1
The pupation and eclosion number in independent and mixed rearing conditions

The fly and rearing type	Pupation number	No pupation number	Eclosion number	No pupation number
<i>D. suzukii</i> (independent)	140	60	128	72
<i>D. suzukii</i> (mixed)	59	41	52	48
<i>D. melanogaster</i> (independent)	134	66	122	78
<i>D. melanogaster</i> (mixed)	68	32	66	34
The reproductive interference could reduce the successful mating rate of <i>D. suzukii</i>				

We found the existence of *D. melanogaster* could impact the normal courtship and mating behavior (see **Additional file e-h**). And the *D. suzukii* successful mating rate was also affected (Table 2), which was significantly decreased in mixed rearing conditions when compared with independent rearing conditions ($\chi^2 = 3.84$, $df = 1$, $P = 0.05$). However, for *D. melanogaster*, the successful mating rate had no significant difference between the two rearing conditions ($\chi^2 = 0.41$, $df = 1$, $P = 0.522$). Besides that, our results showed that the courtship times of *D. suzukii* were not significantly different in independent (728.72 ± 200.07 s) and mixed (1392.25 ± 393.22 s, $t_{13} = 1.64$, $P = 0.125$) condition. The number of courtship

behaviors also had no significant difference ($U = 16$, $P = 0.472$) between the two rearing conditions, the mating times were not significantly different between the independent (1738.1 ± 159.92 s) and mixed (1412.5 ± 182.75 s, $t_{12} = -1.16$, $P = 0.268$) conditions.

As for *D. melanogaster*, the courtship times were also not significantly different in independent (442.54 ± 96.26 s) and mixed (202.3 ± 51.81 s, $t_{21} = 2.02$, $P = 0.057$) conditions. However, the number of courtship behaviors before mating in the mixed rearing condition were significantly fewer than in the independent rearing condition ($U = 102.5$, $P = 0.022$), and the mating times were not significantly different between independent (987.69 ± 60.93 s) and mixed (961.9 ± 64.92 s, $t_{12} = 0.287$, $P = 0.777$) conditions.

Referring to misdirected courtship, in mixed rearing conditions, the *D. suzukii* had significantly more misdirected courtship behavior than *D. melanogaster* ($U = 40$, $P = 0.0057$). Because the male *D. suzukii* had 13 times heterospecific mating attempts towards female *D. melanogaster*. Surprisingly, the male *D. melanogaster* did not have this behavior in our observation. In addition, real heterospecific mating happened twice between male *D. suzukii* and female *D. melanogaster* in behavior observation experiment.

Table 2
The reproductive interference effect on courtship and mating behavior

The fly and rearing type	Courtship time(s)	Mating time(s)	Successful mating rate
<i>D. suzukii</i> (independent)	728.72 ± 200.07	1738.1 ± 159.92	55% (11/20)
<i>D. suzukii</i> (mixed)	1392.25 ± 393.22	1412.5 ± 182.75	20% (4/20)
<i>D. melanogaster</i> (independent)	442.54 ± 96.26	987.69 ± 60.93	65% (13/20)
<i>D. melanogaster</i> (mixed)	202.3 ± 51.81	961.9 ± 64.92	50% (10/20)

Discussion

Similar to a previous study [20], when rearing mixed *D. suzukii* and *D. melanogaster*, our results showed that the offspring of *D. suzukii* significantly decreased (Fig. 1). Datta *et al.* (2008) suggested that existence of *D. melanogaster* could change the oviposition preference, resulting in reduced egg laying by *D. suzukii*. The male *D. melanogaster* may could produce a pheromone, *cis*-vaccenyl acetate (*cVA*) [23], which is natural repellent to female *D. suzukii* searching for oviposition sites. Our results suggested that, besides the oviposition preference change, reproductive interference by *D. melanogaster* was likely to be alternative factor resulting in the offspring of *D. suzukii* significantly decreasing.

Reproductive interference between invasive and native species has received much attention, and is a kind of interspecific sexual interaction where the female reproductive success of one species is reduced because of the interference by another species [21]. In our behavior experiment, we found that the presence of female and male *D. melanogaster* had reproductive interference towards male *D. suzukii*, in which male *D. suzukii* might choose *D. melanogaster* female as the mating target (see **Additional file f**). Usually, reproductive interference is distinguished by seven types: signal jamming, heterospecific rivalry,

misdirected courtship, heterospecific mating attempts, erroneous female choice, heterospecific mating and hybridization [24]. Three types of this reproductive interference, including misdirected courtship, heterospecific mating attempts and heterospecific mating, were observed in our study.

In tested adults, 95% (19 of 20 groups) of *D. suzukii* males showed misdirected courtship behavior. Usually, misdirected courtship is performed or initiated by males, which are often indiscriminate in mate choice, as they invest less energy in reproduction [25], in our study, we found that misdirected courtship was initiated by male *D. suzukii* as well. These males may fail to recognize conspecific females and may prefer heterospecific females or even males [26, 27]. The previous study reported that heterospecific females with larger body size had more attraction to males because a large body size indicates high fecundity [28, 29], which is contrary to our observations where male *D. suzukii* were attracted by female *D. melanogaster* that are smaller than female *D. suzukii*. Therefore, we deduce that male *D. suzukii* fail to recognize conspecific females, and female *D. melanogaster* possess other features that can attract male *D. suzukii*. A study has shown that *D. melanogaster* sex pheromones are cuticular hydrocarbons (CHC) that mediate chemical communication for both sex and species recognition [30], which may make female *D. melanogaster* more attractive, and this CHC may prevent conspecific female scent attraction for male *D. suzukii*.

Consistent with previous reports [31], in our observation experiments, all of heterospecific mating attempts followed the misdirected courtship, while *D. melanogaster* males showed no heterospecific mating attempts. The behavior of heterospecific mating has been reported by many studies, such as Takafuji (1988), Fujimoto *et al* (1996), Takafuji *et al* (1997) [32–34], while fewer *D. suzukii* males showed heterospecific mating with *D. melanogaster* females. Usually, reproductive interference can be regarded as a kind of “mistake” for incomplete species recognition systems. Thus, time, energy, nutrients, or gametes were wasted in heterospecific sexual interactions, causing the fitness loss of individuals involved [22]. The frequency of conspecific courtship and mating, fecundity of females, and fertility of eggs can be influenced by reproductive interference [22].

In addition, more potential factors resulting in population of *D. suzukii* decreasing by *D. melanogaster* were also explored. However, inconsistent with our expectations, the lifetime of *D. suzukii* did not become shorter when reared with *D. melanogaster*. Our results suggest that competition with *D. melanogaster* might not lead to the adults of *D. suzukii* having physical damage, thus affecting the longevity of adults. Therefore, the population of *D. suzukii* decrease was unlikely to be caused by shortening the lifetime of *D. suzukii* adults when reared with and competing with *D. melanogaster*. Besides, the larval interspecific competition between *D. suzukii* and *D. melanogaster* also might not be a factor affecting *D. suzukii* population decreases, due to the mixed rearing having no significant effect on rates of pupation and eclosion (Table 1).

Conclusions

Generally, in this study, we found that the *D. suzukii* population was significantly decreased when rearing with *D. melanogaster*, which was similar to previous research. The potential factors including longevity, larval interspecific and reproductive interference were studied. The experimental results showed that factors of longevity and larval interspecific competition had no significant effect on the population of *D. suzukii*. However, the existence of *D. melanogaster* had reproductive interference effects on male *D. suzukii*, in which the rate of choosing conspecific females as mating targets was significantly reduced, and the successful mating rate also apparently decreased. Therefore, this phenomenon can be further researched, including what features that *D. melanogaster* has that cause male *D. suzukii* not to recognize the conspecific females. We guess *Drosophila melanogaster* may release some substance, such as sex hormone that distracted male *D. suzukii*.

Methods

Fly stocks

Adults of *Drosophila suzukii* were first collected from Hui Wang blueberry orchard in Nanling, Anhui Province, China (30.92°N, 118.34°E). *Drosophila melanogaster* were collected at Anhui Normal University, Anhui Province, China (31.33°N, 118.37°E). In laboratory conditions, adults of the two species were independently reared in nylon cube cages (35 × 35 × 35 cm) at temperature 25 ± 1 °C, with a light: dark photoperiod of 14:10, and relative humidity of 60 ± 5%. Additionally, both flies were fed with solid food diet [35], which was changed out daily.

Longevity and fecundity

In this experiment, newly emerged (< 24 hours) individuals of the two species, *Drosophila suzukii* and *Drosophila melanogaster*, were selected. For laying eggs, one pair of *D. suzukii* or *D. melanogaster* was placed into pre-loaded transparent tubes with 20 ml solid food. Additionally, treatments with the two species mixed and reared together were also conducted. The next day, adult flies were anesthetized by CO₂ and then transferred into another transparent tube with brand new solid food until the paired flies died. The death times of adult flies in each group were recorded. All of the replaced tubes were kept in incubators set at conditions with the same light: dark photoperiod (14:10), temperature (25 ± 1 °C), and humidity (60 ± 5%) and were checked daily. The daily number of offspring was counted, and ten repetitions for each fly were conducted.

Larval competition

We hypothesized that larval interspecific competition would lead to a population change for *D. suzukii*. Therefore, we reared the two fly species in two conditions, to confirm the larval interspecific competition effect on adult emergence. Because interspecific competition is a density-dependent effect on the population dynamics, when one species density increases, it causes another species density to reduce [21]. Furthermore, the species with the higher initial density is likely to exclude the species with lower

initial density [36, 37]. Therefore, in our experiments, we kept the same density in the two rearing conditions to make sure that the competition ability of the two flies was equal in density.

Both flies were fed on yeast water for 8 h, and then two cups containing solid food were used for egg collection. Respectively, twenty larvae of two flies were picked out into a cup with 10 g solid food for independent rearing after egg incubation. For the mixed rearing condition, ten larvae from two flies were put into the same solid food cup. All the cups were kept in incubators set at the following conditions: light: dark photoperiod (14:10), temperature (25 ± 1 °C), and humidity ($60 \pm 5\%$). Cups were checked daily, and the daily numbers of pupation and eclosion instances were counted. Respectively, ten of twenty repetitions for each fly were conducted for eclosion counting and the rest were for pupation counting.

The courtship behavior observation of two flies in independent and mixed conditions

In order to ensure that the flies used in behavior observations were unmated, we picked out the pupae that were going to emerge into EP tubes. All tubes were kept in incubators set at the following conditions: light: dark photoperiod (14:10), temperature (25 ± 1 °C) and humidity ($60 \pm 5\%$). The observation started 24 hours after eclosion of flies. For the independent condition, one pair of *D. suzukii* or *D. melanogaster* was put into a quartz cylinder (height: 0.5 cm, diameter: 3 cm) with a 0.5 mm quartz lid. The right amount of solid food was put in before each observation experiment, so that flies can take food in time. However, in mixed conditions, one pair of both fly types was put together to make observations. We recorded a 40-minute video of courtship and mating behavior with a microscope (VHX-5000, Osaka Japan, Keyence Corporation). The frequency of courtship behavior and total mating time were recorded. We performed 20 observation replicates for the independent and mixed conditions for the two fly species.

Date analysis

We performed a normality test on all collected data. The longevity, offspring numbers, courtship time, and mating time data fit a normal distribution, so these data were analyzed by student test (*t*-test). However, for the data that did not fit the normal distribution, such as the frequency of correct courtship, misdirected courtship, and heterospecific mating attempts were analyzed by the Mann-Whitney U test; the successful mating rate, pupation, and emergence rate were analyzed by the Chi-square test. All these tests were performed with R 3.6.1.

Abbreviations

SWD

Spotted-wing drosophila;

cVA

cis-vacacenyl acetate;

Is

Independent rearing *D. suzukii*;

Ms
Mixed rearing *D. suzukii*;
Im
Independent rearing *D. melanogaster*;
Mm
Mixed rearing *D. melanogaster*;
CHC
cuticular hydrocarbons

Declarations

Authors' contributions

YC, MZ, WH, and JL conduct the experiments. YC contributed to data curation and statistical analysis, and first drafted the writing. PL and HH contributed to idea and design of the study, and comments and revisions; All authors contributed critically to the drafts. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset analyzed during the current study is presented in Additional file as well as Dryad repository, https://datadryad.org/stash/share/ovg5_qZtvaHuupqejQOi4kgviw3ky_vZ6vFJ8uwx-oU;
https://datadryad.org/stash/share/NbDe_Pn5EkX9N93Q092MGLiPPDFy4-JZMW4DdgjSYBg

Ethics approval and consent to participate

The behavioral research in our study were in compliance with the law of the people's republic of China on the protection of wildlife and were approved by the Laboratory Animal Ethics Committee of Anhui Normal University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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