

# Potential Reproductive Advantage of Short-over Long-Winged Adult Males of the Cricket *Velarifictorus ornatus*

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**Abstract** A trade-off between flight capability and reproduction is well known in adult females of the wing-dimorphic cricket *Velarifictorus ornatus*, but it is not clear whether such a trade-off exists in adult males of the species. In the present study, we investigated sexual maturation time, mating frequency, and the fertilization success of spermatophores after sequential mating in long-winged (LW) and short-winged (SW) adult males of *V. ornatus* to evaluate the potential reproductive advantage of the SW over the LW male morph. We found that the SW males of *V. ornatus* attained sexual maturity earlier and produced heavier spermatophores during the early stage after adult emergence than their LW counterparts. Additionally, within a 24-h mating period, the SW males showed a higher mating frequency, greater spermatophore weight, and shorter intermating time interval compared with their LW counterparts. Although females copulated with the two male morphs produced eggs of similar size, fertilization success by SW males was significantly higher than by the LW males. These results provide support for a trade-off between dispersal capability and reproduction success in wing-dimorphic males of *V. ornatus*.

**Keywords** *Velarifictorus ornatus* · Wing polymorphism · Males · Trade-off · Fertilization success

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## Introduction

Due to resource limitation, the evolution of life-history traits is often constrained such that an increase in one trait would lead to a decrease in one or more correlated traits (Biere 1995; Stearns 1976; Schlichting and Pigliucci 1998). In adult females of wing-polymorphic insects, the long-winged (LW) morph allocates more resources than short-winged (SW) morph to develop flight muscles and fewer resources to develop the ovaries, and thus LW females usually have well-developed flight muscles, and are flight-capable (Zera and Denno 1997). In contrast, SW females show underdevelopment of flight muscles without flight capability while attain higher reproductive capability than their LW counterparts (Tanaka 1993; Zera and Denno 1997). The trade-off between flight capability and reproduction has been extensively studied in adult females of wing-dimorphic insects and fully validated in many species, including aphids, planthoppers, stink bugs, and crickets (Zera and Denno 1997; Guerra 2011).

There exists two competing hypotheses about the evolutionary significance of wing dimorphism in male insects. The genetic correlation hypothesis holds that wing dimorphism in male insects derives from the genetic correlation between female and male, i.e., SW females occur because they improve population fitness, whereas SW males occur because of the genetic correlation between females and males (Roff and Fairbairn 1994). Alternatively, as the trade-off hypothesis posits, a trade-off between flight capability and reproduction also exists in males of wing-dimorphic insects (Zera and Denno 1997). Several studies yielded evidences supporting the trade-off hypothesis between flight capability and reproduction (Guerra 2011). For example, compared to short-winged males, long-winged males of *Gryllus texensis* have decreased courtship

propensity and are less aggressive in agonistic encounters (Guerra and Pollack 2007, 2010). Similarly, SW males of the planthopper species *Prokelisia dolus* had higher mating competitiveness and fertilization success than their LW counterparts (Langellotto et al. 2000), and SW males of *P. dolus* are more efficient than LW males at mate searching in natural environments favorable for the occurrence of SW individuals (Langellotto 2001). Importantly, sexual selection might favor apterous males in several natural populations of water strider species (Fairbairn and Preziosi 1996). Several other studies have also provided support to the trade-off hypothesis, and the advantages that SW males have over their LW counterparts include the faster development of reproductive organs (Socha and Hodková 2006; Zeng and Zhu 2012), earlier sexual maturity time (Fujisaki 1992), greater mating success rate (Socha 2004), higher fertilization success (Socha 2008), and higher mating frequency (Zeng and Zhu 2012). These studies suggested that short-winged males have reproduction advantage over longed-winged males, but the reproduction advantage appears to vary across insect taxa.

Although the reproduction advantage of short-winged males over longed-winged males is well documented, several experiments failed to detect the trade-off between flight capability and reproduction in males of wing-dimorphic insects. In the small planthopper *Laodelphax striatellus*, for example, no difference was detected between LW and SW males in terms of testis development rate or adult life (Mishiro et al. 1994). In the pea aphid, *Acyrtosiphon pisum*, different male wing morphs exhibited similar mating competitiveness in the presence of females (Sack and Stern 2007). Roff and Fairbairn (1994) found no difference in the testis mass of SW and LW male sand crickets, *Gryllus firmus*, nor was there any differences found between males of the two morphs in their ability to sire offspring when pairs of males were permanently confined with single females. Similarly, Holtmeier and Zera (1993) found no difference in the ability of SW and LW male of *G. rubens* to sire offspring when males of the two morphs were paired in similar competitive interactions.

LW male insects have an obvious advantage over their SW counterparts regarding the expansion of their distribution. The fact that whether SW males do not always have a reproductive advantage over their LW counterparts may be attributed to multiple factors. To obtain flight capability, the reproductive characteristics of male insects may not change as much as those of female insects during the evolutionary process (Trivers 1972). SW males may also have a reproductive advantage over their LW counterparts in other aspects; the reproductive success of males is not only affected by the development of reproductive organs, but also dependent on mate acquisition. In males of the sand field cricket *G. firmus*, for example, wing dimorphism

has no perceptible effects on fertilization success (Roff and Fairbairn 1994) while SW males spent more time attracting females and thus gain more mating opportunities (Crnokrak and Roff 1995).

The field cricket, *Velarifictorus ornatus*, exhibits distinct wing dimorphism. A physiological trade-off clearly exists between flight capability and reproduction in LW and SW adult females of *V. ornatus*, whereas no significant difference was observed between the two wing morphs with regard to the development rates of testes and accessory glands (Zhao et al. 2010). In the current study, we examined differences between two male wing morphs of *V. ornatus* in sexual maturation time, mating frequency, and the fertilization success of spermatophores produced after sequential mating. The aim of the study was to determine whether SW males have a reproductive advantage over their LW counterparts due to factors other than development of reproductive organs.

## Materials and Methods

### Insects and Rearing Method

Nymphs of *V. ornatus* were collected from the suburb of Zhuzhou, China (27°48'N, 113°12'E) in May 2005. The experimental population was established by rearing nymphs for many generations in an artificial climate chamber (GX-HE302-300, Ningbo Jiangnan Instrument Factory, Zhejiang, China). All the insects used in the study were the progeny of the experimental population. The rearing method was that described by Zhao et al. (2008, 2010). Development of nymphs of *V. ornatus* is affected mainly by changing photoperiod. When the photoperiod changes from short to long days, it promotes the development of nymphs significantly (Zhao et al. 2008). To promote the progress of the experiment, nymphs were reared at 25 °C first under a 12 h light: 12 h dark photoperiod for 30 days from the day of hatching and then continuously under a 16 h light: 8 h dark photoperiod.

### Sexual Maturation Time

After adult emergence, LW and SW males of *V. ornatus* were reared individually in plastic containers (13 × 13 × 8.5 cm). Previous study showed that the pre-oviposition period of adult females of *V. ornatus* is 9–10 days (Zhao et al. 2010), and we thus selected virgin SW females that had emerged as adults 8 days ago for use in the mating experiments. These 8-days-old females were individually transferred into one of the plastic containers and thereby paired with a virgin adult male that had emerged 2, 3, 4, 6, 8, or 10 days ago. Mating success was

defined as the transfer of a spermatophore by the male to the female during the mating process; mating failure was defined as the absence of spermatophore transfer within the observation time (15 min each time). The spermatophore was gently obtained using tweezers immediately after successful mating and then weighed. The time from pairing to successful mating and the weight of spermatophores were recorded.

### Mating Capability in 24 h

The results of experiments on sexual maturation time showed that the mating success rate reached 100 % in SW males 6 days after adult emergence. Therefore, we paired 8-days-old virgin LW and SW males with 8-days-old virgin SW females. A SW or LW male was first placed in a plastic container, and a virgin SW female was then transferred into the same container. After each successful mating, the female was removed and another virgin SW female was transferred into the same container to provide the male with another mating opportunity. The experiments lasted for 24 h. When a female was removed from a container immediately after successful mating, the spermatophore was gently dislodged with tweezers and then weighed. The time interval between every two mating events was recorded.

### Fecundity of Unmated Female

After adult emergence, 15 LW and 15 SW female crickets were individually reared in plastic containers as described above in “Insects and Rearing Method”. Cricket eggs were laid mainly on degreased cotton. The cotton was replaced once every 5 days, six times in total. The used cotton was individually placed in a container under controlled moisture conditions. The hatching of nymphs was examined daily. The number of unhatched eggs on the cotton was counted 50 days later, and the results were used to calculate egg production and hatchability for each female cricket.

### Effect of Multiple Matings on Fecundity of Female

SW males of *V. ornatus* were individually reared after adult emergence, and 8-days-old males were divided into four groups of treatment. For group 1, each male was paired with an 8-days-old virgin SW female; the male was removed after successful mating, and the female was continuously reared individually. In group 2, each male was allowed to mate with a female; the male was then removed, and the female was reared individually; 24 h later, the male was returned to the same container to mate with the female, and after the second successful mating, the male was removed and the female was reared individually. In group 3, each male was allowed to mate with the female

three times as performed in group 2; the male was then removed, and the female was reared individually. In group 4, a male and a female were continuously reared in one container for 48 h; the male was then removed, and the female was individually reared. The mated females were reared as described above in “Insects and Rearing Method”. Egg production and hatchability for each female cricket were investigated referred to the methods described above in “Fecundity of Unmated Female”.

### Comparative Fertilization Success

Fifteen LW and 15 SW virgin males were paired with virgin SW females 8 days after adult emergence. The mated female was removed after each successful mating, and another virgin SW female was transferred into the same container. The LW and SW males were allowed five sequential mating. The female mated to the male during the first, third, and fifth pairings was removed and held individually with degreased cotton, which was replaced once every 5 days, six times in total. Egg production and hatchability for each female cricket were calculated using the methods described in “Fecundity of Unmated Female”. The second and fourth females in the mating sequence were immediately separated from the male after receiving a spermatophore. The spermatophores were gently removed with tweezers and weighed.

### Statistical Analysis

The mating success of LW and SW adult males was compared using Chi square test. The weight of spermatophore was analyzed by ANCOVA with body weight as the covariate. The number of eggs and the egg hatchability of females mated once were compared with females mated multiple times using ANOVA. Generalized linear mixed effects model (GLMM) was used to analyze the number of eggs and egg hatchability per short-winged females which mated to the male during the first, third, and fifth pairings. The time from pairing to successful mating was analyzed using ANOVA. The total weight of the spermatophores, mating frequency, time interval between and fecundity of unmated female were analyzed using Student's *t* test. All the statistical analyses were performed using SPSS 19.0 (IBM Inc., New York, USA).

## Results

### Sexual Maturation Time

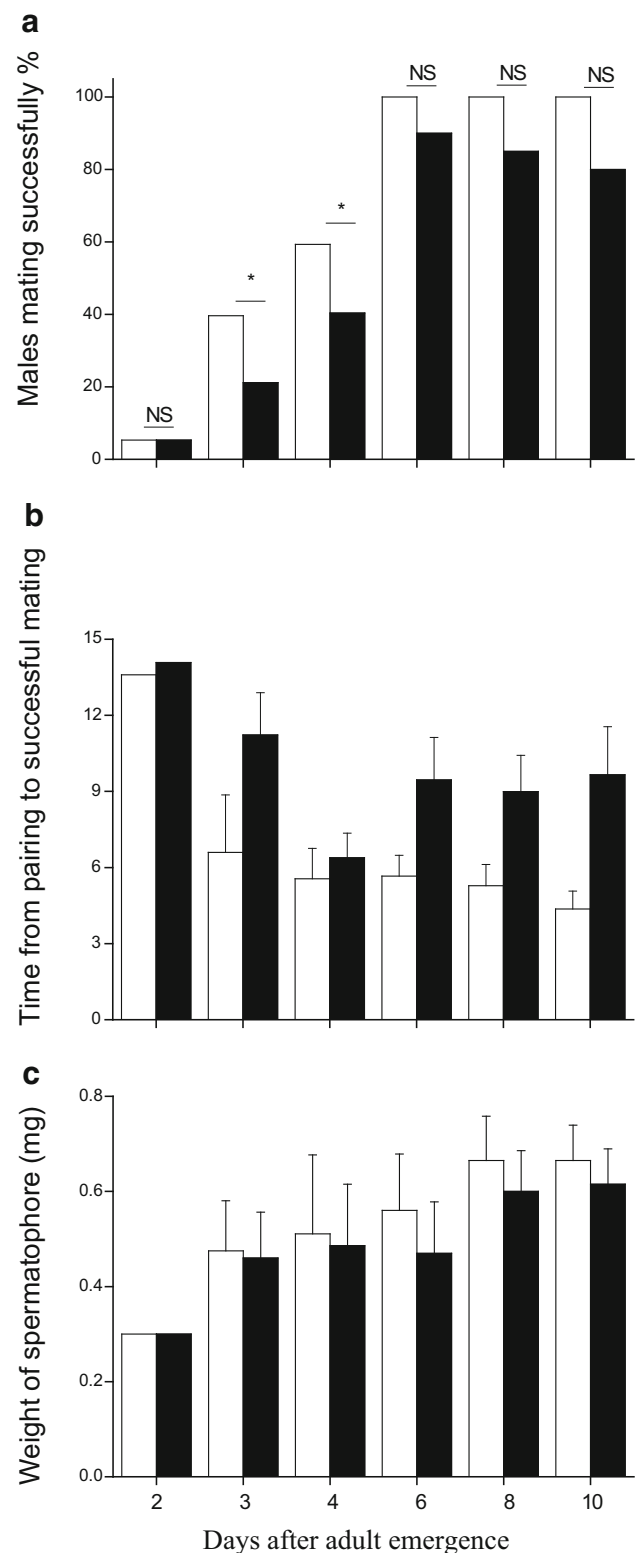
Although the LW and SW males were able to mate 2 days after adult emergence, their mating success rates were quite

low at this early stage (Fig. 1a). An increase occurred in the mating success rate of the two wing morphs on 3 and 4 days after adult emergence, at which times the rate of SW males was significantly higher than that of LW males ( $\chi^2$  test,  $P < 0.05$ ; Fig. 1a). The mating success rates increased sharply for both SW and LW males  $>6$  days old, being slightly, but not significantly, higher for SW males than LW males ( $\chi^2$  test,  $P > 0.05$ ; Fig. 1a).

Only one individual of each wing morph mated 2 days after adult emergence, and we excluded the results obtained at 2 days from the ANOVA analysis. The results of the ANOVA analysis revealed that the amount of time from pairing to successful mating was different between the male wing morphs ( $P < 0.01$ ; Fig. 1b), age had no effect on the time to successful mating in either male wing morphs (ANOVA,  $P > 0.05$ ; Fig. 1b), and there was no interaction between wing morph and age (ANOVA,  $P > 0.05$ ; Fig. 1b). Additionally, the size of spermatophores not only differed between the LW and SW males, but also varied with age (ANOVA,  $P < 0.001$ ; Fig. 1c). A separate analysis of spermatophores produced by male wing morphs of different ages showed that the weight of spermatophores was greater in SW compared with LW males starting 3 days after emergence ( $t$  test,  $P < 0.05$ ; Fig. 1c).

### Mating Capability in 24 h

The first spermatophore of SW males weighed, at  $0.67 \pm 0.07$  mg, significantly heavier than that of LW males, at  $0.55 \pm 0.15$  mg (ANCOVA with body weight as covariate,  $P < 0.05$ ; Fig. 2a). Likewise, the second and third spermatophores were heavier in SW than those of the LW males (ANCOVA with body weight as covariate,  $P < 0.05$ , Fig. 2a). The weight of spermatophores produced by both male wing morphs decreased with the number of copulations that the males experiences (Fig. 2a). There was no significant weight difference between the fourth spermatophore produced by the two male morphs (ANCOVA with body weight as covariate,  $P > 0.05$ , Fig. 2a). Because some LW males did not produce a fifth spermatophore, we did not perform a statistical analysis on the weight of the fifth or subsequently produced spermatophores. The total weight of spermatophores of the SW males was significantly greater than that of the LW males (ANCOVA with body weight as covariate,  $P < 0.001$ ; Table 1).



**Fig. 1** Sexual maturation rate of short-winged (*empty bars*) compared with long-winged (*solid bars*) males of *Velarifictorus ornatus* evaluated using percent males mating successfully (**a**), time from pairing to successful mating (**b**), and spermatophore weight (**c**). *Asterisks* indicate a significant difference between long- and short-winged females of the same age ( $P < 0.05$ ), with NS indicating no significant difference ( $P > 0.05$ ). Chi square was used to test mating success; ANOVA and ANCOVA were used to test the time from pairing to successful mating and spermatophore weight, respectively. Sample sizes in (A) were  $N = 39$  (2 d), 58 (3 d), 59 (4 d), 20 (6 d), 20 (8 d), and 20 (10 d) for short-winged males and 38 (2 d), 54 (3 d), 57 (4 d), 18 (6 d), 17 (8 d), and 16 (10 d) for long-winged males. Sample sizes in (B) and (C) were  $N = 2$  (2 d), 22 (3 d), 35 (4 d), 20 (6 d), 20 (8 d), and 20 (10 d) for short-winged males and 2 (2 d), 12 (3 d), 21 (4 d), 18 (6 d), 17 (8 d), and 16 (10 d) for long-winged males

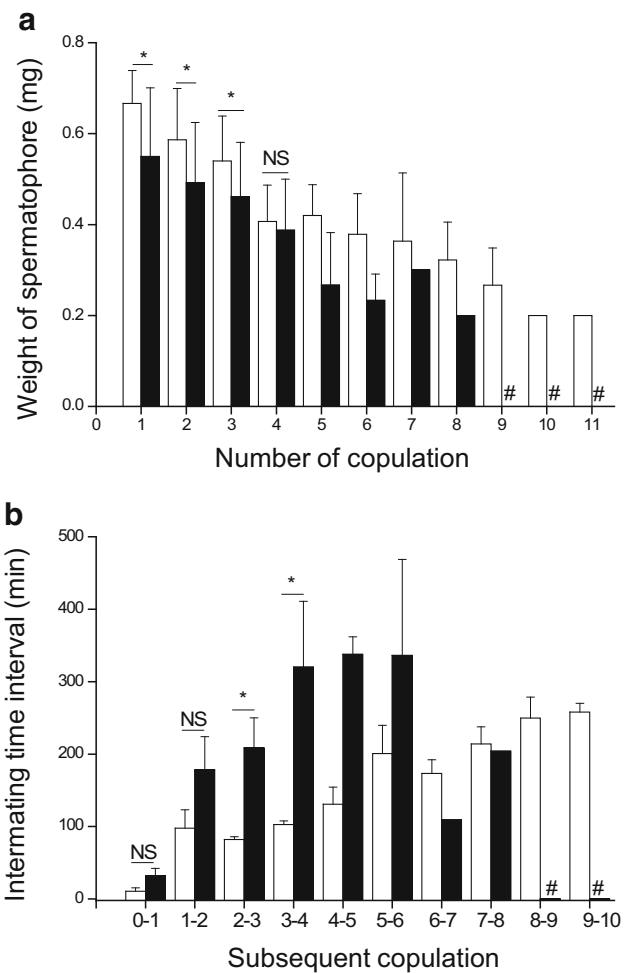
There was no significant difference between wing morphs with regard to the time between pairing and successful copulation by SW and LW males for the first and the second mating ( $t$  test,  $P > 0.05$ ; Fig. 2b). However, the time between pairing and successful mating was shorter in SW compared with LW males for the third copulation ( $t$  test,  $P < 0.05$ ; Fig. 2b). In addition, the time between pairing and successful copulation showed an upward trend in both LW and SW males with the increased in mating (Fig. 2b). Finally, the total number of copulations in 24 h by SW males was significantly higher than that by LW males ( $t$  test,  $P < 0.05$ ; Table 1).

**Fecundity of Unmated Female**

Unmated females of both SW and LW morphs normally oviposit within 30 days after adult emergence, with similar egg production ( $t$  test,  $P > 0.05$ , Table 2). The eggs of unmated females did not hatch within 50 days after oviposition (Table 2), indicating that despite normal oviposition, unfertilized eggs of unmated females of *V. ornatus* were unable to hatch.

**Effect of Multiple Matings on Fecundity of Female**

The number of eggs produced by a female increased as the number of copulations increased; although the trend was not statistically significant (ANOVA,  $P > 0.05$ ; Fig. 3a). Nevertheless, the number of copulations affected egg hatchability, being only  $25.6 \pm 6.7$  % after one mating and  $43.7 \pm 3.9$  after two copulations (ANOVA,  $P < 0.05$ ; Fig. 3b). Similarly, egg hatchability was higher after multiple matings compared with only one mating (ANOVA,  $P < 0.05$ ; Fig. 3b). Nonetheless, egg hatchability was not different among the different levels of multiple matings (ANOVA,  $P > 0.05$ ; Fig. 3b).



**Fig. 2** Comparison of the mean (+SD; initial  $n = 15$ ) spermatophore weight (**a**) and intermating time interval (**b**) between short-winged (*empty bars*) and long-winged (*solid bars*) male *Velarifictorus ornatus* for multiple male copulations occurring during a 24-h period. The time span between pairing the sexes and the first mating is denoted by “0-1”, “1-2” denotes the time span between the first and second mating, and so forth. *Asterisks* indicate a significant difference between long- and short-winged males for a given copulation number (**a**) or time span (**b**) ( $P < 0.05$  by ANOVA for spermatophore weight and by a Chi square test for the intermating time interval), with NS indicating no significant difference ( $P > 0.05$ ). The # symbol indicates no data

**Comparative Fertilization Success**

The results of the GLMM analysis revealed that there is no significant difference in egg production between long-winged and short-winged males (GLMM,  $P > 0.05$ ; Fig. 4a), but male wing morph took significant effect on egg hatchability (GLMM,  $P < 0.05$ ; Fig. 4b). Egg hatchability per female was not different whether the female mated with a LW or a SW male for the first mating (GLMM,  $P > 0.05$ ; Fig. 4b) and the fifth mating (GLMM,  $P > 0.05$ ; Fig. 4b), whereas for the third mating, egg hatchability per female was

**Table 1** Comparison of total weight of spermatophore and mating frequency in 24 h between SW and LW male *Velarifictorus ornatus*

Wing morph	Total weight of spermatophore (mg)	Mating frequency (times)
SW	3.6 ± 0.7 <sup>†</sup> a <sup>‡</sup>	7.9 ± 1.6 <sup>†</sup> a <sup>‡</sup>
LW	1.8 ± 0.7 b	4.2 ± 1.5 b

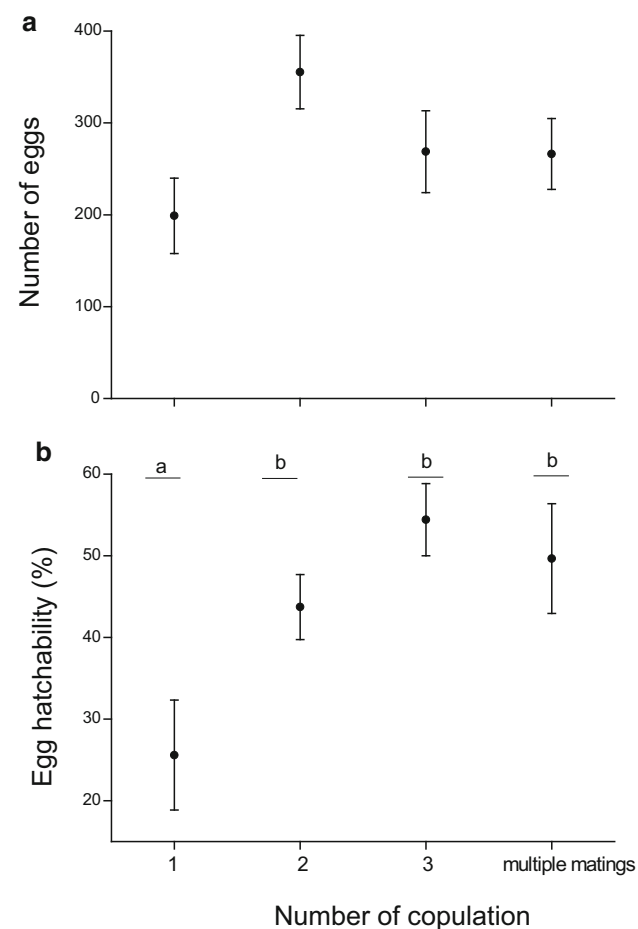
<sup>†</sup> Mean ± SD, 15 individuals per group

<sup>‡</sup> Different letters indicate a significant difference between SW and LW males by a *t* test ( $P < 0.05$ )

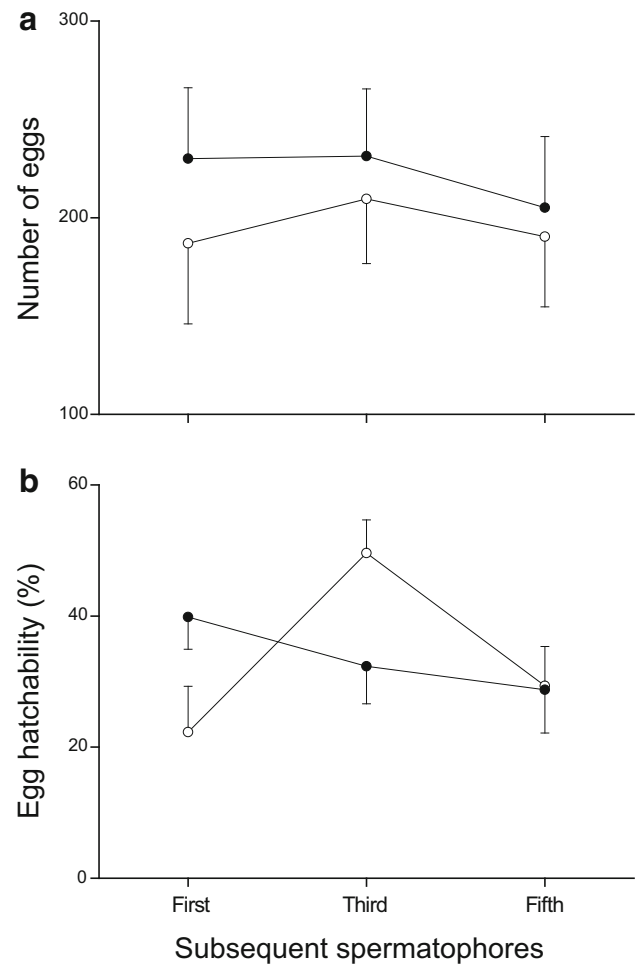
**Table 2** Comparison of egg production and hatching between unmated, SW and LW females of *Velarifictorus ornatus*

Wing morph	egg production	Egg hatching (%)
LW	123.5 ± 69.3	0
SW	138.5 ± 79.1	0

Mean values did not differ significantly by a *t* test ( $P > 0.05$ ,  $n = 15$ )

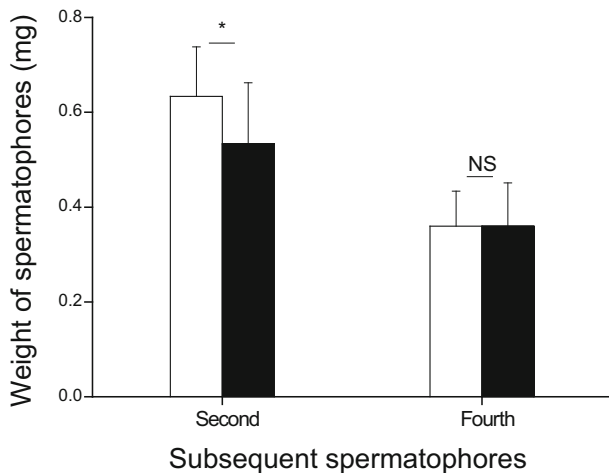


**Fig. 3** Effect of mating frequency on the number of eggs (a) and egg hatchability (b) in short-winged females of *Velarifictorus ornatus*. Different letters indicate a significant difference between mating times by ANOVA ( $P < 0.05$ ,  $n = 15$ )



**Fig. 4** Comparison of the number of eggs (a) and egg hatchability (b) per short-winged female copulating with the short-winged (white circles) or long-winged (black circles) males during the first, third and fifth pairings

higher for females mated with a SW than those mated with a LW male (GLMM,  $P < 0.05$ ; Fig. 4b). The weight of the second spermatophore produced by the SW males was significantly heavier than those by the LW males (ANCOVA with body weight as covariate,  $P < 0.05$ ; Fig. 5), although the weights of the fourth spermatophore were not significantly different between those from the LW and SW males (ANCOVA with body weight as covariate,  $P > 0.05$ ; Fig. 5).



**Fig. 5** The weight of spermatophores transferred to short-winged females copulating with a sequence of five short-winged (*empty bars*) or long-winged (*solid bars*) males. The *asterisk* indicates a significant difference between long- and short-winged males at the same point in the sequence (ANCOVA with body weight as covariate,  $P < 0.05$ ,  $n = 15$ ), with NS indicating no significant difference ( $P > 0.05$ ,  $n = 15$ )

## Discussion

The mating success rate varied with age for both male wing morphs of *V. ornatus*, and both LW and SW males showed a low mating success rate 2 days after adult emergence, and the rate markedly increased at 3 and 4 days after adult emergence. However, mating success rate was higher for SW than for LW males at the older ages (Fig. 1a), until the males reached 6 days after adult emergence, when the differences between the two male morphs disappeared (Fig. 1a). Meanwhile, SW males spent less time obtaining mating opportunities than their LW counterparts at 3 days after adult emergence (Fig. 1b) and produced heavier spermatophores (Fig. 1c). SW males mature sexually earlier compared to LW males may be related to flight muscle development during the early stage after adult emergence. After adult emergence, the flight muscles of LW males increased significantly and reached maximum on day 3 and then decreased on the day 5. In contrast, the weight of flight muscle of SW males remained constant and was significant lighter than that of LW males (Zhao et al. 2010). These were in accord with the result of higher mating success for SW compared with LW males on the day 3 and 4 after adult emergence (Fig. 1a). During the early stage after adult emergence, LW males devote much energy to flight muscle development, whereas SW males allocate resources preferentially to reproductive organs rather than to flight muscle (Zera and Denno 1997), which might have resulted in the significant higher mating success of SW males than LW males on the day 3 and 4 after adult emergence (Fig. 1a). From the day 5 after adult emergence, the flight

muscle of LW males began to histolysis, allowing the LW males to allocate more energy into development of reproductive organs and thus erasing the difference in mating success between LW and SW males from day 5 after adult emergence.

Adult female and male crickets are capable of multiple matings. In the spring field cricket, *G. veletis*, for example, males can mate  $6.8 \pm 0.8$  times in 24 h (Burpee and Sakaluk 1993). In the present study, SW males of *V. ornatus* mated  $7.8 \pm 1.6$  times in 24 h, more than their LW counterparts, which mated  $4.2 \pm 1.5$  times (Table 1), and produced heavier spermatophores than LW males during a 24-h period (Table 1), thus producing spermatophores more efficiently than do LW males during the same period. Additionally, SW *V. ornatus* males obtain more mating opportunities than their LW counterparts, which seem to be attributable to the differences in their sing songs (Zhao et al. unpublished data). To obtain mating opportunities, male crickets produce a calling song to attract females, and the calling song changes to a courtship song when the female is close (Kumashiro et al. 2003). A difference exists in the time spent on calling by two morphs of males of the cricket *Gryllus firmus* to attract females—SW males spend more time calling to attract females and obtain mating opportunities (Crnokrak and Roff 1995). Both cricket calling (Wagner 2005; Mowles 2014) and spermatophore formation (Wagner 2005) require energy consumption. SW *V. ornatus* males, by having higher mating frequency and producing heavier and more spermatophores, spend more energy to produce sing songs and spermatophores than LW males, who spend less energy to produce sing songs and spermatophores because they mate less frequently and produce a lighter spermatophore weight. Since SW males have no need to develop flight muscles, they allocate more energy to produce sing songs and spermatophores, resulting in a higher mating success rate while LW males need to develop flight muscles and thus have to allocate less energy to produce sing songs and spermatophores, leading to a lower mating success rate. This is consistent with what has been observed in the cricket species *V. asperses* (Zeng and Zhu 2012).

Mating incurs a high cost, such as the high energy consumption of mating behaviors, the increased risk of predation, and the spread of disease and risk of injury during the mating process (Daly 1978). Thus, the benefits of multiple matings must outweigh those of the first mating or no mating (Wang and Davis 2006). Although unmated females of *V. ornatus* oviposited normally in the present study, their eggs failed to hatch (Table 2) indicating that mating behavior is necessary for *V. ornatus* to produce fertile eggs. Egg production of *V. ornatus* females was not different between females that mated only once and those mated multiple times (Fig. 3a). However, egg hatchability

was lower for females that mated only once than those mated multiple times, although egg hatchability did not change with more than two matings (Fig. 3b). Therefore, the mating frequency of *V. ornatus* females strongly affects egg hatchability rather than egg production, an observation consistent with the findings in *Nysius huttoni* (Wang and Davis 2006). We could thus infer that, when *V. ornatus* females mate with LW or SW males only once, the fertilization success of the male wing morphs would vary with the number of sperm that they are capable of producing.

Postmating factors, such as sperm load (Langellotto et al. 2000), sperm age (Tarín et al. 2000), sperm competition (Parker and Pizzari 2010), spermatophore size (Sakaluk 1997), and cryptic female choice (Eberhard 1996), affect the capability of male insects to inseminate females. In *Grylloides sigillatus*, for example, LW males produce smaller spermatophores and therefore have a lower fertilization success compared with their SW counterparts (Sakaluk 1997). In *V. ornatus*, the first spermatophore produced by SW males was heavier than that produced by their LW counterparts (Fig. 2a), but there was no difference in fertilization between the two wing morphs (Fig. 4a). This phenomenon may be associated with sperm age or motility in spermatophores produced by LW and SW males. Because considerable amount of energy is needed for maintaining sperm motility, sperm motility in male insects markedly decreases with time after adult emergence (Dowling and Simmons 2012). Therefore, old sperms usually have, relative to new sperm, weakened competitive vigor (Roche et al. 1968), lower fertilization success (Lanman 1968), low rate of successful storage by females (Reinhardt and Siva-Jothy 2005), and a low survival rate of fertilized eggs (Siva-Jothy 2000). After emergence, SW males of *V. ornatus* matured faster sexually than their LW counterparts (Fig. 1), and thus formed sperms sooner than their LW counterparts. Due to late sperm production, sperm were younger with higher motility in the spermatophore of 8-days-old LW compared with SW adult males of the same age, while sperms were older with decreased motility in the spermatophore of SW due to early sperm production. This may explain why, although the spermatophores produced by SW males were heavier than those produced by LW males, the two male wing morphs of *V. ornatus* exhibited similar fertilization success.

In contrast with the first spermatophore, the third spermatophore of SW males had a higher fertilization success than that of the LW males (Fig. 4b). SW males also had a higher mating frequency over 24 h and greater total spermatophore weight than LW males (Table 1). Furthermore, SW males had a shorter time interval between the second and third mating compared with their LW counterparts (Fig. 2b), indicating that SW males produce spermatophores more

efficiently after completing two copulations than their LW counterparts. The third spermatophore of SW males was heavier than that of the LW males (Fig. 3a), which would mean improved female fertilization rate by the SW males according to Sakaluk (1997). In contrast with the third spermatophore, the fifth spermatophores produced by both male morphs exhibited similar fertilization success, without a significant difference (Fig. 4). Because spermatophore production consumes substantial energy (Wagner 2005), the weight of the spermatophore transferred decreased by the fifth mating and the time interval between the production of spermatophores increased (Fig. 2), indicating that the spermatophore production efficiency of both male wing morphs decreased as the mating times increased. In addition, the size of the fourth spermatophore was similar between the two male morphs (Fig. 5), resulting in no significant difference in fertilization success for the fifth spermatophore between the two male wing morphs of *V. ornatus*.

This study provided evidences supporting a trade-off between flight capability and reproduction in males of the wing-dimorphic cricket *V. ornatus*, including earlier sexual maturity, higher mating frequency, greater spermatophore weight, higher fertilization success for SW males than LW males. But many studies suggested that the trade-off can be attenuated by a short bout of flight (Guerra and Pollack 2009; Zeng et al. 2014). In order to forage or search for a mate and new habitats, the LW males are likely to fly under natural conditions, if the flight behavior of LW males can eliminate this trade-off in *V. ornatus*. We also observed a significant difference in fertilization success between the two male wing morphs after repeated mating, which may be associated with spermatophore reformation capability and sperm motility in the spermatophores of LW and SW males after mating. More detailed studies and specific experiments are necessary to further test these hypotheses.

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#### Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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