



## How healthy is your mate? Sex-specific consequences of parasite infections in the moth *Helicoverpa armigera*

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Parasites can play an important role in host sexual selection. According to the 'Hamilton & Zuk hypothesis', females acquire benefits from choosing parasite-free or parasite-resistant males based on their secondary sexual traits (e.g. colourful plumage) as indicators of heritable parasite resistance. However, females may also gain benefits by avoiding sexually transmitted parasites or acquiring more parental care, higher quality sperm and nuptial gifts provided by uninfected males. Here we tested how the presence of the *Ophryocystis elektroscirrha*-like parasite (OE-like) affected longevity, reproduction and mating frequency in the moth *Helicoverpa armigera*. We found that OE-like infection affected the sexes differently: the life span of uninfected males was reduced when paired with infected females, while these females had higher reproductive output than when paired with infected males. These results suggest some form of terminal investment by infected males. When we assessed the effect of OE-like infections on female calling behaviour and sex pheromone signal, we found that the OE-like parasite did not affect the female's pheromone signal in quality or quantity, but it did reduce her reproductive output, suggesting that infected females reallocate resources to maintain an attractive sex pheromone signal. In mate choice experiments, when females were the choosing sex, infected females mated significantly more often with uninfected than with infected males. Since these females produced approximately 12% more offspring, and uninfected females did not prefer uninfected or infected males, these results indicate female choice for uninfected males. Unexpectedly, when we compared the calling behaviour of the two females that were caged together, we found that infected females called significantly earlier than uninfected females, while this was not the case when females were housed alone. Thus, OE-like infection affects reproductive strategies in both males and females, but in different ways, which suggests sex-specific parasite-mediated selection.

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Since the 'parasite-mediated sexual selection hypothesis', also known as 'Hamilton & Zuk hypothesis', was first proposed in 1982, the role of parasites in sexual selection and mating strategies has received much attention (Ashby & Boots, 2015; Hamilton & Zuk, 1982; Poulin & Vickery, 1995; Wittman & Fedorka, 2014). Avoidance of infected mates by females has been observed in vertebrates and invertebrates (Borgia & Collis, 1989; Houde & Torio, 1992; Kavaliers & Colwell, 1995; Klemme & Karvonen, 2016; Martin & Johnsen, 2007; Mazzi, 2004; Worden, Parker, & Pappas, 2000), although the mechanisms behind parasite-mediated mate choice

are still under debate. The 'Hamilton & Zuk hypothesis' states that females acquire benefits from choosing parasite-free or parasite-resistant males, and females can distinguish males by their secondary sexual traits (e.g. colourful plumage), which are thus indicators of heritable parasite resistance (Ehman & Scott, 2002; Folstad & Karter, 1992; Hamilton & Zuk, 1982). Additionally, females choosing uninfected males may gain other benefits, such as reducing the likelihood of parasite transmission through mating (e.g. ectoparasites and sexually transmitted parasites) (Able, 1996; Borgia & Collis, 1989; Knell & Webberley, 2004), or uninfected males may provide better parental care, higher quality sperm and more/better nuptial gifts than infected males (Andersson, 1994;

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Buzatto, Kotiaho, Assis, & Simmons, 2019; Hamilton, 1990; Worden et al., 2000).

Empirical evidence supporting the 'parasite-mediated sexual selection hypothesis' has been reported mostly for birds and fish (Houde & Torio, 1992; Martin & Johnsen, 2007; Mazzi, 2004; Read & Weary, 1990; Ryan, 1988), where acoustic and visual signals predominate, while chemical signals have hardly been explored (Johansson & Jones, 2007; Penn & Potts, 1998). However, chemical signals are extensively used in mate choice in a variety of taxa ranging from vertebrates to invertebrates (Johansson & Jones, 2007; Symonds & Elgar, 2008). Chemical signals, analogous to the colourful plumage of birds, can be costly to produce and might honestly reveal an individual's health status and parasite load (Beltran-Bech & Richard, 2014; Harari, Zahavi, & Thiéry, 2011; Johansson & Jones, 2007; López, Amo, & Martín, 2006). For example, female mice, *Mus musculus*, can distinguish the odour of parasitized males from that of unparasitized males, and discriminate against the odour of parasitized males (Kavaliers & Colwell, 1995; Ehman & Scott, 2002). In insects, several studies have shown that parasitic infection can change sexual signals and mating behaviours in species such as in crickets, beetles and moths (Adamo, 2014; Burand, Tan, Kim, Nojima, & Roelofs, 2005; Fedorka & Mousseau, 2007; Jenkins, Hunter, & Goenaga, 2011).

In this study, we aimed to assess how parasitic infection affects the sexual signal, mating behaviour and sexual selection in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera, Noctuidae). This moth is a worldwide pest and has a variety of host plants (Czepak & Albernaz, 2013; Fitt, 1989; Zalucki, Daghli, Firempong, & Twine, 1986). Females and males can mate multiple times over their lifetime, but both sexes only mate once per night (Yan et al., 2013). In moths, females usually emit species-specific sex pheromones to attract conspecific males from a distance (Allison & Cardé, 2016; Wyatt, 2003). Moth chemical communication systems are species specific and hypothesized to be under stabilizing selection, as any deviation in the signal or the response may reduce the opportunity of finding conspecific partners (Gerhardt & Brooks, 2009; Groot et al., 2006; Löfstedt, 1993). Recently, moth sex pheromones have been suggested to honestly advertise the quality of females, because in some species they seem to be condition dependent and costly to produce and maintain (Burand et al., 2005; Chemnitz, Jentschke, Ayasse, & Steiger, 2015; Harari et al., 2011).

When moths are exposed to parasitic infection, males and females may differentially allocate resources in a range of traits, including life history, immunity and mating traits (Schärer, Rowe, & Arnqvist, 2012; Stoehr & Kokko, 2006; Zuk & Stoehr, 2002). Males are generally hypothesized to maximize their reproductive effort and thus be more susceptible to infections than females, as males may invest fewer resources in immune defence and more resources in maintaining secondary sexual traits (Barthel, Staudacher, Schmaltz, Heckel, & Groot, 2015; Gipson & Hall, 2016; Lindsey & Altizer, 2009; Stoehr, 2007). In contrast, females may maximize their reproductive success by living longer, so likely invest more in immunity to increase longevity and egg production (McKean & Nunney, 2005; Rolff, 2002). As a result, parasitic infections may affect survival and reproduction differently in males and females (Ashby & Boots, 2015; Lindsey & Altizer, 2009; McKean & Nunney, 2001).

The neogregarine protozoan *Ophryocystis elektroscirrha* (OE) is a well-known naturally occurring parasite in monarch butterflies, *Danaus plexippus* (McLaughlin & Myers, 1970). The OE parasite is mainly transmitted vertically, when infected females scatter spores onto their eggs or milkweed leaves, which are subsequently ingested by caterpillars (Altizer, Oberhauser, & Brower, 2000; Leong, Kaya, Yoshimura, & Frey, 1992; McLaughlin & Myers, 1970). However, the OE parasite can be transmitted paternally or

horizontally, that is, through mating or by consumption of spore-infected food (Altizer, Oberhauser, & Geurts, 2004; De Roode, Jacobus, Chi, Rarick, & Altizer, 2009). In *D. plexippus*, OE infections negatively affect host fitness, for example decreasing adult eclosion, life span, mating success, reproduction and flight performance (Altizer & Oberhauser, 1999; Bradley & Altizer, 2005; De Roode et al., 2009).

Recently, we found OE-like parasite spores on *H. armigera* in Australia and China (Gao, Muijderman et al., 2020). In that study, we concluded that OE and the OE-like parasite are different species, based on genetic differences and the inability to reciprocally infect host species (Gao, Muijderman et al., 2020). The occurrence of OE-like infections varied among *H. armigera* populations, with an infection rate of 19% in Australia and 2% in China. Similar to OE-infected monarch butterflies, OE-like infections in *H. armigera* can result in up to ca. 22% of adults becoming stuck in their pupal cases or in having crinkled wings (Gao, Muijderman et al., 2020).

Here, we hypothesized that the presence of the OE-like parasite negatively affects the fitness, sexual behaviours and mate choice in *H. armigera*. We first assessed the effects of OE-like infections on longevity, reproduction and mating frequency in both sexes of *H. armigera*. We subsequently examined the effect of OE-like infections on female calling behaviour and sex pheromone composition, as well as on male and female mate choice.

## METHODS

### Study Insect and OE-like Parasite

The population of *H. armigera* originated from laboratory-reared larvae at the school of Biological Sciences, the University of Queensland, Brisbane, Australia, which were originally collected from the field in 2015, and shipped to the Max Planck Institute for Chemical Ecology (MPICE), Jena, Germany in December 2017, after which they were brought to the laboratory at the University of Amsterdam in 2018. At the University of Amsterdam, *H. armigera* larvae were reared individually on artificial pinto bean diet (Burton, 1970) in climate chambers at 25 °C, 60% relative humidity (RH) and 14:10 h light:dark with lights off at 1100 hours. We checked pupae daily, sexed newly emerged adults and placed these adults individually into 37 ml plastic cups (Solo, Lake Forest, IL, U.S.A.) containing cotton soaked with 10% sucrose.

To obtain infected moths, we inoculated third-instar larvae of *H. armigera* with OE-like spore solutions, as described in detail in Gao, Muijderman et al. (2020) and summarized here. OE-like parasites were originally harvested on laboratory-reared *H. armigera* from the University of Queensland in December 2017. To infect the larvae of *H. armigera*, 1 µl of OE-like spore solution (156 ± 3 SE spores/µl) was pipetted onto a piece of 1 cm<sup>2</sup> artificial diet, onto which a third-instar larva was placed, and this was then put into a 37 ml plastic cup. After consuming the artificial diet, each larva was transferred to cups with normal diet until pupation. We checked pupae daily for eclosion, sexed newly emerged adults and checked them for the presence of OE-like spores in the pupal cases or the abdomens of the adults under a microscope. Adults that showed OE-like spores in the pupal cases or the abdomens were marked as infected and used in the experiments. Uninfected adults used in the experiments originated from the general rearing which was never exposed to the OE-like parasite and was checked for the presence of OE-like infection.

### Longevity and Reproduction

To determine the effects of OE-like infection on longevity, fecundity and fertility of individual moths, we conducted an

experiment with a full-factorial design with respect to the infection status of the moths: we set up four single-pair mating treatments, with each mating pair in a 473 ml plastic beaker (Solo, Lake Forest, IL, U.S.A.) covered with gauze and provided with 10% sugar water: (1) an uninfected female mated with an uninfected male ( $U \times U$ ,  $N = 27$ ); (2) an uninfected female mated with an infected male ( $U \times I$ ,  $N = 29$ ); (3) an infected female mated with an uninfected male ( $I \times U$ ,  $N = 25$ ); and (4) an infected female mated with an infected male ( $I \times I$ ,  $N = 24$ ). We used 0- to 3-day-old virgin moths in this experiment and moths were randomly assigned to each treatment.

In all four treatments, we observed matings with the aid of a red light at 30 min intervals, starting at the onset of scotophase. Since matings generally last 1–3 h, this interval ensured they were all seen. When the pairs did not mate on the first night, we repeated the observations on successive nights until they mated. We measured longevity, fecundity and fertility of individual moths as follows. After mating, once the pairs had separated (on the same night), males and females were put into different beakers (473 ml) and fed with 10% sugar water every 2 days until death. Eggs were collected daily and placed in separate closed beakers (473 ml). The numbers of hatched and unhatched eggs in each beaker were checked daily and counted under a microscope. We measured fecundity by counting the total number of eggs laid by each female during her life span, and calculated fertility as the total percentage of eggs that hatched.

#### Mating Frequency

To evaluate the effect of OE-like parasite infection on mating frequency, one female and one male in four mating combinations as described above were paired for 5 consecutive nights:  $U \times U$  ( $N = 32$ );  $U \times I$  ( $N = 25$ );  $I \times U$  ( $N = 28$ );  $I \times I$  ( $N = 33$ ). We dissected the females after 5 nights. The number of successful matings could be determined by counting spermatophores in the bursa copulatrix. We used 0- to 3-day-old virgin moths in this experiment.

#### Calling Behaviour

To determine the effect of OE-like infection on female calling behaviour, we observed virgin *H. armigera* females over 4 consecutive nights. Specifically, when they hatched, we placed uninfected and infected females individually into transparent plastic beakers (473 ml) covered with fine nylon gauze. We provided the moths with cotton soaked in 10% sugar water and observed them under red light at 30 min intervals throughout the scotophase (i.e. between 1100 and 2100 hours). Calling behaviour was obvious as females clearly extruded the pheromone glands from their abdomen. Observations took place in a separate climate chamber, but in the same climatic conditions in which the moths were reared. We observed a total of 31 uninfected females and 36 infected females. In our observation, two infected females on the first, third and fourth nights and one infected female on the second night did not exhibit calling behaviour.

#### Pheromone Composition

To evaluate the effect of OE-like infection on female sex pheromone composition, we dissected the pheromone glands of 5-day-old females (uninfected:  $N = 30$ ; infected:  $N = 32$ ) from the calling experiment at the peak time of calling, i.e. 7–8 h into the scotophase. We conducted gland extractions as detailed in Groot, Claßen, Staudacher, Schal, and Heckel (2010) and summarized here: we placed glands in conical vials containing a solution of 50  $\mu$ l hexane and 200 ng pentadecane as internal standard and then removed

them after 30 min. We analysed all pheromone samples in a HP7890 Gas Chromatograph (GC) with a 7683 automatic injector. The hexane extracts were reduced to 2  $\mu$ l under a gentle stream of  $N_2$ , after which each sample with 1  $\mu$ l octane was injected into the GC. The sex pheromone peaks were identified and integrated based on their retention times, which were compared to a synthetic pheromone blend of *H. armigera*. This synthetic pheromone blend was injected into the GC before and after 30 samples. We calculated the amount of each pheromone component in the extract relative to the amount of internal standard.

#### Mate Choice

To determine the effect of OE-like infection on mate choice, we conducted two-choice experiments in  $33 \times 33 \times 33$  cm square gauze cages. We placed three adult moths in each cage, one chooser and two potential mates of the opposite sex. For female choice, either an uninfected (in total:  $N = 43$ ) or an infected (in total:  $N = 33$ ) virgin female was released into one cage before the scotophase and given an uninfected and infected male in each cage. The two males in each cage were of the same age and body size and distinguished by randomly clipping a small part of the left or right wing. For male choice, either an uninfected (in total:  $N = 28$ ) or infected (in total:  $N = 37$ ) virgin male was provided with an uninfected and infected female in each cage in the same way as described for female choice. All the moths that were used in the experiment were 1–6 days old. In each cage, we provided the moths with cotton soaked in 10% sucrose solution, and we used a red light to observe matings at 30 min intervals throughout the scotophase under the same climate room conditions as used for moth rearing. In male choice, in addition to mating observations, we recorded the calling behaviour of the two females in the cage every 30 min.

#### Statistical Analyses

We performed all statistical analyses in R software version 3.4.1 (R Core Team, 2018). Survival curves between the mating treatments were analysed using a Cox proportional hazards model (package: survival), where the dependent variables were either male or female longevity, and the independent variable was mating treatment. To determine whether the OE-like parasite differentially affected fecundity (i.e. total number of eggs laid) between females in the four mating treatments, a one-way ANOVA was conducted, while fertility (i.e. percentage of eggs hatching) differences between females were analysed with a generalized linear model (GLM) with Poisson error distribution. To compare the effect of mating treatments on longevity, fecundity and fertility, we used a Tukey post hoc test at the 5% probability level for multiple comparisons (package: multcomp). The association between mating frequency and the mating treatments was tested using a pairwise Fisher test and the obtained  $P$  values were adjusted using a Holm–Bonferroni correction.

To test the effect of OE-like infection on calling behaviour in uninfected and infected females, we used a generalized linear mixed-effects model (GLMM) with a binomial error distribution (package: lme4), where female calling (coded as 0/1) was the binomial response variable, infection status and time were the fixed effect predictors and individuals were modelled as a random effect. The association between females calling (calling or not calling during the night) and female infection status (infected or not infected) was tested by a chi-square contingency test. Finally, the effect of female infection status on the onset time of calling and duration of calling per night was analysed with a Mann–Whitney  $U$  test.

To assess the effect of OE-like infection on pheromone compositions, the total amounts of pheromone of uninfected and infected

females were compared by a Welch's *t* test. Subsequently, we conducted a multivariate analysis of variance (MANOVA), wherein the response variable consisted of the relative amounts of the compounds in the female pheromone blend and the explanatory variable was female infection status. The relative amounts of the compounds in the female pheromone blend were first log ( $x+1$ ) transformed to normalize the data.

In the male choice experiment each male made a binary choice between two females. One female was chosen and the other was not chosen. Each choice thus reflected relative preference for one female over the other. To model the response variable male choice, we randomly selected one of the two females that he could have chosen to obtain a data set that consisted of unchosen and chosen females (as 0/1) that were either infected or uninfected. This ensured that the sample size was equal to the number of choices that were made. To ensure that the explanatory variable 'onset time of calling' reflected the relative difference between the females, each female obtained a score that reflected this difference. For example, if the uninfected female called 30 min earlier than the infected female, she obtained a score of  $-0.5$  while the female that called later obtained a score of  $0.5$ , resulting in the explanatory variable 'difference in onset time of calling' which was associated with each randomly selected female of each choice pair. So, to model male choice we predicted for one female from each choice pair whether she was mated or not mated as a function of the difference, in the onset time of calling, infection status of the female and infection status of the male. This model was analysed using a GLM with a binomial error distribution. Since we found an effect of onset time of calling in male choice, in a separate analysis we further analysed the difference in the onset time of female calling between uninfected and infected females by using a Welch's *t* test. Female choice was analysed in a similar way as male choice, i.e. the dependent variable in the GLM was whether the male was chosen, and the independent variables were female infection status and male infection status.

## RESULTS

### Longevity and Reproduction

The different mating treatments did not affect longevity in females (Coxph:  $\chi^2_3 = 2.8, P = 0.43$ ; Fig. 1a) but did in males (Coxph:

$\chi^2_3 = 7.9, P = 0.049$ ). Specifically, uninfected males that had mated with infected females had significantly shorter life spans than uninfected males that had mated with uninfected females (Tukey post hoc test:  $P = 0.03$ ; Fig. 1b).

The mating treatments affected fecundity ( $F_3 = 5.1, P = 0.003$ ) and fertility ( $F_3 = 33.1, P < 0.0001$ ) of females. Specifically, the fecundity of uninfected females was similar when mated with either uninfected or infected males (Tukey post hoc test:  $P = 0.99$ ). However, infected females that had mated with infected males produced significantly fewer eggs than infected females that had mated with uninfected males (Tukey post hoc test:  $P = 0.013$ ; Fig. 2a). The fertility of uninfected females was similar when mated with either uninfected or infected males (Tukey post hoc test:  $P = 0.794$ ). However, the fertility of infected females that had mated with uninfected males was significantly higher than that of infected females that had mated with infected males (Tukey post hoc test:  $P < 0.001$ ; Fig. 2b).

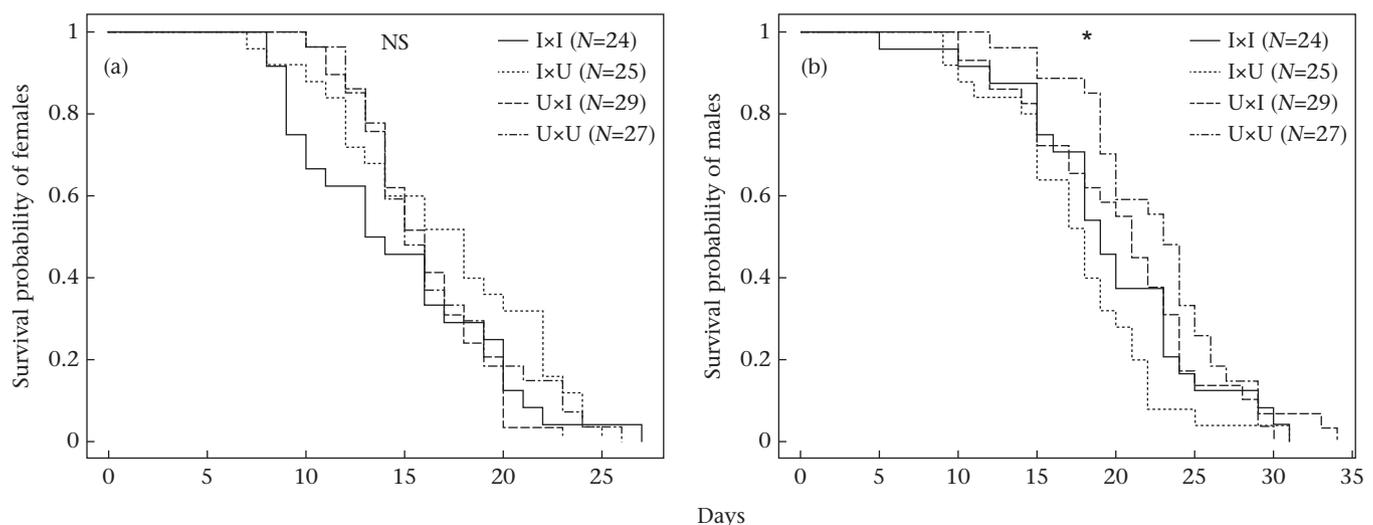
### Mating Frequency

When uninfected males and females were paired for 5 nights, 94% ( $N = 30$  of 32 pairs) of them mated at least once, while 41% ( $N = 13$  of 32 pairs) mated more than three times. In comparison, 84% ( $N = 21$  of 25 pairs) of the uninfected females paired with infected males mated at least once, while 44% ( $N = 11$  of 25 pairs) mated more than three times; 89% ( $N = 25$  of 28 pairs) of infected females paired with uninfected males mated at least once, while 29% ( $N = 8$  of 28 pairs) mated more than three times; and 73% ( $N = 24$  of 33 pairs) of infected pairs mated at least once, while only 9% ( $N = 3$  of 33 pairs) mated more than three times (Fig. 2c).

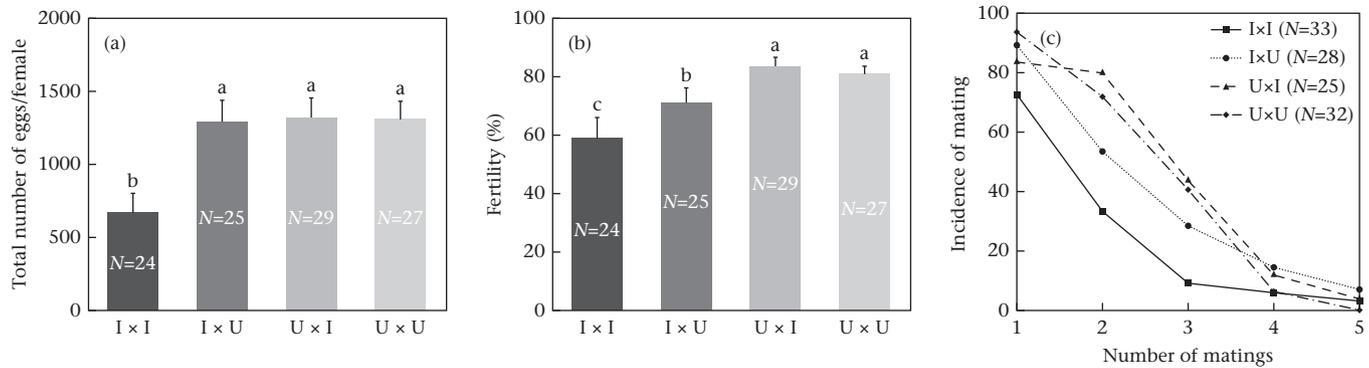
When comparing mating frequency between the treatments, the probability of mating for infected pairs was significantly lower than for uninfected pairs (pairwise Fisher test:  $P = 0.046$ ) and for infected females paired with uninfected males (pairwise Fisher test:  $P = 0.003$ ).

### Female Calling and Pheromone Composition

The calling patterns of uninfected and infected females did not differ over 4 consecutive nights (Fig. 3a). Specifically, there was no association between the number of females calling per night and the infection status of females during the first ( $\chi^2_1 = 1.12$ ,



**Figure 1.** Longevity of (a) females and (b) males in relation to four mating treatments. I × I: infected females mated with infected males; I × U: infected females mated with uninfected males; U × I: uninfected females mated with infected males; U × U: uninfected females mated with uninfected males. \* $P < 0.05$ .



**Figure 2.** Effects of OE-like parasite on (a) fecundity, (b) fertility and (c) mating frequencies of *H. armigera* when paired with the same partner for 5 nights in four mating treatments: I × I: infected females mated with infected males; I × U: infected females mated with uninfected males; U × I: uninfected females mated with infected males; U × U: uninfected females mated with uninfected males. Different letters represent significant differences between the treatments.

$P = 0.291$ ), second ( $\chi^2_1 = 0.15$ ,  $P = 0.697$ ), third ( $\chi^2_1 = 0.03$ ,  $P = 0.853$ ) and fourth nights ( $\chi^2_1 = 0.76$ ,  $P = 0.382$ ; Fig. A1a). In addition, the onset and duration of calling times were similar between uninfected and infected females over the 4 consecutive nights (Fig. A1b, c). Uninfected and infected females also produced similar amounts of pheromone (Welch's  $t$  test:  $t_{59.7} = 0.17$ ,  $P = 0.867$ ) as well as similar ratios of pheromone components (MANOVA:  $F_{8,53} = 1.73$ ,  $P = 0.114$ ; Fig. 3b).

#### Mate Choice

In the female choice experiment, uninfected females did not mate significantly more with uninfected males than with infected males ( $\chi^2_1 = 0.21$ ,  $P = 0.65$ ). However, infected females mated significantly more with uninfected males than with infected males ( $\chi^2_1 = 8.29$ ,  $P = 0.004$ ; Fig. 4a). In the male choice experiment, male choice was not affected by either male infection status ( $\chi^2_1 = 0.24$ ,  $P = 0.63$ ) or female infection status ( $\chi^2_1 = 0.02$ ,  $P = 0.90$ ). However, when we compared the onset time of calling of the two females that were caged together, we found that this did affect male choice ( $\chi^2_1 = 6.94$ ,  $P = 0.008$ ) and that infected females called significantly earlier than uninfected females (Welch's  $t$  test:  $t_{65.9} = 2.77$ ,  $P = 0.007$ ; Fig. 4b and c).

#### DISCUSSION

Effects of OE-like infection on *H. armigera* differed between the sexes, specifically in male longevity and female reproduction. In mate choice experiments, infected females choosing parasite-free males gained fitness benefits, with approximately 12% more offspring (i.e. fertility) compared to when choosing infected males. Male choice was not influenced by female infection status: neither the uninfected nor the infected males mated significantly more with uninfected or infected females. Interestingly, male choice was significantly affected by the difference in the onset time of female calling, and infected females called significantly earlier when housed together with uninfected females. OE-like infections did not affect the calling behaviour or sex pheromone signal of uninfected females, or of infected females held individually.

#### Longevity and Reproduction

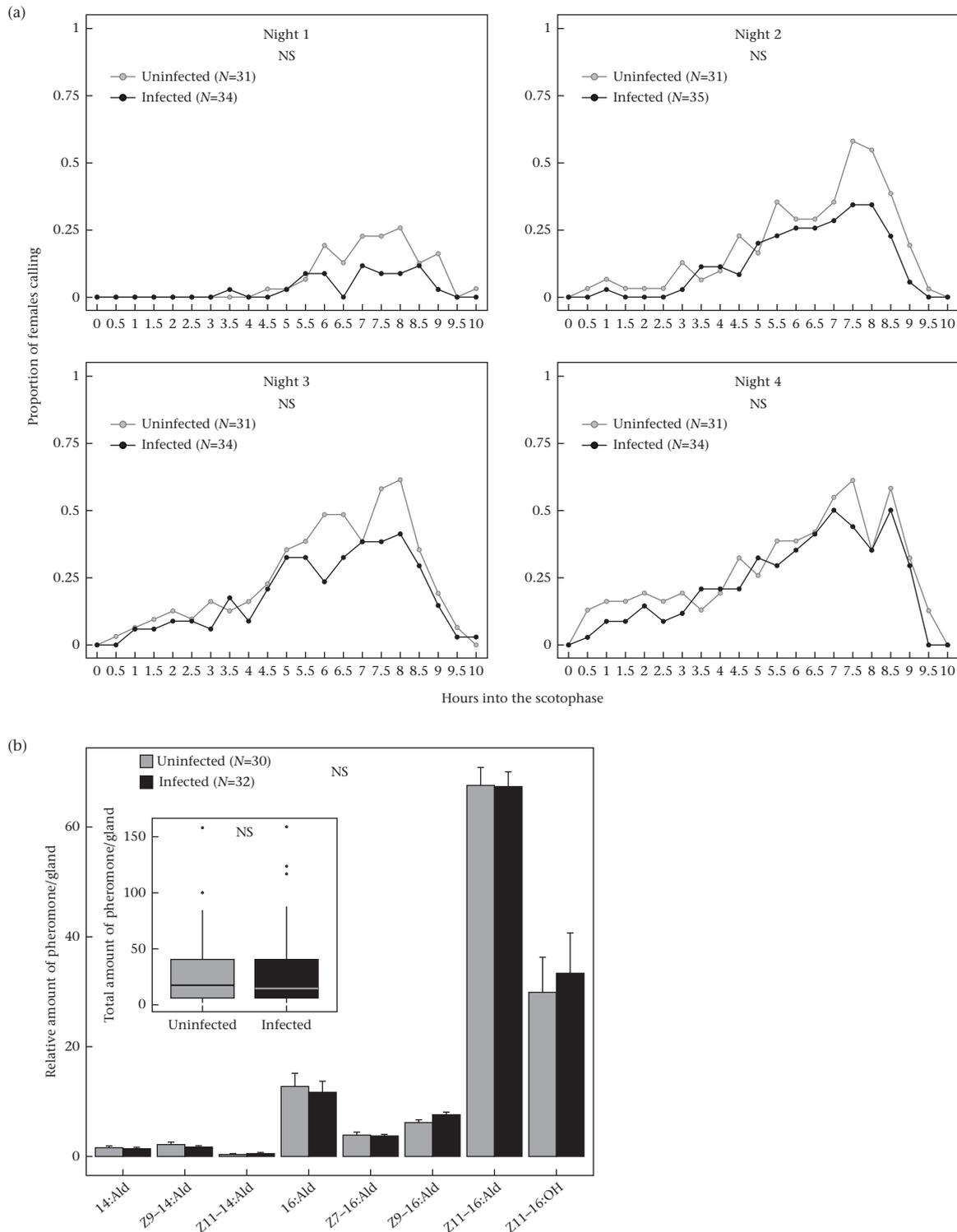
In our experiments, infections affected longevity and reproduction differently between the sexes and in unexpected ways. Female longevity was unaffected by OE-like infection, while uninfected males that had mated with infected females had significantly

shorter life spans compared to uninfected or infected males that had mated with uninfected females. Interestingly, infected females that had mated with uninfected males had higher reproductive output compared to infected females that had mated with infected males. In Lepidoptera, male moths produce spermatophores that may be up to ca. 5% of their body weight (Blanco, Rojas, Groot, Morales-Ramos, & Abel, 2009). One explanation for this observation may be that uninfected males show a possible form of terminal investment strategy (e.g. larger spermatophores) when mating with an infected female, so that there is a trade-off between longevity and reproduction for uninfected males when paired with infected females (Adamo, 1999; Javoš & Tammuru, 2004; Khan & Prasad, 2013; Staudacher, Menken, & Groot, 2015). Another explanation may be that OE-like parasites manipulate infected females in some way to mate with uninfected males to increase the rate of parasitic transmission and survival (Lafferty, 1999). As the reproductive output of uninfected females was not affected by the infection status of their mating partners, a male's infection status seems to be inconsequential for females in good condition.

In other species, parasitic infections have been found to reduce the mating opportunities and competitive abilities of males, but not of females (De Roode, Gold, & Altizer, 2007; Jaenike, 1988; Thomas et al., 1995). However, we found fitness effects for both sexes when both parents were infected, as the mating frequency and reproductive output were lowest in pairs where both partners were infected. This may be because in polygamous species, such as noctuid moths, females as well as males may gain benefits from multiple matings (Gao, Van Wijk et al., 2020; Scharf, Peter, & Martin, 2013). A reduction in mating rates caused by the OE-like parasite may thus decrease not only the male's but also the female's lifetime reproductive output.

#### Sexual Attractiveness

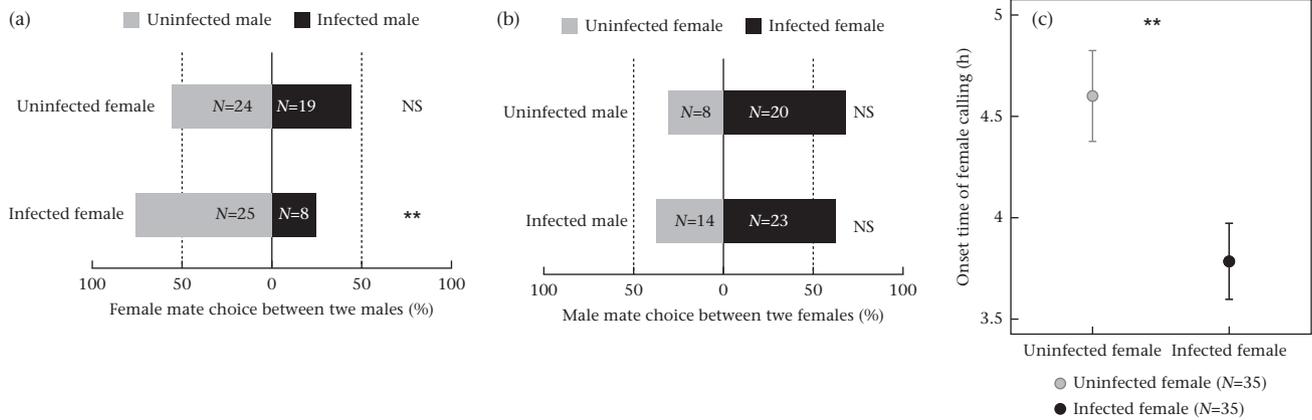
Previous studies indicate that infections may reduce sexual attractiveness, which may be due to a trade-off between sexual signalling and immunocompetence (Folstad & Karter, 1992; McKean & Nunney, 2001; Peters, Delhey, Denk, & Kempenaers, 2004; Verhulst, Dieleman, & Parmentier, 1999). Sex pheromones have been confirmed to be condition-dependent sexual signals in several species, such as in lizards, beetles, flies and moths (Barthel et al., 2015; Martín & López, 2010; Rantala, Kortet, Kotiaho, Vainikka, & Suhonen, 2003; Shelly, Edu, & Pahio, 2007; Worden et al., 2000). For example, immune challenge by a tapeworm significantly reduced the attractiveness of the male sex pheromone in grain beetles, *Tenebrio molitor* (Worden et al., 2000). In contrast,



**Figure 3.** (a) Calling patterns on 4 consecutive nights and (b) pheromone composition (relative amount of pheromone compounds) of *H. armigera* females. Insert: total amount of pheromone. Compounds: 14:Ald: tetradecanal; Z9-14:Ald: (Z)-9-tetradecenal; Z11-14:Ald: (Z)-11-tetradecenal; 16:Ald: hexadecanal; Z7-16:Ald: (Z)-7-hexadecenal; Z9-16:Ald: (Z)-9-hexadecenal; Z11-16:Ald: (Z)-11-hexadecenal; Z11-16:OH: (Z)-11-hexadecenol.

we found that OE-like infection did not affect the female sex pheromone signal in *H. armigera*. One reason for this may be that OE-like infection of *H. armigera* has developed too recently to have high costs. However, as OE-like infection did not reduce female longevity, but did reduce female reproductive output, a possible explanation for our results might be that these infected virgin

females increased terminal investment in their attractiveness for mating opportunity prior to reproduction (Kivleniece, Krams, Daukste, Krama, & Rantala, 2010; Sadd et al., 2006). Animals can adjust their reproductive effort to maximize lifetime reproductive success, as life history theory predicts that organisms should trade current for future reproduction by changing resource allocation



**Figure 4.** Effect of OE-like infection on mate choice in *H. armigera*. (a) Female choice, (b) male choice and (c) average onset time of female calling ( $\pm$  SE) in the male mate choice experiment. \*\* $P < 0.01$ .

(Clutton-Brock, 1984; Kokko, 1998; Siefferman & Hill, 2005). To maintain an attractive sex pheromone signal, infected females may reallocate resources that are presumably allocated to other life history traits, such as life expectancy or reproduction (Johansson & Jones, 2007; Steiger & Stöckl, 2014). Recent studies indeed suggest that moth sex pheromones are costly to maintain (Foster & Johnson, 2011; Harari et al., 2011; Steiger & Stöckl, 2014).

#### Mate Choice

Infections may affect mate choice, because the chooser's and/or the potentially chosen mate's behaviour may be affected. The fact that we found infected females mating significantly more with uninfected than with infected males when given a choice may be due either to female choice or to uninfected males being more competitive than infected males. In general, in mating systems female choice is difficult to disentangle from the effects of male–male competition (Edward & Chapman, 2011; Paul, 2002). However, since we found that uninfected females did not show a preference for uninfected or infected males, it seems more likely that our results are due to female choice rather than that males differed in their competitiveness. Additionally, males are less likely to be choosy because their investment in the offspring is usually smaller than the female's investment. Furthermore, our finding that infected females choosing parasite-free males gained benefits, that is, approximately 12% more offspring, compared to infected females that had mated with infected males, is in line with this explanation.

As the reproductive success of infected pairs was significantly smaller than that of all other combinations (Fig. 2a and b), we expected that infected males would choose uninfected females in the male choice experiment. However, our results indicated that male choice was influenced not by female infection status, but instead by the onset time of female calling. Probably, males cannot discriminate between uninfected and infected females, as their sex pheromone signal did not differ. The fact that infected females called significantly earlier than uninfected females when paired together in the cages, which was not the case when uninfected and infected females were held individually, indicates that females interact with each other and affect each other's behaviour. Previous studies have shown that females alter their calling behaviour based on the presence/absence of conspecifics, most likely to increase their chance of accessing males (Burand et al., 2005; Reherrmann, Altesor, McNeil, & González, 2016; Yang, Dong, & Chen, 2009). Our results imply that infected females

could be able to adjust their mating strategy by calling earlier to increase their mating probability when in competition.

#### Conclusions

We found that the OE-like parasite negatively affected the fitness and reproductive success of its host *H. armigera*, but that these effects differed for males and females. Based on our results, we hypothesize that uninfected males have a terminal investment strategy and mate with any female to increase their reproductive success. In contrast, infected females seem either to put more effort into mate assessment to avoid mating with infected partners or to advance their onset time of calling to increase their mating opportunities and thus gain more offspring. These adaptive changes could be especially important for polygamous species with intense sexual conflict and competition (Alonzo & Warner, 2000; Kokko & Jennions, 2014; Lorenzi, Araguas, Bocquet, Picchi, & Ricci-Bonot, 2019), such as noctuid moths. Mating strategies may differ between males and females due to sex-specific effects of parasites, potentially leading to sex-specific selection (Sharp & Vincent, 2015).

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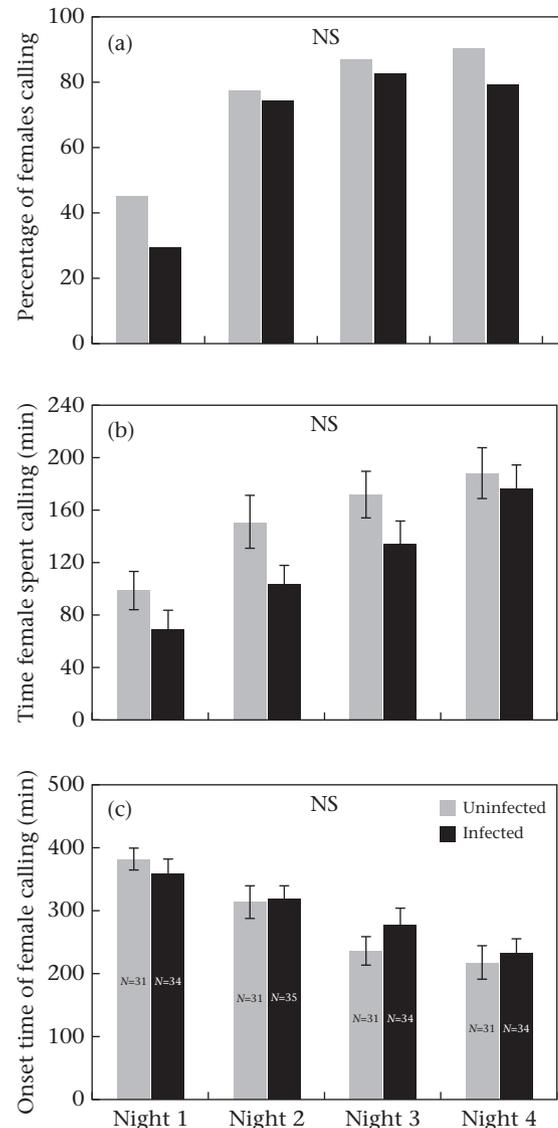
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## Appendix



**Figure A1.** Female calling behaviours on 4 consecutive nights when uninfected or infected. (a) Percentage of females calling; (b) time spent calling; (c) onset time of female calling.