

Potential for pre-release diet supplements to increase the sexual performance and longevity of male Queensland fruit flies

Diana Perez-Staples, Aaron M. T. Harmer, Samuel R. Collins and Phillip W. Taylor

Centre for the Integrative Study of Animal Behaviour, Macquarie University, Sydney, New South Wales 2109, Australia

- Abstract**
- 1 Recent studies have shown that continuous access to a protein source (yeast hydrolysate) can greatly enhance the sexual performance of male Queensland fruit flies (*Bactrocera tryoni*; ‘Q-flies’). However, in Sterile Insect Technique programmes used to eradicate or suppress wild populations, mass-reared Q-flies are typically fed only sucrose and water for up to 2 days before release.
 - 2 We investigated whether adding a protein source to the diet of male Q-flies for a 24- or 48-h window after emergence and then removing it is sufficient to enhance mating probability, latency to mate, copula duration, probability of sperm storage, number of sperm stored, female remating tendency and longevity of male Q-flies.
 - 3 Protein-fed males were more likely to mate than males fed only sucrose, especially when young. Protein-fed males also had shorter mating latencies and longer copulations than protein-deprived males.
 - 4 Females mated by protein-fed males were more likely to store sperm, stored more sperm and were less likely to remate than were females mated by protein-deprived males. Females were also less likely to remate if their first mate had been large.
 - 5 Overall, providing male Q-flies access to a protein source for a 24- or 48-h window early on in their adult life was sufficient to greatly enhance all assessed measures of performance. Although 24-h access was sufficient for a notable enhancement, further benefits were evident in males provided 48-h access.
 - 6 The results are discussed in terms of the practical implications for Sterile Insect Technique programs used to eradicate or suppress wild Q-fly populations.

Keywords *Bactrocera tryoni*, diet, longevity, mating, remating, sperm, Tephritidae.

Introduction

Tephritid fruit flies are among the world’s most economically devastating insect pests, with dozens of species infesting a vast array of commercial and domestic crops (Drew, 1989; White & Elson-Harris, 1992; Aluja, 1994). In many regions, the Sterile Insect Technique (SIT) is a central component of the overall management strategy (Gilmore, 1989; Schwartz *et al.*, 1989; Kakinohana, 1994). In SIT, millions of flies are mass-reared, reproductively sterilized and then released into the field. The released males mate with wild females, transferring nonviable sperm and accessory gland fluids that inhibit further sexual

activity (Jang, 1995; Jang *et al.*, 1999; Radhakrishnan & Taylor, 2007, 2008). Females mated by sterile males have greatly reduced reproduction (if any), reducing both crop damage and the number of wild flies in the next generation. For SIT to be effective, it is essential that the released male flies rapidly attain sexual maturity, compete vigorously against wild males for copulations with wild females, and survive long enough to apply continuous pressure on wild populations.

Adult nutrition plays a decisive role in the maturation, sexual performance and longevity of many tephritid flies and is an especially promising prospect for the enhancement of tephritid flies released in SIT (Yuval *et al.*, 2002, 2007). In particular, numerous studies have shown that continuous inclusion of protein in the adult diet can provide a considerable boost to sexual development or performance. For example, *Ceratitis fasciventris* and *Ceratitis capitata* fed a protein-rich

Correspondence: Dr Diana Perez-Staples, LABIOTECA, Universidad Veracruzana, AP250, Xalapa, Veracruz. 91090 Mexico. Tel. & fax: +52 228 8422773; e-mail: diperez@uv.mx

diet display higher rates of calling, mating and oviposition than adults fed a protein-poor diet (Kaspi *et al.*, 2000; Manrakhan & Lux, 2006). Protein supplements have been shown to enhance overall male sexual performance in mass-reared *C. capitata* (Blay & Yuval, 1997; Papadopoulos *et al.*, 1998; Taylor & Yuval, 1999; but see also Shelly & Kennelly, 2002; Shelly *et al.*, 2002; Shelly & McInnis, 2003). Likewise, in *Anastrepha striata*, *Anastrepha serpentina* and *Anastrepha obliqua*, (but not *Anastrepha ludens*), males fed protein-rich diets exhibit higher calling frequency and mating success than males fed protein-poor diets (Aluja *et al.*, 2001). *Bactrocera dorsalis* males deprived of protein completely, or when immature, have poor mating performance (Shelly *et al.*, 2005). For some tephritids, ingestion of protein can also affect longevity. Access to protein has been reported to increase longevity in *A. serpentina* (Jácome *et al.*, 1999), whereas, in *C. capitata*, the results have been highly variable (Kaspi & Yuval, 2000; Shelly & Kennelly, 2002; Maor *et al.*, 2004). Recent laboratory studies on fertile and sterile Queensland fruit flies (Tephritidae: *Bactrocera tryoni*; 'Q-fly') have found that continuous post-teneral access to a protein source, yeast hydrolysate, can increase mating propensity and longevity in both males and females (Perez-Staples *et al.*, 2007b).

Under recent SIT protocols, mass-reared Q-flies are typically released into the field 1–2 days after emerging from pupae as adults. During this pre-release period, they are usually provided sucrose and water but not a source of protein (Anonymous, 1996). In the field, tephritid flies feed on a variety of substances, including honeydew, leaf exudate, bird faeces and bacteria (Drew *et al.*, 1983; Drew, 1987; Murphy *et al.*, 1994). However, the nutritional quality of foods that tephritids encounter in Australia's desert and oasis landscape (Dominiak *et al.*, 2006) may vary considerably, and protein is presumed to be scarce. If released sterile flies fail to obtain the protein that they need to mature, perform sexually and survive, and hence fail to compete adequately with wild males for matings with fertile wild females, then SIT will be severely compromised. One simple, low-cost, remedy to this problem may be to reduce the need for released sterile flies to find protein in nature by supplementing their diet prior to release.

In other tephritids, studies providing flies life-long continuous access to dietary protein (Kaspi & Yuval, 2000, Perez-Staples *et al.*, 2007b) have been used as the basis for recommendations to SIT programmes in which flies are held for only a couple of days before release into the field. However, access to protein for just a couple of days in SIT programmes may be insufficient to provide much benefit. Also, providing protein and then withdrawing it, as occurs upon release in SIT, might be worse than never providing any at all. For example, *C. capitata* that are maintained on high protein diets for 5 days and then have the protein source withdrawn suffer accelerated mortality (apparently due to metabolic adjustment to the diet) (Kaspi & Yuval, 2000). Before recommending that SIT protocols be changed to incorporate protein in pre-release diets, it is very important to first test: (i) that sufficient benefit accrues from such short windows of protein availability and (ii) that brief access and

then withdrawal of protein does not harm the flies. The present study investigates whether the provision of a protein source (yeast hydrolysate) during a 1–2-day pre-release period and then removing it can yield persistent benefits in mating probability, copula latency, copula duration, sperm numbers, remating inhibition and longevity of male Q-flies.

Materials and methods

General methods

Q-flies were obtained as pupae from the Fruit Fly Production Facility located at Elizabeth Macarthur Agricultural Institute (EMAI) in New South Wales, Australia. Adult flies emerged in the laboratory at Macquarie University (Sydney). On the day that adults emerged, three cages of flies were provided with a dish of dry granular sucrose as food as well as water in moistened cotton wool. One cage of flies was supplied with a supplementary dish of yeast hydrolysate (MP Biomedicals, Aurora, Ohio) as a protein source for 24 h (24-h treatment), another cage of flies was supplied with yeast hydrolysate for 48 h (48-h treatment), and the third cage received no protein supplement. The day after emergence, 150 males from each of the cages were separated by aspirator and placed in 5-L cages with their corresponding diets. Males were not transferred on the day of emergence to prevent damage to the delicate wings of newly-emerged flies. To prevent supplemented males from feeding on residual protein or their own faeces that could have contained protein, all males from each of the three treatments were transferred using an aspirator to clean cages 4 days after the protein dishes were removed.

All cages were maintained, and experiments carried out, at 24–26 °C and 65–75% RH under an LD 14 : 10 h photoperiod. The lights were on full intensity for 12 h and flies also experienced simulated dawn and dusk as the lights stepped on and off in four stages over the course of 1 h.

Mating experiments

The sexual performance of male flies from each of the three treatments was compared in mating experiments carried out when they were 8, 12, 16, 20, 24 and 28 days of age. Q-flies begin mating during an approximately 30-min window around dusk (Barton-Browne, 1957; Tychsens, 1977). On each day of mating trials, 3–4 h before the onset of simulated dusk in the laboratory, we transferred 15 males from each treatment into individual 1-L clear plastic cages with a mesh screen (50 × 100 mm) for ventilation. One 13–17-day-old virgin female that had been fed both yeast hydrolysate and granular sucrose was placed in each cage. Virgin females of this age and diet show high levels of sexual receptivity (Perez-Staples *et al.*, 2007b). Pairs of flies were observed continuously from 15 min before the onset of simulated dusk until the last pair had separated. We noted the time at which copulations began relative to when the lights started to dim out and also noted at what time they ended for later calculation

of copula latency and copula duration. The experiment was replicated three times using batches of flies obtained from EMAI at least 2 weeks apart.

The day after matings, we removed all pairs that had failed to copulate and froze them in individually labelled vials (-20°C). Males from pairs that had mated were also frozen and, to test female remating tendency, were replaced by 13–17-day-old virgin males that had been provided continuous access to both sucrose and yeast hydrolysate. Similar to females, virgin males of this age and diet show a high level of sexual receptivity (Perez-Staples *et al.*, 2007b). The once-mated females and their second prospective mate were observed for mating from 15 min before the onset of simulated dusk. As soon as intromission was observed, pairs were rapidly chilled with aerosol freezing spray (containing 1,1 difluoroethane; Dick Smith Electronics/Electrolube, Australia) and the male's aedeagus was severed with a pair of fine scissors to prevent further sexual activity and to ensure that no sperm were transferred (Harmer *et al.*, 2006). The males used in the remating trials were then frozen and the females were dissected to determine the presence and number of sperm from their first mating.

Sperm numbers stored by females were assessed using methods based on those of Taylor *et al.* (2000, 2001). Females were first subdued using CO_2 . Their entire reproductive tract was then removed into a drop of deionized water by tearing the membrane at the base of the ovipositor with one pair of forceps at the same time as pulling gently on the ovipositor with a second pair. The two spermathecae and ventral receptacle were excised and placed together in a 8.0- μL drop of deionized water on a microscope slide under a stereomicroscope (SZX12, Olympus Corp., Japan). Each storage organ was then broken apart using entomological pins and the sperm were dispersed by stirring the drop of water vigorously with an entomological pin for approximately 30 s. A 18 \times 18 mm cover slip was then carefully lowered over the drop and released so that the drop of liquid dispersed to the edges. The corners of the cover slip were secured with a small drop of clear nail polish and the slide was set aside to dry. Rather than counting all of the sperm in each sample (Harmer *et al.*, 2006; Perez-Staples *et al.*, 2007a), we adopted the more time-efficient approach of subsampling the slides (Taylor *et al.*, 2000, 2001). Sperm was sub-sampled by counting 16 fields (as four rows of four fields) across each cover slip under a phase contrast microscope (Leica DME, Germany) at \times 100 magnification. To derive a conversion factor to convert our subsample counts to estimates of total numbers of sperm under the cover slip, we counted all sperm under 15 cover slips and then ran a linear regression between the sub-sample counts and full counts. We then fitted the intercept through 0 and this provided the conversion factor of total sperm = $6.676 \times$ subsample count ($R^2 = 0.969$, $F_{1,14} = 443.89$, $P < 0.001$).

Mated females were frozen individually in labelled vials immediately after dissections. Once the three replicates were completed, the right wing of each frozen fly was removed and mounted onto double-sided adhesive tape on a microscope slide. After affixing a label next to each wing, a second slide was pressed onto the tape to protect the wings from dust. Each wing was then photographed using a Firewire

digital camera (3 megapixels; Jenoptik ProgRes C10, Germany) through the phototube of a stereomicroscope (SZX12, Olympus) at \times 20 magnification. As a size-related trait (Norry *et al.*, 1999), wing length was measured (in mm) from the intersection of the anal and median band to the margin of the costal band and the $R_4 + 5$ vein (Perez-Staples *et al.*, 2007a) using Image Tools software, version 2.25 (University of Texas, San Antonio, Texas).

Longevity

On the day that adults emerged, three cages were provided with the dietary treatments described above. The day after emergence, ten males from each of the dietary treatments were aspirated into 1-L clear plastic cages with a mesh screen for ventilation. This was repeated three times for each diet. As described above, flies were provided with either 24 h or 48 h of access to yeast hydrolysate and sucrose or only sucrose. Eight days after emergence, all males were transferred to identical clean cages with sucrose and water. The longevity of males was noted daily during a 28-day period. Dead flies were removed daily. Two replicates were carried out.

Statistical analysis

Variables with binary outcomes, including probability of mating, probability of sperm storage (mating experiment) and probability of surviving to 28 days (longevity experiment), were assessed using logistic regression, with significance tested using likelihood ratio tests (G).

Each of the continuous measures assessed required transformation to meet assumptions of parametric analyses. Copula latency, the time from the onset of simulated dusk until intromission, was transformed to natural logarithms. To include a single negative value of -2 min (i.e. a male that started mating before the start of simulated dusk), we added 3 min to all values before applying the transformation. Copula duration was transformed to natural logarithms and the total number of sperm found in the female sperm storage organs was transformed to square root (excluding cases in which no sperm were found). Continuous measures were analyzed by analysis of covariance (ANCOVA).

In all logistic and ANCOVA models, first-order interactions were initially considered. Nonsignificant interactions were excluded from the analysis, and the models were re-run. Effects of nominal predictors in ANCOVA on continuous outcomes are presented as back-transformed least square means (LSM), correcting for other factors in the model.

Results

Mating propensity

Male age had a significant influence on mating propensity for each of the diet treatments, and followed a polynomial trend that increased to a peak at 15–20 days and subsequently declined (Fig. 1, Table 1). There were also strong effects of

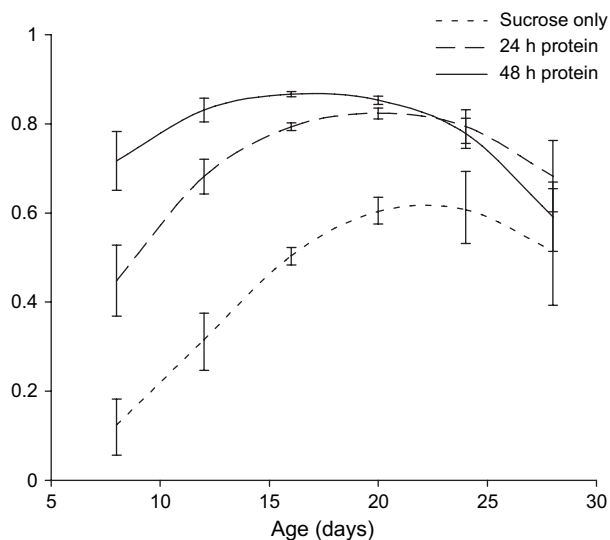


Figure 1 Mating probability of males on the three dietary treatments according to their age. Males were fed: (i) sucrose only; (ii) sucrose and 24 h of yeast hydrolysate (protein); or (iii) sucrose and 48 h of yeast hydrolysate. Bars represent 95% confidence intervals.

diet treatment on mating propensity but these varied with age. Specifically, the advantages of protein-feeding were most apparent when the males were young and, as males aged, the differences among the dietary treatments diminished (Fig. 1). Female size and male size had no significant effect on mating propensity.

Copula latency

The latency from the onset of dusk until copulation varied among the three tested diets ($F_{2,423} = 7.382$, $P = 0.001$). Tukey's HSD tests ($\alpha = 0.05$) revealed that males provided yeast hydrolysate for 48 h had copula latencies (back-transformed LSM = 16.66 min) that were significantly shorter than those of protein-deprived males (back-transformed LSM = 21.83 min). The intermediate copula latencies of males provided yeast hydrolysate for 24 h (back-transformed LSM = 18.86 min) were not significantly different from either of the other treatments. The initial model including all data was

Table 1 Predictors of mating probability for males from 8–28 days of age, fed 24 or 48 h of protein or none at all

| Source | d.f. | G | P |
|---------------------|------|--------|---------|
| Replicate | 2 | 11.162 | 0.004 |
| Male age | 1 | 16.981 | < 0.001 |
| Diet | 2 | 73.057 | < 0.001 |
| Male size | 1 | 0.732 | 0.392 |
| Female size | 1 | 0.108 | 0.743 |
| Male age × Diet | 2 | 19.331 | < 0.001 |
| Male age × Male age | 1 | 33.278 | < 0.001 |

Data were analysed using logistic regression with likelihood ratio tests.

strongly influenced by a single outlier (a protein-deprived male that mated before the onset of simulated dusk). To confirm whether the analysis remained robust, we re-ran the analysis after omitting this data point. Exclusion of this single outlier yielded significant differences not only between males provided yeast hydrolysate for 48 h (back-transformed LSM = 13.10 min) and protein-deprived males (back-transformed LSM = 19.11 min), but also between males provided yeast hydrolysate for 24 h (back-transformed LSM = 15.28 min) and protein-deprived males. There were no significant differences between males provided yeast hydrolysate for 24 or 48 h. Although there were significant differences between replicates ($F_{2,423} = 6.153$, $P = 0.002$), male age ($F_{1,423} = 0.764$, $P = 0.383$), male size ($F_{1,423} = 0.035$, $P = 0.851$) and female size ($F_{1,423} = 0.542$, $P = 0.462$) were not significant predictors of copula latency.

Copula duration

Copula duration ($N = 435$, range 2–475 min) varied significantly with male diet ($F_{2,420} = 16.790$, $P < 0.001$). Tukey's HSD tests revealed significant differences in all comparisons among the three diet treatments. Males provided yeast hydrolysate for 48 h had longer copulations than all other males (back-transformed LSM = 82.76 min), males provided yeast hydrolysate for 24 h had intermediate durations (back-transformed LSM = 62.64 min), whereas protein-deprived males had the shortest copula durations (back-transformed LSM = 39.69 min). Again, although there were significant differences between replicates ($F_{2,420} = 10.715$, $P < 0.001$), male age ($F_{1,420} = 0.501$, $P = 0.480$), male size ($F_{1,420} = 0.831$, $P = 0.363$) and female size ($F_{1,420} = 0.005$, $P = 0.947$) were not significant predictors of copula duration.

Sperm numbers

The likelihood that some sperm was found in mated females varied across the three replicates ($G_2 = 27.300$, $P < 0.001$) and diet treatments ($G_2 = 17.336$, $P < 0.001$) but did not vary with male age ($G_1 = 0.152$, $P = 0.696$), male size ($G_1 = 0.098$, $P = 0.755$) or female size ($G_1 = 0.297$, $P = 0.586$). Sperm was more likely to be found in females mated by males provided yeast hydrolysate for 48 h (88%) than in females mated by males provided yeast hydrolysate for 24 h (79%) ($G_1 = 9.521$, $P = 0.002$) or females mated by males fed only sucrose (70%) ($G_1 = 15.590$, $P < 0.001$). However, the difference in sperm storage probability between females mated by males provided yeast hydrolysate for 24 h and females mated to males fed sucrose alone was not statistically significant ($G_1 = 2.593$, $P = 0.107$).

For those females that did store sperm ($N = 417$, range 8–3585), the total number of sperm stored varied significantly with male age and diet (Table 2). Similar to mating probability, the total number of sperm stored followed a polynomial form, increasing with male age to a peak at 15–20 days, and then declining (Fig. 2). Tukey's HSD tests revealed significant differences between protein-deprived males (back-transformed LSM = 477) and males fed protein for either 24 h

Table 2 Predictors of sperm numbers (transformed to square roots) from analysis of covariance, for males from 8–28 days of age, fed 24 or 48 h of protein or none at all

| Source | d.f. | F | P |
|---------------------|-------|--------|---------|
| Replicate | 2,326 | 18.497 | < 0.001 |
| Male age | 1,326 | 16.332 | < 0.001 |
| Diet | 2,326 | 4.674 | 0.010 |
| Male size | 1,326 | 0.258 | 0.612 |
| Female size | 1,326 | 0.784 | 0.377 |
| Male age × Male age | 1,326 | 42.723 | < 0.001 |

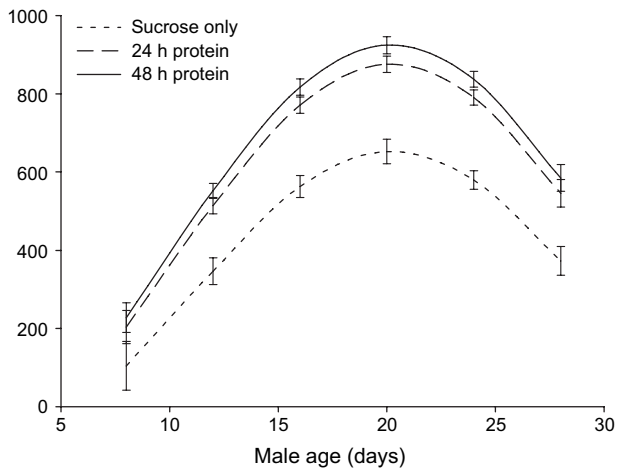
(back-transformed LSM = 670) or 48 h (back-transformed LSM = 713) but no significant difference between males on the two protein supplementation treatments.

Female remating tendency

The probability that a female remated did not vary across replicates, nor with male age, female size or the size of the second prospective mate (Table 3). However, females that had been mated by males fed only sucrose were more likely to remate than were females mated by males provided yeast hydrolysate for 24 h ($G_1 = 5.033$, $P = 0.025$) or 48 h ($G_1 = 9.314$, $P = 0.002$) (Fig. 3, Table 3). There is weak evidence suggesting that females mated by males provided yeast hydrolysate for only 24 h were more likely to remate than females mated by males provided yeast hydrolysate for 48 h ($G_1 = 3.052$, $P = 0.081$). In addition to these effects of male diet, females were more likely to remate if their first mate had been small (Fig. 3, Table 3).

Longevity

The likelihood that males survived up to 28 days varied significantly with male diet ($G_2 = 22.08$, $P < 0.001$, $N = 165$)

**Figure 2** Total number of sperm from predicted formula (back-transformed) stored by females according to her mate's age and diet. Males were fed: (i) sucrose only; (ii) sucrose and 24 h of yeast hydrolysate (protein); or (iii) sucrose and 48 h of yeast hydrolysate. Bars represent 95% confidence intervals.**Table 3** Predictors of female remating probability when paired with males from 8–28 days of age fed 24 or 48 h of protein or none at all

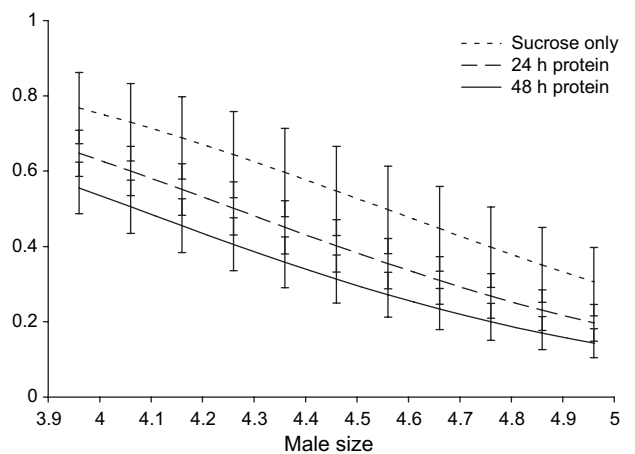
| Source | d.f. | G | P |
|--------------------|------|--------|-------|
| Replicate | 2 | 2.592 | 0.274 |
| Male age | 1 | 0.042 | 0.837 |
| Diet | 2 | 11.890 | 0.003 |
| Male size | 1 | 7.483 | 0.006 |
| Female size | 1 | 0.457 | 0.499 |
| Remating male size | 1 | 0.400 | 0.527 |

Data were analysed using logistic regression with likelihood ratio tests.

and did not vary between the replicates ($G_1 = 0.660$, $P = 0.416$). Pairwise likelihood ratio tests revealed that males fed sucrose alone were less likely to survive to 28 days of age (61.4%) than were males provided supplementary yeast hydrolysate for 48 h (93.2%) ($G_1 = 18.16$, $P < 0.001$) or 24 h (89.8%) ($G_1 = 11.86$, $P < 0.001$). There was no significant difference in the likelihood to survive to 28 days between males provided either 24 or 48 h or yeast hydrolysate ($G_1 = 0.24$, $P = 0.621$).

Discussion

We found that male Q-flies provided protein supplements for just 24 or 48 h after emerging as adults had greatly enhanced mating propensity. Although 24 h of access to yeast hydrolysate was sufficient to return a large boost in performance, there were additional advantages to providing this protein source for a full 48 h. Similarly, Shelly *et al.* (2005) found that males of the oriental fruit fly (*B. dorsalis*) had higher mating propensity at 21–25 days of age when they had been provided access to yeast hydrolysate for the first 12

**Figure 3** Remating probability of females decreases with male size for all male dietary treatments. Males were fed: (i) sucrose only; (ii) sucrose and 24 h of yeast hydrolysate (protein); or (iii) sucrose and 48 h of yeast hydrolysate. Bars represent 95% confidence intervals.

days after emerging. Given that the shorter 24–48-h periods considered in the present study are much more readily incorporated into SIT protocols, it would be of interest to test these shorter periods of access to yeast hydrolysate for oriental fruit flies and other tephritids.

In addition to the overall effects of diet, we found marked effects of age on the mating propensity of males in each of the diet treatments. Specifically, mating propensity increased with age for male Q-flies in all treatments to a peak at 15–20 days. Subsequently, there was a distinct decrease in mating propensity in all diet treatments. Similar distinct ages of peak mating propensity have been reported for male medflies, *C. capitata*, which exhibit greatest mating propensity at two peak ages of 4 and 11 days with lower values between these times and at greater ages (Liedo *et al.*, 2002).

Access to yeast hydrolysate for a brief period after emerging also reduced mating latency; males receiving yeast hydrolysate tended to mate sooner after the onset of simulated dusk than males with no access to a protein source. With respect to overall mating propensity, although 24 h of access to yeast hydrolysate was sufficient to return a large boost in performance, there were additional advantages to providing this protein source for a full 48 h. Both mating propensity and copula latency may generally reflect male ability to invest in mating effort. To date, there have been no studies of how diet influences sexual advertisement (i.e. pheromonal and acoustic calling) or courtship behaviour in Q-flies, although such studies do now appear to be warranted.

Male Q-flies with 24 or 48 h access to yeast hydrolysate had longer copulations than protein-deprived males in the present study, and these results are consistent with the effects of dietary protein on copula duration in other recent studies. For example, Perez-Staples *et al.* (2007b) found that both fertile and irradiated male Q-flies with continuous access to yeast hydrolysate and sucrose in separate dishes had longer copulations than males with no access to a protein source. Furthermore, Prabhu *et al.* (2008) found that male Q-flies receiving continuous access to diets containing various proportions of yeast hydrolysate and sucrose had longer copulations than males receiving sucrose alone. Increased copula duration appears to be a consistent response to dietary protein in Q-flies.

In addition to achieving copulations, for effective SIT it is also important that males succeed in transferring an abundant ejaculate. By transferring an abundance of sperm and other ejaculate components, males are better equipped for the post-copulatory challenges of sperm competition against the ejaculates of other males. In wild females mated by multiple males (e.g. one released sterile and one wild fertile male), more eggs are likely to be fertilized by the sperm of males that have a greater number of sperm present. SIT will hence benefit if released sterile males are able to transfer more sperm. In the present study, female Q-flies that were mated by males provided access to dietary protein for 24 or 48 h were more likely to store sperm, and stored more sperm, than females mated by males deprived of access to dietary protein. Hence, the addition of protein to the diet will likely enhance the ability of released flies to compete in post-copulatory sexual selection.

Although it is obviously important that released male Q-flies succeed in attracting, courting and copulating wild females, it is also important that these copulations be effective at inhibiting sexual receptivity of the mated females. If females mated by released sterile males have a high tendency to remate then they are available to be mated by fertile wild males and might then produce viable offspring. The importance of remating inhibition is exacerbated by the tendency of female Q-flies to store far fewer sperm when they mate with sterile rather than fertile males (Harmer *et al.*, 2006). If females do mate twice (i.e. once with a sterile male and once with a fertile male), then fertile sperm will typically massively outnumber the few sterile sperm present and these twice-mated females will tend to have high fertility. It is hence extremely important that released sterile males be effective at inhibiting further sexual activity of their mates.

Harmer *et al.* (2006) demonstrated that, despite the deficiency in sperm numbers stored, sterile and fertile males are similar in their ability to inhibit further sexual activity of their mates. However, all of the flies tested in the study by Harmer *et al.* (2006) had continuous access to protein. The results of the present study indicate that the flies released in SIT may have limited ability to induce sexual inhibition in females if they have difficulty finding protein in the field. Specifically, female Q-flies mated by males that had been provided sucrose only were more likely to remate the next day than were females mated by males that had been provided access to yeast hydrolysate for 24 or 48 h after emerging as adults.

As in *C. capitata* (Jang, 1995; Jang *et al.*, 1999), mating-induced sexual refraction in female Q-flies is mediated by accessory gland fluids transferred to the female as part of the ejaculate (Radhakrishnan & Taylor, 2007, 2008). The findings of the present study hence suggest that access to dietary protein may increase the titre or quality of male accessory gland fluids in the male's ejaculate.

We found that a brief period of post-teneral access to yeast hydrolysate improved the chances of male Q-flies surviving until 28 days of age compared with males fed sucrose only. Our studies were carried out under laboratory conditions in which ample sucrose was available as an alternative source of nutrition. It is likely that these effects would be further accentuated under the more challenging nutritional and environmental conditions encountered by flies in the field. Our results support strategies taken in pupal releases in which a combined protein, sugar and water feeding station is left in the field to provide nutrition to newly-emerged flies (Dominiak *et al.*, 2000).

There are some minor inconsistencies among different studies with respect to the effects of protein deprivation on longevity of fertile male Q-flies. Perez-Staples *et al.* (2007b) found no effects of protein deprivation on longevity of fertile male Q-flies, although longevity of irradiated males was appreciably increased by the presence of dietary protein. Similarly, Prabhu *et al.* (2008) found no difference in the longevity of fertile male Q-flies fed sucrose only or supplemented with up to 50% yeast hydrolysate mixed together with the sucrose. This may be related to variation

between the studies in the holding environment (e.g. the number of flies that are held together) and the vulnerability of supplied flies to protein deprivation. The positive effects of access to dietary protein to longevity in the present study do not appear to be universal.

There is also considerable variation among studies on how nutrition affects longevity in other tephritids. For example, some studies have reported a survival cost for *C. capitata* males provided protein and then starved (Kaspi & Yuval, 2000; but see also Shelly & Kennelly, 2002), whereas others have found an increased life span when protein is introduced into the diet (Butov *et al.*, 2003). In *Anastrepha suspensa* and *B. dorsalis*, Teal *et al.* (2004) and Shelly *et al.* (2005), respectively, report no significant differences in the survival rate for males fed sugar only or sugar and protein and then only sugar. In the present study, although males were not starved, removal of protein with continuing access to sucrose did not have an adverse effect on survival up to 28 days. Rather, males provided protein for 24 or 48 h were more likely to survive to 28 days than were protein-deprived males. Similarly, for *C. capitata* females, Carey *et al.* (2002) found that the longest-lived flies were those subjected to 1 day of protein followed by 30 days of sugar.

In summary, providing male Q-flies with yeast hydrolysate, a rich protein source, for the current 24–48 h pre-release holding period proved to be a highly effective dietary treatment for increasing overall performance in all measures that were considered. Compared with males denied access to post-teneral protein (i.e. the current release protocols), males provided just 24–48 h access to yeast hydrolysate as a nutritional supplement had: (i) enhanced ability to secure copulations; (ii) reduced mating latency; (iii) longer copulations; (iv) greater probability of sperm storage by mates; (v) more sperm stored by mates; (vi) increased ability to induce sexual inhibition in females; and (vii) increased longevity. Pre-release access to protein hence shows considerable promise as a means of improving the ability of Q-flies released in SIT to successfully compete with wild males and effectively disrupt the propagation and spread of wild populations.

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