



# Asymmetrical reproductive interference between two sibling species of tea looper: *Ectropis grisescens* and *Ectropis obliqua*

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## Abstract

*Ectropis grisescens* Warren and *Ectropis obliqua* (Prout) are two morphologically similar sibling species with overlapping ranges. In this study, manipulative laboratory experiments were conducted to examine the possibility of reproductive interference in sympatric populations of *E. grisescens* and *E. obliqua* and the potential consequences of the mating interaction. Our results showed that the presence of males or females of different species could incur mating interference and significant reduction of  $F_1$  offspring. The reduction was not significant relevant to the initial relative abundance of *E. grisescens* and *E. obliqua*. Detailed observations of mating opportunity showed that female mating frequencies of both species were not significantly affected by the absolute species density, but the mating success of *E. obliqua* females with conspecific males depended on species ratio. In addition, adding males to the other species resulted in lower number of offspring suggesting that the males' behaviour might be linked with mating interference. Males of both *E. grisescens* and *E. obliqua* could interfere the intraspecific mating of the other species, but the impact of the mating interference differed. These combined data indicated that asymmetric reproductive interference existed in *E. grisescens* and *E. obliqua* under laboratory conditions, and the offspring of the mixed species were significantly reduced. The long term outcome of this effect is yet to be determined since additional reproductive factors such as oviposition rate and progeny survival to adulthood may reduce the probability of demographic displacement of one species by the other in overlapping niches.

**Keywords:** tea looper, sibling species, reproductive interference, mating interaction, species exclusion

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## Introduction

*Ectropis grisescens* Warren and *Ectropis obliqua* (Prout) (Lepidoptera: Geometridae: Ennominae) feed on tea leaves

and tender buds. They infest thousands of hectares of tea a year, severely reducing growth, and impacting the tea production in the following year (Zhang & Tan, 2004; Zhang *et al.*, 2014). *E. grisescens* has been reported from Anhui, Fujian, Henan, Hunan, Hubei, Jiangxi and Zhejiang provinces in China (Jiang *et al.*, 2014). It is probably indigenous to China, as there are no reports of *E. grisescens* abroad (Warren, 1894; Swinhoe, 1902; Wehrli, 1939–1954). *E. obliqua* was first found and named in Japan, and now in Korea and in China (Wehrli, 1939–1954; Sato, 1984; Kim *et al.*, 2001). *E. obliqua*

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has been reported from Anhui, Jiangsu and Zhejiang provinces and Shanghai city in China (Jiang *et al.*, 2014). *E. griseescens* and *E. obliqua* are morphologically similar and hard to discriminate in tea plantations. Because of these similarities, *E. obliqua* is likely mistaken for a variant of *E. griseescens* as it was when reported for the first time (Warren, 1894).

Despite their morphological similarities, *E. obliqua* and *E. griseescens* are two sibling species in the same genus in China (Jiang *et al.*, 2014; Zhang *et al.*, 2014). Our previous research of tea loopers from 17 tea fields in different parts of China shows that the two species, although being morphologically similar, had clear reproductive isolation and variable mitochondrial cytochrome oxidase I (COI) sequences (Zhang *et al.*, 2014). Recently, by microscopic examination of some morphological traits can separate the two species. In addition, a genetic distance between *E. obliqua* and *E. griseescens*, based on COI sequences, of about 3.7% (Jiang *et al.*, 2014). These phylogenetic analyses revealed that *E. obliqua* and *E. griseescens* COI sequences fall into two taxonomic clades, and form separate monophyletic groups (Jiang *et al.*, 2014; Zhang *et al.*, 2014). Previous attempts to cross *E. griseescens* and *E. obliqua* did produce fewer female offspring than intraspecific mating, which resulted in an unbalanced sex ratio in the hybrid  $F_1$  generation. The hatching rate, survival to adult, and percent of normal adults was much lower than with intra-species mating. Moreover, a self-cross of  $F_1$  generation adults produced infertile eggs or no eggs (Xi *et al.*, 2014; Zhang *et al.*, 2014). These findings can be explained as closely related species often have incompletely isolated mate recognition systems (Reitz & Trumble, 2002). Laboratory experiments have shown that *E. griseescens* and *E. obliqua* are capable of producing viable  $F_1$  hybrids but that these females are sterile as their  $F_2$  generation was not viable (hybrid breakdown), indicating, most likely, a post-zygotic reproductive barrier (Xi *et al.*, 2014; Zhang *et al.*, 2014).

Asymmetric mating behaviour of both males and females may lead to different consequences in reproductive performance contributing to facilitation or prevention of competitive displacement of indigenous species by invaders (Scott Schneider *et al.*, 2004; Crowder *et al.*, 2010a, b; Hochkirch & Lemke, 2011; Chapple *et al.*, 2012; Luan & Liu, 2012; Wang *et al.*, 2012; Luan *et al.*, 2013). Such sexual interaction plays a critical role in biological invasion and has been called reproductive interference. Reproductive interference is defined as any kind of interspecific sexual interaction, caused by incomplete species recognition systems, which can reduce the fitness of individuals of at least one of the species involved (Gröning & Hochkirch, 2008). Reproductive interference is one of the major factors mediating species exclusion among insects (Reitz & Trumble, 2002). Reproductive interference is generally asymmetric (one species is affected more than the other) and is positively frequency-dependent (Gröning & Hochkirch, 2008; Kishi *et al.*, 2009). Reproductive interference can affect the population dynamics, abundance, habitat choice, geographical distribution of the species involved, is more likely to lead to species exclusion or niche partitioning (Gröning *et al.*, 2007; Hochkirch *et al.*, 2007; Liu *et al.*, 2007; Gröning & Hochkirch, 2008; Kishi *et al.*, 2009; Luan *et al.*, 2014; Sun *et al.*, 2014). Studies on such interspecific interactions in species can give information about the maintenance of speciation, as they might drive speciation due to reinforcement of pre-mating barriers (Servedio & Noor, 2003; Seehausen, 2004; Keranen *et al.*, 2013). However, the ecological significance of reproductive interference has

mostly been discussed in the context of biological invasions, and is still often undervalued.

Previously we found that *E. griseescens* and *E. obliqua* occur in separate tea plantations, despite being reported from the same provinces (Zhang *et al.*, 2014). Here additional collections were undertaken and mixed populations of two species were found in Hangzhou, Wuhan, Xinyang, and Nanchang (Jiang *et al.*, 2014; partial data is not published). Previous studies indicated that interspecific sexual interaction (reproductive interference) might explain this distribution pattern (e.g. Gröning *et al.*, 2007; Hochkirch *et al.*, 2007; Thum, 2007; Noriyuki *et al.*, 2012). In this study, we tested whether asymmetric reproductive interference occurs in *E. griseescens* and *E. obliqua* when they co-exist under laboratory conditions and examined the reproductive consequences of these interferences, and whether reproductive success is dependent in the abundances of sexual interacting species.

## Materials and methods

### Collection and culturation of *E. griseescens* and *E. obliqua*

The population of *E. griseescens* used in this study was collected in 2012 from Songyang (119°42'E, 28°49'N), Zhejiang; whereas the population of *E. obliqua* was obtained from Yuhang (119°90'E, 30°39'N), Zhejiang, China. All species were maintained under the same conditions in the laboratory of the Plantation Centre of Tea Research Institute of Chinese Academy of Agricultural Sciences (CAAS), Hangzhou, China. Cultures of both species were fed with tea fresh leaves in laboratory conditions for a year. All the experiments were conducted at  $24 \pm 1^\circ\text{C}$ , 13:11 h (L:D) and  $75 \pm 5\%$  relative humidity (RH).

### Collection of newly emerged virgin adults

In order to ensure that the adults used were virgin, a few days prior to experiments, tea looper pupae from each culture were collected singly and their sex determined using a stereo microscope before adults emerged. The male and female pupae of each population were placed singly into transparent, colourless glass cages. Newly emerged adults were randomly chosen and prepared for experiments.

### Crossing experiments between *E. griseescens* and *E. obliqua*

Crossing experiments were conducted in transparent, colourless glass cages with three combinations of the two species (table 1): (i) *E. obliqua* in single culture (two pairs), eight replicates; (ii) *E. griseescens* in single culture (two pairs), eight replicates; and (iii) *E. griseescens* and *E. obliqua* in mixed culture (one pair of each species), twelve replicates. All the females were allowed to lay eggs for 5 days and then removed. Females that did not lay eggs during this period, or females that laid eggs that did not hatch in 10 days, were considered sterile. The eggs laid by each female in a replicate were calculated by the total eggs weight divided by average egg weight. Then the number of developing larvae and pupae, and emerged adults was recorded every 24 h for 30–35 days. These records were used to calculate the rates of hatching, pupation and emergence, the proportions of normal adults, and the female progeny ratios.

Table 1. Number of  $F_1$  eggs laid, their hatching, pupation, emergence rate and female progeny ratio, in crossing experiments of *E. griseascens* and *E. obliqua*.

Treatments	$N^*$	Number of eggs laid/female	Hatching rate	Pupation rate	Emergence rate	Female progeny ratio
<i>E. griseascens</i> + <i>E. griseascens</i>	8	424 ± 8.35a	0.67 ± 0.02a	0.94 ± 0.01a	0.81 ± 0.03a	0.46 ± 0.04a
<i>E. obliqua</i> + <i>E. obliqua</i>	8	391 ± 11.32a	0.72 ± 0.02a	0.91 ± 0.02a	0.88 ± 0.03a	0.53 ± 0.03a
<i>E. griseascens</i> + <i>E. obliqua</i>	12	392 ± 15.42a	0.48 ± 0.07b	0.91 ± 0.02a	0.70 ± 0.08a	0.40 ± 0.06a

$N^*$ , number of replicates.

Data in the table are mean ± SE, and means on the same line followed by different letters indicate significant differences ( $P < 0.05$ ).

Table 2. Number of  $F_1$  eggs laid, their hatchability, progeny survival to adulthood and female progeny ratio, in reproductive interference with different mix of *E. griseascens* and *E. obliqua*.

Treatments	$N^*$	Number of eggs laid/female	Hatching rate	Survival to adult	Female progeny ratio
(1 <i>E. obliqua</i> ♀ + 1 <i>E. obliqua</i> ♂) + (1 <i>E. griseascens</i> ♀ + 1 <i>E. griseascens</i> ♂)	8	379.14 ± 18.64a	0.46 ± 0.089a	0.66 ± 0.071a	0.41 ± 0.073a
(1 <i>E. obliqua</i> ♀ + 1 <i>E. obliqua</i> ♂) + (2 <i>E. griseascens</i> ♀ + 2 <i>E. griseascens</i> ♂)	8	378.25 ± 14.72a	0.56 ± 0.067a	0.69 ± 0.032a	0.48 ± 0.039a
(2 <i>E. obliqua</i> ♀ + 2 <i>E. obliqua</i> ♂) + (1 <i>E. griseascens</i> ♀ + 1 <i>E. griseascens</i> ♂)	8	313.50 ± 19.91b	0.64 ± 0.049a	0.69 ± 0.055a	0.46 ± 0.039a

$N^*$ , number of replicates.

Data in the table are mean ± SE, and means on the same line followed by different letters indicate significant differences ( $P < 0.05$ ).

#### Mating interactions in mixed cultures with *E. griseascens* and *E. obliqua*

In order to study the difference in offspring in the mixed of *E. griseascens* and *E. obliqua*, three treatments were conducted with different initial relative abundance for each of the two species in transparent colourless glass cages (table 2). One pair of one species adults was supplemented with one or two pairs of the same or the other species. Eight replicates were used for each treatment. Number of eggs, larvae, pupae and adults were counted. The hatching rate, progeny survival to adulthood and female progeny in  $F_1$  were calculated.

#### Mating opportunity of *E. griseascens* and *E. obliqua*

In order to study whether the presence of individuals of different species affected female mating opportunity with males of the same species, three independent treatments with different proportions of species were compared in transparent colourless glass cages: (iv) only a single conspecific couple were introduced into a cage (number of the conspecific pairs : number of heterospecific pairs = 1:0); (v) one pair of each species in each replicate were introduced into a cage (1:1) and (vi) two pairs of one species and one pair of the other species were introduced into a cage (2:1 or 1:2). The proportion of mated females was compared among the three treatments. The species were discriminated by the marked wings of male adults between individuals. Each treatment started in the middle of the day and we recorded whether intraspecific (conspecific) and interspecific (heterospecific) mating occurred in the evening. Eight replications were arranged for each trial.

#### Mating interference in males of *E. griseascens* and *E. obliqua*

In order to test whether male behaviour is responsible for mating interference, two parallel treatments were conducted.

Changes in the mean number of intraspecific mating and production of progeny for the first 5 days after emergence were recorded after a pair of *E. obliqua* ♂ × ♀ was respectively supplemented with one, two and three virgin males of *E. obliqua* or *E. griseascens*. The same experiment was conducted with a pair of *E. griseascens* ♂ × ♀ supplemented with one to three virgin males of *E. griseascens* or *E. obliqua*. The experiment was replicated 8 times for each combination. We recorded if successful mating occurred in the first evening. Number of eggs, larvae, pupae and adults, and female progeny in  $F_1$  were determined as described in the crossing experiments.

#### Data analysis

For crossing experiments between *E. griseascens* and *E. obliqua*, some parameters were compared among treatments using one-way analysis of variance (ANOVA) and means were separated by Duncan's multiple range test.

For mating interactions in mixed cultures with *E. griseascens* and *E. obliqua*, data were analysed by one-way ANOVA and Duncan's multiple range tests of variance.

For mating opportunity of *E. griseascens* and *E. obliqua*, mating success of individual female from each species was analyzed by using a generalized linear model (GLM) with a binomial error structure that indicated whether each individual female was successfully mated (no mating = 0, mating = 1). Absolute species density (number of conspecific pairs/cage) and relative species density (number of conspecific pairs/total number of species in cage) were represented as main effects.

For mating interference in males of *E. griseascens* and *E. obliqua*, all parameters were analyzed using by one-way ANOVA followed by Duncan's multiple range test, and percentages were transformed by arcsine square root before analysis.

All statistical analyses were done using the IBM SPSS statistical software version 21.0.

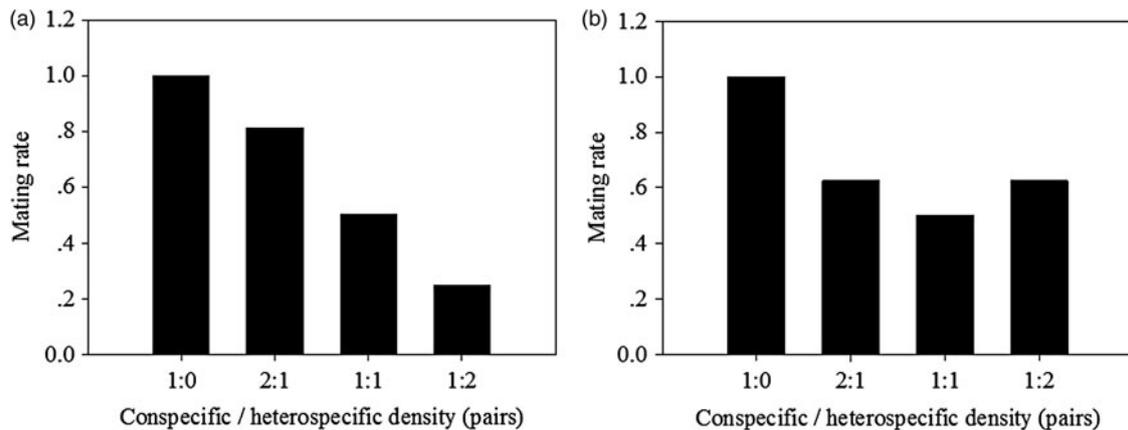


Fig. 1. Mating rate in relation to relative species density in (a) *E. obliqua* and (b) *E. griseescens* females.

## Results

### Crossing experiments between *E. griseescens* and *E. obliqua*

The presence of males and females of different species was found to disturb the reproduction of mated females (table 1). While there was no significant difference in the number of eggs laid per female of each treatment ( $F_{2,24} = 1.921$ ,  $P = 0.168$ ), significant hatching reduction was observed in mixed cultures ( $F_{2,24} = 6.398$ ,  $P = 0.006$ ). The pupation rate and emergence rate of  $F_1$  were both lower in mixed cultures than that in pure culture, even these parameters were not significantly different between pure and mixed cultures ( $F_{2,25} = 0.640$ ,  $P = 0.536$ ;  $F_{2,25} = 2.109$ ,  $P = 0.142$ ). Moreover, there was no significant difference in female ratio in  $F_1$  ( $F_{2,25} = 0.579$ ,  $P = 0.568$ ), the female ratio of  $F_1$  was less than that from pure parents. Therefore, the less  $F_2$  progeny were produced.

### Mating interactions in mixed cultures with *E. griseescens* and *E. obliqua*

Comparisons among all the three treatments indicated there were no significant differences in the hatching rate of  $F_1$  ( $F_{2,21} = 1.498$ ,  $P = 0.246$ ), survival to adult ( $F_{2,21} = 0.110$ ,  $P = 0.896$ ) and female progeny ratio of the  $F_1$  ( $F_{2,21} = 0.481$ ,  $P = 0.625$ ), although these parameters were lower when *E. griseescens* and *E. obliqua* equally presented (table 2). Significantly fewer eggs were observed in the third treatment (two pairs of *E. obliqua* and one pair of *E. griseescens*) compared with the other two treatments ( $F_{2,21} = 4.426$ ,  $P = 0.025$ ).

### Mating opportunity of *E. griseescens* and *E. obliqua*

Next, we tested the mating opportunities of two species. The mating rate of *E. obliqua* females was not affected by the absolute species density, but was significantly affected by the relative species density in the given treatment (fig. 1a, GLM, absolute density, the parameter estimate  $\pm$  SE =  $3.17 \pm 3.44$ , d.f. = 1,  $P = 0.36$ ; relative density,  $1.93 \pm 0.97$ , d.f. = 1,  $P < 0.05$ ). Interestingly, mating success of intraspecific male was relatively higher in the 1:0 trial but was the lowest in the 1:2 trial. Nevertheless, *E. griseescens* females mating success was not affected by the absolute or relative species density (fig. 1b, GLM, absolute density, the parameter

estimate  $\pm$  SE =  $-1.36 \pm 5.33$ , d.f. = 1,  $P = 0.70$ ; relative density,  $0.98 \pm 0.87$ , d.f. = 1,  $P = 0.26$ ).

### Mating interference in males of *E. griseescens* and *E. obliqua*

We found an influence of additional *E. griseescens* males on reproductive success of a pair of *E. obliqua*, but no effect of additional *E. obliqua* males on *E. griseescens* matings. As shown in fig. 2, the intraspecific mating rate of *E. obliqua* decreased with the addition of *E. griseescens* males (fig. 2a;  $F_{6,49} = 16.62$ ,  $P < 0.001$ ). Adding *E. obliqua* males into *E. obliqua* pair decreased the number of larvae slightly, however, the negative effects only became statistically significant when 3 *E. griseescens* males were introduced to *E. obliqua* pair (fig. 2b). For the total number of  $F_1$  progeny reaching adulthood, we observed a slight, but statistically insignificant decrease in the number of  $F_1$  adults produced by *E. obliqua* pair when 1, 2, or 3 additional *E. obliqua* males were present. However, adding *E. griseescens* males in with the *E. obliqua* pair reduced the number of  $F_1$  adults produced significantly (fig. 2c;  $F_{6,49} = 39.07$ ,  $P < 0.001$ ). The proportion of  $F_1$  female progeny (sex ratio) was not significantly affected by any additional (1–3) *E. obliqua* males, while the sex ratio was significantly reduced with an increasing trend of negative influence as more *E. griseescens* males were added into the *E. obliqua* pair (fig. 2d;  $F_{6,49} = 9.89$ ,  $P < 0.001$ ).

Mating interference was also examined by introducing different numbers (1–3) of *E. griseescens* males or *E. obliqua* males into *E. griseescens* pair. We recorded the number of intraspecific mating between the two species. Results (fig. 3) showed that *E. griseescens* did not engage in interspecific mating in most cases, except for a combination of *E. griseescens* pair with one *E. obliqua* male (fig. 3a). This only interspecific mating group subsequently produced the lowest numbers of larvae and adults and smallest female ratio in  $F_1$  generation, but the influence was not statistically significant. Except for this group, adding more *E. obliqua* males or *E. griseescens* males to the *E. griseescens* pair did not show any negative impact on mating and reproduction (fig. 3).

## Discussion

Our results from the laboratory experiments revealed that reproductive interference occurs in *E. griseescens* and *E. obliqua*, and the interaction is asymmetric possibly due to different

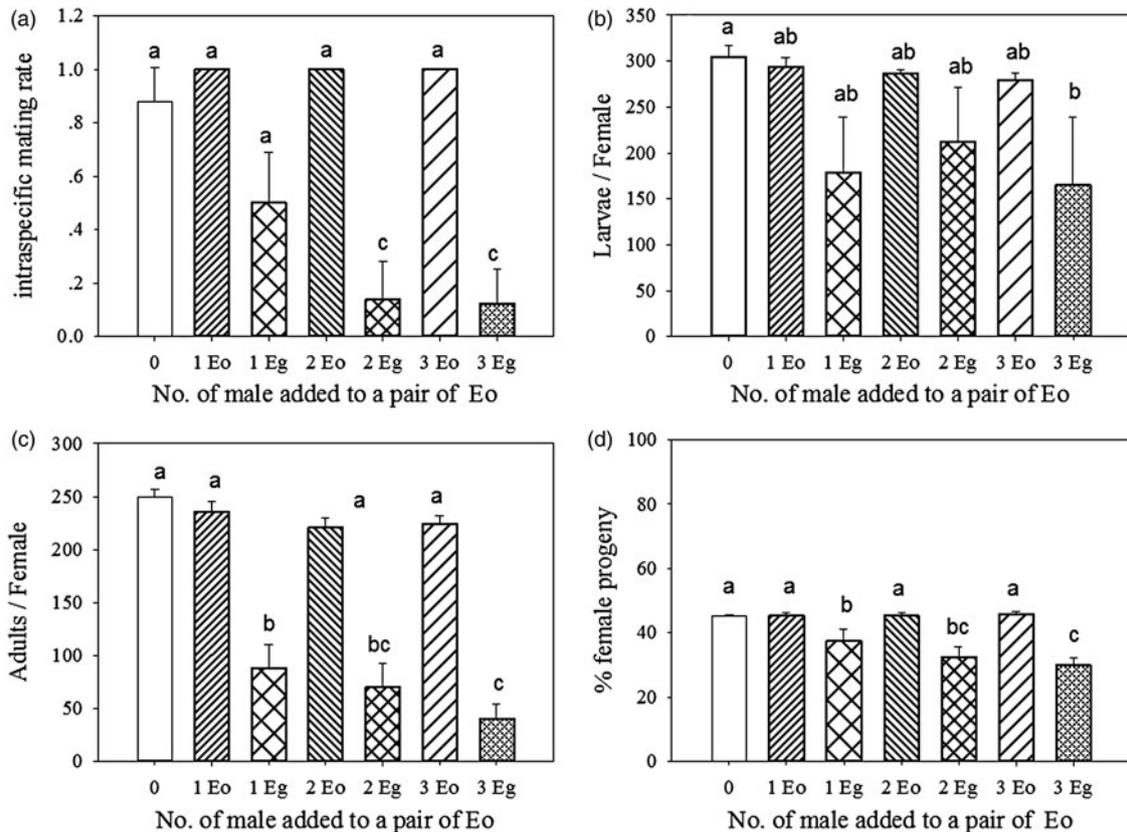


Fig. 2. *E. obliqua* (Eo) performance as affected by *E. griseescens* (Eg) males. (a) Intraspecific mating rate, (b) mean larvae, (c) mean adults and (d) proportion of female progeny within *E. obliqua* when a pair of *E. obliqua* ♂ × ♀ was supplemented with 1, 2 or 3 ♂ of *E. obliqua* or *E. griseescens*. Error bars represent standard error of the means and different letters above bars indicate significant differences ( $P < 0.05$ ).

mating behaviour between the two sibling species leading to different consequences of reproductive performance. In the current research, mixed populations of *E. griseescens* and *E. obliqua* were found in the tea plantations of Chun'an, Hangzhou, Wuhan, Xinyang and Nanchang. Therefore, reproductive interactions between *E. griseescens* and *E. obliqua* are likely to occur naturally because of their overlapping niches.

Studies on insects mating strategy may provide insight into the evolution of mating behaviour, competitive interactions and speciation (Rowe & Arnqvist, 2002; Chapman *et al.*, 2003). Females can recognize conspecific males or reject misdirected courtships from unsuitable males, and females always exert more influence over mating than males (Gröning & Hochkirch, 2008). We observed that the frequency of *E. obliqua* females successfully mating with conspecific males was decreased as the proportion of individuals of the other species increased (fig. 1a). In contrast, the frequency of *E. griseescens* female mating was not affected by the species density ratio (fig. 1b). Results from our other experiments support the conclusion of an asymmetric mating interaction between *E. griseescens* and *E. obliqua*. Adding more males of *E. griseescens* into the *E. obliqua* pair tend to induce more interspecific crosses (*E. obliqua* ♀ × *E. griseescens* ♂) (fig. 2a), but the similar phenomenon (*E. griseescens* ♀ × *E. obliqua* ♂) was not observed in mating interaction cross between *E. griseescens* female and *E. obliqua* male (fig. 3a). These results suggest that males of *E. griseescens* have a greater ability to cause mating interference than *E.*

*obliqua* males. As suggested above, the reduced mating success of *E. obliqua* in the mixed treatments may be the consequence of asymmetric mate recognition ability, e.g. females of *E. griseescens* were often highly sensitive in recognition and rejection males of *E. obliqua*, while *E. obliqua* females were less selective against males of *E. griseescens*. Such asymmetric mating preference phenomenon might be the result of reproductive isolation and could be a clue for the direction of evolution. Further studies will be expected to reveal the factors determining the differences in mating behaviour and mating systems that produce an asymmetric mating interaction between species.

Mate quality and number of mates are the key factor affecting female reproductive success (Bleu *et al.*, 2012; Himuro & Fujisaki, 2012). Our mating interference experiments suggested that mating interactions between *E. griseescens* and *E. obliqua* were frequency-dependent. When a pair of *E. obliqua* ♂ × ♀ was supplemented with different numbers of virgin males of *E. griseescens*, the overall number and female ratio of  $F_1$  offspring was reduced dramatically, and the more males of *E. griseescens*, the fewer offspring (fig. 2). However, the overall number and female ratio of  $F_1$  offspring did not change much when a pair of *E. griseescens* ♂ × ♀ was supplemented with virgin males of *E. obliqua* (fig. 3). Mating attempts by *E. griseescens* males reduced the fecundity of *E. obliqua* females much more than mating attempts by *E. obliqua* males reduced the fecundity of *E. griseescens* females. Consequently, we suggest that the fecundity of females of both species would

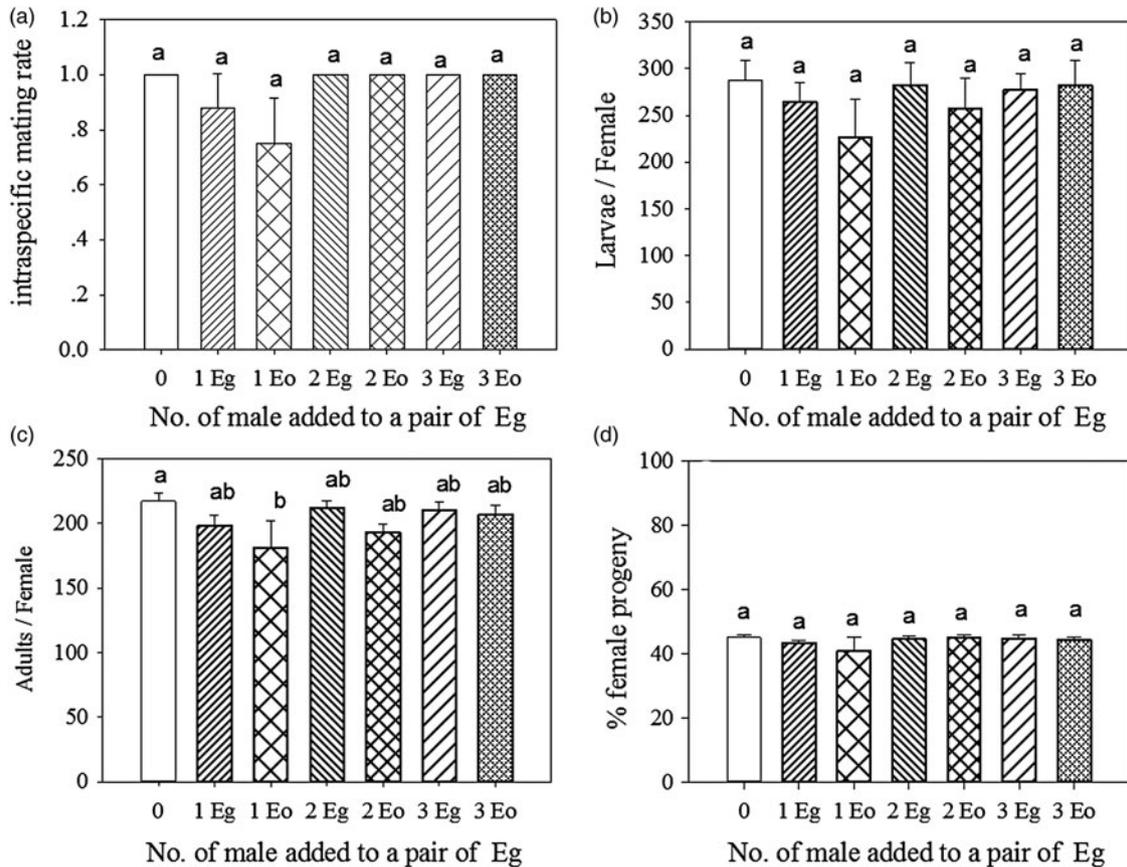


Fig. 3. *E. grisescens* (Eg) performance as affected by *E. obliqua* (Eo) males. (a) Intraspecific mating rate, (b) mean larvae, (c) mean adults and (d) proportion of female progeny within *E. grisescens* when a pair of *E. grisescens* ♂ × ♀ was supplemented with 1, 2 or 3 ♂ of *E. grisescens* or *E. obliqua*. Error bars represent standard error of the means and different letters above bars indicate significant differences ( $P < 0.05$ ).

decrease as the number of the other species males increase, possibly due to this male frequency-dependent effect on mating success. The crossing experiments we conducted demonstrated that the presence of males and females of other species could reduce offspring sex ratio and the number of  $F_1$  offspring (table 1). Furthermore, offspring from differently initial relative abundance of *E. grisescens* and *E. obliqua* did suffer the impact of interspecific crossing, and was less affected by the initial relative abundance (table 2). Overall, the presence of the other species males can affect the intraspecific mating success, and offspring were decreased after adding either male only or both males and females in laboratory.

Asymmetric mating interaction has mainly been discussed in the context of biological invasive species or hybrid zones, for example, whiteflies (Liu *et al.*, 2007), spider mites (Ben-David *et al.*, 2009), ground-hopper (Gröning *et al.*, 2007), beetle (Kishi *et al.*, 2009), and ladybird beetle (Soares & Serpa, 2007). Although the hypothesis of species exclusion still needs to be tested experimentally, it has been suggested that reproductive interference might also offer opportunities for pest control (Ardeh *et al.*, 2004). For example, competitive exclusion has been successfully applied in biological control of exotic Homoptera (Reitz & Trumble, 2002). With increasing public awareness of the ecological problems of conventional insecticides, integrated pest management techniques (multiple

tactics used in eradication programs) are needed (Suckling *et al.*, 2013). Mating disruption, reducing the number of offspring, is an effective, very selective, and environmentally-benign tactic, especially for moth pests (Judd & Gardiner, 2005; Harari *et al.*, 2007; Suckling *et al.*, 2013). For this reason, based on the experimental data, *E. obliqua* seemed to be the inferior species. *E. grisescens* has a stronger capacity for excluding *E. obliqua* than vice versa, and this capacity is associated with asymmetric reproductive interference favouring *E. grisescens*. The basic study of mating interference is significant for evolution of species, control of heredity, and prevention of insect pests. This study established a foundation for exploring the possibility to reduce the fitness of *E. obliqua* by releasing *E. grisescens* into *E. obliqua*-dominated areas.

Mating behavioural interactions between closely related species resulting from incompletely isolated mate recognition systems may lead to reproductive interference that is often asymmetric and can have serious population consequences (geographic separation) for the species (Liu *et al.*, 2007; Gröning & Hochkirch, 2008; Crowder *et al.*, 2010a, b; Noriyuki *et al.*, 2012). Reproductive interference has been commonly found under laboratory conditions, but is not always found in field conditions (Coyne & Orr, 1989; Thum, 2007). Under natural conditions, environmental factors and several ecological mechanisms of the location might influence

interactions between species and produce different results (Dame & Petren, 2006; Hochkirch & Gröning, 2012). Our study shows reproductive interference in laboratory conditions and these two species with the same life history and behavioural traits have the potential for interaction in the field, but further studies are needed to determine if mating interference will occur in field conditions. Ultimately, linking detailed laboratory studies with long-term field survey datasets may provide the key to predicting patterns of species exclusion or coexistence.

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