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Sequential mate choice in the South American fruit fly: the role of male nutrition, female size and host availability on female remating behaviour

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After mating, female sexual receptivity usually decreases and returns, depending on various factors, one of which can be the quality of the first or second male. Here, we tested in the South American fruit fly *Anastrepha fraterculus* if female remating is conditioned by the quality of the second male, when females encounter sequential mates (under no-choice conditions) and when females have the opportunity to choose between well-nourished and malnourished males, both for mating and for remating. Additionally, we tested if other factors such as female size and the opportunity to oviposit influenced females' remating propensity. We found that females remate more often when they encounter a male of good nutritional condition, under both choice and no-choice conditions, and when females had access to a host to oviposit. Other traits of the sequential mate, such as male mating status (virgin or mated), male density (one or three males) and female size, had no effect on female remating behaviour. Plasticity in female post-copulatory mating under both choice and no-choice conditions indicates that *A. fraterculus* females are able to discriminate between males with different nutritional qualities without needing to compare them directly. Females were more choosy when remating, probably as a result of previous sexual experience, yet it remains to be seen which cues females use to distinguish between males. We discuss our results in the context of female pre- and post-copulatory mating decisions.

KEY WORDS: protein diet, mate choice, remating, oviposition behaviour, Tephritidae.

INTRODUCTION

Mate choice and the cues used by females to choose between males have been studied in many species (Gibson & Langen 1996; Jennions & Petrie 1997; Candolin 2003; Wong & Candolin 2005). Choosing among potential mates, especially if there is

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great variability in male quality, can be costly in terms of energy and time for sampling but also the most rewarding tactic in terms of potential direct/indirect benefits to females (Janetos 1980; Real 1990). In species with a lek mating system, females are able to assess several males that could differ in their quality (Bradbury 1981). Most studies have focused on how male factors like quality or quantity of male resources and paternal care, male genetic quality and male attractiveness per se affect female choice (Andersson 1994). Other factors extrinsic to males such as the cost to females of choosiness and female condition may also affect female mate choice (Gray 1999). After mating, there is a decrease in female receptivity, and after the sexual refractory period, receptivity is restored, and females can gauge male attractiveness again. There is considerable debate as to how females choose their subsequent mates (e.g. Gibson & Langen 1996), but there is evidence that suggests females choose their sequential mates relative to the attractiveness of the first male (Bakker & Millinsky 1991; Downhower & Lank 1994). Male characteristics, such as size, age and nutritional and mating status, may influence female remating decisions (Pitnick 1991; Karl & Fischer 2013; but see Schafer & Uhl 2004). For example, in *Drosophila*, the addition of protein in the male diet increases male mating success with non-virgin females, suggesting that male quality is an important factor that can influence females' post-copulatory decisions (Frike et al. 2008).

There is also evidence that male sexual experience influences female choice. Several studies have found that females prefer mating with either sexually experienced or virgin males (Sivinski 1984; Schlaepfer & McNeil 2000; Harris & Moore 2005; Ivy et al. 2005; Pérez-Staples et al. 2010; but see Shelly & Whittier 1993) and that females can use chemical cues such as male pheromones or cuticular hydrocarbons to distinguish between virgin or previously mated males (Teal et al. 2000; Ivy et al. 2005; Weddle et al. 2013). However, there have been relatively fewer studies on how male sexual experience influences female remating decisions. For example, in the tropical butterfly *Bicyclus anynana*, females had a higher remating frequency when exposed to virgin (and younger) males over non-virgin (and older) males (Karl & Fischer 2013).

Female remating choice criteria can also be influenced by the availability of males, as choosiness will increase when there is greater variation in male quality (Real 1990). This may be particularly important in species that form leks, where females evaluate several males simultaneously. Mate choice is density-dependent, and multiple mating will also depend on male encounter rates regardless of the adaptive strategy used by polyandrous females (Kokko & Rankin 2006). Female intrinsic factors, such as reproductive state, age or size, among others, can also influence female remating. For example, virgin females have been found to be less choosy than mated females (Gabor & Halliday 1996; Judge et al. 2010), whereas the likelihood to remate can decrease with female age (Abraham et al. 2016a). Also, as larger females are considered more fecund, large females have been found to have a higher remating probability than smaller females (e.g., Schafer & Uhl 2004). Extrinsic factors, such as the availability of oviposition sites, may also influence female remating decisions in some species but not in others (Sivinski & Heath 1988; Landolt 1994; Carsten & Papaj 2005; Aluja et al. 2009; but see Landeta-Escamilla et al. 2016).

The family Tephritidae comprises several species of fruit flies that usually mate in leks (Prokopy & Hendrichs 1979; Malavasi et al. 1983; Shelly & Kaneshiro 1991). The South American fruit fly *Anastrepha fraterculus* (Wied.) (Diptera Tephritidae) is a phytophagous species that oviposits in a wide variety of hosts. The males of this species attract females by forming leks on the undersides of leaves, females visit the leks in response to

olfactory cues (pheromone release by males), males display an elaborated courtship (calling behaviour), and females assess male quality and exert mate choice (Malavasi et al. 1983; Segura et al. 2007). Female receptivity decreases after the first mating (De Lima et al. 1994; Abraham et al. 2011a), and females mated with protein-deprived males remate sooner. Also, the accessory gland products (a component of the ejaculate) of protein-deprived males are less effective in inhibiting female receptivity (Abraham et al. 2011b, 2012). In *A. fraterculus*, as in *A. obliqua*, females mated with virgin or multiply mated males stored similar numbers of sperm, indicating that males can replenish sperm supplies or alternatively partition the sperm load in sequential matings (Pérez-Staples & Aluja 2006; S. Abraham unpublished data). The female refractory period (RP, time between first and second copulation) is relatively long, depending on female strain. Laboratory females remate sooner than wild females, with an average RP of 12 days vs 15 days respectively, but rematings can occur as early as 48 hr after mating. Female polyandry varies from two to three matings in a month. Female fecundity after a single mating is similar to that after two matings, that is, females do not enhance their fecundity by remating. *Anastrepha fraterculus* is a complex of cryptic species composed of several morphotypes (Cáceres et al. 2009; Hernández-Ortiz et al. 2012; Rull et al. 2013). When crosses between Argentinean and Peruvian morphotypes were analysed, female morphotype determined their remating behaviour, showing that females control, at least in part, their remating propensity (Abraham et al. 2014). Finally, other factors, such as the opportunity to oviposit, may influence remating behaviour, although this factor has not yet been studied in *A. fraterculus*.

Here, we used laboratory *Anastrepha fraterculus* to determine if male quality influences female remating propensity. Particularly, we determined whether male diet (protein-fed vs protein-deprived), male mating status (virgin vs previously mated) or the number of males offered for remating (one vs three males per female) affected female remating propensity when females were first mated with virgin protein-fed males. Second, as in various tephritid flies, nutritional status is an important male trait that determines male mating success with virgin females (Aluja et al. 2001; Shelly & Kennelly 2002; Prabhu et al. 2008; Pérez-Staples et al. 2009), we determined whether virgin and non-virgin females discriminated among potential mates based on male nutritional status. Finally, we determined whether other factors, such as female size and the opportunity to oviposit influenced female remating propensity and the sexual RP.

We hypothesised that the characteristics of the sequential male, male density, the presence of oviposition host and female size would affect female remating decisions. Based on evidence for higher mating success for protein-fed males, higher pheromone production of sexually experienced males and positive density-dependent mating behaviour, we expected females to be more likely to remate if they encountered a protein-fed, non-virgin male, or three males, compared with a protein-deprived male, virgin male or one male. On the other hand, we predicted that females would remate more if they had access to an oviposition host or if they were larger and likely more fecund.

MATERIALS AND METHODS

Insects

Anastrepha fraterculus were obtained as pupae from a laboratory colony established in 2006 at the LIEMEN-PROIMI, Tucumán, Argentina. The colony was started with adults reared from infested guavas collected in the field in the vicinity of Tafi Viejo, Tucumán province, northwestern

Argentina. Rearing followed methods described by Jaldo et al. (2001) and Vera et al. (2007). On the day of emergence, flies were sorted by sex and were transferred to 14 L plastic containers in groups of 150 adults with water and food provided ad libitum. Flies were fed with adult diet consisting of sugar (57.9%) (Ledesma S.A., Jujuy, Argentina), hydrolysed yeast (14.5%) (Yeast Hydrolyzed Enzymatic, MP Biomedicals®), hydrolysed corn (27.3%) (Gluten Meal, ARCOR®, Tucumán, Argentina) and vitamin E (0.3%) (Parafarm®, Buenos Aires, Argentina) (w/w) (Jaldo et al. 2001). Protein-deprived males (see below) were given only sugar. Flies were tested at 10–19 days of age.

Experimental procedure

Female remating under no-choice conditions. The day before the start of the experiments, virgin protein-fed males of 10–19 days-old were transferred individually to 500 mL plastic containers. The container was sealed at the top with a voile cloth, which had a small opening to allow fly removal. Males were provided only with water.

On the day of the experiment, the water was removed, and one female per container was added for a period of 3 hr from the onset of lights in the laboratory (from 7:00 to 10:00 hr). Females were virgin, protein-fed and the same age as the males. Flies were observed continuously to detect pairs in copula. At 10:00 hr, non-mated flies and mated males were discarded. Mated females were kept singly in 500 mL plastic containers with water, food and an oviposition substrate consisting of a slice (2 cm diameter, 1 cm thick) of agar (30 g of agar in 500 mL of water) wrapped in Parafilm® for 3 days. Four days after first mating, half of the females were offered one of two possible treatments as follows:

- Male diet: protein-fed vs sugar-fed males (protein-deprived), both virgin and the same age as the females (14–23 days-old).
- Male mating status: virgin vs previously mated males (males mated 5 days before), both protein-fed and the same age as the females.
- Male density: one vs three males, all of them virgin, protein-fed and the same age as the females.

Four repetitions were carried out for each of the male treatments except for male mating status, for which three repetitions were carried out.

Effect of male diet under choice conditions on female mating and remating likelihood. As described in the results section, females were more likely to remate if presented a protein-fed male than a sugar-fed male. Consequently, we investigated female mating patterns when offered a choice between these male types for both initial and repeat matings. On the day before the trial, protein- and sugar-fed males were marked with a dot of paint on their notothorax (Politec®). Different colours were used for the male types, and the colours were alternated between replicates. On the same day, females were transferred singly to 500 mL plastic containers. The container was sealed at the top with a voile cloth, which had a small opening to allow fly removal. Females were provided only with water.

On the day of the experiment, the water was removed, and two males per container were added (one protein-fed male and one sugar-fed male) for a period of 3 hr from the onset of lights in the laboratory (from 7:00 to 10:00 hr). The number of mating pairs and male colour were recorded. At 10:00 hr, non-mated flies and mated males were discarded. Mated females were kept singly with water, food and an oviposition substrate (see above) for 3 days. Four days after first mating, two males were offered per female (one protein-fed male and one sugar-fed male) for a period of 3 hr. The number of remating females and male identity were recorded. Two repetitions of 100 females each were performed.

Effect of host presence and female size on female remating behaviour. The day before the experiment started, females were transferred singly to 500 mL containers with water and food. Virgin females were offered one sexually mature virgin male for a period of 3 hr from the onset of lights in the

laboratory (from 7:00 to 10:00 hr). Flies were observed continuously to detect pairs in copula. Once separated, the male was removed from the container and discarded, and mated females remained singly with water and food. This procedure of offering one virgin male to each female for 3 hr was repeated every 48 hr. Half of the mated females ($N = 32$) were offered an oviposition substrate (described above) that was replaced every 72 hr. The other half of the females ($N = 32$) did not have access to an oviposition substrate. The trial lasted 20 days with a total of 11 observation dates (i.e. females were given 10 opportunities to remate). At the end of the trial, all females (remating and non-remating) were anaesthetised with ice and preserved at -20°C for morphometric analyses. Female head widths were measured following Rodríguez et al. (2002) and Sciarano et al. (2007) using a dissecting microscope (Arcano ZTX 1065) fitted with an ocular micrometer.

Data analysis

In analysing female remating under no-choice, female remating percentages were compared between repetitions with a one-way ANOVA, using repetition as the class variable. As there was no significant variation between repetitions, data were pooled for each of the three experiments (male diet: $F = 0.17$; $df = 3, 4$; $P = 0.910$; male mating status: $F = 0.03$; $df = 2, 3$; $P = 0.971$; number of males: $F = 0.94$; $df = 3, 4$, $P = 0.501$). For the no-choice experiments, we used χ^2 tests of independence to analyse if remating likelihood depended on male condition (mating status or diet) or density, the null hypothesis being that remating likelihood did not depend on male condition or density. For the choice experiment, we used χ^2 tests of independence to test whether female remating was independent of the first male's diet. The frequency of females remating with either a sugar-fed or protein-fed male was analysed by a χ^2 of goodness of fit, where random mating would be expected if there was no effect of male diet on female remating behaviour. RPs were compared between remated females with and without access to an artificial host by a t -test of independent samples. We used a nominal logistic regression to determine if female likelihood to remate (dependent variable) depended on the availability of the oviposition host (independent variable) and the size of the female (independent variable). Analyses were carried out using STATISTICA (Statsoft 2007).

RESULTS

Female remating under no-choice conditions

Effect of male diet on female remating likelihood. Four repetitions were pooled for the analysis (N females offered protein-fed males for remating: $r_1 = 23$, $r_2 = 34$, $r_3 = 27$, $r_4 = 17$; N females offered protein-deprived males for remating: $r_1 = 24$, $r_2 = 34$, $r_3 = 29$, $r_4 = 20$). Significantly more females remated with protein-fed males than with protein-deprived males ($\chi^2 = 6.16$; $df = 1$; $P = 0.013$) (Fig. 1A).

Effect of male mating status on female remating likelihood. Three repetitions were pooled for the analysis (N females offered virgin males for remating: $r_1 = 29$, $r_2 = 41$, $r_3 = 35$; N females offered mated males for remating: $r_1 = 28$, $r_2 = 43$, $r_3 = 35$). There was no significant difference in the likelihood of females remating with a virgin or mated male ($\chi^2 = 0.50$; $df = 1$; $P = 0.479$) (Fig. 1B).

Effect of male density on female remating likelihood. Four repetitions were pooled for the analysis (N females offered one male for remating: $r_1 = 28$, $r_2 = 18$, $r_3 = 12$, $r_4 = 17$; N females offered three males for remating: $r_1 = 28$, $r_2 = 18$, $r_3 = 11$, $r_4 = 19$). There was

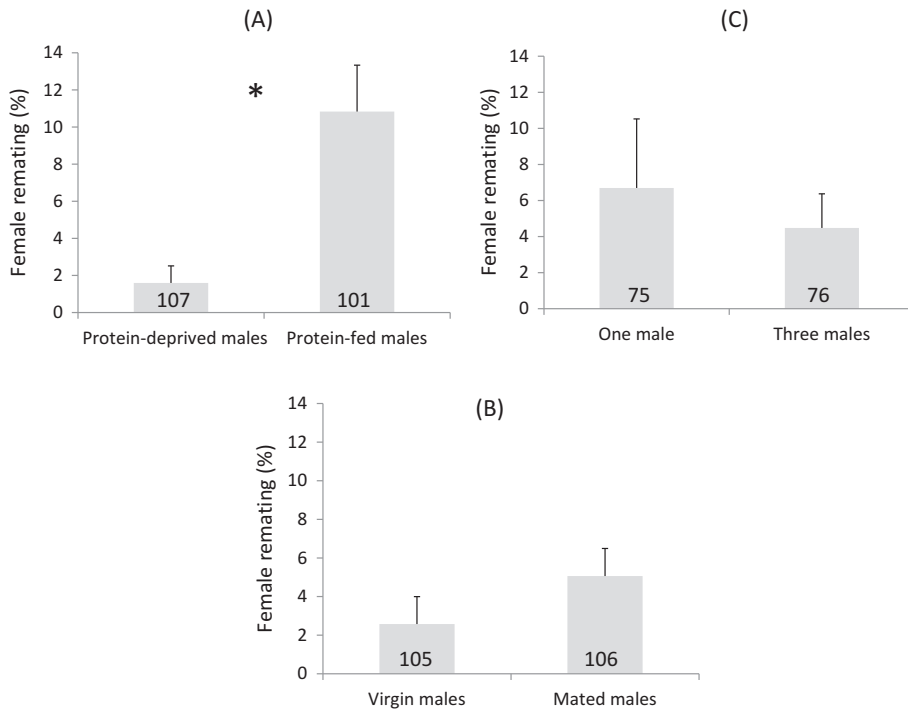


Fig. 1. — Remating percentage of *Anastrepha fraterculus* females under no-choice conditions 72 hr after first mating with virgin sexually mature protein-fed males. Second males were: (A) protein-fed or protein-deprived; (B) virgin or previously mated; or (C) presented singly or in groups of three. The asterisk indicates significant differences. Numbers inside bars indicate sample sizes.

no significant effect of male density on female likelihood to remate ($\chi^2 = 0.0003$; $df = 1$; $P = 0.985$) (Fig. 1C).

Effect of male diet under choice conditions on female mating and remating likelihood

Of the 200 females, 120 females mated. Of these 120 females, 65.8% chose to mate with protein-fed males and 34.2% with sugar-fed males; this difference was statistically significant ($\chi^2 = 12.03$; $df = 1$; $P = 0.002$). However, there was no significant difference in female remating between females first mated with protein or sugar-fed males ($\chi^2 = 3.46$; $df = 1$; $P = 0.062$). Five out of 79 females (6.3% of remating) that first mated with protein-fed males remated with protein-fed males, and none of them chose sugar-fed males. Seven out of 41 (17.1%) females first mated with sugar-fed males remated, and only one of them did so with a sugar-fed male. That is, when given a choice, there was a significant difference in the type of male that females remated with. Of the 12 remated females, 11 remated with protein-fed males and one with a sugar-fed male ($\chi^2 = 8.33$; $df = 1$; $P = 0.015$).

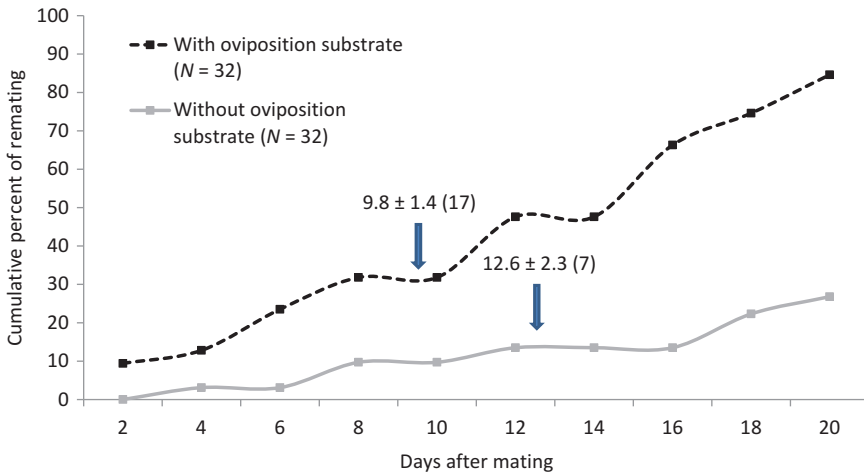


Fig. 2. — Cumulative remating curves in *Anastrepha fraterculus* females first mated with virgin sexually mature protein-fed males, with and without oviposition substrate. Arrows indicate the sexual RP between the first and second copulation [mean \pm SE (*N*)].

Effect of host presence and female size on female remating behaviour

Cumulative curves of remating females, with and without access to oviposition substrate, are shown in Fig. 2. Presence of an oviposition host had a significant effect on the probability of females remating ($\chi^2 = 9.188$, *df* = 1, *P* = 0.0024). Females provided an oviposition host were more likely to remate. However, there was no significant difference in the RP between the groups of females (*t*-test of independent samples, *t* = -1.04, *df* = 22, *P* = 0.310). Female size had no effect on female likelihood to remate ($\chi^2 = 0.037$, *df* = 1, *P* = 0.8472). There was no significant effect of the interaction between presence of an oviposition host and female size ($\chi^2 = 0.391$, *df* = 1, *P* = 0.531).

DISCUSSION

Female remating behaviour in *A. fraterculus* is modulated not only by the characteristics of the mating partner (Abraham et al. 2011b, 2013) but also by the nutritional status of the sequential mate and the presence of a host for oviposition. Contrary to our predictions, traits such as male mating status, male density and female size had no effect on female remating behaviour. In other tephritids, male nutritional status strongly influences male mating success (Shelly & Kennelly 2002; Prabhu et al. 2008; Pérez-Staples et al. 2009; Liedo et al. 2013). Here, male nutritional status also affected female post-copulatory mating decisions. More females remated when they were exposed to protein-fed males (under no-choice conditions) and, when they had the opportunity to choose, did so almost entirely with protein-fed males irrespective of the first male's nutritional status. Plasticity in female post-copulatory mating under both choice and no-choice conditions indicates that females are able to discriminate between males with different nutritional qualities without needing to compare them

directly. As these results are from a laboratory strain, they may not completely mirror patterns existing in wild populations.

Overall, the evidence suggests that ingestion of protein at the adult stage in tephritid fruit flies increases male sexual competitiveness in parameters related to both their pre- and post-copulatory success (Taylor & Yuval 1999; Pérez-Staples et al. 2008b; Liedo et al. 2013; Taylor et al. 2013). Protein-fed males in several tephritids have greater mating success and copulation duration (Blay & Yuval 1997; Kaspi et al. 2000; Shelly & Kennelly 2002; Prabhu et al. 2008; Pérez-Staples et al. 2009), greater probability of sperm production, transfer and storage (Taylor & Yuval 1999; Pérez-Staples et al. 2008b; Costa et al. 2012) and greater ability to inhibit female receptivity to remating (Blay & Yuval 1997; Yuval et al. 2002; Pérez-Staples et al. 2008a, 2008b; Aluja et al. 2009; Gavriel et al. 2009; Costa et al. 2012). A previous study in *A. fraterculus* revealed that wild females mated with protein-fed males had longer copulation durations, lower remating rates, longer RPs, higher probability of storing sperm and higher quantity of sperm stored; for laboratory flies, virgin females mated with protein-fed males had longer copulation durations and longer RPs (Abraham et al. 2011b).

Moreover, diet affects not only the quantity of sperm stored but also the size of the male reproductive organs. In a related tephritid, the Queensland fruit fly, *Bactrocera tryoni*, the sizes of the accessory glands, testes, ejaculatory apodeme and ejaculatory duct were all larger in protein-fed males compared with protein-deprived males (Vijaysegaran et al. 2002; Pérez-Staples et al. 2011; Weldon & Taylor 2011). For *A. fraterculus*, male diet influences their accessory gland proteins (AGPs): AGPs of protein-deprived males injected in females are less efficient in inhibiting female receptivity than females injected with AGPs of protein-fed males (Abraham et al. 2012). Therefore, ejaculate components, sperm quantity and AGPs are all affected by male diet and seem to be related to a decrease in female receptivity after mating in this species (Abraham et al. 2011b, 2012). The higher mating success of protein-fed males was also observed here with previously mated females. Similarly, males fed high-yeast diets had higher mating success with non-virgin females in *D. melanogaster* (Frike et al. 2008). Depending on the level of polyandry in the population, male ability to obtain matings with non-virgin females may be particularly important, as, in the wild, most encounters will probably be with previously mated females (Lewis et al. 2013).

The cues used by females to distinguish between males of different nutritional qualities are not known. One or several morphological, acoustic, olfactory, tactile or behavioural cues (Candolin 2003) could be used to signal mate quality. In particular, male courtship displays and pheromone emission have been shown to affect male mating success (Droney 1996; Briceño & Eberhard 2002; Kotiaho 2002; South et al. 2011). Since male *A. fraterculus* have an elaborate pre-copulatory courtship and use pheromones to attract females to lekking sites, non-virgin females would have ample opportunity to gauge male nutritional condition in terms of both courtship and/or pheromones. Cuticular hydrocarbons (CHCs) could also be used by females to recognise the quality of potential partners and aid in mate choice (Blomquist & Bagnères 2010). In fact, in this morphotype of *A. fraterculus*, the CHC profile changes with gender and age (Vanicková et al. 2012). Protein-fed males probably have a different quantity/quality of CHCs. In male decorated crickets, diet affects CHCs expression and mating success (Rapkin et al. 2017), likewise in *D. melanogaster*, CHCs profiles change strongly with diet and age; females fed a high yeast diet had almost a twofold increase in the amount of CHCs with age, irrespective of the amount of dietary sugar, although this did not translate into differences in female attractiveness (Fedina et al. 2012).

Neither male sexual experience nor male density had an effect on female remating behaviour. We do not know if these traits are irrelevant for mate choice or if females are not able to distinguish between these traits. Contrary to our results, in the butterfly *B. anynana*, females had a higher remating frequency when exposed to virgin over non-virgin males (Karl & Fischer 2013). Further studies could test if there are any differences in chemical or behavioural cues between virgin and non-virgin males. With respect to male density, in *A. fraterculus*, as in *A. serpentina*, females did not remate more often when they faced a higher male density, which could indicate that female remating decisions are, at least in part, under female control (Landeta-Escamilla et al. 2016). In fact, in a previous study with this same species, remating decisions varied with female morphotype but not with male morphotype (Abraham et al. 2014), suggesting that the likelihood of remating depended on the female and not the male. Likewise, female but not male age determined remating in *A. ludens* (Abraham et al. 2016a). Thus, the evidence points to a strong female role in modulating remating decisions.

Contrary to what was observed in other tephritids, such as *A. ludens*, *A. obliqua* and *A. serpentina* (Aluja et al. 2009; Landeta-Escamilla et al. 2016), the presence of an oviposition substrate strongly increased the likelihood of remating in *A. fraterculus*, similar to *A. suspensa*, *Toxotrypana curvicauda*, *Rhagoletis juglandis* (in the Tephritidae family) and the seed beetle *Callosobruchus maculatus* (Sivinski & Heath 1988; Fox & Hickman 1994; Landolt 1994; Carsten & Papaj 2005). In *R. juglandis*, for example, egg load (defined as the number of mature oocytes in a female's ovary) is strongly influenced by the presence of a host (Alonso-Pimentel et al. 1998), and mating probability depends on egg load (Carsten & Papaj 2005). In *T. curvicauda*, females remate more often in the presence of papaya fruit, but remating occasionally occurs before oviposition, suggesting that remating does not follow sperm depletion (Landolt 1994).

Here, results do suggest that females maintained with an oviposition substrate could remate as a result of sperm or ejaculate depletion. *Anastrepha fraterculus* females that showed willingness to remate (that is, exhibited a typical behaviour of accepting to mate by extending and lifting the ovipositor after being mounted by the male) but were prevented from doing so, had decreased egg hatch compared with females allowed to remate (Abraham et al. 2011a). In this same species, females that received an ejaculate were less likely to remate than those prevented from receiving an ejaculate (Abraham et al. 2016b). Both these results suggest that *A. fraterculus* remate to replenish sperm or ejaculate supplies. While we do not know how other components of the ejaculate, such as AGPs, can be depleted with consecutive matings, evidence in the Oriental fruit fly *Bactrocera dorsalis* suggests that males can quickly replenish accessory glands even during mating, while in *B. tryoni*, the recovery of accessory gland size is evident a few hours after mating (Radhakrishnan & Taylor 2008; Wei et al. 2015). In *A. obliqua* multiply mated males are less likely to inhibit females from remating, suggesting that ejaculate but not sperm depletion could affect female remating (Pérez-Staples & Aluja 2006; Pérez-Staples et al. 2008a).

Nevertheless, female sperm limitation in *A. fraterculus* per se cannot fully explain female remating behaviour. In previous studies with this same species, females prevented from remating had a lower egg hatch but not lower sperm reserves (Abraham et al. 2011a, 2016b). However, egg hatch does not depend solely on sperm numbers. Other factors such as sperm viability or AGPs can affect fertilisation and egg development. Thus, these results suggest that sperm quantity does not determine whether a female will remate or not. Other factors such as AGPs can determine females' likelihood

of remating (Jang 2002; Radhakrishnan & Taylor 2007; Radhakrishnan et al. 2009; Abraham et al. 2012).

In summary, female remating in *A. fraterculus* is a complex behaviour influenced by several factors, including (1) the nutritional quality of the first male to mate with the female (Abraham et al. 2011b); (2) the nutritional status of the sequential male mate (3); the opportunity to oviposit; and (4) female intrinsic characteristics, such as female fecundity and morphotype (Abraham et al. 2011a, 2014). In a broad ethological context, after mating and once receptivity is renewed, females will again visit lek sites where they will once more choose between males. Results suggest that male nutritional status has a marked influence on female mate choice, perhaps mediated through increased male courtship rates or pheromone emissions or altered chemical composition of the pheromone signal or CHC profiles. The study of the interaction among different factors (male and female size, nutritional condition, age and strain) could shed light over which aspects carry more weight in determining female remating.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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