Yeast Hydrolysate Deprivation and the Mating Success of Male Melon Flies (Diptera: Tephritidae)

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Yeast hydrolysate deprivation and the mating success of male melon flies (Diptera: Tephritidae)

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Abstract

Recent studies have examined the effects of adult diet on the mating success of male tephritid fruit flies. In most cases, comparisons involve males that have either been fed yeast hydrolysate continuously or not fed yeast hydrolysate at all. As such, this research does not address the more likely ecological scenario of temporal variability in protein availability faced by wild flies. The present study monitored the effect of short-term yeast hydrolysate deprivation on the mating success of male melon flies, Zeugodacus cucurbitae (Coquillett) (Diptera: Tephritidae). Males attained sexual maturation while provided a sugar-yeast hydrolysate mixture (full diet) and were then either maintained on the full diet (control males) or transferred to a sugar-only diet (treated males). Mating trials conducted in field tents showed that treated males achieved fewer successful matings than control males when tested 3 or 7 d after feeding on the sugar-only diet. However, treated males had similar mating success as control males when tested only 1 d after the diet shift. A second experiment employed the same protocol, but yeast hydrolysate-deprived males were given access to cue-lure, a male attractant known to enhance mating success, 1 d before testing. Cue-lure exposure enhanced the mating success of males deprived of yeast hydrolysate for 1 or 3 d but had no effect among males that fed on sugar alone for 7 d before testing. These findings are compared with those obtained previously on a related species, Bactrocera dorsalis (Hendel).

Key Words: Zeugodacus cucurbitae; sexual selection; nutrition; cue-lure; sexual signaling

Resumen

Estudios recientes se han examinado los efectos de la dieta de los adultos sobre el éxito de apareamiento de los machos de la mosca de la fruta tephtrítida. En la mayoría de los casos, las comparaciones involucran a machos que han sido alimentados con hidrolizado de levadura (YH) continuamente o no alimentados con YH en absoluto. Como tal, esta investigación no se trata del escenario ecológico más probable de la variabilidad temporal en la disponibilidad de proteínas enfrentada por las moscas silvestres. El presente estudio monitoreó el efecto de la privación de YH a corto plazo sobre el éxito de apareamiento de los machos de la mosca de melón, Zeugodacus cucurbitae (Coquillett) (Diptera: Tiphritidae). Los machos alcanzaron la maduración sexual mientras que fueron proveídos con una mezcla de azúcar-YH (dieta completa) y luego se mantenían en la dieta completa (machos de control) o se transfirieron a una dieta sólo de azúcar (machos tratados). Se realizaron los ensayos de apareamiento en tiendas de campaña mostraron que los machos tratados lograron menos apareamientos exitosos que los machos de control cuando se probaron 3 o 7 días después de alimentarse con la dieta de solo azúcar. Sin embargo, los machos tratados tuvieron un éxito de apareamiento similar al de los machos de control cuando se probaron sólo 1 día después del cambio en la dieta. Un segundo experimento utilizó el mismo protocolo, pero a los machos privados de YH se les dio acceso a señuelo-señal, un atractivo masculino conocido para mejorar el éxito de apareamiento, 1 día antes de la prueba. La exposición al señuelo-señal aumentó el éxito de apareamiento de los machos privados de YH durante 1 ó 3 días, pero no tuvo efecto entre los machos que se alimentaron con azúcar sólo durante 7 días antes de la prueba. Estos resultados se comparan con los obtenidos previamente en una especie relacionada, Bactrocera dorsalis (Hendel) (Diptera: Tiphritidae).

Palabras Clave: Zeugodacus cucurbitae; selección sexual; nutrición; señuelo-señal; señalización sexual

A considerable amount of research has recently focused on investigating the influence of adult nutrition on the survival and mating ability of male tephritid fruit flies (Diptera: Tephritidae) in order to improve the effectiveness of the sterile insect technique used against this group of pests. Regarding mating performance, the majority of studies report that, in competitive scenarios, males fed a standard mixture of sugar and yeast hydrolysate (yeast hydrolysate, a material with high amino acid content, Fanson & Taylor 2012) achieved significantly more successful copulations than males fed sugar only (e.g., Ceratitis capitata (Wiedemann) [Kaspi et al. 2000]; Ceratitis rosa (Karsch) [Quilici et al. 2013]; Anastrepha fraterculus (Wiedemann) [Liendo et al. 2013]; Anastrepha obliqua (Macquart) [Liedo et al. 2013]; Anastrepha serpentina (Wiedemann) and Anastrepha striata Schiner [Aluja et al. 2001]; Anastrepha suspensa (Loew) [Pereira et al. 2009]; Bactrocera dorsalis (Hendel) [Shelly et al. 2005]; Bactrocera tryoni (Froggatt) [Pérez-Staples et al. 2009]; Zeugodacus (formerly Bactrocera) [Virgilio et al. 2015] cucurbitae (Coquillett) [Haq et al. 2010a] [all Diptera: Tephritidae]). Although the factors underlying this result are often unidentified, several studies report increased sexual signaling (i.e., pheromone calling or wing-fanning) by yeast hydrolysate-fed males relative to males fed sugar only (e.g., Kaspi et al. 2000; Aluja et al. 2001). Data obtained from individually marked males of C. capitata revealed a direct relationship between male signaling activity and mating success. Therefore, it appears reasonable to conclude that heightened signaling of yeast hydrolysate-fed males is a key determinant of increased mating competitiveness. Moreover, yeast hydrolysate feeding by male tephritids has been reported to increase the probability and amount of sperm transfer during copulation as well as inhibit remating by females (e.g.,
Shelly: Mating of male melon flies


Studies that emphasize the importance of adult nutrition generally involve comparisons of extreme conditions prior to testing, i.e., male tephritids are supplied either yeast hydrolysate continuously or deprived of yeast hydrolysate. Such data are useful in development of optimal procedures for pre-release treatment of sterile males but do not address the more likely ecological scenario of temporal variability in protein availability faced by wild flies. Studies that have examined short-term food or yeast hydrolysate starvation in tephritids have uniformly described profound effects on male sexual behavior. For example, Landolt & Sivinski (1992) found that pheromone calling by A. suspensa males declined monotonically over starvation intervals of 1 to 21 h. Epsy & Heath (1993) also found that males of this same species, when fed only water for 24 h, emitted less pheromone than males continuously provided sugar-yeast hydrolysate or sugar-only diets. Short-term starvation also was reported to decrease mating success in C. capitata. Males maintained on sugar only or a sugar-yeast hydrolysate then starved for 24 h achieved significantly fewer successful copulations than males continuously fed on the respective diets (Shelly & Kennelly 2003). Finally, Shelly et al. (2007) found that males of B. dorsalis switched from a sugar-yeast hydrolysate diet to a sugar-only diet exhibited decreased mating success after 3 or 5 d of feeding on sugar exclusively.

The goal of the present study was to examine the effect of short-term yeast hydrolysate deprivation on the mating success of male melon flies, Z. cucurbitae, and compare these results with an earlier study by Shelly et al. (2007) conducted with the oriental fruit fly, B. dorsalis.

Materials and Methods

STUDY INSECTS

Flies used in this study were derived from a laboratory colony started with 300 to 400 adults reared from zucchini (Cucurbita pepo L. [Cucurbitaceae]) collected in commercial fields near Kapolei, Oahu, Hawaii, USA. The colony was maintained in a screen cage (60 × 40 × 30 cm) and provided a food mixture of sugar and yeast hydrolysate at a 5:1 volumetric ratio (“full diet”) and water ad libitum. Retail-purchased zucchinis were rinsed and provided for oviposition. Infested vegetables were placed in opaque plastic boxes that rested on wire-mesh screening over a layer of vermiculite for pupation. Larvae developed in situ, and non-infested zucchinis were added as needed to insure superabundant food. Pupae were sifted and placed in a paper bag for emergence in a Plexiglas cage (40 × 30 × 30 cm). To obtain flies for the experiments, adults were separated by sex using an aspirator within 3 d of emergence, well before reaching sexual maturity at 14 to 18 d of age (Shelly, unpublished data), and placed in cubical (30 cm per side) screen cages (approx. 250 flies per cage) with food and water as described above. All life stages were held at 23 to 27 °C and 50 to 80% RH under natural light with photoperiod of 12:12 h L:D. All flies used in the study were 7 to 9 generations removed from the wild.

MARKING FLIES

To distinguish males in different treatments, flies were marked using fluorescent dye following procedures used in the sterile insect technique (FAO/IAEA/USDA 2014). Pupae were coated with fluorescent orange or green dye (blaze orange or signal green, DayGlo Corporation, Cleveland, Ohio, USA). Upon adult emergence, dye was usually visible on the face and under the wings with a dissecting microscope under UV (black) light. However, where external dye was not conspicuous, the head was crushed with forceps to examine the collapsed ptilinum, which picks up dye particles upon emergence from the puparium. Dye color was alternated between male types over successive test dates to control for any inadvertent effects associated with color.

FIELD TENT MATING TRIALS

Two experiments were performed in which males subject to different feeding regimes competed for copulations. In both experiments, control males (as well as females) were fed the full diet and water their entire adult life and were not provided cue-lure. Treated males were subject to different intervals of yeast hydrolysate deprivation and tested either without (Experiment 1) or with (Experiment 2) access to cue-lure prior to testing. In both experiments, all individuals were sexually mature (20 to 29 d old), and for a given trial, control and treated males were the same age (+ 1 day).

Experiment 1 examined the effect of yeast hydrolysate deprivation alone on male mating success, i.e., treated males were not exposed to cue-lure. Treated males were fed the full diet and water until 1, 3, or 7 d prior to testing at which time the full diet was replaced with a Petri dish containing granular sugar. On the appropriate day (between 10:00 AM and 12:00 PM), treated males were counted and transferred (at 20 to 22 d of age) to new holding cages, where the sugar diet was introduced. As described below, individual mating trials involved 80 control and 80 treated males, but 90 treated males were transferred to allow for any pre-test mortality. To maintain uniform handling, 90 control males were transferred to new cages at the same time as the treated males, although no diet change was involved.

Experiment 2 followed the same procedure, but the treated males were given access to cue-lure 1 d before use. Cue-lure (4-(p-acetoxoyphenyl)-2-butanone) is a synthetic analogue of the naturally occurring compound raspberry ketone (4-(p-hydroxyphenyl)-2-butanone) (Vargas et al. 2010). Zeugodacus cucurbitae males are attracted to and feed on both chemicals and display enhanced mating success for 1 to 3 d after feeding (Shelly & Villalobos 1995; Shelly & Nishimoto 2017; Shelly 2000; see Kumar et al. 2013 for similar findings with Bactrocera tryoni [Froggatt]). Half ml of cue-lure was applied to a cotton wick (4 cm long, 1 cm diam) resting in a Petri dish. The cue-lure-bearing wick was introduced into the cage between 10:00 AM and 10:30 AM and removed after 3 h. This procedure is known to enhance the mating success of Z. cucurbitae males (maintained on a full diet) up to 3 d after cue-lure exposure (Shelly & Nishimoto 2017).

Mating trials were conducted during Jun to Sep 2016 in 2 nylonscreen field tents (2.5 m ht, 3 m diam) placed outside our laboratory in Halawa, Oahu, USA. Three potted guava plants, Psidium guajava L. (Myrtaceae), (2–3 m high) were placed in the center of each field tent to provide leaf surfaces for sexual activities. In Experiments 1 and 2, 80 control males, 80 treated males, and 80 females were released per tent. Both sexes were released approximately 2 h before sunset (sexual activity in Z. cucurbitae is restricted to sunset [Suzuki & Koyama 1980]). Tents were checked every 10 to 15 min from approximately 1 h before sunset to 0.5 h past sunset, using a flashligh, when necessary, to locate mating pairs under low light. Mating pairs were collected by gently coaxing them into plastic vials. At the end of a trial, the vials were placed in a freezer, and dye color was scored for individual males on the following day using a black light and a dissecting microscope. Unmated flies remaining in the tent were killed with a fly swatter prior to the next test day. For both experiments, 2 trials were run per day on 5 dates for each category of treated males. Air temperatures ranged from 24 to 28 °C during the mating trials.
LABORATORY TRIALS

Three additional experiments were performed in the laboratory to measure mating activity and wing fanning activity, respectively, of control and treated males in a non-competitive environment. In Experiment 3, mating frequency was measured in a non-competitive assay to assess the mating propensity of yeast hydrolysate-deprived treated males relative to continuously yeast hydrolysate-fed control males. Fifteen control males or 15 treated males along with 15 females were placed in individual plexiglass cages 2 h before sunset, and matings were counted 30 min after sunset. Treated males were protein-deprived for 7 d immediately prior to testing and were not exposed to cue-lure. Two cages were established with control and treated males, respectively, per test day on 4 different d (i.e., 8 total cages for each male treatment). None of the flies were marked; all of the flies were 27 to 30 d old.

Wing-fanning activity of control and treated males was monitored in Experiments 4 and 5. In both experiments, 10 control or 10 treated males along with 3 mated females were placed in individual plexiglass cages adjacent to a west-facing window 2 h before sunset, and counts of wing-fanning males were made at 1 min intervals for 45 min immediately preceding sunset. Three plexiglass cages were observed per day in both experiments. In Experiment 4, the 3 cages contained, respectively, control males, treated males deprived of yeast hydrolysate for 1 d and not provided cue-lure, or treated males deprived of yeast hydrolysate for 1 d and provided cue-lure (1 d before testing following above protocol). In Experiment 5, the 3 cages contained, respectively, control males, treated males deprived of yeast hydrolysate for 7 d and not provided cue-lure, or treated males deprived of yeast hydrolysate for 7 d and provided cue-lure. Females, which were mated 1 to 3 d before testing and hence non-receptive, were introduced to stimulate male sexual activity; no female rematings were observed in these experiments. Observations were made over 8 different d for both experiments (i.e., N = 8 cages per treatment per experiment). As in the preceding experiment, none of the flies were marked, and flies were between 27 and 30 d old.

DATA ANALYSIS

Mating success of control and treated males in field tent trials was compared using the paired t-test. Comparisons of percent mating success among treated males in different treatment categories were made using t-tests with arc sine transformed data. Numbers of matings in non-competitive conditions and counts of wing-fanning males were analyzed using parametric tests (t-test and ANOVA, respectively). Upon detection of significant between-group variation, the Holm-Šídák method for multiple comparisons was used to identify pairwise differences (P = 0.05). In all the above cases, data met the parametric assumptions of normality and equal variances. Means ± 1 SE are presented. Statistical analyses were performed using SigmaPlot 11.0 (Systat Software, San Jose, California, USA).

Results

FIELD TENT MATING TRIALS

Treated males not exposed to cue-lure and deprived of yeast hydrolysate for 3 or 7 d prior to testing achieved significantly fewer matings per replicate than control males fed yeast hydrolysate continuously (Table 1). One d of yeast hydrolysate deprivation did not reduce mating performance significantly. After cue-lure exposure (1 d before testing), treated males achieved significantly more matings per replicate than control males after only 1 d of yeast hydrolysate deprivation (Table 1). After 3 d of the sugar-only diet, cue-lure-exposed treated males had similar mating success as control males, but after 7 d of sugar-only, the treated males achieved significantly fewer matings per replicate than control males.

Results of the 2 field tent experiments are summarized in Figure 1, which describes the outcome of the mating trials in terms of the relative mating success of treated males. As the data on mating counts imply, cue-lure feeding enhanced the relative mating success of males that were yeast hydrolysate-deprived for 1 (t = 3.1; P = 0.007) or 3 (t = 3.3; P = 0.004) d but had no effect after 7 d (t = 0.4; P = 0.72) of feeding on sugar only.

| Table 1. Number of matings by control and treated Z. cucurbitae males in competitive mating trials conducted in field tents. Control males were fed sugar-yeast hydrolysate (YH) (full) diet continuously and never provided cue-lure (CL). Treated males were yeast hydrolysate-deprived (i.e., fed sugar only) for varying intervals in both Experiments 1 and 2 and provided cue-lure in Experiment 2 only. Values are means ± 1 SE; N = 10 replicates in all cases. |
|---|---|---|---|---|
| Experiment 1 – No cue-lure provided |  |
| Duration of YH deprivation (d) | Control | Treated | Paired t | P value |
| 1 | 20.2 ± 1.4 | 19.3 ± 1.3 | 0.2 | 0.0834 |
| 3 | 24.7 ± 1.2 | 18.2 ± 0.7 | 4.3 | 0.002 |
| 7 | 28.0 ± 1.4 | 17.9 ± 0.8 | 5.1 | < 0.001 |
| Experiment 2 – Cue-lure provided |  |
| Duration of YH deprivation (d) | Control | Treated | Paired t | P value |
| 1 | 16.8 ± 1.0 | 24.5 ± 1.1 | 4.5 | 0.001 |
| 3 | 20.7 ± 1.3 | 22.7 ± 1.0 | 1.0 | 0.335 |
| 7 | 26.8 ± 1.2 | 17.1 ± 1.4 | 4.2 | 0.002 |

Fig. 1. Relative mating success of treated Z. cucurbitae males in field tent experiments as a function of duration of yeast hydrolysate (YH)-deprivation and cue-lure (CL) exposure, where treated males were cue-lure-deprived in Experiment 1 (solid circles) and cue-lure-fed in Experiment 2 (open circles). Values are means ± 1 SE; N = 10 replicates in all cases.
control males that were continuously yeast hydrolysate-fed. On average, 10.0 + 0.4 matings were observed per cage for yeast hydrolysate-deprived males compared to 10.5 + 0.5 for control males ($t = 0.8; P = 0.42; df = 14$).

In Experiment 4, where treated males were deprived of YH for 1 d before testing, wing fanning levels differed significantly among treatment groups ($F_{2, 21} = 4.47; P = 0.02$), with cue-lure-fed treated males having significantly higher signaling activity than control males or treated males not provided cue-lure (Fig. 2). Signaling did not differ significantly between these latter 2 groups.

In Experiment 5, where treated males were deprived of yeast hydrolysate for 7 d before testing, wing fanning levels differed significantly among treatment groups ($F_{2, 21} = 24.1; P < 0.001$), with control males having significantly higher signaling activity than either cue-lure-deprived or cue-lure-fed males (Fig. 2). Signaling did not differ significantly between these latter 2 groups.

### Discussion

Feeding on yeast hydrolysate, which is rich in amino acids, appears critical to the mating success of *Z. cucurbitae* males. In competition with yeast hydrolysate-fed males, males that have fed only on sugar during their adult life either do not mate at all (McInnis et al. 2004) or mate only infrequently (Haq et al. 2010b). The present study further shows that, even after attaining sexual maturation on a full diet, intervals of 3 to 7 d of yeast hydrolysate deprivation reduced mating competitiveness. The no-choice trials conducted in the laboratory showed that yeast hydrolysate-deprived males are capable of mating, as nearly identical numbers of copulating pairs were observed, on average, in cages containing continuously yeast hydrolysate-fed males or males deprived of yeast hydrolysate for 7 d. However, measurement of wing fanning activity revealed that, after 7 d of feeding on sugar only, males signaled significantly less frequently than control males. Thus, it appears that yeast hydrolysate deprivation resulted in lower wing fanning levels, which, in turn, resulted in reduced mating success in competitive trials.

![Fig. 2. Wing-fanning activity of control and treated males in laboratory cages.](image)

Previous studies demonstrated that yeast hydrolysate-fed *Z. cucurbitae* males displayed heightened mating success for 1 to 3 d after feeding on cue-lure (Shelly & Nishimoto 2017), apparently owing to elevated signaling levels and not production of a more attractive pheromone (Shelly & Villalobos 1995). In the present study, males deprived of yeast hydrolysate for only 1 d and supplied cue-lure obtained significantly more matings and wing fanned significantly more frequently than control males. However, after 7 d of feeding on sugar only, cue-lure feeding had no effect on either mating success or wing fanning activity. Although wing fanning was not measured for the 3 d interval of yeast hydrolysate deprivation, results suggest that cue-lure feeding boosted wing fanning of these males. Among males deprived of yeast hydrolysate for 3 d, males not provided cue-lure obtained significantly fewer copulations than control males, whereas males given cue-lure had similar mating success as control males.

The findings presented here offer direct comparisons with those described previously for *B. dorsalis* and the male lure methyl eugenol (Shelly et al. 2007). We found that in the absence of male lure, yeast hydrolysate deprivation has identical effects on male mating success in the 2 species. At 1 d of yeast hydrolysate deprivation, both species showed no effect on mating success but at 3 or 7 d of deprivation mating success was reduced significantly. For males fed yeast hydrolysate continuously, ingestion of methyl eugenol increased the mating success of *B. dorsalis* for considerably longer post-feeding intervals compared with *Z. cucurbitae*. It appears that *Bactrocera dorsalis* males fed methyl eugenol have a mating advantage over non-methyl eugenol-fed males for at least 35 d after feeding on the lure which contrasts markedly with the 1 to 3 d advantage noted for *Z. cucurbitae* after cue-lure feeding (Shelly & Dewire 1994; Shelly & Villalobos 1995; Shelly & Nishimoto 2017). For males deprived of yeast hydrolysate, methyl eugenol had a stronger compensatory effect than cue-lure. Even after 7 d of feeding on sugar alone, *B. dorsalis* males given methyl eugenol had a significant mating advantage over fully fed methyl eugenol-deprived individuals. By contrast, *Z. cucurbitae* males given cue-lure exhibited significantly greater mating success compared with control males after 1 d of yeast hydrolysate deprivation but not after 3 or 7 d deprivation.

Although wild flies from a recently established strain were used in the current study, the results have potential relevance to the implementation of the sterile insect technique in controlling infestations of *Z. cucurbitae*. Earlier studies showed that the ability to achieve successful matings requires yeast hydrolysate in the pre-release diet (McInnis et al. 2004; Haq et al. 2010b). However, data from this study suggests that after release, sterile males must be capable of locating protein sources in the field to insure adequate sexual competitiveness over time. Moreover, studies on several tephritid species (e.g., Kaspi & Yuval 2000; Taylor et al. 2013; Utgés et al. 2013) have demonstrated that adult flies maintained on a diet containing yeast hydrolysate and later subjected to a period of complete starvation results in lower survival than flies reared on sugar alone prior to starvation. Obviously, these concerns are trivial if food is plentiful in the environment and, like sterile *C. capitata* males (Garvel et al. 2010), released males of *Z. cucurbitae* are able to locate food sources. However, if releases occur during food shortages, then yeast hydrolysate-fed males may suffer rapid mortality offsetting any mating advantage associated with that diet. Ongoing tests with sterile, mass-reared males of *Z. cucurbitae* are currently being investigated to address the above issues.

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