Female remating inhibition and fitness of *Bactrocera dorsalis* (Diptera: Tephritidae) associated with male accessory glands

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Abstract

Polyandry is widespread among insects but male accessory gland products can influence the propensity of former mates to copulate later with other males. In addition, females may receive nutritional supplements in accessory gland fluids that substantially increase fitness regardless of whether re-mating has been inhibited. In this study, we investigated polyandry in female *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) and the relationship between remating and male accessory gland contents. Similar to other fruit flies, 2 kinds of accessory glands were observed in male *B. dorsalis*. Male mesodermal accessory glands expanded significantly in length and area when copulation finished. A remating refractory period occurred in *B. dorsalis* females, but it did not differ in duration following copulations with either virgin or non-virgin males. Besides, we found that virgin females lived longer, but produced much fewer eggs than mated females. Remating with a refractory period resulted in more eggs being laid and offspring produced than continuous exposure to 2 mates. In addition, females in the continuous presence of 2 males produced significantly more offspring than females with only 1 male present. We also observed that increases in male and female age reduced the rate of fertilization. These results indicated that multiple matings increased the fitness of *B. dorsalis* females, although remating inhibition existed in *B. dorsalis* fruit flies. It is the great reproductive ability of *B. dorsalis* that enable flourishing populations to occur in wild.

Key Words: fitness; male accessory glands; refractory period; remating inhibition; reproductive success

Resumen

El re-apareamiento está muy extendido entre los taxa de animales, y las glándulas accesorias masculinas han demostrado que estan involucradas en el apareamiento y el re-apareamiento de los insectos. Para que el macho sea eficaz en aparearse, los fluidos de las glándulas accesorias primero deben ser rellenadas. Durante el apareamiento, la hembra podria recibir beneficios substanciales de aumento del acondicionamiento por medio de los fluidos de las glándulas accesorias masculinas independientemente de si el re-apareamiento han sido inhibido, lo que ha sido demostrado en muchas especies de insectos. En este estudio, la capacidad de las hembras de *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) para aparearse fue investigado y se analizó la relación entre el re-apareamiento y el contenido de las glándulas accesorias masculinas. Se evaluaron los niveles de la aptitud de las hembras de *B. dorsalis* apareadas sólo una vez o más de una vez con cantidades variables de tiempo entre apareamientos consecutivos mediante la comparación de la longevidad de las hembras y los números de huevos y progenies. Los resultados mostraron que las glándulas accesorias masculinas mesodérmicas se expandieron significativamente en longitud y en área después del apareamiento. El éxito significativamente reducido del re-apareamiento en las hembras apareadas reveló en una vez que existía una inhibición en el re-apareamiento de las hembras de *B. dorsalis*, mientras que no se observaron diferencias en la capacidad de apareamiento entre los machos vírgenes y no vírgenes. Las hembras vírgenes vivieron más tiempo que las hembras apareadas. La presencia continua de los machos con hembras en una proporción de 2:01 causó que las hembras produjeren significativamente más progenies que cuando la relación fue de 1:1.

Palabras Clave: aptitud; glándulas accesorias masculinas; período refractario; inhibición de apareamiento; éxito reproductive

In insects, mating success is determined by many factors, such as the adult body size, diet and mating experience of both males and females (Aluja et al. 2008; Del Castillo et al. 2001; Miyatake et al. 1999; Radhakrishnan & Taylor 2008). Females of many insect species may mate multiple times for various reasons, including generating genetic variance in offspring, gaining access to male-guarded resources and obtaining nutrients from male ejaculates (Ridley 1988). The occurrence of female remating has been widely reported as a widespread phenomenon in insects (Arnqvist & Nilsson, 2000). However, in other species females rarely if ever mate more than once (Thornhill & Alcock 1983). In these cases females may have nothing to gain by further copulations, and strive to avoid physical injury during copulation or be under the influence of mating-inhibiters produced by male accessory glands and included in the ejaculate (Eady et al. 2007; Edvardsson & Tregenza 2005; Aluja et al. 2009). Decreased receptivity of female Diptera after mating has been observed in almost all examined species (Chapman et al. 1998), and male accessory gland fluids (AGFs) play a role in inhibiting remating in Tephritid fruit flies, such as *Ceratitis*

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capitata, *Bactrocera cucurbitae* and *B. tryoni* (Jang et al. 1999; Kuba & Itô 1993; Miyatake et al. 1999; Radhakrishnan & Taylor 2007, 2008).

In mated male insects, inability to fully recover reproductive reserves, such as sperm and accessory gland fluids that might act to inhibit female remating or serve as a nutritional resource for mates, can decrease the efficacy of subsequent copulations. Decreases and increases in male accessory gland sizes are indicators of depletion and recovery of their contents after mating, as has been demonstrated in the Queensland fruit fly *B. tryoni* (Radhakrishnan & Taylor 2008), *Drosophila melanogaster* (Bangham et al. 2002) and the stalk-eyed fly, *Cyrtodiopsis dalmanni* (Baker et al. 2003; Rogers et al. 2005). If there is a sufficient recovery period—beyond the refractory period—between consecutive matings, a previously mated male could transfer similar quantities of sperm as a virgin (Pérez-Staples & Aluja 2006).

Polyandry, with its potential mix of hazards and rewards, enhances fertility (Ridley 1988; Taylor et al. 2008), but shortens female lifespan in a number of Tephritids (Blanckenhorn et al. 2002; Chapman et al. 1998; Chinajariyawong et al. 2010). The most obvious potential fitness benefit of multiple matings is to increase the female's lifetime reproductive success including fecundity, fertility, and quality of offspring (Arnqvist & Nilsson 2000; Blanckenhorn et al. 2002; Yanagi & Miyatake 2003). Female multiple mating occurs in oriental fruit fly *B. dorsalis* (Hendel) (Tephritidae), a destructive pest of many fruits and vegetables. *B. dorsalis* has become widely distributed because of international trade in fresh fruits and vegetables, and because of its high reproductive rate and broad host range. While the mating behavior of the *B. dorsalis* has been investigated (Lin et al. 2004; Ren et al. 2008; Shelly 2000a; Shelly 2000b), details concerning the consequences of remating in both males and females remain unclear. Therefore, we investigated: 1) whether the size of male accessory glands decrease after mating and how long they take to recover and; (2) the effects of multiple mating on female lifespan and fecundity.

Materials and Methods

FLY STOCKS

Wild *B. dorsalis* were obtained as pupae from citrus orchard in Hainan province of China, and maintained under laboratory conditions. The larvae were reared on artificial diet containing water, honey, sugar, vitamin C and yeast extract (Wang et al. 2013). Adults were held in 40 × 30 × 30 cm stock cages enclosed with a fine synthetic mesh. All cages were kept under constant conditions of 27 ± 0.5 °C, 75 ± 5% RH and 14:10 h L: D. Food was supplied ad libitum by inserting a glass vial containing adult diet on a sponge into the side of cages, and food vials were replaced daily (Wang et al. 2013). The experimental group was exposed to simulated dawn and dusk as the lights were switched on and off in 3 stages over a 1 h period at the beginning and end of each day. Preliminary experiments indicated that *B. dorsalis* matured on day 8 after emergence with most flies mating at this age. Therefore, all flies were tested at 8 days old. The newly emerged adult flies (day 1) were removed and housed in a new cage with diet, and an aspirator was used to separate males and females on day 4 after emergence before complete sexual maturation (no mating of mixed-sex flies was observed in the first 5 days in preliminary experiments). Peak mating activity occurred in a 30-min period during the simulated dusk.

DETERMINING VARIATIONS IN MALE ACCESSORY GLAND SIZES AFTER MATING

Adult male flies were assigned to 4 groups based on their mating status: 0 h after mating (n = 30), 16 h after mating (n = 30), unmated-paired (UP, n = 30) and unmated-unpaired (UU, n = 30). All experiments were conducted according to the methods established by Radhakrishnan & Taylor (2008). Males in the UP group were paired individually with a virgin female of the same age at 9:00 in the morning, and removed and dissected at dusk before mating to exclude the possible influence of female and sex pheromone on accessory glands' size. Males in the UU group were maintained in a separate cage with no encounters with females and were dissected at dusk. All of the test cages were placed into an incubator to avoid contacting sex pheromone emitted from flies in other cages.

All males were dissected in phosphate buffered saline (0.02 M, pH = 7.4) on a microscopic slide. Images of the accessory glands were captured using a binocular dissecting microscope (OLYMPUS, SZX-ILLK200, Tokyo, Japan) connected via a QIMAGING digital camera to a computer with image-pro discovery software. It was difficult to measure the size of the ectodermal accessory glands (EAG), and since most of the male accessory gland products transferred to female comes from the mesodermal accessory glands (MAG) (Radhakrishnan & Taylor 2008), we focused on the MAG when determining the accessory gland size variation. Length of each accessory gland was measured by tracing a midline that longitudinally bisected each organ with Image J 1.44b (US National Institutes of Health, Bethesda, MD, USA), and mean length of the glands was used in analyses. Mean area was calculated from the longitudinal surface area by tracing the outline of each tubule using the same software. Body size was determined by measuring the distance between the humeral cross-vein and subcostal vein and the end of the radial 4 + 5 vein in the wings (Pérez-Staples et al. 2007).

REMATING ABILITY OF FEMALE *B. DORSALIS*

Female remating was determined over a span of 3 days using the method described by Radhakrishnan & Taylor (2008). Six groups designated M_1 to M_6 were maintained based on their mating status. Groups M_1, M_2 and M_3 each consisted of 100 virgin males and 100 virgin females, 8-10 days old, respectively. In M_4, non-virgin males that had mated on day 8 were given an opportunity to mate a second time with virgin females on day 9. In M_5, non-virgin females that had mated with virgin males on day 8 were given an opportunity to mate again with virgin males on the second day 9. In M_6, females that had mated once with non-virgin males on day 9 were given an opportunity to mate again with virgin males on day 10. This part of work was designed to investigate the influence of mating status on female remating. All experiments were performed in 40 × 30 × 30 cm cages and started at 9:00 a.m., which allowed sufficient time to adapt to the new conditions and the new companions. When matings occurred, we checked the cages every 10 min to count the number of mating pairs and then carefully removed them into a transparent plastic vial. To analyze the mating latency (time from the beginning of simulated dusk until intromission) and copulation duration, vials containing the mating pairs were placed under dim red light and monitored by video (SONY, HDR-CX180E, Shanghai, China). The duration of mating latency and copulation were recorded by checking the video every 10 min. Three replicates were maintained for each experiment.

EFFECTS OF REMATING ON FEMALE FITNESS

Flies were assigned randomly into 6 groups from G_1 through G_6. In G_1, 30 virgin females were reared individually in 7 cm diam × 10 cm height containers. In G_2, one hundred 8-day old virgin females and males were mixed together in a new stock culture cage and allowed to mate at dusk; and 30 mated females were transferred randomly into an experimental container without exposure to males. In G_3, 30 females

that had continuously mated twice with virgin males (similar to M_5) were maintained without further exposure to males. In G_4, 30 females that had mated with non-virgin males were maintained without further exposure to males (similar to M_4). In G_5, 30 virgin females and 30 virgin males each were selected randomly from the separated by sex stock cultures, and placed in pairs into 30 containers on day 8. In G_6, 30 virgin females and 60 virgin males were selected randomly and transferred into 30 containers at a ratio of 1♀ : 2♂ on day 8. All of these flies were kept in constant conditions as previously described. An oviposition device (OD) consisting of 1.5 mL orange juice and 16 pinholes on the surface was used to collect eggs. Food and ODs were changed every day. Because the peak egg-laying period of *B. dorsalis* is at dusk (Yuan et al. 2003), the number of eggs from all females were counted on the second day, and the eggs from 20 females (chosen randomly) were transferred to fresh larval diet maintained at 27.5 ± 0.5 °C. The number of hatched larvae in the larval diet was counted to determine hatchability and fertility 2 days later. Flies were monitored daily until all females died. During this period, males in the G_5 and G_6 containers that died were replaced with males from the same cohort. Flies in group G_6 were monitored daily to record mating numbers in order to investigate the refractory period and remating. Female longevity was measured from the day of the first mating because any mating effect would be manifested only after copulation.

STATISTICAL ANALYSIS

Data analysis was conducted using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The means in groups of mating status were subjected to analyze one-way analysis of variance, and means were separated by least significant difference test when $P < 0.05$. Sample sizes of female fitness varied across analyses because of missing data (e.g., some females escaped) or excluded data, i.e., females that laid fewer than thirty eggs (< 2%) or produced less than 5 offspring in total (Taylor et al. 2008) were considered as having had an insufficient mating. Survival was analyzed using cumulative survival curves with the treatments as fixed discrete factors. Fecundity and fertility of each group were recorded daily and pooled per week for further analysis. The fitness and reproductive cost were expressed either as mean oviposition rate and number of eggs-laying in the lifetime.

Results

DETERMINING VARIATIONS IN MALE ACCESSORY GLANDS AFTER MATING

We dissected 2 types of male *B. dorsalis* accessory glands: one pair of long mesodermal accessory glands (MAG) and 3 pairs of convoluted ectodermal accessory glands (EAG), which appeared spongy and sometimes branched (Fig. 1). The length and area of the MAG varied significantly among the groups ($F = 21.580$; df = 3, 110; $P < 0.001$ Fig. 2A; $F = 7.158$; df = 3, 110; $P < 0.001$, Fig. 2B). After mating both length and area of MAG were significantly larger when compared to virgin males. However, differences were not observed in both length and area between males in unmated-paired (UU) and unmated-unpaired (UP) groups ($P = 0.156$, Fig. 2A; $P = 0.978$, Fig. 2B).

REMATING ABILITY OF FEMALE *B. DORSALIS*

Female reproductive success was estimated in 6 groups of flies with various sexual histories ($F = 93.911$; df = 5, 17; $P < 0.001$, Fig. 3). There was a good repeatability in 3 independent experiments ($F = 0.023$, df = 2, 18; $P = 0.977$). There were no significant differences in the tendencies of virgin females to mate with virgin males on day 8, 9 or 10 (M_1, M_2 and M_3), and the same was true for virgin females paired with non-virgin males (M_4). However, virgin females had greater mating success

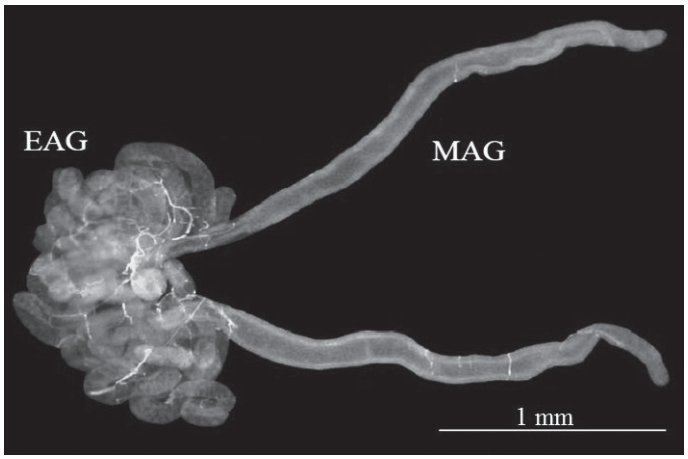

Fig. 1. Male accessory glands of *Bactrocera dorsalis*. One pair of long tube mesodermal accessory glands (MAG) and 3 pairs of long, convoluted, complex and fragile ectodermal accessory glands (EAG).

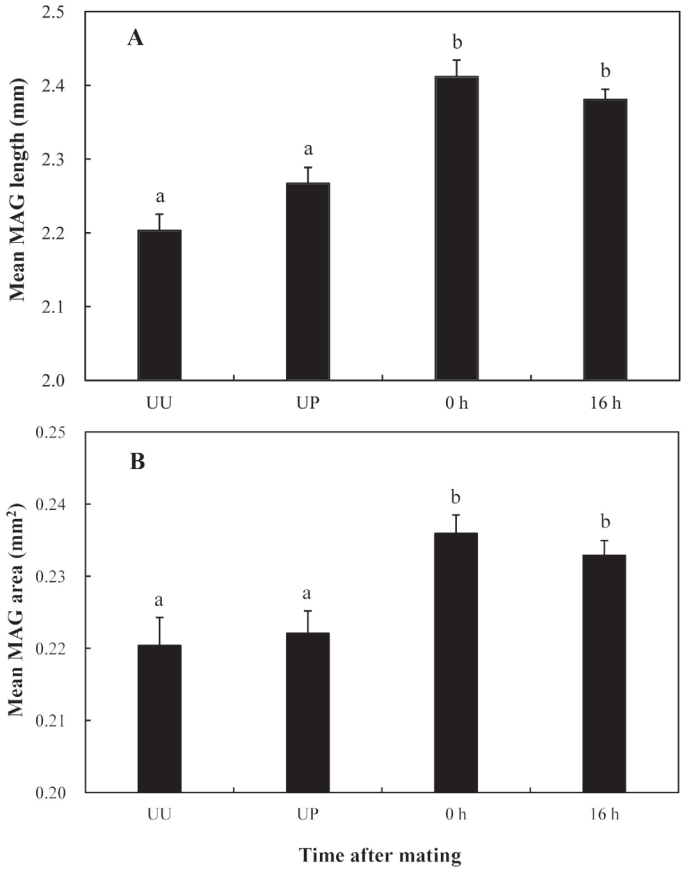

Fig. 2. Size of male mesodermal accessory gland (MAG) of *Bactrocera dorsalis*. A, mean length (± S.E.), and B, mean area (± S.E.) of glands. UP means unmated males that were paired with virgin females before the onset of the simulated dusk (when mating occurs), and UU means unmated males and that were not paired with females. 0 h is the size of the MAG just after mating; 16 h is the size of the MAG at 16 h. Different letters above error bars indicate significantly different means.

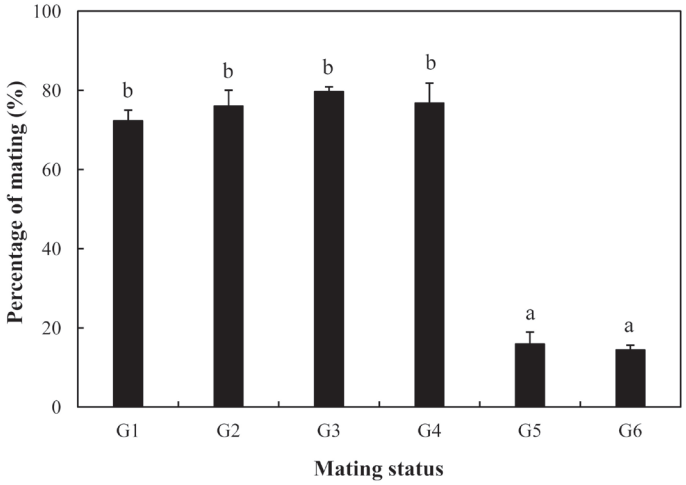

Fig. 3. Mean percentage of successful matings among *Bactrocera dorsalis* of different mating statuses. M_1, matings of 8-day old virgin females with of 8-day old virgin males on day 1; M_2, matings of 9-day old virgin females with 9-day old virgin males on day 2; M_3, matings of 10-day old virgin females with 10-day old males on day 3; M_4, matings on day 2 of virgin females with males that had mated on day 1; M_5, both first and second matings with virgin males; M_6, first mating to non-virgin male and remating to virgin male. Different letters indicate significantly different proportions.

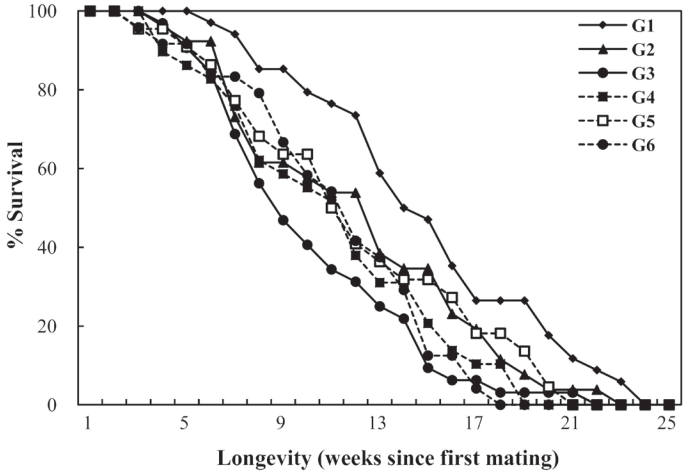

Fig. 4. Percent survival of *Bactrocera dorsalis* females as a function of mating status and duration of time elapsed since first mating. G_1, virgin females housed alone (n = 30); G_2, females mated once (n = 26); G_3, females mated twice with different virgin males (n = 32); G_4, females mated with males that had mated on the previous day (n = 29); G_5, females housed with males at a sex ratio of 1: 1 (n = 23); G_6, females housed with males at a sex ratio of 1♀: 2♂ (n = 24).

than females that had already mated, and already mated females had a small, but similar mating success, to remate regardless of whether their first mating was with a virgin (M_5) or a non-virgin male (M_6) (Fig. 3). There were no significant differences in mating success and durations of copulation among different treatments (F = 1.25; df = 5, 17; P = 0.346; F = 1.017; df = 5, 17; P = 0.45, respectively). Female longevity did not differ in those that had mated once in G_2, 2 times on consecutive days in G_3 or intermittently in G_6. Among a total of 25 females in G_6 (1 of 30 had been killed, 2 had escaped and 2 had laid less than 30 eggs), 19 mated more than once, 5 mated more than twice, and 1 female mated 4 times.

EFFECTS OF REMATING ON FEMALE FITNESS

Female longevity was significantly influenced by mating (F = 3.691; df = 5, 163; P = 0.003, Fig. 4). The virgin females housed alone lived significantly longer than mated females in all groups, while no significant differences were found among females in different mating experiments. Among all the groups, there were significant differences in the mean numbers of eggs laid per female in her lifetime (F = 21.621; df = 5, 163; P < 0.001, Fig. 5A). Mated females laid more eggs than virgin females in group G_1, while no significant differences were observed among females housed alone after having mated regardless of whether they mated once, twice or mated with non-virgin males. Egg numbers were similar regardless of whether females were maintained with 1 or 2 males (G_5 vs G_6). There were no significant differences in longevity and number of eggs laid per lifetime between G_3 females (2 matings to virgin males) and females in G_6 (2 and 3 intermittent matings to non-virgin males) although the time between matings in G_6 was longer than in G_3.

Mean hatchability differed significantly between groups (F = 19.313; df = 4, 92; P < 0.001, Fig. 6A). Females that mated with previously mated males had the lowest hatchability (eggs of virgin females in group G_1 never hatched and were excluded from analysis). Significant differences in hatchability were not observed among females that mated once, twice or those housed with males at a sex ratio of 1: 1. Egg laid by females in group G_6 had the highest hatchability. Females laid most of their eggs during the first 10 weeks of the female's reproductive life and hatchability was also highest during this period (Fig. 5B and Fig. 6B).

Production of offspring (larvae) differed among the various sexual environments (F = 26.941; df = 4, 92; P < 0.001, Fig. 7). Females housed with males produced more offspring, and females housed with higher number of males (1♀: 2♂) produced still more offspring because of the higher hatchability of egg laid by female in G_6. Intriguingly, there was no significant difference in the number of offspring produced by females that mated either with virgin or with non-virgin males. Mating of females on 2 consecutive days in group G_3 did not increase offspring number. Regarding oviposition and hatchability, most of the offspring were produced in the first 10 weeks of the female's reproductive life (Fig. 5B and Fig. 6B).

Discussion

Various proteins are biosynthesized in male accessory glands, and these proteins then form part of the seminal fluid proteins. Recent studies suggest that components of accessory gland fluids inhibit female remating in a number of dipteran species (Gavriel et al. 2009; Miyatake et al. 1999). The size of accessory glands are correlated to the quantity of proteins secreted (Ravi Ram & Ramesh 2002). The shapes of the male accessory glands in *B. dorsalis* are similar to those of other tephritids, such as, *C. capitata* (Marchini et al. 2003), *B. oleae* (Marchini & Del Bene 2006) and *B. tryoni* (Radhakrishnan et al. 2009). The size of MAGs in male *B. dorsalis* measured in this study showed significantly increased length and area after mating (Fig. 2). Such variation was not observed in previous studies of other species (Bangham et al. 2002; Rogers et al. 2005; Radhakrishnan & Taylor 2008), which found mating-related decreases in accessory glands size. Possibly an initial mating stimulated protein synthesis resulting in enlargement. Copulation in *B. dorsalis* is relatively lengthy (mean duration > 8 h). If accessory gland fluids are released early in mating there might be ample time for the gland to be replenished by the end of the coupling. In this study, males (mated and unmated) were the same age, so this variation must result from the mating behavior. Migration of AGF into females occurs within 30 minutes in *C. capitata* (Marchini et al. 2003). Transfer and replenishment of acces-

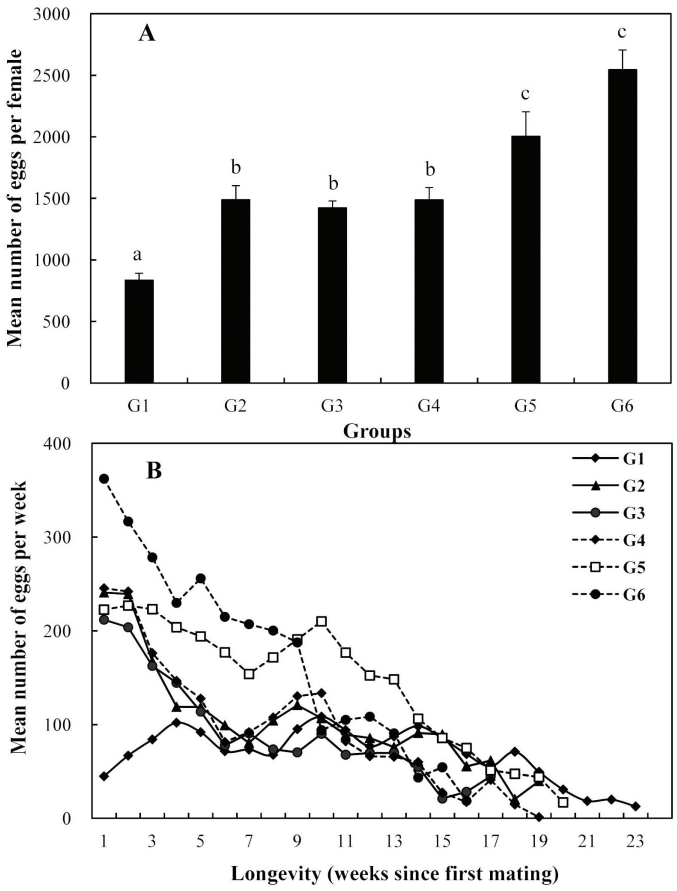

Fig. 5. Fecundities of *Bactrocera dorsalis* females of various mating statuses. A. Lifetime mean number (± S.E.) of eggs laid per female, and B. Mean oviposition rates per week of *Bactrocera dorsalis* females of various mating statuses. G_1, virgin females housed alone (n = 30); G_2, females mated once (n = 26); G_3, females mated twice with different virgin males (n = 32); G_4, females mated with males that had mated on the previous day (n = 29); G_5, females housed with males at a sex ratio of 1: 1 (n = 23); G_6, females housed with males at a sex ratio of 1♀: 2♂ (n = 24). Different letters indicate significant differences.

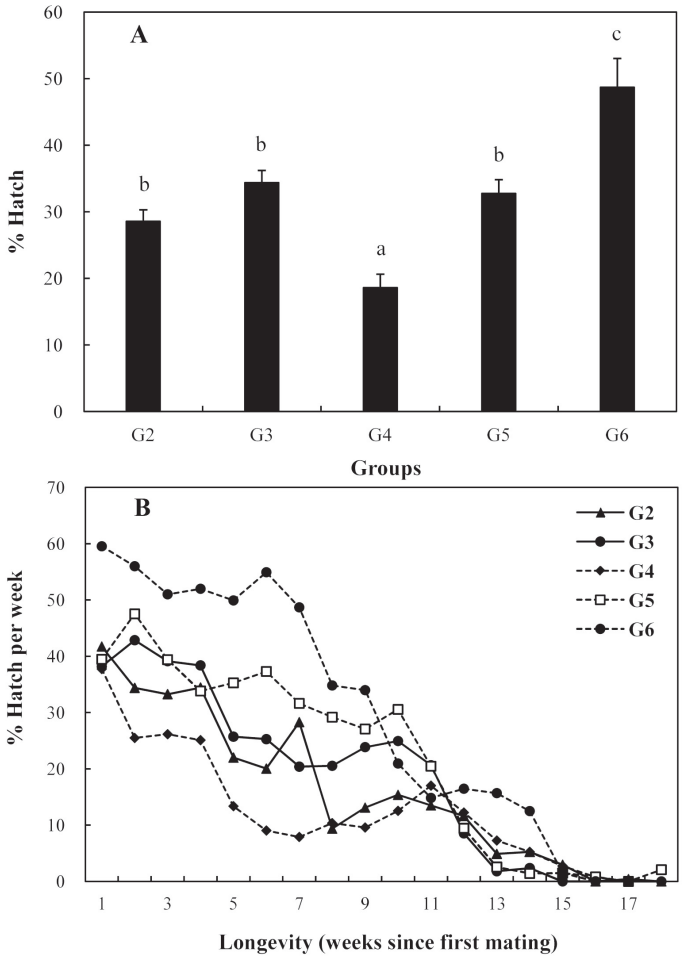

Fig. 6. Fertilities of *Bactrocera dorsalis* females of various mating statuses. A. Lifetime mean percent hatch of eggs laid by females of G_2 – G_6 mating statuses, B. Mean percent hatch of eggs laid each week by females of G_2 – G_6 mating statuses. G_2, females mated once (n = 18); G_3, females mated twice with virgin males (n= 20); G_4, females mated with non-virgin males (n = 18); G_5, females housed with males with a sex ratio of 1: 1 (n = 18); G_6, females housed with males with a sex ratio of 1♀: 2♂ (n = 19). Data for the unfertilized eggs laid by virgin females in group G_1 and for females that produced fewer than 5 eggs in total were not analyzed. Different letters indicate significant differences.

sory gland fluids were also determined in *B. tryoni* (Radhakrishnan & Taylor 2008). The preconditioning of secretions occurs prior to matings for better copulation. Depletion of secretion after mating could also occur in the accessory glands of *B. dorsalis* indeed at the beginning, but it may be masked by an extended and rapid replenishment, which in turn may increase gland size. In many insects, a strong correlation has been observed between the size of accessory glands and the quantity of protein secretions (Ravi Ram & Ramesh 2002; Wigby & Sirot 2009).

In multiple matings, male mating success can depend not only on accomplishing a first copulation, but also on how effectively they can replenish reproductive reserves in time for the next mating event. In keeping with their ability to replenish AGF *B. dorsalis* males could not only remate successfully on successive days but also effectively inhibit female remating on consecutive days. Similar results are reported in other flies such as *B. tryoni* (Harmer et al. 2006; Radhakrishnan & Taylor 2008), *C. capitata* (Miyatake et al. 1999) and *B. cucurbitae* (Kuba and Itô 1993). In addition to AGF, ejaculate size of male *Anastrepha obliqua* was not related to mating history, time elapsed since the last mating, and copulation duration, since, there were always enough spermatozoa stored in the seminal vesicle of males to allocate similar numbers of sperm among successive mates (Pérez-Staples & Aluja 2006). Laboratory studies on a number of tephritid species have suggested that renewal of female receptivity is strongly associated with the quality of the first mate, as well as AGF-induced refractory periods, which range from one day to more than 2 months (Chinajariyawong et al. 2010). Considering the AGF-induced refractory period of female *B. dorsalis*, one day could be too short to recover sexual receptivity. Interestingly, there was no significant difference of females mate success between virgin or non-virgin males (M_5 and M_6) and both had low remating success (Fig. 3). So we inferred that the transferred secretions did not decrease during the second mating, which was also validated by no diminishment of the size of MAG after first mating. There were no differences in either mating success or copulation duration of virgin and non-virgin females of *B. dorsalis*. This was not consistent with *B. tryoni* in which shorter copulation duration was observed in non-virgin than in virgin females (Radhakrishnan & Taylor 2008).

Polyandry is not universally associated with increased female fitness (Martin et al. 2004; Harano et al. 2006). Female insects continually housed with males often suffer a significant reduction in lifespan (Arnqvist & Nilsson 2000). The same can be the case for males (Martin & Hosken 2004). In *B. dorsalis* multiple mating further decreased female lifespan but increased female lifetime reproductive success. Similar

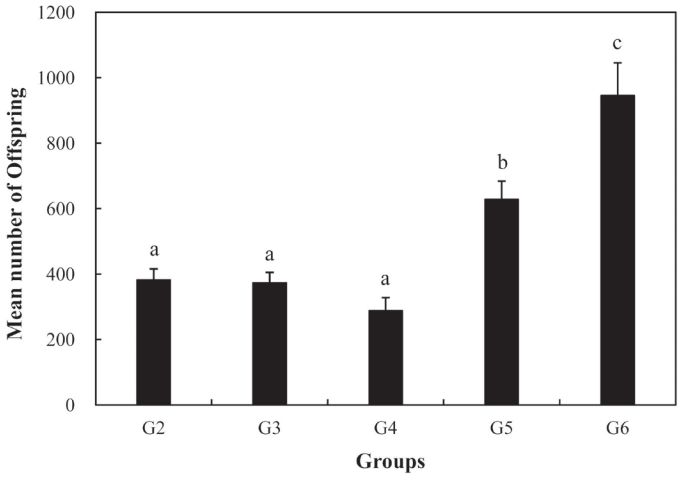

Fig. 7. Lifetime mean numbers (± S.E.) of offspring produced by *Bactrocera dorsalis* females of various mating statuses. G_2, females mated once (n = 18); G_3, females mated twice with different virgin males (n = 20); G_4, females mated with non-virgin males (n = 18); G_5, females housed with males with a sex ratio of 1: 1 (n = 18); G_6, females housed with males with a sex ratio of 1♀: 2♂ (n = 19). Data for the unfertilized eggs laid by virgin females in group G_1 and for females that produced fewer than 30 eggs and/or five offspring in total were not analyzed. Different letters indicate significant differences.

results, that multiple mating resulted in more egg-laying, were found in *B. dorsalis* (Shelly 2000). Unexpectedly, we did not find any significant differences of longevities among various groups of mated females. These results were similar to the relationship between mating, reproduction and longevity in *D. simulans* but not in *D. melanogaster* (Taylor et al. 2008). In the latter, females that mated at high frequency had a shortened lifespan even when egg production costs were excluded (Chapman et al. 1995; Chapman et al. 1998). The life spans of *B. dorsalis* females mated with non-virgin males were similar to other mated females, indicating the same effect of the first and the next day mating on female lifespans. Conversely, studies of *A. striata* and *C. capitata* revealed that female flies mated with non-virgin males suffered longevity costs (Pérez-Staples & Aluja 2004; Chapman et al. 1998). Accessory gland proteins can have a dose-dependent effect on longevity (Chapman et al. 1995), suggesting that the volume of secretions transferred to females was similar in virgin and experienced males. In addition to inducing female refractory behavior, male accessory gland secretion can promote oogenesis and stimulate egg-laying (Avila et al. 2011). This may contribute to the fewer eggs laid by virgin *B. dorsalis* females in the present study. Correspondingly, mating/ ejaculate induced significantly more eggs laid by mated female. Remating frequency was higher under crowded conditions in *C. capitata* (Kraaijeveld et al. 2005; Vera et al. 2002). *Bactrocera dorsalis* females maintained with a male laid more eggs than those housed alone; however, there was no difference between the effects of 1 or 2 male companions (Fig. 5). Similar result was also investigated in *D. simulans* females (Taylor et al. 2008). Females housed with males may encounter more frequent remating opportunities which may lead to more egg-laying.

No differences in egg hatchability between females mated once or twice suggested that females received sufficient numbers of sperm in their spermathecae by mating with a virgin male (G_2 and G_3 in Fig. 6), but not enough in the second mating next day, as evidenced by lower hatchability. Offspring produced after one mating of *B. dorsalis* revealed that sperm can stay available in the spermatheca for as long as 15 weeks. Females that mated twice over a multiple day interval laid more eggs and produced more offspring than females that mated twice on successive days. The mean refractory period of female flies that mated more than once was 20.5 days in this study. Female flies with a refractory period would receive more ejaculate in a subsequent mating, which would contain more accessory gland proteins and sperm to stimuli egg laying. Lastly, there were no significant differences in lifetime reproductive success between females that mated 2 and 3 times. This finding was therefore consistent with the majority of findings across insects (Arnqvist & Nilsson 2000).

Mating confers both costs and benefits. An optimal copulation frequency likely exists for a given species or individual, but this will most certainly be different for males and females (Blanckenhorn et al. 2002). In *B. dorsalis*, we found that the length and area of male accessory glands were enlarged to a certain extent, and that there was a high remating success in male flies, but inhibition of remating in females. While mating and remating decreased longevity, the benefits of female with multiple mating, which produced more offspring, were sufficient to offset the cost of remating, especially when housed at a high sex ratio.

Acknowledgments

This study was supported in part by the Program for Innovative Research Team in University (IRT0976), Natural Science Foundation of Chongqing (cstc2013jjB0176), and the Earmarked Fund for Modern Agro-industry (Citrus) Technology Research System, and the Fundamental Research Funds for the Central Universities (XDJK2013A017) of China.

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