Journal of Insect Science, (2018) 18(2): 25; 1–8
doi: 10.1093/jisesa/iey008
Research article

Influence of Supplemental Protein on the Life Expectancy and Reproduction of the Chinese Citrus Fruit Fly, *Bactrocera minax* (Enderlein) (*Tetradacus minax*) (Diptera: Tephritidae)

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Subject Editor: Muhammad Chaudhury

Received 27 September 2017; Editorial decision 22 January 2018

Abstract

Bactrocera minax (Enderlein) (Diptera: Tephritidae) is a major citrus pest in China, whose artificial rearing technology of the adult is not well documented to date. In this study, we tried to determine if supplementing proteins to the adult diet could result in the enhancement of some fitness parameters of *B. minax*. Four feeds with varying protein source were provided as F0 (water), F1 (sucrose), F2 (sucrose + yeast), and F3 (sucrose + peptone). F0 and F1 being the control, F2 and F3 were protein food types. The results showed that adults fed by F2 and F3 lived longer with 40.1 d and 32.8 d, respectively, had reduced death rates (death peaks were delayed for 5.6 d and 4.1 d, respectively), increased mating frequencies (8.1 and 5.3 per females, 4.7 and 7.3 per males, respectively), and longer mating durations (with 42 d and 34 d). In addition, females recorded an increased adult ovary development, more egg load (with 94.8 and 77.3 brood eggs per ovary) and to greater oviposition rates of 63.2 eggs/female and 19.3 eggs/female. Based on our results, protein supplements enhanced *B. minax* survival, mating, and fecundity. This study does not only provide basic knowledge to implement artificial rearing of *B. minax*, but also deepens our understanding on its physiology that could be used to enhance the management of the pest.

Key words: *Bactrocera minax*, protein compositions, survival, mating, fecundity

Fruit flies (Diptera: Tephritidae) are among the most destructive agricultural pests known to occur worldwide (White and Elson-Harris 1992, Bhattacharya et al. 2013). Fruit flies damage fruits and vegetables by laying their eggs inside the fruits and cause them to rot and drop, thus inducing significant losses in production (Hollingsworth et al. 1997, Bhattacharya et al. 2013). Besides causing direct losses to a wide range of fruits and vegetables, they limit the development of agriculture in many countries because of strict trade quarantines imposed to prevent their spread. *Bactrocera minax* (Enderlein) (*Tetradacus minax*) (Diptera: Tephritidae) is one of the most important quarantinable pests which attacks citrus fruits in China. It also occurs in other Asian countries such as Bhutan, Nepal, and India (EPPO/CABI 1997, Wang et al. 2014). In China, *B. minax* mainly occurs in temperate provinces such as Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Sichuan, and Yunnan (EPPO/CABI 1997, Wang et al. 2014). The pest exhibits about 6 mo's pupal diapause from November to May as a way to fight against harsh environmental conditions (Chen et al. 2016). Such long pupal diapause is a barrier for laboratory rearing and development of control strategies against this pest (Chen et al. 2016). Damage caused by larval feeding on the fruit pulp results in fruit segments partially or completely turning into paste. The losses are estimated to range

between 5 and 20% and can reach up to 50% (Wang et al. 1995, 2009). In a global agricultural system that promotes food security and sustainability, there is a necessity to produce nutritional complements in quality and quantity to meet the rising demands in fruits and vegetables of the population. *B. minax*, therefore, represents a significant threat to the completion of these challenges unless it is well controlled and managed.

Nutrition plays a vital role in the development of tephritid fruit flies. Life span, maturity development, and reproductive capacity are mostly influenced by the quality of the food (Good and Tatar 2001, Fontana et al. 2010). Reproduction and longevity are mutually dependent in sexually reproducing organisms, with an increase in the energy used for reproduction resulting in a subsequent decrease in longevity (Partridge and Andrews 1985). Hence, the nutritional conditions in an environment influence the trade-off between current reproductive effort and life span because of competition for nutrients between somatic maintenance and gamete production (Kirkwood and Rose 1991). Lee and Lee (2005) reported on the benefit of artificial diet on predator's development. Also, the reproductive ability in many Tephritids is strongly influenced by diet (Aluja et al. 2001; Carey et al. 2002, 2008; Harwood et al. 2013; Liedo et al. 2013; Pereira et al. 2013).

Protein is one of the most important nutrients required for ovarian development and vitellogenesis in insects (Raikhel and Dhadialla 1992, Aluja et al. 2001, Taylor et al. 2013). In general, fruit flies fed with protein supplements, for example *Anastrepha ludens* (Loew), *Ceratitis capitata* (Wiedemann), and *Bactrocera cucurbitae* (Coquillett), had their fecundity enhanced while given protein supplements (Mangan 2003, Harwood et al. 2013). Also, protein-enriched foods were shown to promote the longevity of the fruit fly, *Anastrepha serpentina* (Wiedemann) (Jácome et al. 1999). In addition, the protein level of the food has a high influence on egg production (Mangan 2003). For example, while evaluating the effect of larval diet on the development and reproductive rates of male and female Mediterranean fruit fly, Kaspi et al. (2002) found that protein-fed larvae were large in size, grew fast, and had more nutritional reserves upon emergence.

A source of sugar is an important nutrient for fruit flies because it provides energy for survival and reproduction. Hagen (1953) reported that both sexes of the western cherry fruit fly, *Rhagoletis indifferens*, Curran, require sources of sugar for survival. Fontellas and Zucoloto (1999) also found that West Indian fruit fly, *Anastrepha obliqua*, Macquart, did not survive more than 3 d without a sugar (sucrose) source. However, Sacchetti et al. (2014) reported that olive fruit fly, *Bactrocera oleae*, did not survive longer when fed with sugar diet. Similarly, Gavriel et al. (2011) reported that male Mediterranean fruit fly had a short life span and the ability of the female to receive male were not inhibited when fed with granulated sugar only. This implies that fruit flies depend upon these two nutrients for both survival and egg production. To the best of our knowledge, none of the previous studies about the artificial rearing techniques of *B. minax* has been successful due to the high adult mortality rates observed. This unfortunate situation hindered a comprehensive investigation of its biological characteristics and the development of effective prevention measures. For this reason, the understanding of the nutritional factors that could reduce mortality of *B. minax* and enhance its reproduction capacities is of prime importance for the development of an artificial rearing program. Thus, the present study aimed at evaluating the feeding response of adults to different protein supplements through the assessment of their effects on the survival and reproduction of the Chinese citrus fruit fly, *B. minax*.

Materials and Methods

Acquisition of Adult Fruit Flies

Mature larvae of *B. minax* were collected from Orange orchards in Songzi, Hubei, China, between 20 October and 10 November 2015. The collected larvae were taken to the Yangtze university insectary where they were moved into plastic container (21 cm in diameter) containing 15% water content sand (sterilized sand by constant temperature 60°C for 24 h to kill Nematodes) and kept indoor at room temperature (4.8–22.6°C, 75% RH ± 5%) till adult emergence. The plastic container containing fourth stage pupae were placed into big cage (baby mosquito net) to prevent the emerging adult to escape. Adults emerged in late April and early May 2016 and ended on 27 May 2016.

Diet Preparation

In this study, a solid diet was used to feed the flies, the diet was measured in grams; sucrose 4 g (Tianjin Beilian Fine Chemicals Development Co. Ltd.), sucrose + yeast extract (3 g + 1 g, making 3:1) (yeast extract and sucrose + peptone (3 g and 1 g, making 3:1)(Guangdong Huankai Microbial Sci & Tech. Co. Ltd). After measurements, the solid food was mixed evenly and placed on Petri dishes ready to be provided to flies. Yeast extract was used as source of protein, as it contains vitamin content and protein which provide excellent growth conditions for more microbes. Peptone is the microbiological culture media prepared by enzymatic digestion of selected animal tissues, widely used to suit a range of nutritional requirements. Peptone was used as source of protein as it supports excellent growth of various microorganisms. It also contains short chains peptides and broad spectrum amino acids required for the microbial growth.

Experimental Design

The obtained adults were separated in three cohorts. To assess the effects of protein supplementation on the survival of *B. minax*, a total of 60 newly emerged flies comprising 30 males and 30 females were introduced in a wire mesh cage (dimensions: 30 cm × 30 cm × 30 cm) covered with a white cloth at the bottom. Flies were provided with four types of food designated as F0 (water alone), F1 (sucrose alone), F2 (sucrose + yeast extract, mass ratio 3:1), and F3 (sucrose + peptone, mass ratio 3:1). There were five replicates for each food type, 30 males and 30 females were provided each food type resulting into a total of 60 flies per replicate. After preparation, 4 g solid food were provided to the colony into the Petri dish (diameter: 9 cm) and replaced every 24 h. Also, the flies were provided with 7 ml of water twice a day (morning and evening) on a cotton bud (diameter: 0.5 cm) that was replaced on a weekly basis. Male and female flies' mortality was recorded daily.

To assess the effect of protein supplementation on mating and egg production, 30 males and 30 females virgin *B. minax* (0 to 24 h after emergency) were provided each food source (F0, F1, F2, and F3) resulting into a total of 60 flies per replicate. There were five replicates for each food type. Fresh orange fruit was provided for egg laying as they are adapted to oviposition through the thick skin of oranges (CABI, 2017), and the orange was replaced every three days. The frequency of mating (mating times) was observed and recorded every half an hour from 8:00 a.m. to 6:00 p.m.

Finally, 800 females were kept in four separate cages (200 females/cage) where they were provided with four food sources as described above. Twenty insects were dissected every 5 d for a duration of 20 d to assess the ovarian development and egg load (Chou et al. 2012).

Demographic Analysis

Adult life expectancy was obtained by the following expression; "Life expectancy" = (∑Survival days per insect)/Total number of adult tested. On evaluating the effect of protein on flies, cumulative mortality ratio (CMR) and feeding days were compared. First, we calculated the mortality ratio (MR %) by MR % = daily mortality/total lifetime mortality * 100, then CMR % obtained by the following formula; CMR_n (%) = MR_1 + MR_2 + MR_3 + … + MR_n. Where; MR is the mortality ratio; CMR is the cumulative mortality ratio, *n* is the feeding days when food was supplied to the flies.

The adult cumulative mortality in all four types of food (F0, F1, F2, and F3) tested were transformed to probability units and analyzed using the logistic regression (y = a/(1 + Exp (b – k x))) where x is the feed days in each food and y is the cumulative mortality in probability units. Time–mortality data were fitted using probability analysis to estimate the lethal time for 50% (LT_{50}) and 90% (LT_{90}) cumulative mortality in each food treatment tested. To obtain the age-specific cumulative mortality, cumulative mortality data were subjected Arcsine transformation (arcsine square root transformation) (Sokal and Rohlf 1995, Warton and Hui 2011) and then were subjected to analysis of variance (ANOVA one-way) and means were separated by Tukey's test at P = 0.05.

To access mating dynamic the following expression were used; "Lifetime Mating frequency per female" = ∑(daily number of mating times/daily number of surviving females). "Lifetime Mating frequency per male" = ∑(daily number of mating times/daily number of surviving males). The cumulative mating ratio (CMR) for each diet was calculated using the following expression: first, we calculated the mating ratio (MR %) by the following formula, MR% = (lifetime mating times)/(total lifetime mating times) * 100. The MR obtained was used to calculate the CMR by using the following formula; CMR_n (%) = MR_1 + MR_2 + MR_3 + … + MR_n. Where; MR is the daily mating ratio; CMR is the cumulative mating ratio; n is the feeding days (days of food supply to the flies, until death of the last fly).

The adult cumulative mating in four food sources (F0, F1, F2, and F3) tested was transformed to probability units and analyzed using the logistic regression (y = a/(1 + Exp (b – k x))) where x is the feeding days in each food and y is the cumulative mating in probability units. Mating time data were fitted using probability analysis to estimate the time for 16% (mating onset), 50% (mating peaks), and 84% (mating ends) (Gong et al. 2012) cumulative mating in each food treatment tested. Mating peaks are the 50% cumulative mating. To obtain the age-specific cumulative mating ratio, cumulative mating data were subjected to Arcsine transformation (arcsine square root transformation) (Sokal and Rohlf 1995, Warton and Hui 2011) and then were subjected to ANOVA (one-way) and means were separated by Tukey's test at P = 0.05.

To access the female oviposition, the orange provided for oviposition was removed from the cage and cut through the oviposition patch or hole. The eggs found were counted by naked eyes and recorded. The following expression was used to estimate the female egg production per female. Oviposition per female = ∑(total weekly fecundity/weekly average number of females surviving).

To access the female fecundity of *B. minax*, egg load was determined by counting the number of mature oocytes in the egg chambers under stereomicroscope. The estimated standard stages of ovarian development were assigned by relating to particular oogenesis stages. Hence, the ovarian development in *B. minax* observed was "first stage": ovary is original, very small, nondeveloped, nonshaped, and with subsidiary silks. Its diameter is just a little greater than the oviduct. "Second stage": ovary is growing, swelling. and big. Subsidiary silks diminish or lose. Primary formation of ovary and egg yolk precipitation. "Third stage": ovary is growing bigger, and harder egg yolk deposition to form egg. Eggs can be seen in the oviducts. Mature Eggs in ovary, before and after the onset of oviposition (Fig. 1) (Fletcher et al. 1978; Kendra et al. 2006; Chou et al. 2012). To calculate the ratio of different ovary stages the following formula was used: "Ratio of specific ovary stage (%)" = (number of flies with the specific ovary stage)/(total number of flies dissected) * 100.

Data on the effect of diet on adult life expectancy, mortality, age-specific mortality, mating frequency per female, mating

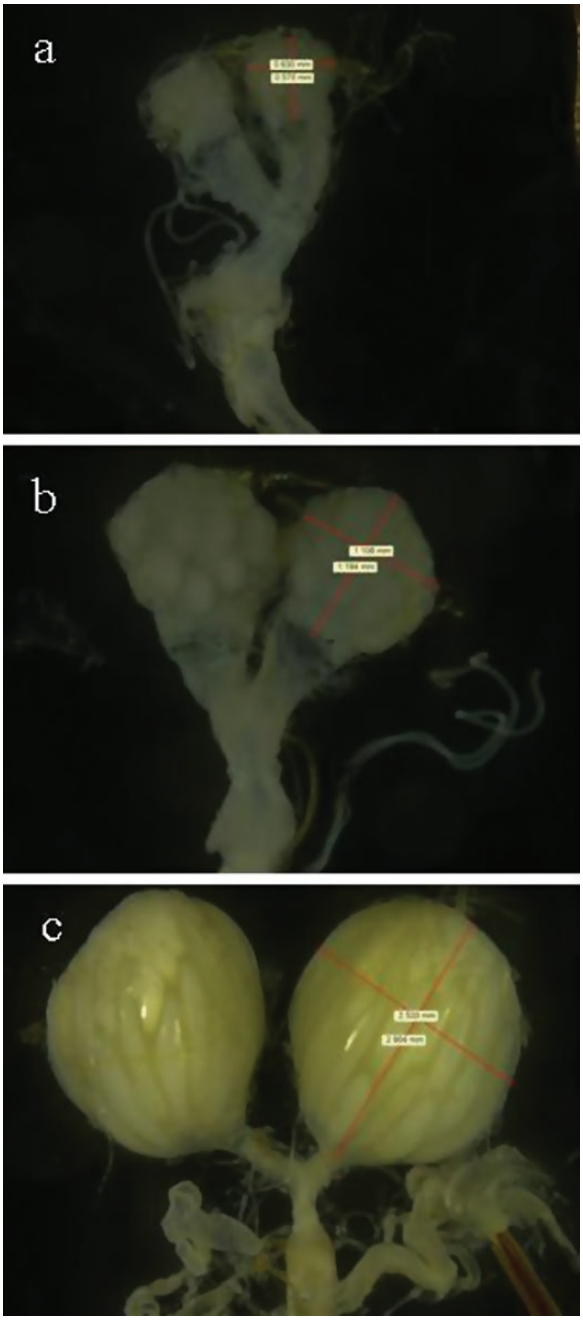

Fig. 1. Grading of the ovarian in female adult of *B. minax*. Stages of ovarian development in adult *B. minax*, Stages 1–2 represent sexually immature females, and refer to steps in the ovary maturation phase. Stage 3 represents mature ovaries, before and after the onset of oviposition. (a) means ovarian stage 1, (b) means ovarian stage 2, (c) means ovarian stage 3.

frequency per male, age-specific mating frequency, ovarian egg load, and female oviposition were subjected to ANOVA (one-way), and means were separated by Tukey's test at $P = 0.05$. Statistical analyses were performed using SPSS 17.0 (SPSS Inc. 2008).

Results

Effect of Protein Supplements on Adult *B. minax* Survival

Life expectancy

Diet influences life expectancy ($F = 89.055$, df = 3, 19, $P = 0.000$) presented in Table 1. Protein supplementation resulted in significant differences in adult life expectancies. The longest adult life expectancy was observed when flies were fed the diet F2 (40.10 ± 1.79 d) followed by the diet F3 (32.76 ± 2.66 d) ($P < 0.000$), the life expectancy on nonprotein diet (F1) observed to be (19.62 ± 0.09 d; $P = 0.024$).

Mortality dynamic

Adult mortality was observed to vary with the nutrient type supplemented. The mortality rate was highest when *B. minax* was fed with F0 and F1 diets (Table 2). F2 and F3 diets had a different effect on their mortality rates; 100% adult mortality was observed at age 65 d and 75 d when flies were fed with F2 and F3, respectively; 100% adult mortality was observed at 20 and 30 d on flies fed with F0 and F1food types, respectively. The flies fed on diet F2 and F3 showed a slow cumulative mortality (Fig. 2).The LT_{50} of adults fed with the diet F0 was the shortest (5.32 d), whereas LT_{50} was longer when flies were fed with diet F2 (14.6 d) followed by diet F3 (LT_{50} = 13.1 d) and diet F1 (LT_{50} = 9.0 d). The use of protein-enriched foods extended the peak of adult mortality (Fig. 2). In addition, when flies were fed with diets F2 and F3, their mortality peaks were delayed by 5.6 d and 4.1 d than that of adults fed with diet F1. LT90 were observed to be longer when flies were fed with F2 (43.8 d) and F3 (31.8 d). Nonprotein diet (F0 and F1) had short LT90 of 7.68 d and 16.16 d, respectively (Table 3).

Effect of Protein Supplements on *B. minax* Mating Behavior

Mating frequency

The food type influenced the mating frequency of *B. minax* ($F_{♀} = 25.617$, df = 3, 19, $P = 0.000$; $F_{♂} = 12.696$, df = 3, 19, $P = 0.000$) (Table 1). Higher mating frequency was observed when flies were fed with F2. No mating was observed when flies were fed on diet F0. Mating frequency was observed to be the lowest with 0.38 per female and 0.31 per male in the lifetime when adults *B. minax* were fed on F1 diet. (F1 vs F2, F3 $P_{♀} = 0.000$, 0.002; $P_{♂} = 0.030$, 0.001) (Table 1). There was a significant increase in mating frequencies when adults' *B. minax* were fed on diet F2 or F3 ($P < 0.05$). Hence, the mean values were 8.07 ($P = 0.000$) for a female and 4.69 ($P = 0.030$) for a male when *B. minax* was fed on the diet F2 and 5.31 ($P = 0.002$) for female and 7.30 ($P = 0.001$) for a male when the diet F3 was used. However, the mating frequency showed no statistical difference between the diets F3 and F2 for both females and males ($P_{♀} = 0.094$; $P_{♂} = 0.283$).

Mating dynamic

No mating behavior was observed for adults fed with diet F0 only (Fig. 3) By contrast, adults fed with diets F1, F2, and F3 started mating at 12, 10, and 9 feeding days, respectively. The mating was observed to end at 18 d for flies fed with diet F1, 51 d for F2, and 42 d for F3. The flies fed with diet F2 mating behavior lasted for 42 d, followed by flies fed with F3 diet lasted for 34 d (Table 4). Among the diets tested, F2 and F3 showed similar mating peaks (50% cumulative mating ratio) (Table 5).

The mating peaks (50% mating ratio) for the fruit flies fed with F2 and F3 diets were observed at 24.6 and 21.7 d old, respectively, whereas for flies fed with diet F1 the peak occurred only at 14.9 d (Fig. 3 and Table 5).

Table 1. Life expectancy, mating frequency, ovarian egg load, and female egg production data for *B. minax* maintained at different food sources (protein diet and non-protein diets)

Food sources	Life expectancy	Mating frequency		Ovarian egg load	Female oviposition
		♀	♂		
F0	5.7 ± 0.1d	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c
F1	19.6 ± 0.1c	0.38 ± 0.2b	0.31 ± 0.1b	15.0 ± 7.6b	0.0 ± 0.0c
F2	40.1 ± 1.8a	8.07 ± 0.3a	4.69 ± 0.4a	94.79 ± 6.3a	63.2 ± 8.7a
F3	32.8 ± 2.7b	5.31 ± 1.5a	7.3 ± 1.9a	77.29 ± 7.0a	19.3 ± 2.5b

The mean ± standard deviation (life expectancy, mating frequency/male or female, number of eggs/ovary, and egg production per female) are represented. **F0**–Diet containing Water alone; **F1**–Diet containing Sucrose alone; **F2**–Diet containing Sucrose and Yeast; **F3**–Diet containing Sucrose and Peptone. Different letters between food types are statistically different after Tukey's test at $P = 0.05$.

Table 2. Age-specific cumulative mortality ratio

Food sources	Age-specific cumulative mortality ratio (%)					
	5 d	10 d	30 d	50 d	65 d	75 d
F_0	37.0 ± 4.1a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
F_1	36.3 ± 12.3a	66.0 ± 8.3b	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
F_2	23.3 ± 2.1a	49.7 ± 0.6b	77.0 ± 3.1b	87.7 ± 2.3b	98.7 ± 0.6b	100.0 ± 0.0a
F_3	25.3 ± 0.6a	49.3 ± 1.5b	82.7 ± 4.4b	96.7 ± 2.1a	100.0 ± 0.0a	100.0 ± 0.0a

Arcsine transformation (arcsine square root transformation used to transform cumulative mortality data and then one-way ANOVA, means were separated by Tukey's test at $P = 0.05$. Different letters between food types are statistically different after Tukey's test at $P = 0.05$.

Effect of protein supplements on adult *B. minax* fecundity

Ovarian development

The timing of ovary development varied in relation to the type of diet the flies were fed with (Fig. 4). When fed with diets F2 and F3, the ovary of the adult *B. minax* developed faster than that of F1 diet. The ovaries of female fed with diets F2 and F3 developed to stage 2 on the 5th day, to stage 3 on the 10th day and were found to have stage 2 and stage 3 ovaries 5 d earlier than those female fed with diet F1. For females fed with diets F3, F2, and F1, the disappearance of stage 1 ovaries occurred at 20 d, 15 d, and 10 d, respectively. The disappearance time in females fed with diets F3 and F2 was shorter than for females fed with diet F1.

Ovarian egg load

The ovarian egg load of *B. minax* appeared to be different depending on the food type provided to adult flies (F = 14.979, df = 3, 46, P = 0.000) (Table 1). For females fed with water only, no egg was observed in the ovary. For females fed with diet F1, brood eggs per ovary were 15.00 ± 7.57 (n = 3), significantly lower than in females fed with diets F2 (94.79 ± 6.28; n = 24; P = 0.000) and F3 (77.29 ± 6.97; n = 45; P = 0.006). The results, however, showed there was no difference between brood eggs of females fed with diet F2 and diet F3 (P = 0.233).

Female oviposition

There were significant differences in egg production when different foods were fed (F = 43.677, df = 3, 19, P = 0.000) (Table 1).

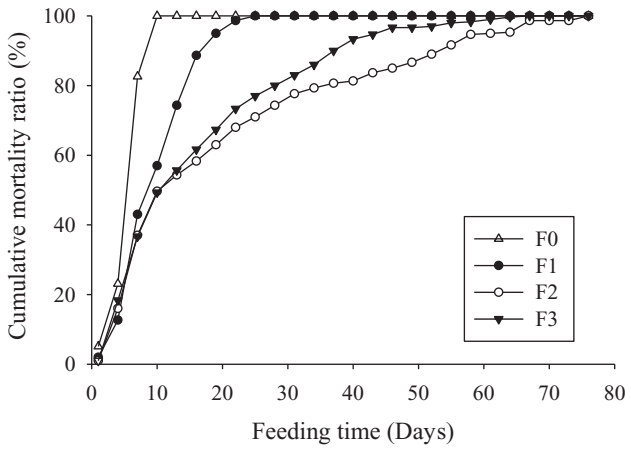

Fig. 2. Effect of different food types on cumulative mortality of adult *B. minax*.

No egg was laid when females were fed with diets F0 and F1. The mean number of eggs laid was of 63.2 ± 8.7 eggs/individual in females fed with diet F2 and 19.3 ± 2.5 eggs/individual in females fed with diet F3. The results showed a significant difference in the amount of eggs laid by females fed with diet F2 and diet F3 (P = 0.000).

Discussion

Sugar and other nutritional components like protein are necessary dietary requirement for energy production and development that greatly affect the life history traits in adults of many insect species (Bateman 1972, Jácome et al. 1999, May et al. 2015). In the present study, it was observed that provision of water or sugar alone resulted in high mortality rates of the adults' *B. minax*. However, provision of sugar and protein enhanced the survival and reproduction of adult *B. minax*. Adult mortality of 100% were observed at age 30 d on the flies fed with nonprotein diet, while at age 75 d 100% mortality was observed for protein-fed adult. These results congruent with Teal et al. (2004) who found that *Anastrepha suspensa* provided with a diet of sugar and protein lived longer than those fed with sugar alone. Sucrose diet facilitates metabolism of insect where the energy obtained extend insect life span (Lardies et al. 2004, Naya et al. 2007). Protein is an important nutritional element for insect reproduction as it contains amino acids necessary for insect oviposition (Lardies et al. 2004, Nash et al. 2014). Alamzeb et al. (2006) reported that protein is necessary for female oocytes maturation to reach vitellogenic stage. Harwood et al. (2013) also reported that protein supplement following eclosion period led to higher survival and reproductive abilities in the Mediterranean fruit fly, *C. capitata* Wiedemann and melon fly, *B. cucurbitae* Coquillett suggesting that provisioning protein-enriched foods extend fruit flies life expectancy and reproduction.

Chinese citrus fruit fly showed different mating frequencies following the food type provided. Protein diet (yeast and peptone) showed increase in mating frequencies. Mating started at early age around nine feeding days after eclosion on protein-fed adult, mating duration on protein-fed flies lasted for 42 d. Shelly et al. (2002) reported that *C. capitata* male mating was more achieved when subjected to protein diet than nonprotein diet. On females, they observed that the females were sighted more near protein-fed males than nonprotein fed males. Joachim-Bravo et al. (2009) investigated on the role of protein on sexual behavior of *C. capitata* and, observed that male fed with high protein diet showed greater number of copulation. In addition, young males and males fed with high protein

Table 3. Curve equation for regression between cumulative mortality and feeding days of *B. minax* adults fed with different food types

Food types	Regression equation	R	$R_{0.05}$	$R_{0.01}$	Feeding days	
					LT_{50}	LT_{90}
F0	Y = 102.057/(1 + Exp(4.665 – 0.869X))	1.000	0.666	0.798	5.3	7.7
F1	Y = 99.255/(1 + Exp(2.817 – 0.315X))	0.994	0.388	0.496	9.0	16.2
F2	Y = 93.472/(1 + Exp(1.364 – 0.103X))	0.967	0.413	0.526	14.6	44.9
F3	Y = 96.843/(1 + Exp(1.612 – 0.128X))	0.982	0.707	0.834	13.1	32.7

Y: represents the cumulative mortality of *B. minax*; X: feeding days (days of food supply to the flies, until death of the last fly) of *B. minax*; LT50: (Lethal Time) is the time required to record 50% adult mortality. LT90: the maximum lethal time required to record 90% adult mortality; R: coefficient of determination; $R_{0.05}$: coefficient of determination at 95%, $R_{0.01}$: coefficient of determination at 99%. (If R-value is greater than 0.05 and 0.01 are statistically significant and R-value less than 0.05 and 0.01 are not statistically significant). The adult cumulative mortality was transformed to probability units and analyzed using the logistic regression ($y = a/(1 + \text{Exp}\ (b - k\ x))$) where x is the feed days in each food and y is the cumulative mortality in probability units. Time–mortality data was fitted using probability analysis to estimate the lethal time for 50% (LT 50) and 90% (LT 90) cumulative mortality in each food treatment tested.

showed greater participation and called more often than older males and males fed with nonprotein diet (Roriz and Joachim-Bravo 2013). Egg production increased proportionally with higher mating frequencies. This trade-off interaction resulted in the reduction of female life expectancy when fed with protein diet as earlier reported by Papanastasiou et al. (2013). The ovary growth was faster when adults *B. minax* were fed F2 and F3 diet than F1. This indicates that protein supplementation resulted in a faster ovary maturation and female egg production. For example, Jácome et al. (1999) reported that female *A. serpentina* requires access to protein-enriched diets in order to produce significant number of eggs.

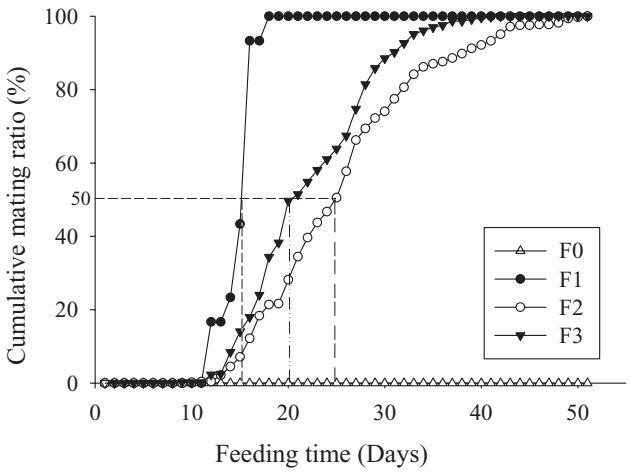

Fig. 3. Cumulative mating of adult *B. minax* fed on different food sources (50% represent adult mating peaks).

For most of the studied biological characteristics of *B. minax*, our results showed that protein source diet in *B. minax* had the highest effect on egg production and survival compared with nonprotein sources (F0 and F1) where flies died shortly. The results are in agreement with previous studies on physiology of fruit flies (Nash et al. 2014, Shinwari et al. 2015). Between the two protein sources tested, addition of yeast extract as source of protein had the highest effect compared with the addition of peptone, yeast extract showed positive response on flies survival and reproduction. The results are in agreement with previous studies on physiology of fruit flies (Nash et al. 2014, Shinwari et al. 2015). Zajitschek et al. (2012) reported that yeast diet had a strong influence on survival and female reproductive fitness. The higher effects of yeast extract can be attributed to its composition (nitrogenous compounds, carbon, sulfur, trace nutrients, vitamin B complex) and also the fact that it's made up of microorganisms from fungi groups. Apart from amino acids, other substances such as B-complex vitamins and mineral were incorporated into the yeast diets. These components play important catalytic roles in the physiology of many insect species (Fraenkel and Blewett 1943, Nash et al. 2014, Zhou et al. 2016) and are known to enhance the fecundity in fruit flies (Ben-Yosef et al. 2010).

Loss of reproductive ability (reproduction senescence) for *B. minax* was observed when flies were subjected to nonprotein diet. This explains the significance of protein on flies' reproduction. Delaying host access and protein sources result to reduced reproduction known as reproduction senescence (Harwood et al. 2013). Bonduriansky et al. (2008) reported that trade-off between survival and reproduction is influenced by time and duration of poor reproductive conditions may decrease the overall fecundity of an organism because of senescence unless the reproductive ability can be extended to advanced ages.

Table 4. Age-specific cumulative mating ratio

Food sources	Age-specific cumulative mating ratio (%)					
	5 d	15 d	18 d	30 d	42 d	51 d
F0	—	—	—	—	—	—
F1	0.0 ± 0.0a	43.3 ± 23.3a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
F2	0.0 ± 0.0a	7.1 ± 1.7a	21.4 ± 4.8b	88.5 ± 4.8b	95.1 ± 1.4b	100.0 ± 0.0a
F3	0.0 ± 0.0a	14.0 ± 2.7a	34.4 ± 7.7b	74.1 ± 7.9a	100.0 ± 0.0a	100.0 ± 0.0a

Arcsine transformation (arcsine square root transformation used to transform mating frequency data and then one-way ANOVA, means were separated by Tukey's test at $P = 0.05$. Different letters between food types are statistically different after Tukey's test at $P = 0.05$.

Table 5. Curve equation from regression analysis between cumulative mating ratio and feeding days for *B. minax* adults fed on different food sources

Food types	Regression equation	*R*	$R_{0.05}$	$R_{0.01}$	Feeding days		
					Mating onset (16%)	Mating peak (50%)	Mating end (84%)
F0	—	—	—	—	—	—	—
F1	Y = 106.933/(1 + Exp(16.30 – 1.087X))	0.972	0.775	0.875	13.4	14.9	16.2
F2	Y = 97.131/(1 + Exp(5.425 – 0.223X))	0.997	0.304	0.393	17.1	24.6	32.7
F3	Y = 99.740/(1 + Exp(5.407 – 0.249X))	0.994	0.339	0.436	15.1	21.8	28.4

Y: represents the cumulative mating ratio of *B. minax*; X: represents the feeding time (days of food supply to the flies, until death of the last fly) of *B. minax*; —: represent unmated; *R*: coefficient of determination; $R_{0.05}$: coefficient of determination at 95%, $R_{0.01}$: coefficient of determination at 99%. (If R-value is Greater than 0.05 and 0.01 are statistically significant and R-value Less than 0.05 and 0.01 are not statistically significant). The adult cumulative mating ratio in four food sources (F0, F1, F2, F3) tested was transformed to probability units and analyzed using the logistic regression ($y = a/(1 + \text{Exp}(b - k\,x))$) where x is the feeding days in each food, y is the cumulative mating ratio of *B. minax* in probability units. Mating time data were fitted using probability analysis to estimate the time for 16% (mating onset), 50% (mating peaks), and 84% (mating ends) cumulative mating in each food treatment tested.

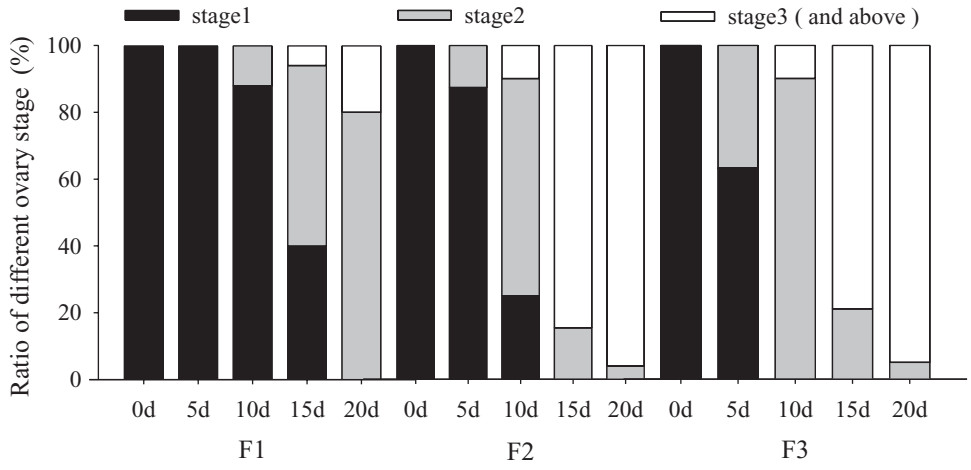

Fig. 4. Ovarian development for female *B. minax* flies fed with different food types. d: indicates feeding days when food was supplied to the flies.

During this study, both sugar and protein appeared to be essential components for the survival and reproduction of *B. minax*. The deprivation of protein and sugar from adult diets resulted in high mortality rates. However, the use of yeast extract as source protein enhanced adult life span, mating frequency, fecundity, and ovarian development in *B. minax*. Same results were obtained by Lee et al. (2008), Fanson et al. (2009), and Fanson and Taylor (2012) as diet had influence on life span and egg production on Queensland fruit fly and *D. melanogaster*. These results suggest that yeast extract is a suitable source of protein in mass rearing of *B. minax*. Nevertheless, more studies are needed to unveil other factors required for a successful artificial rearing of this devastative citrus fly so as to implement an effective management strategy of their populations.

Acknowledgments

This work was supported by the National Science Foundation of China (31572010). We thank Dr. Mazarin Akami from Huazhong Agricultural University for reviewing the final version of our manuscript.

Competing Interests

The authors have no conflict of interest.

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