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Sex differences in developmental response to yeast hydrolysate supplements in adult Queensland fruit fly

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Abstract

Post-teneral dietary supplements have been found to improve mating performance of male *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) (Q-fly) and show considerable promise for enhancement of sterile insect technique (SIT) programmes even when applied within the current 48 h pre-release holding period. However, Q-flies are released as a bisexual strain, and the positive effects of a diet including yeast hydrolysate for males may also boost reproductive development and sexual performance of females. Increased prevalence of mature sterile females can substantially dilute SIT efficacy as mating capacity of sterile males is largely depleted by sterile females rather than the relatively rare wild females. Here, we demonstrate that providing yeast hydrolysate for 48 h after adult emergence, emulating the current pre-release holding period of Q-fly SIT, leads to a significant increase in reproductive development and sexual performance in male Q-flies. In contrast, female Q-flies with access to yeast hydrolysate for 48 h had ovaries that were poorly developed and, particularly at younger ages, were less likely to mate and remate than females with continuous, ad libitum access to yeast hydrolysate. Our findings suggest that addition of yeast hydrolysate into the pre-release diet of Q-flies could be a cost-effective means of releasing a bisexual strain with competitive males but with sexually immature females, thereby rendering it operationally more similar to a unisexual strain.

Introduction

Adult nutrition is an important determinant of sexual development and mating performance in many insects. Synovigenic insects, including most tephritid fruit flies, must acquire resources after adult emergence to trigger and sustain growth of internal reproductive structures before sexual maturity is attained (Wheeler, 1996). For these species, adult diet during the period immediately after emergence can be particularly crucial in determining whether and how quickly sexual maturity is attained. In many sterile insect technique (SIT) programmes used to manage wild tephritid fruit fly populations, adult flies are released when still sexually immature. Accordingly, released flies must succeed in foraging for the resources needed to complete reproductive development before they can mate with, and induce reproductive failure in, wild females. Surprisingly little is known about the natural food sources of tephritid flies, but it is thought that common elements of their diet include honeydew, nectar, bird droppings, and especially bacteria (Drew & Yuval, 2000; Weldon & Taylor, 2011). However, natural sources of protein are thought to be often scarce and poor in essential amino acids (Courtice & Drew, 1984; Ben-Yosef et al., 2010). Given the link between nutrition of released flies and SIT efficacy, there has been substantial interest in the potential value of pre-release diet supplements that might reduce the reliance of released flies on natural food sources.

In mass-rearing facilities, tephritid flies are usually provided sugar (sucrose) and yeast hydrolysate as a rich source of minerals, vitamins, sterols, and amino acids. The positive effects of providing adult tephritid flies a diet containing yeast hydrolysate in addition to sucrose have been widely documented (e.g., Yuval et al., 2002, 2007; Liedo et al., 2011). For example, in the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann, addition of yeast

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hydrolysate to sucrose in the diet of adult females resulted in a 27-fold increase in egg production (Romanyukha et al., 2004) and substantially increased male sexual performance (Taylor & Yuval, 1999; Kaspi et al., 2000). Likewise in the West Indies fruit fly, *Anastrepha obliqua* Macquart, and the Mexican fruit fly, *Anastrepha ludens* (Loew), yeast hydrolysate in the diet increases egg load (Aluja et al., 2001). For male Caribbean fruit flies, *Anastrepha suspensa* Loew, yeast hydrolysate activates key regulatory genes and enzymes required for the synthesis of the sex pheromone by which they attract females (Teal et al., 2007). In *A. ludens* and *A. obliqua* pheromone production increases for males fed yeast hydrolysate compared to sucrose-fed males (Liedo et al., 2011). Inclusion of yeast hydrolysate in the diet provided to males is also associated with increased mating success in *Bactrocera cucurbitae* (Coquillett) (Haq et al., 2010a,b) and *Bactrocera dorsalis* (Hendel) (Shelly et al., 2005).

For the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Q-fly) (Diptera: Tephritidae), Australia's most damaging fruit fly pest, continuous access to yeast hydrolysate (in addition to sucrose and water) from adult emergence has been found to increase sexual development and mating activity of both males and females (Drew et al., 1983; Vijaysegaran et al., 2002; Meats & Leighton, 2004; Meats et al., 2004; Pérez-Staples et al., 2007; Prabhu et al., 2008). On diets of yeast hydrolysate, sucrose, and water, some mass-reared Q-flies begin exhibiting sexual activity after 5–6 days, but a majority are active only after 8–9 days (Pérez-Staples et al., 2007). However, Q-fly SIT programmes typically hold emerged adult flies (in plastic bins at high density) for only 1–2 days before they are released in the field (Anonymous, 1996; Campbell et al., 2008; Worsley et al., 2008). Maintenance of adult tephritid flies for long periods at high density has been shown to have detrimental effects on survival (Gaskin et al., 2002) and mating behaviour (Díaz-Fleischer et al., 2009). Studies in which Q-flies have been maintained with continuous access to yeast hydrolysate and released only when close to maturing (7 days after emerging) have reported low recapture rates, indicating poor survival in the field (Meats et al., 2003). Current evidence hence suggests that enhanced nutrition during a pre-release holding period of sufficient duration for maturation is not likely to be an effective approach for SIT. Subsequently, however, it has been demonstrated that supplementation of male Q-fly with yeast hydrolysate for 24 or (especially) 48 h – the current pre-release holding period – can substantially enhance sexual performance for a month or longer after the supplement is removed. In the laboratory, supplemented Q-fly males obtain more matings, transfer more sperm and are more effective at inhibiting female remating (Pérez-Staples et al., 2008). In field cages, sterile male Q-flies provided 48 h access to yeast hydrolysate secured as many copulations with wild females as did wild males that had been provided continuous access to yeast hydrolysate, greatly exceeding the performance of sterile males provided only sucrose (Pérez-Staples et al., 2009). Males provided 48 h access to yeast hydrolysate also show elevated responsiveness to cue lure traps in field cages, a result consistent with sexual maturation (Weldon et al., 2008).

Unlike some other tephritid SIT programmes (Robinson, 2002; Franz, 2005), Q-flies are released as a bisexual strain. Previous work on the potential value of yeast hydrolysate supplements has focussed mainly on males as the sex tasked with corrupting reproduction of wild females, and there is currently little information on what effect a short pre-release period of access to yeast hydrolysate would have on female tephritids. Interestingly, although Pérez-Staples et al. (2009) found that 48 h access to yeast hydrolysate induced a marked increase in sexual performance of males in field cages, this effect was not evident for females. Drew (1987) and Vijaysegaran et al. (2002) noted that, compared with males, female Q-flies have greater need of dietary constituents of yeast hydrolysate to complete reproductive maturation. This is also consistent with the finding of Pérez-Staples et al. (2007) that female Q-flies deprived of yeast hydrolysate showed a greater reduction in mating performance than did males. In a bisexual SIT release, the impact of released males can be greatly diluted by the presence of millions of sexually receptive sterile females. With regard to the different dietary needs of male and female Q-flies, we propose that providing yeast hydrolysate supplements during the short pre-release period could promote male reproductive maturation and sexual activity, while having a substantially smaller effect (i.e., meeting a much smaller proportion of total dietary need) in females. It might hence be possible to promote a more male-biased operational sex ratio that more closely resembles a single sex release in terms of the ratio of males to females engaged in mating activity. In the present study, we examine the effect of providing yeast hydrolysate in the diet of mass-reared adult Q-flies for 24 or 48 h after adult emergence on the reproductive development and sexual performance of both male and female Q-flies.

Materials and methods

Non-irradiated Q-flies were obtained as pupae from the Industry & Investment NSW Fruit Fly Production Facility located at Elizabeth Macarthur Agricultural Institute (EMAI) in Menangle, New South Wales, Australia. Adult flies emerged in the laboratory at Macquarie University, Sydney, Australia. On the day that adults emerged, four

cages of ca. 300 flies were provided with a 55-mm Petri dish of dry granular sucrose as food as well as water in moistened cotton wool. The first cage of flies was supplied with an additional dish of yeast hydrolysate enzymatic (MP Biomedicals, Aurora, OH, USA) for 24 h (24 h treatment), the second cage of flies was supplied with yeast hydrolysate for 48 h (48 h treatment), the third cage of flies was supplied with yeast hydrolysate constantly throughout the experiment (continuous yeast hydrolysate treatment), and the fourth cage received no yeast hydrolysate supplement. The day following emergence, 130 females and males from each of the cages were separated by aspirator and placed in 5-l cages with their corresponding diets. Flies were not transferred on the day of emergence to prevent damage to the wings of newly emerged flies. To prevent 24 and 48 h treatment females and males from feeding on residual yeast hydrolysate or their own faeces, all flies from each of the four treatments were transferred again using an aspirator to clean cages 4 days after the dishes of yeast hydrolysate were removed.

All cages were maintained, and experiments carried out, at 24–26 °C, 65–75% r.h., and L14:D10 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.

Reproductive development

Reproductive development of male and female flies from the four diet treatments was compared by measuring the size of female and male reproductive organs. Flies were sorted into the various treatments as above, and when 2, 4, 6, 8, 12, 16, 20, 24, and 28 days of age 10 males and 10 females from each treatment were dissected under a stereomicroscope (SZX12; Olympus, Tokyo, Japan). A single cohort of flies was used for this experiment. Photographs of the reproductive organs were taken using a 3 megapixel digital camera (ProgRes C10; Jenoptik Laser Optik Systeme, Jena, Germany) through the phototube of the stereomicroscope. Digital photographs were then measured using ImageJ software, version 1.37 (US National Institute of Health, Bethesda, MD, USA). For males, we measured the area of both testes, area of the long arms of the accessory glands, length and width of the ejaculatory pump, and area of the ejaculatory duct, and for females, we measured the area of both ovaries (Vijaysegaran et al., 2002; Radhakrishnan & Taylor, 2008).

Mating experiments

The sexual performance of both male and female flies from each of the four diet treatments was compared in mating experiments carried out when they were 6, 8, 12, 16, 20, 24, and 28 days of age. Q-fly mating activity takes place at dusk, when copulations (which often continue for several hours) are initiated over a period of ca. 30 min (Barton-Browne, 1957; Tychsen, 1977). On each day of mating trials, 3–4 h before the onset of simulated dusk in the laboratory, we transferred 10 males and 10 females from each treatment into individual 1-l clear plastic cages with a mesh screen (50 × 100 mm) for ventilation. Focal males and females were paired with one 10- to 11-day-old virgin female or male, respectively, that had been provided with continuous access to yeast hydrolysate and granular sucrose. Virgin males and females of this age and diet show high levels of sexual receptivity (Pérez-Staples et al., 2007; Prabhu et al., 2008). Pairs of flies were observed casually up to 1 h and then continuously from 15 min before the onset of simulated dusk until the last pair had separated. We noted the time at which copulations began and also noted at what time they ended for later calculation of copula latency and copula duration. Copula latency was expressed as the time (in min) that elapsed after the earliest recorded copulation by a focal male or female. The experiment was replicated twice, using batches of flies obtained from EMAI that emerged on 18 July 2007 and 30 October 2007.

Pairs that had mated were maintained in the mating cage with their respective diet. On the day after mating trials, we removed all pairs that had failed to copulate and froze them in individually labelled vials (−20 °C). To test remating tendency, on the morning after mating, the non-treatment females and males were removed, placed in individually labelled vials and frozen, and replaced by 10- to 11-day-old virgin females or males that had been provided continuous access to both sucrose and yeast hydrolysate. The once-mated focal flies and their second prospective mates were observed for mating as above before the onset of simulated dusk. At the end of the remating trials when all of the lights had switched off, all pairs were frozen individually in labelled vials.

Once the two 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. Each wing was then photographed using a digital camera through the phototube of a stereomicroscope (as described above). Wing length was measured (in mm) from the intersection of the anal and median band to the margin of the costal band and the R4 + 5 vein using ImageJ software (Pérez-Staples et al., 2008).

Data analysis

Measurements of reproductive organs were averaged when there were two structures (ovaries, testes, accessory

glands). Reproductive organs were analysed by generalized linear models (GLM) using a Gaussian model with identity as a link. Main effects included in the model were diet treatment, age and their interaction. Age was used as a continuous variable. P-values reported are from χ^2 tests. For models where the interaction between age and diet treatment was not significant, post hoc comparisons of differences in mean levels between diets are presented. Two extreme outliers that are best interpreted as errors in organ placement for photography rather than biological variation were excluded for assessment of ejaculatory apodeme length and width. For a similar reason, one extreme outlier was excluded for ejaculatory duct area. Ovary size was rank transformed. Generalized linear models were run using R v. 2.4.1 (R Development Core Team, 2011).

Mating and remating probability of focal males and females were analysed together using logistic regression models, with significance tested using likelihood ratio tests (chi-squared test). Main effects included in the model were replicate, sex, diet treatment, age of focal male or female, wing length of focal male or female, and wing length of the paired female or male (first partner for first matings, second partner for rematings). Age of the focal animal was treated as a continuous variable. The interaction of diet treatment and age of focal animal was included in the model. The interaction of focal animal and partner wing length was also included in the model to detect the possible presence of size-related mating preference. Model parameter estimates were inspected to identify simple effects between factor levels. Analyses were performed using JMP Statistical Software (SAS Institute, Cary, NC, USA).

Continuous measurements such as copula duration and mating latency were analysed using ANCOVA. Replicate, sex, age of the focal animal (used as a continuous variable), diet treatment, the interaction of age and diet, wing length of the focal animal, wing length of the second partner, and their interaction were included in the model. Copula duration and mating and remating latency data were ln-transformed to meet residual distribution requirements for ANCOVA. Mating and remating latency were analysed relative to the earliest mating latency recorded. Figures for each sex are presented separately for ease of comparisons.

Results

Male reproductive development

Diet treatments had strong effects on male reproductive organ size. For all organs measured, males provided continuous yeast hydrolysate had larger organs than males from other diet treatments. Males fed protein for 24 or 48 h also developed larger organs than males fed sucrose only (Figure 1A–E).

The model for testes area explained 17% of all deviance (model deviance = 0.223, null residual deviance = 1.098, d.f. = 360). Testes area was influenced by interaction between male age and diet (male age: Δdeviance = 0.005, d.f. = 1, P = 0.22; diet: Δdeviance = 0.136, d.f. = 3, P<0.001; interaction: Δdeviance = 0.083, d.f. = 3, P<0.001)

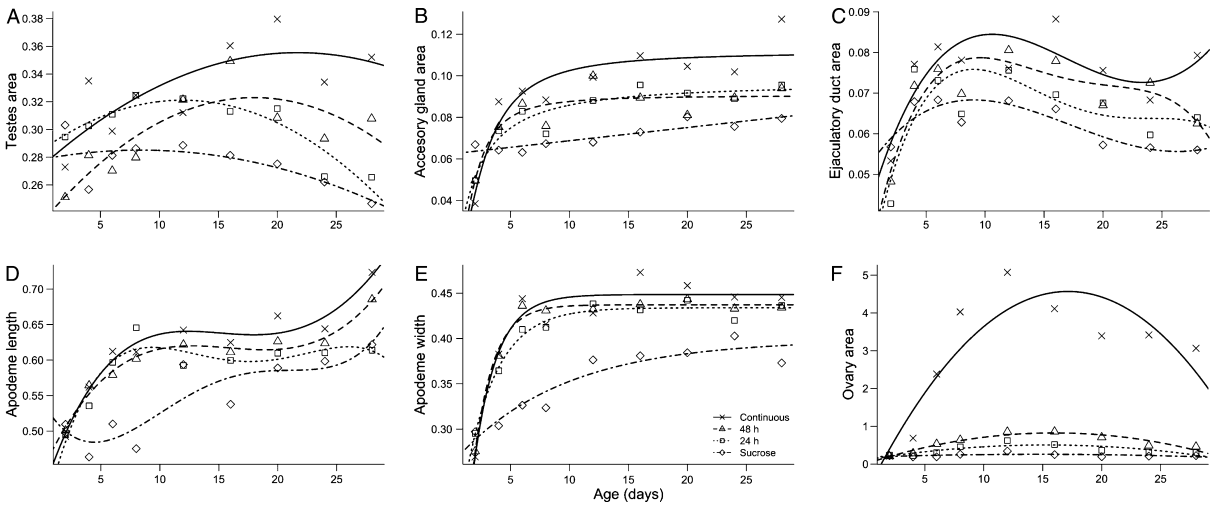

Figure 1 Male and female reproductive organ size from four diet treatments in relation to age. Individuals were fed either (1) sucrose only (sucrose), (2) sucrose and 24 h of yeast hydrolysate (24 h), (3) sucrose and 48 h of yeast hydrolysate (48 h), or (4) sucrose and continuous access to yeast hydrolysate (continuous). (A) Mean testes area (mm^2), (B) accessory gland area (mm^2), (C) ejaculatory duct area (mm^2), (D) ejaculatory apodeme length (mm), (E) ejaculatory apodeme width (mm), and (F) ovary area (mm^2).

Table 1 Male reproductive organ size GLM parameters ± confidence intervals for models with interaction between diet treatment and age

	Diet treatment			
Parameter	24 h	48 h	Continuous	Sucrose
Testes				
a	−0.00025 ± 0.0002	−2.78e-04 ± 0.0002	−1.738e-04 ± 0.00019	−9.463e-05 ± 0.0002
b	0.006 ± 0.006	9.991e-03 ± 0.005	7.556e-03 ± 0.006	1.593e-03 ± 0.006
c	0.285 ± 0.038	2.329e-01 ± 0.031	2.733e-01 ± 0.034	2.784e-01 ± 0.034
Accessory gland area				
a	0.097 ± 0.016	0.090 ± 0.007	0.112 ± 0.010	0.114 ± 0.548
b	0.557 ± 0.567	0.597 ± 0.316	0.951 ± 0.225	−6.544 ± 358.810
c	0.831 ± 0.841	0.480 ± 0.339	0.558 ± 0.262	40.764 ± 333.073
Apodeme length				
a	3.658e-01 ± 0.068	4.428e-01 ± 0.070	4.077e-01 ± 0.075	5.436e-01 ± 0.083
b	7.276e-02 ± 0.030	3.619e-02 ± 0.031	5.187e-02 ± 0.033	−3.073e-02 ± 0.037
c	−7.332e-03 ± 0.004	−2.418e-03 ± 0.004	−3.965e-03 ± 0.004	4.911e-03 ± 0.005
d	2.987e-04 ± 0.0002	5.384e-05 ± 0.0002	1.165e-04 ± 0.0002	−2.412e-04 ± 0.0002
e	−4.227e-06 ± 3.286e-06	−9.604e-08 ± 3.461e-06	−9.614e-07 ± 3.740e-06	3.881e-06 ± 4.177e-06

Males were fed either (1) sucrose and 24 h of yeast hydrolysate (24 h), (2) sucrose and 48 h of yeast hydrolysate (48 h), (3) sucrose and continuous access to yeast hydrolysate (Continuous), or (4) sucrose only (Sucrose).

(Figure 1A). The model was best explained by the equation $y = ax^2 + bx + c$ (Table 1).

For accessory gland area, the model explained 32% of all deviance (model deviance = 0.065, null residual deviance = 0.134, d.f. = 324). Apodeme length was positively influenced by the interaction between male age and diet (male age: Δdeviance = 0.034, d.f. = 1, P<0.001; diet: Δdeviance = 0.023, d.f. = 3, P<0.001; interaction: Δdeviance = 0.008, d.f. = 3, P = 0.0005) (Figure 1B). The model was best explained by the equation $y = a/(1 + b \times e^{-cx})$, where x is age (Table 1).

For ejaculatory duct area, the model explained 0.08% of all deviance (model deviance = 0.009, null residual deviance = 0.091, d.f. = 341). Apodeme length was not influenced by male age (Δdeviance = 0.0004, d.f. = 1, P<0.72), but was positively influenced by diet (Δdeviance = 0.008, d.f. = 3, P<0.001). Post hoc differences in mean levels revealed males fed yeast hydrolysate continuously had significantly bigger duct areas than males fed sucrose only, and than males fed yeast hydrolysate for 24 h. Males fed yeast hydrolysate for 48 h also had significantly bigger duct areas than males fed sucrose only. There were no significant differences between males fed yeast hydrolysate for 48 h and males fed yeast hydrolysate for 24 h. There was no evidence of interaction between male age and diet (Δdeviance = 0.001, d.f. = 3, P = 0.18) (Figure 1C). The model was best explained by a fourth order polynomial: $y = a + bx + cx^2 + dx^3 + ex^4$, where x is age.

For apodeme length, the model explained 47% of all deviance (model deviance = 0.941, null residual deviance = 1.055, d.f. = 357). Apodeme length was positively influenced by the interaction between male age and diet (male age: Δdeviance = 0.627, d.f. = 1, P<0.001; diet: Δdeviance = 0.274, d.f. = 3, P<0.001; interaction: Δdeviance = 0.040, d.f. = 3, P = 0.005) (Figure 1D). The model was best explained by a fourth order polynomial: $y = a + bx + cx^2 + dx^3 + ex^4$, where x is age (Table 1).

For apodeme width, the model explained 38% of all variance (model deviance = 0.638, null residual deviance = 1.040, d.f. = 358). Apodeme width was positively influenced by male age (Δdeviance = 0.390, d.f. = 1, P<0.001) and was also influenced by male diet (Δdeviance = 0.245, d.f. = 3, P<0.001). Post hoc differences in mean levels revealed that males fed yeast hydrolysate continuously had significantly larger apodeme widths than males fed sucrose only. Males fed yeast hydrolysate for 48 or 24 h also had significantly larger apodeme widths than males fed sucrose only. There were no significant differences between males fed yeast hydrolysate continuously, for 48 or for 24 h. There was no evidence of interaction between age and diet (Δdeviance = 0.003, d.f. = 3, P = 0.78). Apodeme width was best explained by an asymptotic exponential model (asymptotic exponential growth curve): $y = a - b \times e^{-cx}$, where a is the maximum value, b is the slope, and c is a constant (Figure 1E).

Female reproductive development

Females with continuous access to yeast hydrolysate had ovarian growth until ca. 15 days of age, after which growth rate declined. Females fed 48 or 24 h of protein hydrolysate had slightly larger ovaries than sucrose fed females,

but did not fully develop ovaries compared with females fed yeast hydrolysate continuously (Figure 1F).

For ovary size, the model explained 53% of all deviance (model deviance = 2 063 379, null residual deviance = 1 824 575, d.f. = 359). Ovary size was positively influenced by the interaction between female age and diet (female age: Δdeviance = 141 661, d.f. = 1, P<0.001; diet: Δdeviance = 182 281, d.f. = 3, P<0.001; interaction: Δdeviance = 98 907, d.f. = 3, P = 0.0003) (Figure 1F). The model was best explained by a polynomial equation: $y = ax^2 + bx + c$ (Table 2).

Mating performance

Mating probability. Mating probability was significantly higher for males than for females ($\chi^2 = 146.586$, d.f. = 1, P<0.001). Mating probability varied significantly between diet treatments ($\chi^2 = 205.535$, d.f. = 3, P<0.001), male and female age ($\chi^2 = 22.704$, d.f. = 1, P<0.001), and the interaction between age and dietary treatment ($\chi^2 = 18.366$, d.f. = 3, P<0.001). The size of the focal individual ($\chi^2 = 2.385$, d.f. = 1, P = 0.12), the individual it mated with ($\chi^2 = 1.321$, d.f. = 1, P = 0.25), and the interaction between the size of both individuals had no effect on mating probability ($\chi^2 = 0.323$, d.f. = 1, P = 0.57). There were no significant differences between replicates ($\chi^2 = 4.99$, d.f. = 1, P = 0.48).

At 6 days after adult emergence, males provided continuous access to yeast hydrolysate were more likely to mate than males provided yeast hydrolysate for 48 or 24 h, but these in turn were more likely to mate than sucrose fed males (Figure 2A). By day 8, males fed 48 or 24 h of protein hydrolysate had comparable mating probabilities to males fed yeast hydrolysate continuously. Subsequently, by 20 days after adult emergence, mating probability in all dietary treatments was similar (Figure 2A).

Female mating probability increased with time for all dietary treatments (Figure 2B). Females provided continuous access to yeast hydrolysate were more likely to mate than females fed other diets. However, females given 48 or 24 h access to yeast hydrolysate did not reach mating levels of females fed yeast hydrolysate continuously, although they did have higher mating probabilities than females fed sucrose only (Figure 2B).

Latency to mate. Mating latency differed significantly between the sexes ($F_{1,926} = 27.775$, P<0.001). Dietary treatment ($F_{3,926} = 33.298$, P<0.0001) and age ($F_{1,926} = 6.579$, P = 0.011) had a significant effect on both male and female mating latency. The interaction between age and dietary treatment had no significant effect on mating latency ($F_{3,926} = 1.769$, P = 0.15). The size of the focal individual ($F_{1,926} = 0.269$, P = 0.61) and the interaction between the focal individual's size and the mate had no significant effect ($F_{1,926} = 0.707$, P = 0.40), whereas the first mate's size did have a significant effect on mating latency ($F_{1,926} = 6.153$, P = 0.013). There were no significant differences between replicates ($F_{1,926} = 3.708$, P = 0.055).

Mating latency of males provided continuous access to yeast hydrolysate was shorter compared with sucrose-fed males and males provided yeast hydrolysate for 48 or 24 h (Figure 2C). Dietary treatment had a more marked effect on female mating latency. Females feeding continuously on yeast hydrolysate had shorter mating latencies than females from other dietary treatments. However, this mating advantage was not apparent for females feeding for 48 or 24 h on yeast hydrolysate (Figure 2D).

Copula duration. Copula duration (ln-transformed) varied significantly with sex ($F_{1,921} = 13.439$, d.f. = 1,921, P = 0.0003), diet treatment ($F_{3,921} = 30.554$, P<0.0001), but not with age ($F_{1,921} = 0.003$, P = 0.96). The interaction of diet treatment and age had a significant effect on copula duration ($F_{3,921} = 2.613$, P = 0.050). Copula duration of sucrose-fed males was significantly shorter at any age than that of males with access to yeast hydrolysate (Figure 2E). However, for females, the effect of feeding on yeast hydrolysate was less clear (Figure 2F). Focal males and females with larger wings had longer copulations ($F_{1,921} = 7.125$, P = 0.008), whereas the mate's size ($F_{1,921} = 0.207$, P = 0.65), or the interaction between the

Table 2 Female ovary size GLM parameters ± confidence intervals

	Diet treatment			
Parameter	24 h	48 h	Continuous	Sucrose
a	−0.002 ± 0.0006	−0.003 ± 0.001	−0.018 ± 0.004	−0.0004 ± 0.0002
b	0.051 ± 0.019	0.102 ± 0.036	0.623 ± 0.107	0.010 ± 0.007
c	0.113 ± 0.109	0.009 ± 0.212	−0.779 ± 0.628	0.184 ± 0.043

Females were fed either (1) sucrose and 24 h of yeast hydrolysate (24 h), (2) sucrose and 48 h of yeast hydrolysate (48 h), (3) sucrose and continuous access to yeast hydrolysate (Continuous), or (4) sucrose only (Sucrose).

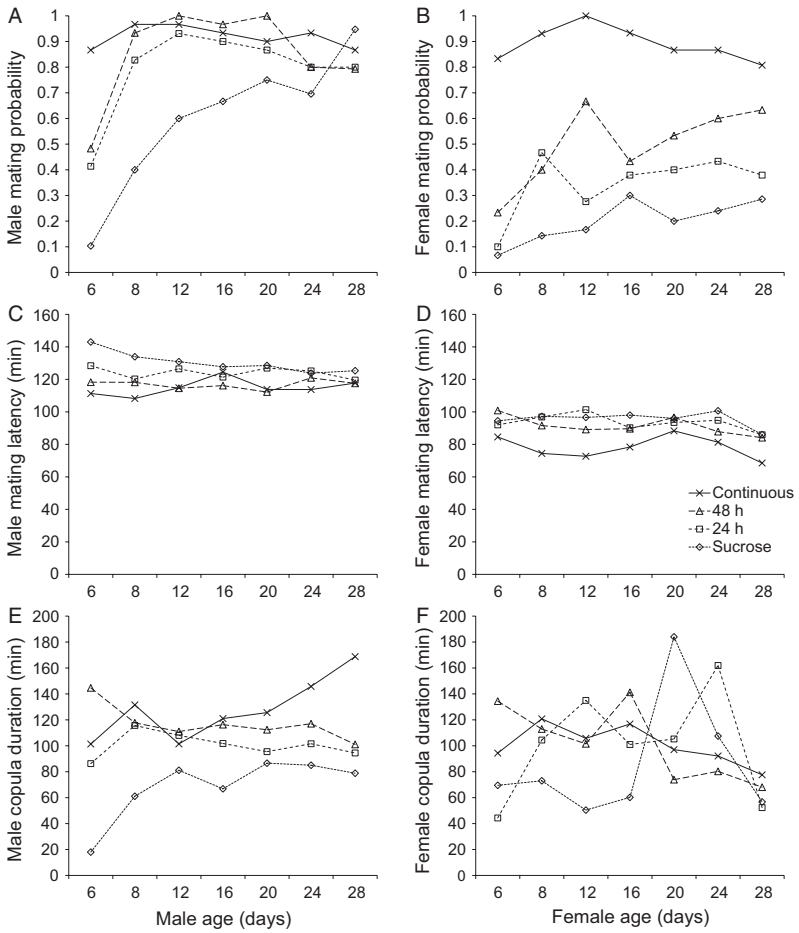

Figure 2 Mating performance of focal individuals from four diet treatments in relation to their age. (A) Male mating probability, (B) female mating probability, (C) male mating latency (relative to earliest recorded time), (D) female mating latency, (E) male copula duration, and (F) female copula duration. Focal males and females were fed either (1) sucrose only (Sucrose), (2) sucrose and 24 h of yeast hydrolysate (24 h), (3) sucrose and 48 h of yeast hydrolysate (48 h), or (4) sucrose and continuous access to yeast hydrolysate (Continuous). Control males and females aged 10–15 days were provided sucrose and continuous access to yeast hydrolysate.

size of the focal individual and the mate had no significant effect ($F_{1,921} = 0.289$, $P = 0.59$).

Remating probability. Remating probability was significantly higher for males than for females ($\chi^2 = 203.002$, d.f. = 1, $P<0.0001$). Remating probability differed significantly between diet treatments ($\chi^2 = 47.591$, d.f. = 3, $P<0.0001$), but male and female age ($\chi^2 = 0.852$, d.f. = 1, $P = 0.36$), or the interaction between age and dietary treatment ($\chi^2 = 4.987$, d.f. = 3, $P = 0.17$) had no significant effect. The size of the focal individual ($\chi^2 = 1.457$, d.f. = 1, $P = 0.23$), the individual it mated with ($\chi^2 = 0.878$, d.f. = 1, $P = 0.35$), or the interaction between the size of both individuals had no effect on remating probability ($\chi^2 = 3.803$, d.f. = 1, $P = 0.051$). There were significant differences between replicas ($\chi^2 = 4.277$, d.f. = 1, $P = 0.039$).

Males provided continuous access to yeast hydrolysate were likely to remate at all tested ages (Figure 3A). At 6 days after adult emergence, males given access to yeast hydrolysate for 48 or 24 h were less likely to remate than males with continuous access, but this difference was not evident after 8 days. Males given access to sucrose only were less likely to remate than males given access to yeast hydrolysate between 6 and 20 days after adult emergence, but by 28 days after adult emergence, they were as likely to remate as males provided access to yeast hydrolysate (Figure 3B). Females that were provided continuous access to yeast hydrolysate were more likely to remate than were females fed any of the other diets (Figure 3B).

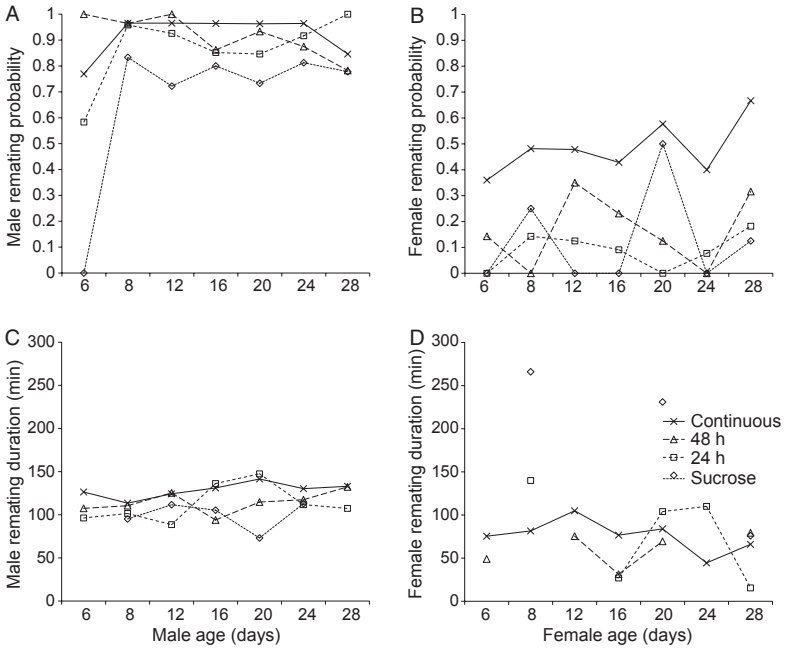

Figure 3 Mating performance of focal individuals from four diet treatments in relation to their age. (A) Male remating probability, (B) female mating probability, (C) male remating duration, and (D) female remating duration. Focal males and females were fed either (1) sucrose only (Sucrose), (2) Sucrose and 24 h of yeast hydrolysate (24 h), (3) sucrose and 48 h of yeast hydrolysate (48 h), or (4) sucrose and continuous access to yeast hydrolysate (Continuous). Control males and females aged 10–15 days were provided sucrose and continuous access to yeast hydrolysate. For treatments where the females did not remate, lines are not connected.

Females given access to yeast hydrolysate for 48 or 24 h were not more likely to remate than females fed sucrose only.

Latency to remate. Remating latency (ln-transformed) did not vary with sex ($F_{1,542} = 1.597$, $P = 0.21$), or diet treatment ($F_{3,542} = 2.266$, $P = 0.08$), whereas age ($F_{1,542} = 99.330$, $P<0.0001$) did affect remating latency significantly, but not the interaction between age and treatment ($F_{3,542} = 1.490$, $P = 0.22$). Neither the size of the focal individual ($F_{1,542} = 0.226$, $P = 0.64$) nor the size interaction between the focal individual and its mate ($F_{1,542} = 0.012$, $P = 0.91$) had a significant effect on remating latency. However, remating latency was significantly faster for focal individuals whose second mate was bigger ($F_{1,542} = 8.564$, $P = 0.004$). There were also significant differences between replicates ($F_{1,542} = 45.928$, $P<0.0001$).

Remating duration. Remating duration (ln-transformed) varied with sex ($F_{1,560} = 45.368$, $P<0.0001$), dietary treatment ($F_{3,560} = 2.981$, $P = 0.031$), but not with age ($F_{1,560} = 0.208$, $P = 0.65$), nor the interaction between age and treatment ($F_{3,560} = 1.130$, $P = 0.34$). The size of the focal individuals ($F_{1,560} = 0.145$, $P = 0.70$), their mates ($F_{1,560} = 0.500$, $P = 0.48$), or their interaction ($F_{1,560} = 2.648$, $P = 0.10$) had no significant effect on remating duration. There were significant differences between replicates ($F_{1,560} = 4.521$, $P = 0.034$). Both sexes that were provided continuous access to yeast hydrolysate had relatively constant copula durations compared with the rest of the dietary treatments (Figure 3C and D).

Discussion

Males

Providing males access to yeast hydrolysate during just the first 2 days after emergence substantially increased their sexual development and mating performance. Benefits of access to yeast hydrolysate were evident in all male reproductive organs: testes, accessory glands, ejaculatory apodeme, and ejaculatory duct. The size of the reproductive organs of males that were given access to yeast hydrolysate for 48 h indicated that they reached sexual maturity at ages that were comparable to males that had continuous access to yeast hydrolysate throughout the developmental period. Vijaysegaran et al. (2002) found that Q-fly male reproductive organs such as the ejaculatory apodeme and accessory glands, but not the testes, increased in size when continuously provided yeast hydrolysate. Fat body reserves and

pheromone production also increased (Vijaysegaran et al., 2002). Here, we found that males provided yeast hydrolysate for 48 h after adult emergence, a period that emulates the 2-day pre-release holding period used in current Q-fly SIT programmes (Anonymous, 1996; Campbell et al., 2008; Worsley et al., 2008), produced sexual organs that were comparable in size to males fed yeast hydrolysate continuously. Producing males that can quickly develop and mature sexually will benefit SIT programmes. The acceleration of male sexual development that results when male Q-flies are given a dietary boost during a short period prior to release should minimize the delay between release and attainment of sexual maturity in the field, where adequate nutrition may be difficult to find (Courtice & Drew, 1984; Ben-Yosef et al., 2010).

As in previous laboratory studies (Pérez-Staples et al., 2008), and in field cage trials utilizing sterile males and wild females (Pérez-Staples et al., 2009), the benefit of yeast hydrolysate feeding for only 48 h was evident in the number of copulations obtained by males. However, here we show that this benefit also extended to remating tendency of the subset of males from each treatment that mated on the first opportunity. SIT programmes will benefit from males that are not only able to compete with wild males but that can also quickly remate with other females. Other benefits of providing 48 h access to yeast hydrolysate included shorter mating latencies and longer copula durations. Earlier studies also revealed positive effects of access to yeast hydrolysate for 48 h on sperm transfer, ability to induce sexual inhibition in females (Pérez-Staples et al., 2008), and on attraction to cue lure-baited traps (Weldon et al., 2008).

Females

Mass-reared females fed yeast hydrolysate for 48 or 24 h had smaller ovaries similar to females fed sucrose only. This was in sharp contrast with females provided continuous access to yeast hydrolysate, which showed rapid development of ovaries that reached peak size at 12 days of age. Similarly, Vijaysegaran et al. (2002) found '…practically no ovarian development…' over 3 weeks for females fed only sucrose in contrast to rapid development of ovaries in females fed yeast hydrolysate, with peak size at 14 days of age. Q-fly females need ca. 0.1 mg of yeast hydrolysate per day to mature their oocytes (Meats & Leighton, 2004). Therefore, it seems likely that providing female Q-flies with yeast hydrolysate for only 48 or 24 h is not adequate for the acquisition of sufficient amino acids and other nutrients to trigger or sustain normal reproductive development.

Mating and remating probability of females provided yeast hydrolysate for 48 or 24 h was comparable to females fed sucrose only, yet much lower than females provided continuous access to yeast hydrolysate. Even when mating probability increased with age, females that were provided yeast hydrolysate for 48 h did not reach the same level of mating success as females that were fed yeast hydrolysate continuously. Compared with males, females gained considerably less benefit from access to yeast hydrolysate for a period that emulates current pre-release protocols.

An important result that can be inferred from our study is that reproductive maturation and sexual maturation are not the same phenomenon. That is, females fed 24 or 48 h of yeast hydrolysate or sucrose only did not develop ovaries and yet a small proportion of females fed these diets mated after 8 days of age, although never in numbers similar to females with continuous access to yeast hydrolysate. This suggests a non-deterministic link between reproductive and sexual development. Females will mate at some degree even when their reproductive organs are quite poorly developed. It suggests that sexual activity may emerge well in advance of reproductive readiness and that different aspects of maturity can proceed at different pace. Care is hence needed when interpreting 'maturity' of field-collected samples, as even flies with low levels of morphological maturity of reproductive organs may still be sexually active.

Q-flies are currently released as a bisexual strain in SIT programmes. Dilution of sterile males by released sterile females has been recognized as a potential limit on the efficacy of SIT since the earliest examples of its application (e.g., Bushland & Hopkins, 1951). This limitation has been overcome in SIT programmes that target *C. capitata* and *B. dorsalis* owing to the development of genetic sexing strains that utilize temperature-sensitive lethality and/or sex-specific pupal colouration, which permits male-only releases (Franz, 2005). Our results suggest that the substantial benefits accrued by males from access to a diet that includes yeast hydrolysate during the first 2 days after emergence will be matched by only limited benefit to females. Thus, although both sexes are produced and released, males will derive vastly greater sexual performance benefits from a pre-release diet that includes yeast hydrolysate.

For regional management programmes that utilize SIT to control insect species with long pre-reproductive periods, it is normally not feasible to hold sterile insects until they become sexually mature. Holding sterile adults for extended periods is limited by interactions between the financial costs of the infrastructure and personnel required to maintain the large number of insects that are released in SIT programmes, and the mating performance and survival of animals that are held at high density during early adult life (Gaskin et al., 2002; Meats et al., 2003; Díaz-Fleischer et al., 2009). Although we tested non-irradiated

mass-reared individuals, field cage studies also match results reported here, as sterile males provided 48 h of yeast hydrolysate had higher mating probabilities than sterile males provided sucrose only. In the case of sterile females with access to yeast hydrolysate for 48 h, they did not have increased mating performance compared with sterile females provided sucrose only (Pérez-Staples et al., 2009). Providing a yeast hydrolysate diet for 48 h in the case of the Q-fly could accelerate male sexual development, thus overcoming many of the disadvantages of releasing males as immature adults, while producing sexually immature females. This may be a cost-effective means of biasing a bisexual strain in favour of relatively greater male than female representation in the mating arena, reaping many of the benefits currently available only to single-sex releases.

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