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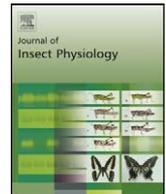
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Male and female condition influence mating performance and sexual receptivity in two tropical fruit flies (Diptera: Tephritidae) with contrasting life histories

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ABSTRACT

Recent recognition of widespread polyandry in insects has generated considerable interest in understanding why females mate multiple times and in identifying factors that affect mating rate and inhibit female remating. However, little attention has been paid to understanding the question from both a female and male perspective, particularly with respect to factors that may simultaneously influence female remating rates. Here, we report on a study aimed at ascertaining the possible interactive effects that male and female size and diet, and female access to a host could have on mating latency, probability, and duration and female refractory period using two tropical fruit fly species with contrasting life histories. Of all factors tested, adult diet played the most significant role. Both *Anastrepha ludens* and *Anastrepha obliqua* males which had constant access to protein and sucrose mated more often, had shorter copulations and induced longer refractory periods in females than males fed a low quality diet (sucrose offered every third day). Female size and the interaction with male diet determined how quickly female *A. ludens* mated for the first time. Smaller females mated sooner with low quality fed males than with high quality fed males while there was no difference for large females, suggesting that male choice may be at play if high quality fed males discriminate against smaller females. Copulation duration also depended on both male and female nutritional condition, and the interaction between male diet and female size and diet. Large and high quality fed females had shorter copulations regardless of male condition. Importantly, for *A. ludens*, female refractory period depended on male size and the nutritional condition of both males and females, which could indicate that for this species, female receptivity does not depend only on the condition of the male ejaculate. For *A. obliqua* refractory period was associated with the interaction between male size and diet and male diet and host presence. We discuss our results in terms of male ability to inhibit female remating and the relative contribution of female condition to this behavior. We also address the importance of studying effects simultaneously on species with contrasting life histories.

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1. Introduction

In the majority of insects, females mate multiple times despite the fact that one or a few copulations are sufficient to fertilize their eggs (Arnqvist and Nilsson, 2000). Multiple mating in female insects can improve and diversify the genetic constituents of their offspring (Zeh and Zeh, 2001; Yasui, 1998), increase lifetime offspring production (Arnqvist and Nilsson, 2000), and prevent inbreeding depression (Tregenza and Wedell, 2002). However, there may also be some costs associated with reproduction and multiple mating (Johnstone and Keller, 2000) such as a reduction in lifespan (Chapman et al., 1998), an increased risk of predation

(Hendrichs and Hendrichs, 1998; see however, Sivinski, 1984) and an increased risk of contracting sexually transmitted diseases (Hurst et al., 1995; Knell, 1999).

Despite the fact that considerable attention has been paid to the occurrence of polyandry in several insect taxa (see review by Arnqvist and Nilsson, 2000) less attention has been devoted to the combined factors that could influence female remating rates. In insects where adults are long-lived and forage in patchy environments, the quality and availability of resources are likely to influence the adult stage of the life-cycle (Jones and Widemo, 2005). This may in turn affect the time to reach sexual maturity for both sexes and thus the latency to copulate and remate as an adult.

Amount and quality of food ingested by a male can play an important role in calling and courting (Field and Yuval, 1999), affect the production of pheromones to attract mates (Epsky and

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Heath, 1993), and the quality and quantity of its ejaculate (Simmons, 2001). Adult diet may be especially critical in determining male ability to inhibit female remating and induce a sexual refractory period during which time females will not be receptive to mate. For example, high quality fed male Mediterranean fruit flies, *Ceratitis capitata*, and Queensland fruit flies, *Bactrocera tryoni*, are more likely to inhibit female remating than low quality fed males (Blay and Yuval, 1997; Pérez-Staples et al., 2008a). Short-term inhibition in females can be determined by sperm numbers (e.g., Mossinson and Yuval, 2003), accessory gland products (Chen, 1984; Gillot, 2003; Wolfner, 2002; Radhakrishnan and Taylor, 2007) and/or physical stimuli during copulation (Miyatake et al., 1999), all of which could depend on male physiological condition.

Phenotypic traits such as size have also been shown to influence female choice (Thornhill and Alcock, 1983; Clutton-Brock, 1988; Andersson, 1994), and ejaculate size (Fox et al., 1995). For example, in *C. capitata* and *B. tryoni*, females are less likely to store sperm and more likely to remate if their first mate is small (Taylor and Yuval, 1999; Pérez-Staples et al., 2008a). However in *Drosophila melanogaster*, Zhang et al. (2008) have shown that male body size is not related to sperm competitive ability.

Aside from diet quality and size, female remating behavior can also be influenced by the access to ovipositional resources such as hosts (Ringo, 1996). As females lay eggs, sperm resources become depleted and females may be more likely to remate. In walnut flies *Rhagoletis juglandis*, papaya flies *Toxotrypana curvicauda*, and the adzuki bean beetle, *Callosobruchus chinensis* females that were provided with an opportunity to lay eggs in hosts are more likely to mate and remate than females not given a host (Landolt, 1994; Harano et al., 2006; Carsten and Papaj, 2005). The proximity to a host may also accelerate female sexual maturity (Aluja et al., 2001a) and potentially increase remating opportunities.

Individual physiological and phenotypic conditions have usually been manipulated in a single sex and have been studied separately. Given the possibility of complex mechanisms involved in determining female remating rates, here we compared the contributions of three potential influences, male and female adult size, diet, and host usage on mating latency, mating frequency, copulation duration and duration of female refractory periods in two tropical fruit fly species with contrasting life histories: *A. ludens* and *A. obliqua*. To our knowledge this is the first report where female sexual receptivity has been studied simultaneously with varying female and male condition. *Anastrepha obliqua* and *Anastrepha ludens* are closely related sympatric species with different life strategies (Aluja et al., 2009). While *A. ludens* is a generalist fruit fly that exploits hosts that often exhibit extended fruiting periods and are found at low densities, *A. obliqua* is more of a specialist exploiting hosts with greater number of fruits that become suitable for oviposition during a brief window of time (Díaz-Fleischer and Aluja, 2003). *A. obliqua* is an egg-limited species, that responds strongly to host maturation, while *A. ludens* is a time-limited species that does not respond so strongly to such an environmental stimulus (Díaz-Fleischer and Aluja, 2003). The availability of an artificial oviposition substrate influences egg load and ovarian maturity in *A. obliqua* whereas in *A. ludens* that is not the case (Aluja et al., 2001a).

A. ludens males attract females by wing fanning and emitting a pheromone during dusk from 1700 to 1900 h on the underside of non-host trees (Aluja et al., 2000). Males may call alone or from leks formed on adjacent leaves (Aluja et al., 1983). Copulations last 73.4(+6.6) min (Aluja et al., 2000) and females lay clutches of eggs (up to 40 eggs/clutch). Male diet does not seem to influence mating likelihood or copula duration (Aluja et al., 2001b [but see Aluja et al., 2008a]). *A. obliqua* has a bimodal pattern of calling activity, doing so mostly in leks from the leaves of non-host and host trees

in the morning and afternoon (Aluja and Birke, 1993; Aluja et al., 2000). Males have been shown to copulate up to three times in field-cage experiments with an over-supply of virgin females (Aluja et al., 2001b). Copulations last on average (\pm SE) 47.1 \pm 0.9 min (Aluja et al., 2001b), and females lay eggs singly (Aluja et al., 2000). For *A. obliqua* male diet has a strong effect on mating frequency (Aluja et al., 2001b), and there is some indication that multiply mated males cannot inhibit female remating (Pérez-Staples et al., 2008b).

Here, we predicted that large, high quality fed females that were provided with ample oviposition substrates would exhibit the highest mating rate and shortest refractory period compared to all other females. Given the differences in ovipositional strategies between the two species, we further predicted a more marked effect of host presence on *A. obliqua* latency to mate, sexual maturity and remating rates compared to *A. ludens*.

2. Methods

2.1. Source and handling of flies

Wild *A. ludens* and wild *A. obliqua* larvae were obtained from naturally infested Marsh grapefruit (*Citrus paradisi* Macfayden) and naturally infested tropical plums (*Spondias mombin* L.), the former collected in Tuzamapan and Las Trancas and the latter in San Rafael and Llano Grande both in the state of Veracruz, Mexico. Once collected in the field, fruit was transported to the laboratory, weighed, and placed in plastic containers with vermiculite to allow larval exit and to provide a suitable medium for pupation. Pupae were recovered and processed following methods described in Aluja et al. (2003).

Pupae were weighed using an analytical balance (Ohaus® model AP2105) and classified as small or large depending on the full spectrum of weights. For *A. ludens*, small pupae weighed 15–20 mg and large pupae weighed 21–25 mg. For *A. obliqua* small pupae weighed 10–15 mg and large pupae weighed 16–20 mg. Once adults emerged, they were separated by sex and weight class and placed in 30 cm \times 30 cm \times 30 cm Plexiglas cages with 50 individuals each. Males and females were fed either a 3:1 mixture of sucrose and protein (Yeast Hydrolysate Enzymatic, ICN Biomedicals, USA) offered *ad libitum* (high quality fed) or a diet of sucrose (Baker Analyzed R) offered every third day (low quality fed).

2.2. Experimental design and protocol

In a first laboratory experiment five factors were manipulated simultaneously to test for the potential effect of phenotypic and physiological condition on female refractory period: female size (2 levels), male size (2 levels), female diet (2 levels), male diet (2 levels), and presence or absence of an oviposition device or host (2 levels). Hosts consisted of a 2.5 cm, green colored, agar sphere (Bioxon, Becton Dickinson de México S.A. de C.V., Mexico) as described in Jácome et al. (1995).

When females (adults) reached sexual maturation at 10 days of age they were marked on the back of the thorax with a distinctive dot of acrylic paint following methods described in Pérez-Staples and Aluja (2004). Two days later, five individuals of each sex, size and diet were transferred to a 13 cm \times 13 cm \times 25 cm plastic container with or without an artificial host. This resulted in five pairs of 32 combinations of factors. In the case of *A. ludens*, it was only possible to complete two replicates with five pairs of adults per cage; three replicates were set up using cages with three pairs of flies. For analyses, the experimental unit across the entire experiment was therefore mean per treatment cage and not individual females. Each treatment was replicated five times, so

160 cages were observed simultaneously. Cages were held at constant environmental conditions (12L:12D photoperiod; 27 °C; and 75% R.H.).

A. ludens were observed daily for 120 consecutive days (from July to November 1996) from 4:00 pm until the last copulation ended. While *A. obliqua* were observed for 150 consecutive days (from October to March 1997) from 8:00 am until 6:30 pm or until the last copulation ended. Every day, one observer registered the number and duration of copulations and the identity of females in copulating pairs. For calculation of mating latency, the number of days until the first copulation of each female in every treatment cage was recorded. Female refractory period was recorded as the number of days elapsed between copulations.

2.3. Statistical analyses

For each species, mating latency, the total number of copulations obtained, female refractory period and copula duration were analyzed each by means of a factorial ANOVA with female and male size and diet, and host availability as independent variables. All dependent variables were log transformed plus 1 to meet assumptions of ANOVA models. Only significant interactions are presented. Mating latency was calculated as the mean number of days for the five females from release into cages until the first copulation. Copula duration was calculated as the mean duration of all copulations in a treatment cage. Female refractory period was calculated as the mean number of days from the first copulation to the next.

3. Results

3.1. Mating latency

The latency to copulate for *A. ludens* ranged from 11 to 53 days with a mean (\pm S.E.) of 20.4 ± 0.28 days. The interaction between female size and male diet had a significant effect ($F_{1,128} = 5.59$; $P = 0.019$) on the average mating latency of females. Small females took longer to begin mating with high quality fed males for the first time than with low quality fed males, while there was no difference in mating latency for large females paired with males of either diet (Fig. 1). Male diet ($F_{1,128} = 4.19$; $P = 0.043$), and female diet ($F_{1,128} = 4.48$; $P = 0.036$) also had a significant effect on mating latency. High quality fed males took a day longer to copulate for the first time than low quality fed males, while high quality fed females

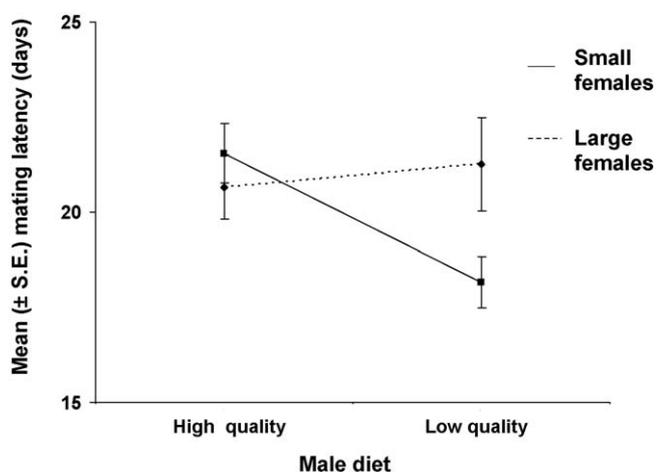


Fig. 1. Mean (\pm S.E.) female mating latency (number of days elapsed from release into cages until first copulation) for small (continuous line) or large (interrupted lines) *Anastrepha ludens* females. Data are shown untransformed to ease interpretation ($N = 160$ cages, 32 treatments \times five replicates).

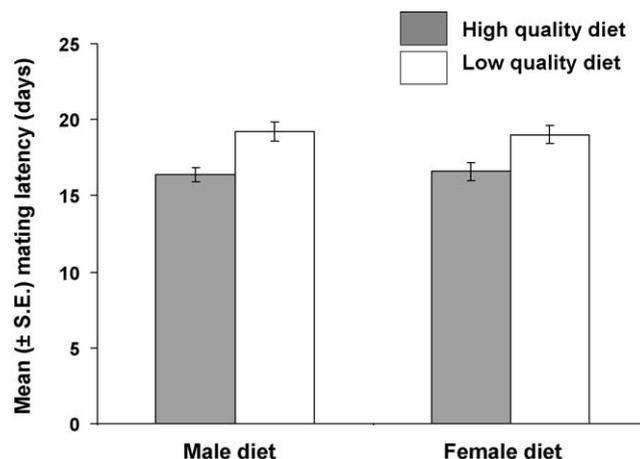


Fig. 2. Mean (\pm S.E.) female mating latency (number of days elapsed from release into cages until first copulation) for *Anastrepha obliqua* females mated to males fed a high quality (grey bars) or a low quality (clear bar) diet. Females were fed a high quality (grey bars) or a low quality (clear bar) diet. Data are shown untransformed to ease interpretation ($N = 160$ cages, 32 treatments \times five replicates).

copulated 2 days earlier than low quality fed females. Host presence ($F_{1,128} = 0.24$; $P = 6.21$), male size ($F_{1,128} = 1.87$; $P = 0.17$), and female size ($F_{1,128} = 1.20$; $P = 0.27$) had no effect on mating latency.

For *A. obliqua*, the latency to copulate ranged from 13 to 33 days with a mean (\pm S.E.) of 17.79 ± 0.42 days. Male and female diet were the most important factors determining mating latency, as both male diet ($F_{1,128} = 11.77$; $P < 0.001$) and female diet ($F_{1,128} = 10.05$; $P = 0.002$) had a significant effect on mating latency. Female *A. obliqua* mated sooner to males fed a high quality diet than to males fed a low quality diet (Fig. 2). Male size ($F_{1,128} = 0.63$; $P = 0.42$), female size ($F_{1,128} = 0.44$; $P = 0.50$) or host presence ($F_{1,128} = 0.02$; $P = 0.88$) had no effect on the latency to mate.

3.2. Mating frequency

Both *A. ludens* and *A. obliqua* females mated multiply. *A. ludens* females mated more often than *A. obliqua*, the majority of which mated only once in their lifetime (Fig. 3).

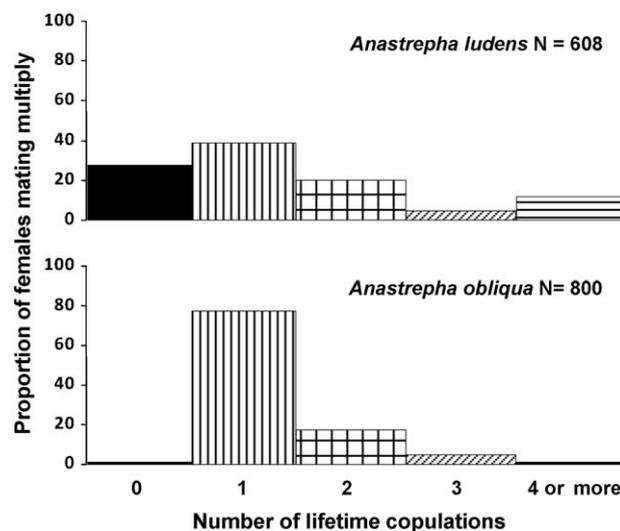


Fig. 3. Overall proportion of *Anastrepha ludens* ($N = 608$) and *Anastrepha obliqua* ($N = 800$) females engaging in 0, 1, 2, 3 and 4 or more copulations during their lifetime under laboratory conditions.

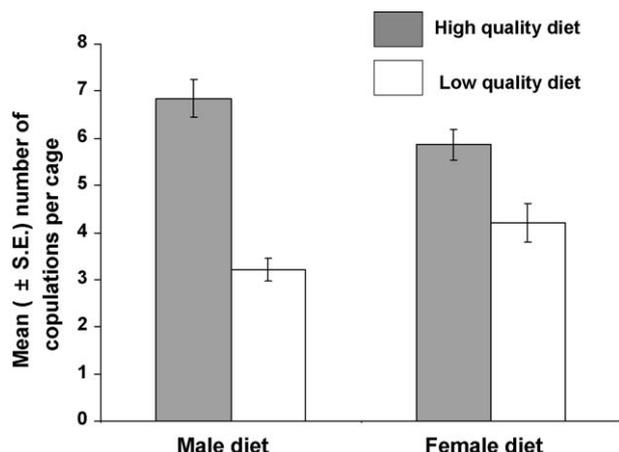


Fig. 4. Mean (\pm S.E.) number of copulations per *Anastrepha ludens* female per cage. Males were fed a high quality (left black bar) or low quality (left clear bar) diet and females were fed a high quality (right black bar) or low quality (right clear bar) diet. Data are shown untransformed to ease interpretation ($N=160$ cages, 32 treatments \times five replicates).

For *A. ludens* both male diet ($F_{1,128} = 83.06$; $P < 0.0001$), and female diet ($F_{1,128} = 28.39$; $P < 0.0001$) had a significant effect on the total number of copulations per cage. *A. ludens* females copulated more when caged with males fed a high quality diet than when caged with males fed a low quality diet (Fig. 4). Similarly, females fed a high quality diet copulated more than females fed a low quality diet (Fig. 4). Male size ($F_{1,128} = 0.78$; $P = 0.38$), female size ($F_{1,128} = 2.39$; $P = 0.12$) or host presence ($F_{1,128} = 3.13$; $P = 0.07$) had no effect on the total number of matings.

For *A. obliqua*, only male diet had a significant effect on the total number of copulations per cage ($F_{1,128} = 29.91$; $P < 0.0001$). Females mated more often with males fed a high quality diet (6.66 ± 0.18 mean \pm S.E.) than with males fed a low quality diet (5.53 ± 0.10 mean \pm S.E.). Female diet ($F = 1.76$, $P = 0.18$), male size ($F_{1,128} = 0.21$; $P = 0.64$), female size ($F_{1,128} = 0.13$; $P = 0.71$) and host presence ($F_{1,128} = 0.01$; $P = 0.93$) had no effect on the number of matings per cage.

3.3. Copula duration

For *A. ludens* female diet ($F_{1,128} = 4.41$; $P = 0.038$) had a significant effect on average mating duration. Females fed a high quality diet mated for shorter periods of time (44.8 ± 1.62 S.E.) than females fed a low quality diet (51.2 ± 1.62 S.E.). Female size ($F_{1,128} = 2.65$; $P = 0.10$), male size ($F_{1,128} = 0.31$; $P = 0.57$), male diet ($F_{1,128} = 0.42$; $P = 0.51$), and host presence ($F_{1,128} = 0.39$; $P = 0.57$) had no effect on copulation duration.

For *A. obliqua*, male diet ($F_{1,128} = 24.53$; $P < 0.0001$) and female diet ($F_{1,128} = 20.99$; $P < 0.0001$) had a significant effect on mean copulation duration. Females mated for longer periods with males fed a low quality diet than with males fed a high quality diet (Fig. 5). Similarly, copulation duration was shorter for females fed a high quality diet than for females fed a low quality diet (Fig. 5). The interaction between female size and male diet also had a significant effect on copulation duration ($F_{1,128} = 6.11$; $P = 0.015$), with large females exhibiting a greater difference in copula duration when mating with either low quality fed or high quality fed males compared to small females. Male size ($F_{1,128} = 0.29$; $P = 0.59$), female size ($F_{1,128} = 0.55$; $P = 0.45$), and host presence ($F_{1,128} = 0.31$; $P = 0.57$) had no effect.

3.4. Refractory period

Refractory period (time elapsed from the first to the second copulation) ranged from a minimum of 1–40 days and averaged

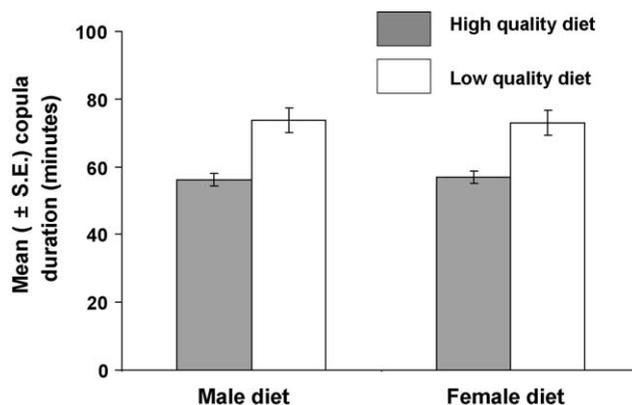


Fig. 5. Mean copula duration (\pm S.E.) per female per cage for *A. obliqua* males fed a high quality (left black bars) or a low quality (left clear bar) diet and females fed a high quality (right black bars) or a low quality (right clear bar) diet. Data are shown untransformed to ease interpretation ($N = 160$ cages, 32 treatments \times five replicates).

12.84 ± 0.32 S.E. for *A. ludens*, while for *A. obliqua* the range of refractory periods spanned from 1 to 73 days from the first copulation to the next and averaged 17.35 ± 1.04 S.E.

Male size ($F_{1,128} = 6.44$; $P = 0.012$), female diet ($F_{1,128} = 4.93$; $P = 0.028$) and the interaction between male diet and female diet ($F_{1,128} = 6.56$; $P = 0.012$) had a significant effect on female *A. ludens* refractory period. Females housed with smaller males exhibited longer refractory periods (13.9 ± 0.77 S.E.) than females housed with larger males (11.78 ± 0.69 S.E.). Females fed a high quality diet exhibited longer refractory periods when mated to males fed a high quality diet than when mated to males fed a low quality diet, while females fed a low quality diet exhibited similarly long refractory periods irrespective of the nutritional condition of their mate (Fig. 6). Host presence ($F_{1,128} = 2.93$; $P = 0.089$), female size ($F_{1,128} = 1.28$; $P = 0.26$), and male diet ($F_{1,128} = 1.78$; $P = 0.18$) had no effect on refractory period.

For *A. obliqua*, host presence ($F_{1,128} = 2.06$; $P = 0.15$), female diet ($F_{1,128} = 0.01$; $P = 0.90$), and female size ($F_{1,128} = 1.30$; $P = 0.25$) had no effect on the length of the refractory period. However, male diet ($F_{1,128} = 11.24$; $P = 0.001$), the interaction between male size and male diet ($F_{1,128} = 7.5$; $P = 0.007$), and the interaction between male diet and host presence ($F_{1,128} = 4.17$; $P = 0.04$) did have a

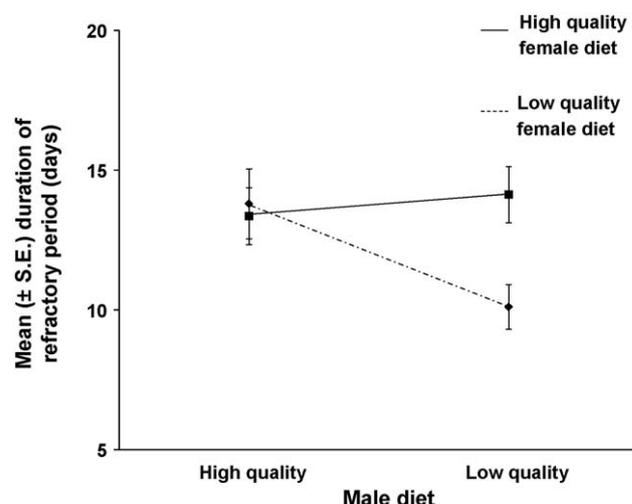


Fig. 6. Mean (\pm S.E.) refractory period (number of days elapsed from the first to the second copulation) of high quality fed (continuous line) or low quality fed (interrupted line) *Anastrepha ludens* females mated to either high quality or low quality fed males. Data are shown untransformed to ease interpretation ($N = 160$ cages).

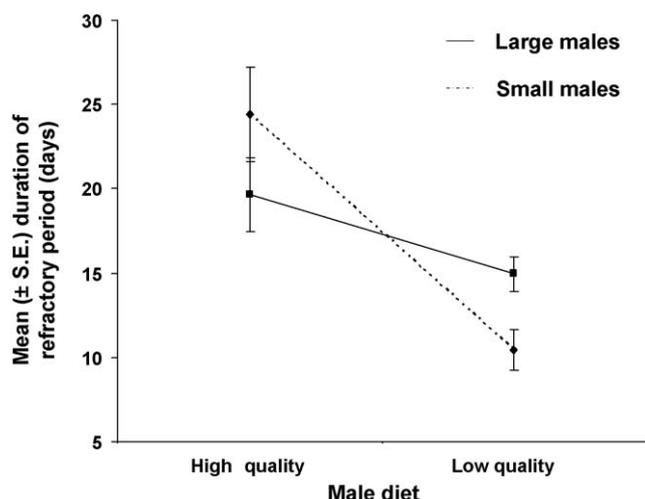


Fig. 7. Mean (\pm S.E.) refractory period (number of days elapsed from the first to the second copulation) of high quality fed (continuous line) or low quality fed (interrupted line) *Anastrepha obliqua* females mated to either high quality or low quality fed males. Data are shown untransformed to ease interpretation ($N=160$ cages, 32 treatments \times five replicates).

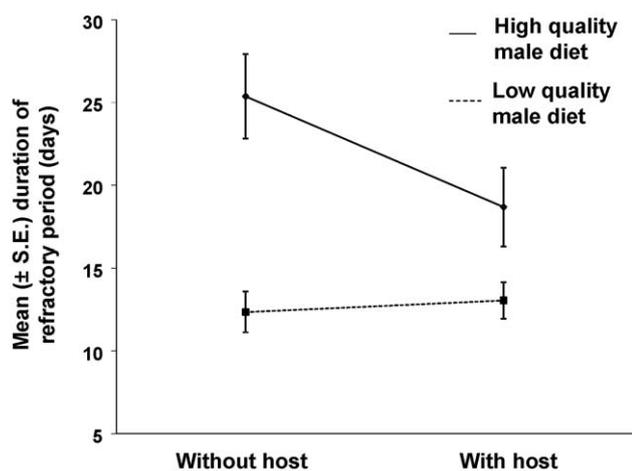


Fig. 8. Mean (\pm S.E.) refractory period (number of days elapsed from the first to the second copulation) for *Anastrepha obliqua* females mated to high quality fed (continuous line) or low quality fed (interrupted line) males in the presence or absence of an artificial host. Data are shown untransformed to ease interpretation ($N=160$ cages, 32 treatments \times five replicates).

significant effect on the length of the female refractory period. Males fed a high quality diet induced longer refractory periods in mated females than males fed a low quality diet (Fig. 7), and this effect was more pronounced in larger versus smaller males (Fig. 7). Females provided a host had shorter refractory periods than females without a host, however only for females mated to high quality fed males (Fig. 8).

4. Discussion

Our results show that sexual receptivity/refractory periods in *A. ludens* and *A. obliqua* are influenced by a hitherto poorly understood constellation of factors that many times interact in complex ways and are influenced by both female and male condition. Importantly, they also show that the patterns observed are some times species-specific, which can be explained by the differences in life-history characteristics that distinguish *A. ludens* from *A. obliqua*. Of all factors tested, adult diet played the most significant role. Both *A. ludens* and *A. obliqua* males which had constant access

to protein and sucrose mated more often, had shorter copulations and induced longer refractory periods in females than males fed a low quality diet (sucrose offered every third day). These findings agree with recent reports by us on related studies (Aluja et al., 2008a [and also Aluja et al., 2001b]) and send clear signals to managers of mass rearing facilities producing sterile flies for area-wide control of these pestiferous species. We will discuss these findings along five broad themes: similarities/contrasts between species, mating latency, copulation duration, mating frequency, and refractory period.

4.1. Similarities/contrasts between species

The most important similarity between *A. ludens* and *A. obliqua* was the consistent and strong effect of male adult diet on most mating performance parameters. With the exception of some variables in the case of *A. ludens*, females mated sooner, more often and had longer refractory periods when mated with males fed a high quality diet. Males fed a high quality diet could engage in higher quality calling activity than those lacking energetic reserves (Papadopoulos et al., 1998; Field and Yuval, 1999), reach sexual maturity sooner (e.g., Aluja et al., 2001a; Pérez-Staples et al., 2007), and could transfer a higher quantity or quality of ejaculate which in turn influences female sexual receptivity (Simmons, 2001; but see Shelly and Kennely, 2002). Nutritional condition could also be used by females as an indirect measure of male quality. Indeed, pheromone composition in tephritids is strongly influenced by male diet (Epsky and Heath, 1993; Tan and Nishida, 1996; Shelly et al., 2004a). Previously, diet has been shown to be important for the sexual performance of both these species (Pérez-Staples et al., 2008b; Aluja et al., 2008a), although for *A. ludens* male diet may be less critical (Aluja et al., 2001b).

Interestingly, factors reputed to influence mate choice and overall performance in many Diptera such as adult size played a lesser role in shaping the sexual response of both males and females. A bigger size has been shown to be an advantage in winning fights with intruders (e.g., Burk, 1983), competing with other males (Aluja et al., 2008a), increased flight ability (Sharp et al., 1983; Bloem et al., 1994) and mating frequency (Churchill-Stanland et al., 1986). However, under our laboratory conditions (small cages), size was not important for obtaining copulations but was important for inducing female refractory periods. Nevertheless, it has been found that female remating propensity may, or may not, be influenced by the suitability of additional available mates (Shelly et al., 2004b; Shelly and Edu, 2008 respectively). Recently, working in large field enclosures (3 m diam \times 3 m height) we reported that in the case of *A. ludens* diet, not size, determined male copulation success over time (continuous 4-day observation period) which coincides with our findings here (Aluja et al., 2008a). We also found that both size and diet determined the success of territory defence by resident males, but that only size influenced the likelihood of success by invading males (Aluja et al., 2008a). We are fully aware that here we worked in small enclosures in which females were enclosed with males without respite for the duration of the experiment. Accordingly, they were constantly exposed to close-range male courtship with little opportunity for female choice. Thus the high remating rates of *A. ludens* may reflect an artefactual “convenience polyandry”, whereby females remate just to be left alone. In nature remating will only take place when females make the effort to visit a male mating aggregation. Furthermore, because of this forced proximity, it is hard to decide if males are inhibiting female receptivity or overcoming this inhibition, as it is the same males doing both all the time. This caveat aside, our experimental conditions allowed us to flush out interesting differences in two fruit fly species with contrasting life histories. For example, despite the “forced

conjugalit y", *A. obliqua* females tended not to remate as frequently as *A. ludens*, which suggests a real biological difference between the species.

Here, contrary to predictions, the presence of a host had no effect on the latency or likelihood to mate. In *R. juglandis*, and *T. curvicauda*, female mating likelihood does increase when hosts are available (Landolt, 1994; Carsten and Papaj, 2005). Nevertheless, for *A. obliqua*, host presence decreased female refractory period but only for females mated to high quality fed males. Thus, female remating inhibition decreases after having received a high quality ejaculate and having had the opportunity to lay eggs in hosts. Females given access to hosts also have higher egg loads (Aluja et al., 2001a). Thus, females under these beneficiary conditions could quickly oviposit and become sperm-limited remating faster than females not given access to hosts. The lack of a similar effect for *A. ludens* is not surprising, as the availability of an artificial oviposition substrate does not influence egg load or ovarian maturation in this species (Aluja et al., 2001a) and oviposition patterns over time (D az-Fleischer and Aluja, 2003).

4.2. Mating latency

Mating latency for *A. obliqua* depended on male and female diet. When both males and females were fed on a high quality diet they mated sooner for the first time than when they were fed a low quality diet. This concurs with previous results in this species where inclusion of protein into the adult diet has been shown to influence female ovarian development and sexual maturity (Aluja et al., 2001b). For *A. ludens*, the interaction between female size and male diet had an effect on the latency to obtain the first copulation. Small females took longer to mate with high quality fed males than with low quality fed males whereas no effect of male diet was found for larger females. Small females may mature slower than larger females but the interaction with male diet suggests that male choice may be occurring. High quality fed males may be discriminating against smaller females. In various insect taxa, larger females are more fecund so males prefer mating with larger females (Hieber and Cohen, 1983; Johnson and Hubbell, 1984). Large females quickly obtained copulations regardless of the males' nutritional condition.

4.3. Copulation duration

Male diet and female diet also influenced copulation duration for *A. obliqua*, while for *A. ludens* only female diet was important. Furthermore, for *A. obliqua* the interaction between male diet and female size seems to indicate that copulations with high quality fed males tend to be shorter when females are large. This result could offer some support to the hypothesis that females also play an active role in terminating copulations. Thus, it may be in the females' best interest to engage in a short copulation as mating can be costly due to increased risk of predation and time lost for other vital activities such as oviposition which in the case of *A. obliqua* is closely associated with individual fitness as most native hosts ripen at once and only during very short periods (D az-Fleischer and Aluja, 2003). From the males' perspective, a short copulation can also be advantageous (Aluja et al., 2008b). In *C. capitata*, males fed a better quality diet such as protein engage in shorter copulations (Taylor and Yuval, 1999), whereas in *B. tryoni* the opposite effect occurs (P erez-Staples et al., 2007). Short copulations may result in greater opportunity to find mates and therefore increase the chances of contributing more offspring to the next generation. This will depend on the likelihood of encountering receptive females and the operational sex ratio in the population (Emlen and Oring, 1977; Alonso-Pimentel and Papaj, 1996, 1999). However, in the case of *A. ludens* a short copulation is unlikely to

result in additional matings, as males only mate at dusk during a short period of time and they are unlikely to mate with more than one female per night (Aluja et al., 2000). Finally, longer copulations do not necessarily result in more ejaculate transfer. For example, in *A. obliqua* there is no association between sperm storage and copula duration (P erez-Staples and Aluja, 2006).

4.4. Mating frequency

Both *A. ludens* and *A. obliqua* females mated multiple times. Nevertheless, there were differences in the frequency and the proportion of individuals in the population of each species that engaged in multiple copulations. Indeed, a greater proportion of *A. obliqua* females mated only once compared to *A. ludens*. Life-history characteristics such as the different ovipositional strategies of both species (D az-Fleischer and Aluja, 2003) could partially explain these differences. The costs of multiple mating in terms of time investment may be large for *A. obliqua*, whose females could, as noted before, be foregoing valuable oviposition opportunities when engaging in time consuming courtship rituals and copulations. By contrast, *A. ludens* females have greater opportunities to oviposit as their hosts are available for longer periods of time (D az-Fleischer and Aluja, 2003) and thus could potentially benefit from receiving multiple ejaculates.

4.5. Refractory period

Few studies have simultaneously addressed the effects of female and male diet and size on refractory periods. Here, in *A. ludens* female refractory periods were associated with male size and the interaction between male and female diet. Previous studies have shown that *C. capitata* and *B. tryoni* females mated to low quality fed males are more likely to remate (Blay and Yuval, 1997; P erez-Staples et al., 2008b). Consistent with results for *C. capitata*, high quality fed *A. ludens* females had shorter refractory periods when mated to low quality fed males. However, when females were fed a low quality diet themselves, there was no relationship between the length of the refractory period and a males' diet. If inhibition of female remating depended solely on male nutritional condition then we would have expected low quality fed females mated to low quality fed males to have short refractory periods, when in fact the opposite occurred. Low quality fed females had long refractory periods compared to the refractory period of high quality fed females mated with high quality fed males. Perhaps if females have a low nutritional condition they may not have the adequate resources to produce eggs and thus have no need to remate quickly. This result suggests that female remating inhibition depends not only on substances received in the male ejaculate but also on female physiological condition.

A different scenario was observed for *A. obliqua*, as female condition had no effect on refractory period, whereas the interaction between male nutritional condition and male size did have a significant effect on the length of female refractory period. Surprisingly, small males induced longer refractory periods than larger males. Smaller males may have to invest heavily in the few copulations that they obtain and gain paternity by inducing long refractory periods. If larger males are more successful at obtaining mates they may need to partition ejaculates between mates and may not be able to induce long refractory periods. Certainly there is some evidence to suggest that *A. obliqua* males that mate repeatedly can transfer comparable quantities of sperm but their ability to inhibit female remating decreases with increasing mating frequency (P erez-Staples and Aluja, 2006; P erez-Staples et al., 2008b). However, male size did not determine the amount of matings observed, thus alternative explanations

may be needed. For example, it could be that courtship intensity by males influences female receptivity.

In conclusion, *A. obliqua* females do not remate as frequently as *A. ludens*, which could be explained in part by their different life histories. Being a shorter lived, egg-limited species, *A. obliqua* females are confronted with the need to lay large amounts of eggs in a very limited amount of time after mating. Previous studies have found that *A. obliqua* males are able to supply females with large quantities of sperm even if males have previously mated (Pérez-Staples and Aluja, 2006), thus females may not need to replenish sperm supplies by remating repeatedly. In contrast, *A. ludens*, a longer lived species, may need to mate repeatedly over its lifetime to replenish sperm reserves.

Furthermore, our results demonstrate that mating decisions and more importantly female remating inhibition depend on both male and female physiological condition and interactions thereof. Thus, more studies are required with more species where both female and male condition are manipulated at the same time to more fully understand the dynamics behind female remating decisions in tephritid flies. Also, female mating rate may be quite different in more natural conditions (e.g., Vera et al., 2002; Aluja et al., 2008a,b) where females have the space to avoid male harassment and can more easily evade males.

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