Time-Pattern and Frequency Analyses of Sounds Produced by Irradiated and Untreated Male *Bactrocera tryoni* (Diptera: Tephritidae) During Mating Behavior

R. W. MANKIN,[1] M. LEMON,[2] A.M.T. HARMER,[2] C. S. EVANS,[2] AND P. W. TAYLOR[2]

Ann. Entomol. Soc. Am. 101(3): 664–674 (2008)

ABSTRACT Behavior and sounds associated with mating of mass-reared irradiated and untreated (nonirradiated) *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) males were analyzed from synchronous acoustic and video records. The flies tested were from a population used in sterile release programs that help maintain fruit-fly-free areas in Australia. Males typically produce "calling," "courtship," and "copula" sounds as mating progresses. Calling sounds are variable-duration bursts of wing vibrations produced before the male orients toward the female. Rapid bursts of courtship sounds of relatively constant duration are produced after orientation toward the female. Copula sounds of variable amplitude and duration are produced after mounting. There was a significant negative correlation between courtship wingbeat frequency and wing width of untreated males, and those that copulated had a lower frequency than those that failed. Wingbeat frequencies in flight, calling, courtship, and copula sounds were significantly correlated within flies, possibly because they all are produced by the same flight motor, with amplitudes affected by the positioning of the wings relative to "stops" on the thorax. The temporal patterns of calling and courtship sounds differed significantly between irradiated and untreated males. Irradiation is known to damage insect central nervous system interneurons and thereby alter the coordination and timing of behavioral activities, but this is the first study identifying an effect of irradiation on tephritid calling and courtship sounds. Although differences in temporal patterns of calling and courtship sounds have potential to affect mating competitiveness, no differences were observed in proportions of irradiated and untreated males that copulated in the laboratory.

KEY WORDS *Bactrocera tryoni*, sterile insect technique, mating competitiveness

The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), a major pest in eastern Australia (Sutherst et al. 2000), is excluded from several important fruit-growing regions by areawide management that relies largely on public education, surveillance traps, and the sterile insect technique (SIT) (Dominiak et al. 2003, Meats et al. 2003). The goal of SIT (e.g., Vreysen et al. 2006) is to flood infested areas with reproductively sterile males that successfully compete with wild males for copulation with wild females, whose eggs then fail to develop. The population of wild flies thereby declines in the subsequent generation. The mating competitiveness of sterile males strongly influences the effectiveness of SIT (Robinson et al. 2002). Mass rearing and irradiation used to induce sterility can impair the mating competitiveness of some tephritids used in SIT programs (Cayol 2000, Briceño et al. 2002, Hendrichs et al. 2002); consequently, knowledge of the sexual behavior of fertile and sterile mass-reared *B. tryoni* males is of considerable practical interest.

B. tryoni mate only during a brief ≈30-min period around dusk (Barton Browne 1957, Tychsen and Fletcher 1971, Fletcher and Giannakakis 1973, Tychsen 1977). Initially, males position themselves on the undersides of leaves, release mate-attracting pheromones, and vibrate their wings audibly in trains of pulses 50 ms or longer. When this behavior was first identified (Monro 1953, Tychsen 1977), the sounds produced were thought to be stridulations. Neale (1989) subsequently renamed the wing vibrations as "calling sounds" based on their waveforms and frequencies, and the similarity of these behaviors to the pheromone emission and wing vibration behaviors of *Ceratitis capitata* (Wiedemann) (Webb et al. 1983) and *Bactrocera* (*Zeugodacus*) *cucurbitae* Coquillett (Kanmiya et al. 1987). When females alight on male-occupied leaves, the males orient and court them with further wing vibrations, producing bursts of "courtship

---

[1] Corresponding author: USDA–ARS, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608 (e-mail: richard.mankin@ars.usda.gov).

[2] Centre for the Integrative Study of Animal Behaviour, Macquarie University, 2109 Sydney, Australia.

sounds" (Neale 1989) < 50 ms in duration. The male may then mount, occasionally vibrating his wings to produce "copula sounds" (Neale 1989), and tapping the female with his legs while intromitting. Females can discriminate against males by choosing not to alight on a leaf or by curtailing the interaction during courtship or mounting. Hence, male performance at each of these stages is highly pertinent to SIT.

One effect of irradiation that might lead to impaired male performance is its known interference with the editing of RNA that encodes genes for ion channels in insect central nervous system (CNS) interneurons (Haddad et al. 1997). Impaired functioning of interneurons is known to cause defects in behavioral activity and mobility (O'Farrell 2001). We conducted comparative investigations to identify differences in wingbeat frequencies or pulse-train temporal patterns between irradiated and untreated males that potentially could be used by females to discriminate against irradiated males.

Materials and Methods

Flies. Pupae were obtained between April and June 2005 from general production runs of the New South Wales Department of Primary Industries, Elizabeth Macarthur Agriculture Institute, fruit fly rearing facility. Two bags of pupae were taken from a container of clean pupae after sieving and held to anoxia. After delivery to the irradiation facility at Lucas Heights, one bag was irradiated (70–75 Gy in anoxia), and the other bag was left untreated. The bags were sent to Macquarie University by courier. On arrival at the laboratory, the pupae were transferred to 1-liter plastic emergence jars filled with crumpled paper. When they emerged, most flies climbed onto the paper until their wings had hardened, and then flew up to a 5-liter maintenance cage (clear plastic, with a large mesh window for ventilation) that was inverted over the emergence jar. The maintenance cage above the emergence jar was replaced daily. The flies in each maintenance cage were supplied with granular sucrose and yeast autolysate enzymatic (MP Biomedicals, Aurora, OH) in separate petri dishes for food, and a water-soaked cotton wick for moisture. Within 4 d after emergence, well before sexual maturation of flies from this source (Perez-Staples et al. 2007, Perez-Staples et al. 2008, Prabhu et al. 2008), the flies were sorted by aspirator into separate male and female cages of 50–100 flies each and then maintained on the same diet.

The flies were held and the acoustic and video recordings were conducted in a rearing room maintained at 24–26°C and 60–70% RH. The room had uncovered windows so that the flies experienced a natural dusk. The lights were on for 12 h each day, but always switched off at least 1 h before dusk.

Acoustic and Video Recordings. Recordings were made in a sound-attenuating chamber constructed from the modified shell of a 50-liter refrigerator with new rubber seals on the door. The interior walls were lagged with two sheets of dense, 3-mm rubber and a single sheet of 12-mm soft expanded foam, and covered with 38-mm acoustic foam (Illbruck Acoustic, Inc., Minneapolis, MN). A double window of 5-mm-thick clear acrylic (200 by 300 mm, 35-mm air gap between layers) at the back of the chamber allowed light to enter from a window in the laboratory to provide a natural dusk.

Observation cages were constructed by rolling PVC-coated fiberglass mesh (0.25-mm yarn, 7.2 by 12 strands per cm; Cyclone MINIweave, Dandenong South, VIC, Australia) into 40-mm-long, 42-mm-diameter cylinders and gluing the edges together. One end of each cylinder was glued into the inside of a 42-mm internal diameter, 8-mm-high plastic ring. Mesh was glued over the outer face of the plastic ring, enclosing the end of the cylinder. The mesh cylinder then made up the walls of the cage, and the mesh glued across the end made up the roof. A clear base for video observation was constructed from an identical plastic ring onto which was glued a 5-mm-thick, 50- by 50-mm transparent acrylic plate. To seal the flies in for recording, the open end of the mesh cylindrical cage was lowered so that the open end fitted snugly into the plastic ring on the base.

Recordings were obtained from flies aged 10–14-d. On each day of recording, at least 3 h before dusk, three irradiated and three untreated males were gently transferred to separate test-cage mesh cylinders using an aspirator. An untreated female was transferred to each cylinder, which was set on its base and marked to enable identification of each fly. The six marked test cages were then placed on a bench next to the recording chamber in front of a window. When sexual activity commenced in any cage, it was removed from the bench and placed into the sound-attenuation chamber. The screen roof was positioned directly underneath a microphone (MK-10, Sennheiser, Hanover, Germany), and the base was positioned directly over the lens of an infrared sensitive video camera (model 951130 CCS-Sony-HR N266, Sony Corp., Tokyo, Japan). Supplementary infrared light for video recording was provided by an Infra-Red LED light source (930 nm) placed at the chamber window. A preamplifier (M-Box, Digidesign, Daly City, CA) provided 48-V phantom power for the microphone input. The video and sound tracks were digitized and combined in real time as a QuickTime (Apple, Cupertino, CA) movie using a DRC-1500 PCI-X card (Digital Rapids, Markham, ON, Canada) in a computer with Dual 3.2 GHz Xeon processors and four GB RAM. The audio track was uncompressed (44.1 kHz, 16 bits). The video track was compressed using Mpeg-4 at 320 by 240 pixels and 25 frames per second (standard PAL format). The behavior of pairs was recorded until activity ceased or copulation occurred. On most evenings, we were able to obtain useable recordings from two or more males.

After recording was completed for the evening, all pairs of flies were euthanized using CO_2, transferred to a labeled vial, and stored in a freezer at −19°C. The right wing of each fly was removed and mounted onto double-sided adhesive tape on a labeled microscope

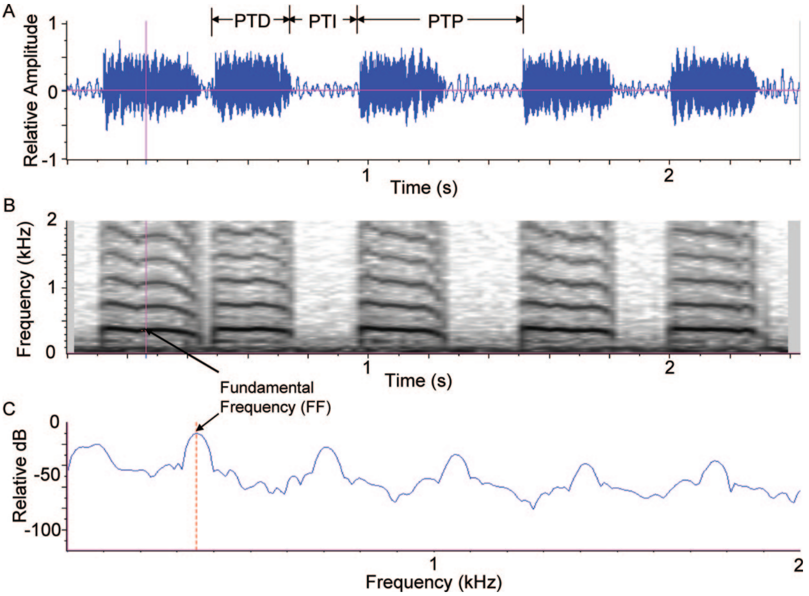

Fig. 1. Example of male *B. tryoni* calling pulse-train oscillogram (A) and spectrogram (B), with power spectrum (C) calculated by FFT of a 2048-point (0.046-s) time slice centered at time marked by a cursor in A and B.. The pulse train duration, interval between pulse trains, and the period between the start of one train and the start of the next are designated as PTD, PTI, and PTP, respectively. Darker shading in spectrogram indicates higher relative spectrum level. The FF marked by curser in C is noted also at intersection of cursor and first spectral band in B.

slide. A second slide was pressed onto the tape to protect the wings from dust. The wing was photographed through the phototube of a dissecting microscope (SZX12, Olympus, Tokyo, Japan) using a digital camera (ProgRes C-10, Jenoptik, Jena Germany) connected to an Apple iMac computer (1.8-GHz G4 PowerPC, 1-GB RAM) that was used to capture, calibrate and measure images. Images were calibrated and four distances between six landmarks on each wing were measured using ImageJ 1.36b (National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/) and saved in a spreadsheet.

Signal Processing. The QuickTime movies were edited in Final Cut Pro HD version 3.0 (Apple, Cupertino, CA) operating on an Apple G5 PowerMac (dual 2.5-GHz processors, 4.5-GB RAM). Sections of interest from each recording were marked and exported to separate files. During exporting, a time code from the parent file was added to each frame, and brightness and contrast were enhanced. Copies of the soundtracks from each of these videos were exported as Audio Interchange File (.aif) files. Because the video and audio shared a common time base, each sound could be synchronized with the corresponding behavior of the flies.

In each recording, the first bout of calling sounds and the first bout of courtship sounds with at least five consecutive pulse trains were analyzed using Raven 1.2 (Cornell Lab of Ornithology, Ithaca, NY). The start, midpoint, and end of each calling pulse train, and the start and end of each courtship pulse train were noted in a spreadsheet. The pulse train duration (PTD) was calculated as the time between the start and end of each train (Fig. 1). The pulse train interval (PTI) was the time difference between the end of a train and the start of the next train. The pulse train period (PTP) was the time difference between the starts of two consecutive trains (=PTD + PTI). Fast Fourier transformations (FFT) were calculated on 2048-point time-slices of the waveforms using a Hamming window, and spectrograms were calculated using sections with 90% overlap. The first harmonic (fundamental frequency, FF) at the start, midpoint, and end of each calling pulse train (Fig. 1), and at the midpoint of each courtship pulse train (Fig. 2) was noted in the spreadsheet. The first harmonic is also the wingbeat frequency (Webb et al. 1976).

Male or female flights and copula sounds occurred in some of the recordings. Because they were irregular in duration and did not occur in discernible temporal patterns, we measured the fundamental frequencies at the midpoints of their pulse trains but did not analyze their pulse train durations or intervals.

Statistical Analysis. JMP 4.0.2 (Sall et al. 2001) was used to calculate the mean and median values of PTD, PTI, PTP, and FF of the pulse trains in each bout. JMP also used for analyses of variance, *t*-tests, and correlation analyses of the median values of measured variables. When not otherwise specified, correlation was measured by the Pearson product moment correlation coefficient.

Results

Frequencies and Temporal Patterns of Wing Vibrations. Male *B. tryoni* produced four sounds in the context of different behaviors during the recording sessions, two of which, calling and courtship sounds,

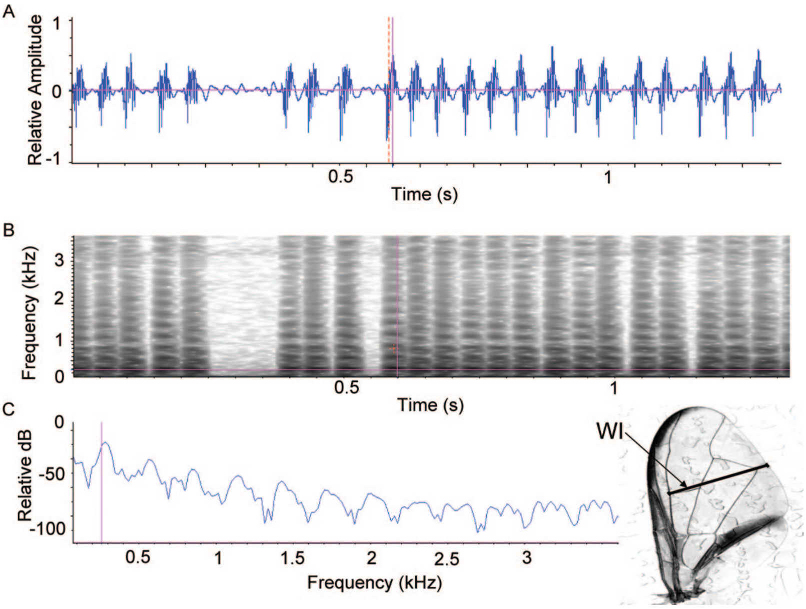

Fig. 2. Example of male *B. tryoni* courtship pulse-train oscillogram (A), and spectrogram (B), with cursor at midpoint of ninth pulse train (at 0.6 s) indicating center of time-slice for power spectrum (C). Inset at side of spectrum shows right wing of male and the position of a wing-width index, WI, measured in correlating wing width with courtship median FF.

were easily distinguished by their different pulse train durations and intervals (Figs. 1 and 2; Table 1). Irradiated and untreated males were similar in distributions of the median fundamental frequencies at midpoints of pulse trains in calling (hereafter designated as calling wingbeat frequency, Fig. 3A) and courtship (hereafter designated as courtship wingbeat frequency, Fig. 3B). They also were similar in distribution of median pulse train duration in calling (Fig. 4A) and courtship (Fig. 5A). In contrast, irradiated and untreated males differed considerably in distributions of the median intervals and periods between pulse trains in calling (Fig. 4B and C) and courtship (Fig. 5B and C).

Male sometimes produced copula sounds of relatively constant frequency and variable amplitude after mounting females (Fig. 6). In observations where cop-

Table 1. Temporal characteristics of wing-vibration pulse trains produced by untreated and irradiated male *B. tryoni* during mating

Pulse-train parameter	Mean ± SE of median	
	Untreated	Irradiated
Calling	(N = 22)	(N = 29)
Median FF at midpoint (Hz)	315.7 ± 6.2	326.8 ± 5.4
Duration (ms)	141.5 ± 22.3	150.7 ± 19.4
[a]Interval (ms) (t = −2.018, P = 0.049)	322.5 ± 69.4	508.2 ± 60.5
[b]Period (ms) (t = −2.31, P = 0.025)	473.6 ± 67.2	679.3 ± 58.5
Courtship	(N = 19)	(N = 30)
Median FF at midpoint (Hz)	304.8 ± 5.0	303.6 ± 4.0
Duration (ms)	27.57 ± 0.96	29.22 ± 0.76
[c]Interval (ms) (t = 6.78, P = 0.013)	11.76 ± 1.50	16.70 ± 1.12
[d]Period (ms) (t = 10.87, P = 0.0019)	39.47 ± 1.59	46.17 ± 1.26

FF at midpoint is the fundamental frequency (wingbeat frequency) at the midpoint of the pulse train, and n is the number of *B. tryoni* pairs observed.

[a–d] Significant differences between magnitudes of parameter values for untreated and irradiated males (t and P listed in parentheses).

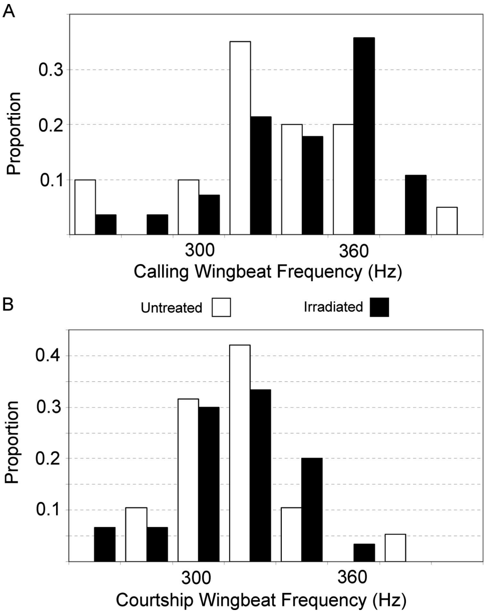

Fig. 3. Distributions of median fundamental frequencies of calling (A) and courtship (B) pulse trains (open versus solid bars for untreated versus irradiated males, respectively).

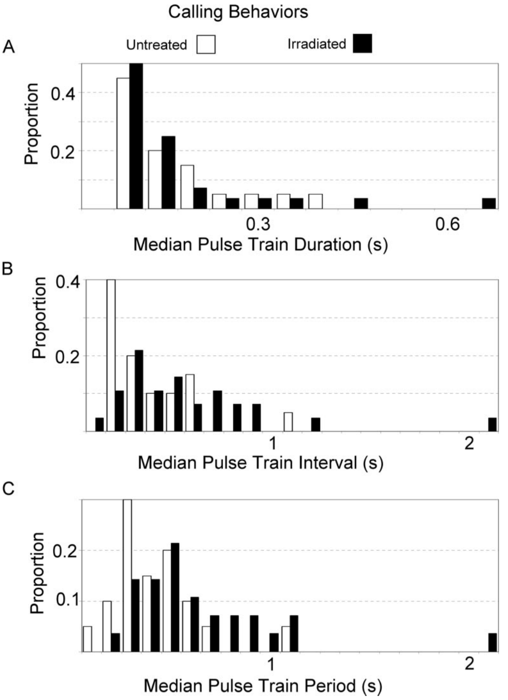

Fig. 4. Distributions of median durations (A), intervals (B), and periods (C) in calling pulse trains of untreated (open bar) and irradiated (closed bar) males.

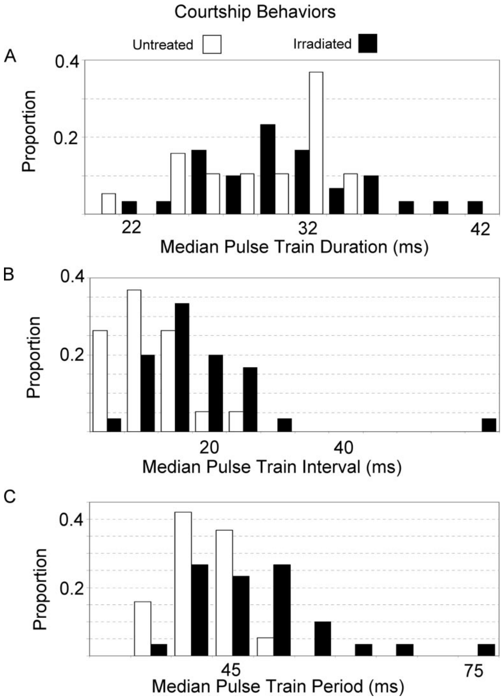

Fig. 5. Distributions of median durations (A), intervals (B), and periods (C) of courtship pulse trains of untreated (open bar) and irradiated (closed bar) males.

ula sounds occurred with calling and/or courtship sounds (Table 2), the FF at the midpoint of the first copula pulse train (hereafter designated as the copula wingbeat frequency) was significantly lower than the calling wingbeat frequency, but it was not different from the courtship wingbeat frequency.

Occasionally, a male (Fig. 7) or a female (Fig. 8) took flight during the observation period, producing a sound that could be identified by its notably lower frequency than for calling, courtship, or copula sounds (Table 2). The wingbeat frequencies in flights of irradiated males, untreated males, and untreated females were not significantly different (mean FF ± SE: irradiated male, 171.0 ± 3.95 Hz; untreated male, 174.6 ± 3.78 Hz; female, 175.1 ± 5.07; df = 2, 51; $F = 0.299$; $P = 0.74$).

Relationships between Wing Size and Wingbeat Frequencies. One of the indices of wing size measured for each tested male, a wing-width index (WI), shown in the inset of Fig. 2, was found to be significantly correlated with courtship wingbeat frequency of untreated males ($r = -0.593$, $N = 19$, $P = 0.0075$) but not irradiated males ($r = -0.11$, $N = 29$, $P = 0.57$). No significant correlations with WI were found for calling, copula, or flight FF of either irradiated or untreated males, but in all of these comparisons, wingbeat frequency was negatively correlated with WI.

Relationships between Calling and Courtship Pulse Trains. Correlations among the frequencies and temporal patterns of calling, courtship, copulation, and flight sounds were considered in separate and combined analyses of irradiated and untreated males. Almost all pairs of calling, courtship, flight, and copula FFs frequencies were significantly correlated (Table 3). These correlations were similar in irradiated and untreated males, so the two groups were pooled for the analysis.

Five other statistically significant correlations were identified in comparisons among pulse train durations and intervals (Table 3), in addition to the two expected correlations between calling PTI and PTP, and between courtship PTI and PTP. The correlations between courtship and calling median PTD, courtship median PTD and calling median PTI, and courtship and calling median PTI were significant only for irradiated males (Table 3).

Mating Success. The proportions of males that produced calling and courtship sounds, as well as the proportion that ultimately copulated, are listed in Table 4. There were no significant differences in the proportions of irradiated and untreated males performing these behaviors. However, the male flight wingbeat frequency was significantly lower for males that copulated than those that failed (169.3 ± 2.5 Hz for 36 successful copulation attempts, and 203.3 ± 8.6 Hz for three unsuccessful copulation attempts, $t = 14.597$, $P = 0.0005$). Similarly, the courtship wingbeat frequency was significantly lower for untreated males that copulated than for those that failed (299.8 ± 4.7 Hz for 16 successful copulation attempts, and 331.7 ± 7.5 Hz for three unsuccessful copulation attempts, $t = 7.238$, $P = 0.016$). The relationship between courtship

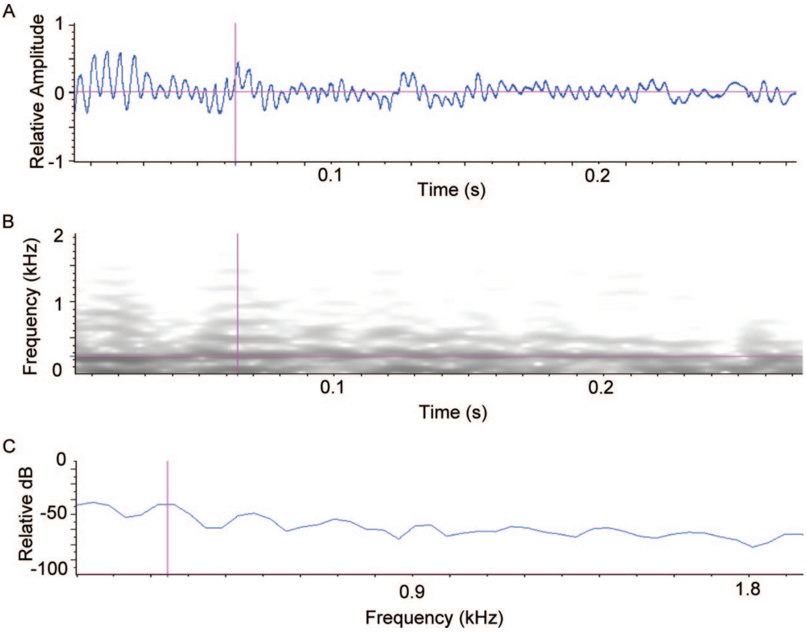

Fig. 6. Example of copula pulse train oscillogram (A), and spectrogram (B), with vertical cursors at 0.064 s indicating center of time-slice for power spectrum (C). The 244-Hz FF is indicated by a horizontal cursor in B and vertical cursor in C. Variation of amplitude in A is likely due to changes in the orientations of the wings relative to the microphone.

wingbeat frequency and copulation was not significant for irradiated males.

Discussion

Comparisons with Mating Behaviors in Related Species. The ranges of wingbeat frequencies and pulse-train durations, intervals, and periods in Tables 1 and 2 are similar in many respects to those reported previously by Neale (1989) for laboratory colonies of *B. tryoni* and a close relative, *B. neohumeralis* (Hardy). The high variation is reminiscent of that seen in wing vibration sounds of *C. capitata* (Briceño et al. 2002), other tephritids (Mankin et al. 1996), and drosophilids (Ewing and Miyan 1986). The calling wingbeat frequency distribution in Fig. 3A overlaps the distributions for both species studied by Neale (1989), who reported frequencies ranging from 250 to 412 Hz for *B. tryoni*, and from 337 to 473 Hz for *B. neohumeralis.* Likewise, the courtship wingbeat frequency distribution in Fig. 3B overlaps the distributions in Neale (1989), who reported frequencies ranging from 277 to 351 Hz for *B. tryoni* and from 331 to 439 Hz for *B. neohumeralis.* The mean of 175 ± 12.4 ms for *B. tryoni* calling PTDs in Neale (1989) is well within the 100–600-ms range of median PTDs in our study (Fig. 4A).

A greater difference was apparent in comparisons of the PTI of *B. tryoni* calling sounds, with Neale (1989) reporting a considerably shorter mean (160 ± 14.5 ms) than found in Table 1 for the median PTI of either irradiated or untreated males, but still within the relatively large overall range of 100-2000 ms in Fig. 4B. The mean reported by Neale (1989) for PTD of courtship sounds was slightly shorter (20.0 ± 1.2 ms) than in the irradiated and untreated males we observed (Table 1; Fig. 5A), but the mean PTI was appreciably longer (26.0 ± 1.3 ms). This value is higher than all PTIs observed for untreated males in our study, and at the upper end of the range observed for irradiated males (Fig. 5B). Similar results held for the PTP (Figs. 4C and 5C). These differences between studies might indicate differences in the populations from which flies were derived, but more likely stem from differences in mass-rearing-imposed selection, maintenance conditions, or testing conditions.

Correlations among Mating and Sound Production Behaviors. In many tephritid species, including *Anastrepha suspensa* (Loew) (Burk 1981, Burk and Webb 1983), *Anastrepha fraterculus* (Wiedemann) (Segura et al. 2007), and *C. capitata* (Taylor and Yuval 1999), larger males have higher mating success than small

Table 2. **Differences in fundamental frequencies (FFs) at midpoints of pulses in comparisons between copula and calling, courtship, and flight sounds in observations where both occurred**

Compared measurement[a] (Hz)	Mean difference[b]	N	t	P
Median calling FF (324.63)	54.63 ± 9.41	8	5.804	0.0007
Median courtship FF (302.55)	25.91 ± 15.38	11	1.685	0.1229
Male flight FF (165.73)	−104.6 ± 8.3	15	−12.583	<0.0001

N is the number of observations compared ± SE.

[a] Mean of the N observations of the specified FF.

[b] Mean difference between FF of compared measurement and FF at midpoint of first copula pulse train.

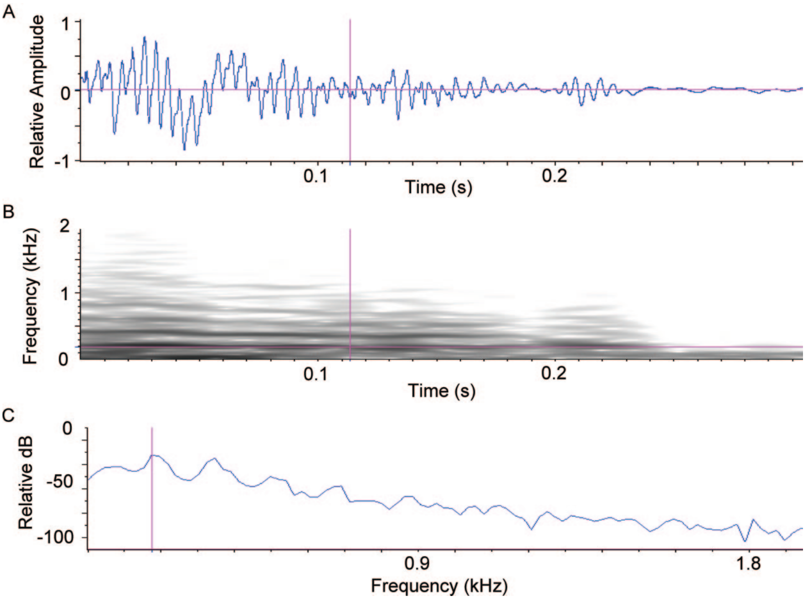

Fig. 7. Example of male flight oscillogram (A) and spectrogram (B), with vertical cursors at 0.1125 s indicating center of time-slice for power spectrum (C). The 176-Hz FF is indicated by horizontal cursor in B and vertical cursor in C.

males, possibly as a result of female discrimination. Several aspects of *B. tryoni* mating behavior observed in this study are consistent with a potential role of acoustic courtship in female selection of larger mates. Consider, for example, the result above (in Mating Success) that the courtship wingbeat frequency of males who copulated successfully was significantly lower than that of unsuccessful males, and also the finding of a negative correlation between the WI (Fig. 2) and courtship wingbeat frequency (in Relationships between Wing Size and Wingbeat Frequencies). Studies with other insects have noted negative correlations between body weight and flight wingbeat frequency (Byrne et al. 1988, Darveau et al. 2005), and positive correlations between wing size and body weight (e.g., Darveau et al. 2005). Additionally, there was a significant correlation between flight and courtship wingbeat frequencies (Table 3). Such a series of

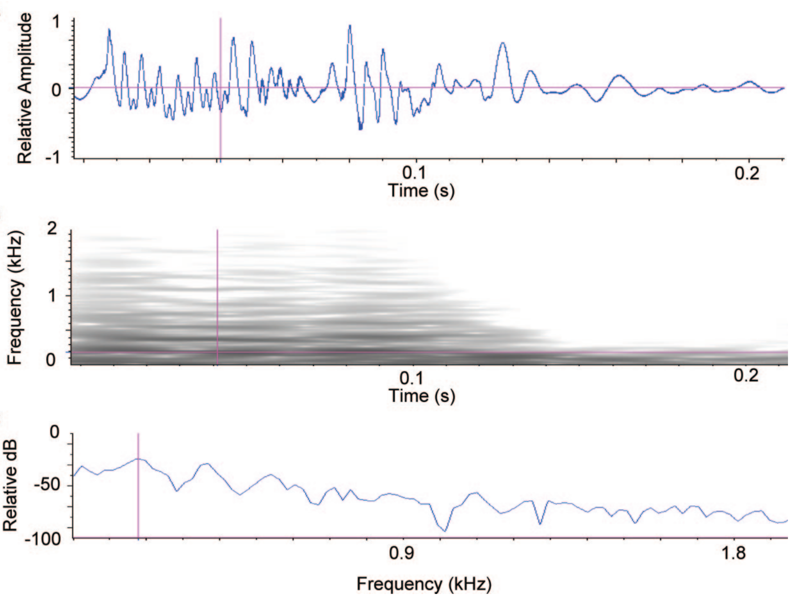

Fig. 8. Example of female flight oscillogram (A) and spectrogram (B), with vertical cursors at 0.41 s indicating center of time-slice for power spectrum (C). The 177-Hz FF is indicated by horizontal cursor in B and vertical cursor in C.

Table 3. Correlations between measured properties of sounds produced during calling, courtship, copulation, and flight: relationships among fundamental frequencies, and courtship and calling pulse train durations, intervals and periods

Measurement	Correlated measurement	N	r_s	P
Fundamental frequency				
Call-start median	Call-midpoint median	48	0.9174	<0.0001
Call-start median	Call-end median	48	0.8876	<0.0001
Call-midpoint median	Call-end median	48	0.9596	<0.0001
Court median	Call-start median	27	0.6487	<0.0001
Court-median	Call-midpoint median	27	0.6367	<0.0001
Court median	Call-end median	27	0.5234	0.0051
Court median	Male flight	31	0.3024	<0.0001
Male flight	Call-start median	21	0.5242	<0.0001
Male flight	Call-midpoint median	21	0.5770	<0.0001
Male flight	Call-end median	21	0.5273	0.0140
Male flight	Copula	14	0.5083	<0.0001
Copula	Call-start median	8	0.6717	0.0002
Copula	Call-midpoint median	8	0.7362	0.0007
Copula	Call-end median	8	0.8217	0.001
Pulse-train				
Call median PTI	Call median PTD	48	−0.312	0.0309
Court median PTP	Court median PTD	49	0.391	0.0055
Court median PTD	Call median PTD (irradiated only)[a]	18	0.550	0.0179
Court median PTD	Call median PTI (irradiated only)[b]	18	−0.520	0.0269
Court median PTI	Call median PTI (irradiated only)[c]	18	0.628	0.0053
Call median PTI	Call median PTD	48	−0.312	0.0309

N is number of *B. tryoni* ranked pairs; r_s is Spearman's rank correlation coefficient; except where specified, irradiated and untreated males were combined.

[a] $N = 9$, $r_s = -0.237$, $P = 0.539$ for untreated.

[b] $N = 9$, r_s −0.085, $P = 0.828$ for untreated.

[c] $N = 9$, r_s −0.395, $P = 0.293$ for untreated.

correlations is consistent with a hypothesis that a relatively lower courtship wingbeat frequency is associated with relatively larger male size. Consequently, a female *B. tryoni* may have potential to assess male size by assessing the fundamental frequency of the courtship sound, and, like a female *A. suspensa* (Burk and Webb 1983), may be using this information in deciding whether to copulate with a courting male.

It was not possible to confirm a direct relationship between female preference and male size in this study, however. Although untreated males that copulated did have a slightly higher wing-width index than those that failed this difference was not significant (WI = 1.705 ± 0.034 for four males that did not copulate, and WI = 1.726 ± 0.0135 for 26 males that copulated, $t = 0.326$, $P = 0.57$). It should be noted also that, as in other studies that examined the effect of male *B. tryoni* wing length on mating success (Perez-Staples et al. 2007, Perez-Staples et al. 2008, Prabhu et al. 2008), there was no significant difference in the wing length of males that copulated and those that failed. Such results suggest that additional factors besides wing size play a role in the decision by a female *B. tryoni* to copulate or not.

Table 4. Comparison of the proportions of *B. tryoni* performing calling, courtship, and copulatory behaviors in 31 tests of three untreated and three irradiated males in separate cages with untreated females

Behavior	Mean untreated	Mean irradiated	t	P
Calling	0.61 ± 0.05	0.58 ± 0.06	0.394	0.348
Courtship	0.33 ± 0.05	0.38 ± 0.06	−0.571	0.285
Copulation	0.44 ± 0.06	0.42 ± 0.06	0.261	0.398

A possible explanation for the strength of the relationships identified among the calling, courtship, and flight fundamental frequencies in Table 3 can be seen in previous studies on the biomechanics (Boettiger and Furshpan 1952, Miyan and Ewing 1985) and neuromuscular control (Machin and Pringle 1959, Nachtigall and Wilson 1967) of insect flight. In Diptera and several other insect orders, the wings are moved indirectly by asynchronous muscles attached to the thorax. The muscle-thorax flight motor operates as a resonant structure, oscillating at a rate dependent on the inertia and the stiffness of the attached load (Machin and Pringle 1959). The amplitude of dipteran wing vibrations is determined partly by the positioning of pleural sclerites which act as mechanical stops to the motion of structures that drive the wings (Boettiger and Furshpan 1952). If the behaviors that produce calling and courtship sounds involve the setting of the pleural sclerites to fixed stops that restrict the wing-vibration amplitude to a small, narrow range, the wing loading becomes negligible, and the vibration frequency will approach the maximal level of an undamped flight motor (e.g., King et al. 1996). It can be predicted, then, that the frequencies of the calling and courtship sounds of each male approach the resonance frequency of its flight motor, and the frequencies of copula and flight sounds decrease proportionately from this level as the vibration amplitude increases. Such a prediction is consistent with the results of Table 3. Similar mechanisms may also underlie the sounds of other tephritids, including *C. capitata* (Webb et al. 1983), that produce calling sounds with low-amplitude vibrations, and bees that produce low-amplitude, high-frequency vibrations for pollen sonication, defense, and soil compaction (King et al. 1996).

The phenomenon of sonication suggests the possibility of a role for tephritid calling-sound production that has not been discussed previously. The calling sounds of *B. tryoni* are structurally similar to those of several other tephritids where roles in mate attraction have been proposed or confirmed, including *B. cucurbitae* (Kanmiya et al. 1987), *A. suspensa* (Sivinski et al. 1984), and *C. capitata* (Webb et al. 1983, Mankin et al. 2004). During calling, *B. tryoni* males periodically rub the rear of their abdomen with the hind legs and then rub the wings with these legs (Neale 1989; unpublished data). This behavior very likely serves to disperse a sex pheromone that is produced in the posterior ventral wall of the rectum and released from the anus by muscular contraction (Fletcher 1968). The pheromone transferred to the wings would be volatilized more rapidly by the wing movements than by passive evaporation from the abdomen (Webb et al. 1983, Briceño and Eberhard 2000). In addition, in *B. tryoni* and several other species of the subfamily Dacinae (Kuba and Sokei 1988, Neale 1989), pheromone may be volatilized by vibration-induced transfer of pheromone to hairs on the wings, which then rub against bristles on the third abdominal tergite. A third possibility is that some of the energy produced by the flight motor is transferred to the thorax and abdomen when the wing motion is stopped by the pleural sclerites. The abdominal vibrations produced by the wing-sclerite contact could serve as an additional mechanism of pheromone dispersal from the abdomen. The vibration of stamens and anthers during sonication by bees is considered to be an effective method for releasing pollen from flowers (King and Buchmann 1996), and vibrations of surfaces containing liquids are known to be a useful method of producing aerosols (e.g., Zentner 1961).

Differences between Sounds of Irradiated and Untreated Males. The differences that we observed between wing-vibration behaviors of irradiated and untreated males were primarily in the timing of pulse train intervals (Table 1) and durations (Table 3) of calling and courtship. Conceivably, these timing differences could be the result of irradiation insults to neural timing mechanisms that have been proposed to control wing vibrations during courtship in other dipterans (e.g., Ewing and Miyan 1986). Known effects of irradiation on the CNS include interference with the editing of RNA that encodes genes for ion channels in interneurons (Haddad et al. 1997), which ultimately leads to defects in behavioral activity and mobility (O'Farrell 2001). Thus, it is not at all surprising that many studies have found irradiated mass-reared tephritids to be somewhat less competitive than their fertile mass-reared or wild counterparts. Excessive doses have massively debilitating effects on sexual competitiveness of tephritid flies (*C. capitata:* Hooper 1971, 1972; Lux et al. 2002; *A. obliqua:* Toledo et al. 2004; *B. tryoni:* Bhatti and Shipp 1972). However, even the irradiation doses that are widely accepted as suitable for SIT can reduce sexual competitiveness relative to untreated flies (Hendrichs et al. 2002, Lux et al. 2002, Rull et al. 2005).

Lux et al. (2002) found that irradiated male *C. capitata* were less likely to call and overall seemed "less sexually motivated." Hence, the reduced sexual competitiveness of irradiated flies in that study may have resulted from low levels of sexual effort. Results of the current study suggest, in addition, that effects of irradiation on male calling and courtship sounds also could play a role in reduction of sexual competitiveness. For example, the PTIs and PTPs of calling and courtship sounds produced by irradiated males were significantly longer than those of untreated males (Table 1), perhaps reflecting a diminished ability to maintain or recover from the effort of sound production. This is the first study to have identified an effect of irradiation on the calling and courtship sounds of a tephritid fly.

Nonetheless, the observed differences in temporal patterns of wing vibrations produced by irradiated and untreated male *B. tryoni* did not result in differential mating success in the test cages (Table 4). Similarly, Harmer et al. (2006) and Perez-Staples et al. (2007) found no differences in mating tendency of irradiated and untreated *B. tryoni* when tested in 1-liter cages in the laboratory. However, laboratory mating behavior bioassays do not necessarily provide an accurate assessment of male competitiveness in the field (Hendrichs et al. 2002). Lux et al. (2002) found, for example, that the effects of irradiation dose on the competitiveness of male *C. capitata* were more apparent in large field cages than in small laboratory cages. Consequently, it currently remains unclear whether the observed effects of irradiation on the mating sounds of mass-reared male *B. tryoni* have any bearing on their sexual competitiveness under natural conditions.

Acknowledgments

We gratefully acknowledge the assistance of staff at New South Wales Department of Primary Industries, especially Laura Jiang and Selliah Sundaralingam who generously provided access to fly stocks from the factory producing flies for use in the sterile insect technique. This research was made possible by funds from The Australia & Pacific Science Foundation and Macquarie University Research and Development Grant (to P.W.T.).

References Cited

Barton Browne, L. 1957. The effect of light on the mating behaviour of the Queensland fruit fly *Strumeta tryoni* (Frogg.). Aust. J. Zool. 5: 145–158.

Bhatti, M. A., and E. Shipp. 1972. Mating competition of irradiated and normal males with normal females of the Queensland fruit fly, *Dacus tryoni* (Frogg.). Nucleus 9: 1–2.

Boettiger, E. G., and E. Furshpan. 1952. The mechanics of flight movements in Diptera. Biol. Bull. 102: 200–211.

Briceño, R. D., and W. G. Eberhard. 2000. Male wing positions during courtship by Mediterranean fruit flies (*Ceratitis capitata*) (Diptera: Tephritidae). J. Kans. Entomol. Soc. 73: 143–147.

Briceño, R. D., W. G. Eberhard, J. C. Vilardi, P. Liedo, and T. E. Shelly. 2002. Variation in the intermittent buzzing songs of male medflies (Diptera: Tephritidae) associated with geography, mass-rearing, and courtship success. Fla. Entomol. 85: 32–40.

Burk, T. 1981. Signaling and sex in acalyptrate flies. Fla. Entomol. 64: 30–43.

Burk, T., and J. C. Webb. 1983. Effect of male size on calling propensity, song parameters, and mating success in Caribbean fruit flies, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 76: 678–682.

Byrne, D. N., S. L. Buchmann, and H. G. Spangler. 1988. Relationship between wing loading, wingbeat frequency and body mass in homopterous insects. J. Exp. Biol. 135: 9–23.

Cayol, J. P. 2000. Changes in sexual behavior and in some life history traits of tephritid species caused by mass-rearing processes, pp. 83–860. *In* M. Aluja and A. Norrbom [eds.], Fruit flies (Tephritidae): phylogeny and evolution of behavior. CRC, Boca Raton, FL.

Darveau, C.-A., P. W. Hochachka, K. C. Welch, Jr., D. W. Roubik, and R. K. Suarez. 2005. Allometric scaling of flight energetics in Panamanian orchid bees: a comparative phylogenetic approach. J. Exp. Biol. 208: 3581–3591.

Dominiak, B. C., A. E. Westcott, and I. M. Barchia. 2003. Release of sterile Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), at Sydney Australia. Aust. J. Exp. Agric. 43: 419–528.

Ewing, A. W., and J. A. Miyan. 1986. Sexual selection, sexual isolation and the evolution of song in the *Drosophila repleta* group of species. Anim. Behav. 34: 421–429.

Fletcher, B. S. 1968. Storage and release of a sex pheromone by the Queensland fruit fly, *Dacus tryoni* (Diptera: Trypetidae). Nature (Lond.) 219: 631–632.

Fletcher, B. S., and A. Giannakakis. 1973. Factors limiting the response of females of the Queensland fruit fly, *Dacus tryoni,* to the sex pheromone of the male. J. Insect. Physiol. 19: 1147–1155.

Haddad, G. G., Y. Sun, R. J. Wyman, and T. Xu. 1997. Genetic basis of tolerance to O_2 deprivation in *Drosophila melanogaster.* Proc Natl. Acad. Sci. U. S.A. 94: 10809–10812.

Harmer, A.M.T., P. Radhakrishnan, and P. W. Taylor. 2006. Remating inhibition in female Queensland fruit flies: effects and correlates of sperm storage. J. Insect Physiol. 52: 179–186.

Hendrichs, J., A. S. Robinson, J. P. Cayol, and W. Enkerlin. 2002. Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies. Fla. Entomol. 85: 1–13.

Hooper, G.H.S. 1971. Competitiveness of gamma-sterilized males of the Mediterranean fruit fly: effect of irradiating pupal or adult stage and of irradiating pupae in nitrogen. J. Econ. Entomol. 64: 1364–1368.

Hooper, G.H.S. 1972. Sterilization of the Mediterranean fruit fly with gamma radiation: effect on male competitiveness and change in fertility of females alternately mated with irradiated and untreated males. J. Econ. Entomol. 65: 1–6.

Kanmiya, K., A. Tanaka, H. Kamiwada, K. Nakagawa, and T. Nishioka. 1987. Time-domain analysis of the male courtship songs produced by wild, mass-reared, and by irradiated melon flies, *Dacus curcubitae* Coquillett (Diptera: Tephritidae). Appl. Entomol. Zool. 22: 181–194.

King, J. J., and S. L. Buchmann. 1996. Sonication dispensing of pollen from *Solanum laciniatum* flowers. Funct. Ecol. 10: 449–456.

King, M. J., S. L. Buchmann, and H. Spangler. 1996. Activity of asynchronous flight muscle from two bee families during sonication (buzzing). J. Exp. Biol. 199: 2317–2321.

Kuba, H., and Y. Sokei. 1988. The production of pheromone clouds by spraying in the melon fly, *Dacus curcubitae* Coquillett (Diptera: Tephritidae). J. Ethol. 6: 105–110.

Lux, S. A., J. C. Vilardi, P. Liedo, K. Gaggl, G. E. Calcagno, F. N. Munyiri, M. T. Vera, and F. Manso. 2002. Effects of irradiation on the courtship behavior of medfly (Diptera: Tephritidae) mass reared for the sterile insect technique. Fla. Entomol. 85: 102–112.

Machin, K. E., and J.W.S. Pringle. 1959. The physiology of insect fibrillar muscle. II. Mechanical properties of a beetle flight muscle. Proc. R. Soc. Lond. B. 151: 204–225.

Mankin, R. W., J. B. Anderson, A. Mizrach, N. D. Epsky, D. Shuman, R. R. Heath, M. Mazor, A. Hetzroni, J. Grinshpun, P. W. Taylor, and S. L. Garrett. 2004. Broadcasts of wing-fanning vibrations recorded from calling male *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) increase captures of females in traps. J. Econ. Entomol. 97: 1299–1309.

Mankin, R. W., A. Malavasi, and C. Aquino. 1996. Acoustical comparisons of calling songs from *Anastrepha* species in Brazil, pp. 37–41. *In* B. A. McPheron and G. J. Steck [eds.], Fruit fly pests. St. Lucie Press, Delray Beach, FL.

Meats, A. W., R. Duthie, A. D. Clift, and B. C. Dominiak. 2003. Trials on variants of the sterile insect technique (SIT) for suppression of populations of the Queensland fruit fly in small towns neighboring a quarantine (exclusion) zone. Aust. J. Exp. Agric. 43: 389–395.

Miyan, J. A., and A. W. Ewing. 1985. How Diptera move their wings: a re-examination of the wing base articulation and muscle systems concerned with flight. Philos. Trans. R. Soc. Lond. B 311: 271–302.

Monro, J. 1953. Stridulation in the Queensland fruit fly, *Dacus* (*Strumenta*) *tryoni.* (Frogg.). Aust. J. Sci. 16: 60–62.

Nachtigall, W., and D. M. Wilson. 1967. Neuro-muscular control of dipteran flight. J. Exp. Biol. 47: 77–97.

Neale, C. F. 1989. Some aspects of the specific mate recognition systems of *Dacus tryoni* and *Dacus neohumeralis.* M.S. thesis, University of Queensland, Brisbane, Australia.

O'Farrell, P. H. 2001. Conserved responses to oxygen deprivation. J. Clin. Invest. 107: 671–673.

Perez-Staples, D., V. Prabhu, and P. W. Taylor. 2007. Post-teneral protein feeding enhances sexual performance of Queensland fruit flies. Physiol. Entomol. 32: 225–232.

Perez-Staples, D., A.M.T. Harmer, S. R. Collins, and P.W. Taylor. 2008. Potential of pre-release diet supplements to increase the sexual performance and longevity of male Queensland fruit flies. Agric. For. Entomol. (in press).

Prabhu, V., D. Perez-Staples, and P. W. Taylor. 2008. Protein:carbohydrate ratios promoting sexual activity and longevity of male Queensland fruit flies. J. Appl. Entomol. (in press).

Robinson, A. S., J. P. Cayol, and J. Hendrichs. 2002. Recent findings on medfly sexual behavior: implications for SIT. Fla. Entomol. 85: 171–181.

Rull, J., O. Brunel, and M. E. Mendez. 2005. Mass rearing history negatively affects mating success of male *Anastrepha ludens* (Diptera: Tephritidae) reared for sterile insect technique programs. J. Econ. Entomol. 98: 1510–1516.

Sall, J., A. Lehman, and L. Creighton. 2001. JMP start statistics—a guide to statistics and data analysis using JUMP and JMP IN software. SAS Institute, Cary, NC.

Segura, D., N. Petit-Marty, R. Sciurano, T. Vera, G. Calcagno, A. Allinghi, P. G. Cendra, J. Claderak, and J. Vilardi. 2007. Lekking behavior of *Anastrepha fraterculus* (Diptera: Tephritidae). Fla. Entomol. 90: 154–162.

Sivinski, J., T. Burk, and J. C. Webb. 1984. Acoustic courtship signals in the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Anim. Behav. 32: 1011–1016.

Sutherst, R. W., B. S. Collyer, and T. Yonow. 2000. The vulnerability of Australian horticulture to the Queensland fruit fly, *Bactrocera* (*Dacus*) *tryoni*, under climate change. Aust. J. Agric. Res. 51: 467–480.

Taylor, P. W., and B. Yuval. 1999. Postcopulatory sexual selection in Mediterranean fruit flies: advantages for large and protein-fed males. Anim. Behav. 58: 247–254.

Toledo, J., J. Rull, A. Oropeza, E. Hernández, and P. Liedo. 2004. Irradiation of *Anastrepha obliqua* Macquart (Diptera: Tephritidae) revisited: optimizing sterility induction. J. Econ. Entomol. 97: 383–389.

Tychsen, P. H. 1977. Mating behaviour of the Queensland fruit fly, *Dacus tryoni* (Diptera: Tephritidae), in field cages. J. Aust. Entomol. Soc. 16: 459–465.

Tychsen, P. H., and B. S. Fletcher. 1971. Studies on the rhythm of mating in the Queensland fruit fly, *Dacus tryoni.*. J. Insect. Physiol. 17: 2139–2156.

Vreysen, M.J.B., J. Hendrichs, and W. R. Enkerlin. 2006. The sterile insect technique as a component of sustainable area-wide integrated pest management of selected horticultural insect pests. J. Fruit Ornam. Plant Res. 14 (Suppl. 3): 107–131.

Webb, J. C., J. L. Sharp, D. L. Chambers, J. J. McDow, and J. C. Benner. 1976. Analysis and identification of sounds produced by the male Caribbean fruit fly, *Anastrepha suspensa.* Ann. Entomol. Soc. Am. 69: 415–420.

Webb, J. C., C. O. Calkins, D. L. Chambers, W. Schwienbacher, and K. Russ. 1983. Acoustical aspects of the behavior of Mediterranean fruit fly, *Ceratitis capitata:* analysis and identification of courtship sounds. Entomol. Exp. Appl. 33: 1–8.

Zentner, R. J. 1961. Techniques of aerosol formation. Microbiol. Mol. Biol. Rev. 25: 188–193.

Received 22 July 2007; accepted 28 December 2007.