

ACOUSTICAL COURTSHIP SIGNALS AND SEXUAL SUCCESS  
IN IRRADIATED CARIBBEAN FRUIT FLIES  
(*ANASTREPHA SUSPENS*A)  
(DIPTERA: TEPHRITIDAE)

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ABSTRACT

The effects of radiation on the two acoustical sexual signals of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), were examined by irradiating flies at 2.5, 5, 7.5, and 10 kR 2-26 h before emergence and noting the effects on sound production and sexual behaviors. The pulse train intervals of the calling song increased with dosage, while the propensity to produce the sound decreased. The pulse trains of control songs consisted of two frequencies. The lower of these frequencies became lower yet with increased radiation but the higher frequency was unchanged. When flies were irradiated 24-48 h before eclosion, low frequency sounds decreased in proportion and often were absent altogether. The fundamental frequency of the precopulatory song was also lower in flies irradiated with higher dosages. The pulse trains were longer in 10 kR irradiated flies, and may be indicative of relative sexual incompetence. Several of the changes in the sound production of irradiated flies may make them less effective as sexual advertisements. These include increased pulse train interval, decreased calling sound propensity, and lowering of the sound frequencies. However, our measures of sexual success, time after introduction until mating, mating attempts, and mating duration showed no significant relationship with radiation dosage.

RESUMEN

Se examinaron los efectos de radiación en las dos señales acústicas sexuales de la mosca de frutas del Caribe, *Anastrepha suspensa* (Loew), irradiando moscas a 2.5, 5, 7.5 y 10 kR 2-26 horas antes de su salida y notando los efectos del sonido producido y el comportamiento sexual. Los intervalos del pulso del canto de llamada aumentó con la dosis, mientras que disminuyó la propensidad a producir el sonido. El pulso del canto del testigo consistió de dos frecuencias. La más baja de estas frecuencias fue todavía más baja con el aumento de radiación, mientras que no cambió la frecuencia alta. Cuando las moscas se irradiaron 24-48 horas antes de su eclosión, disminuyeron en proporción los sonidos de baja frecuencia y a menudo estaban totalmente ausente. La frecuencia fundamental del canto precopulatorio también fue más baja en las moscas irradiadas con dosis altas. El pulso fue más largo en moscas irradiadas con kR y pudiera ser indicativo de incompetencia sexual relativa. Algunos de los cambios en la producción de sonido de moscas irradiadas las pudieran hacer menos efectivas como anunciantes sexuales. Estos incluyen aumento en los intervalos del pulso del canto, inución en la propensidad de los cantos y reducción de las frecuencias del sonido. Sin nuestra medida del éxito sexual, tiempo después de su introducción hasta el acoplamiento, atentados de acoplamiento y la duración del acoplamiento, no indicaron una relación significativa con dosis de radiación.

The failure of sterile insects released to control pests is sometimes ascribed to poor mating interactions due to courtship incompetence in the sterilized insects (Burk and Calkins 1983). Certain Tephritidae have complex acoustical courtship components, in addition to pheromones and postures, so that there are particularly extensive possibilities for the breakdown of sexual communications among irradiated flies (Sivinski et al. 1984).

In *Anastrepha suspensa* (Loew), the Caribbean fruit fly, there are two types of sexual sound signals generated by wing fanning. The calling sound is produced by pheromone emitting males within mating aggregations (leks). Precopulatory sound is made by males as they mount females and attempt intromission. The acoustical properties of these signals were described by Webb et al. (1976). Females use the acoustical characteristics and the intensity of sound signals in choosing mates (Burk and Webb 1983, Webb et al. 1984, Sivinski et al. 1984). The calling sound is a highly structured bifrequent signal composed of repeated pulse trains. It has been shown that prophylactic antibiotic treatment of diet and larval irradiation can alter the structure of this signal (Greany et al. 1977, Sharp and Webb 1977). The high levels of male advertisement, apparent female choice and large variance in male reproductive success could present difficulties for sterile insect release programs. In species such as *A. suspensa*, sterilized males with flawed communicative abilities might be far less competitive than their wild rivals, more so than males in species having less complex mating systems (see Burk and Calkins 1983). Because of this potential sensitivity, the effects of various radiation dosages were examined on the sexual behavior, mating success, and more specifically, the structure of acoustical advertisements.

#### METHODS

Caribbean fruit flies used in this study were from a colony kept for over a decade at the Insect Attractants, Behavior, and Basic Biology Research Laboratory, ARS, USDA, Gainesville, Florida.

Pupae were irradiated with a  $^{137}\text{Cs}$  source (Radiation Machinery Gammator M<sup>®</sup>) at a dosage rate of 1732 Roentgens/min until pupae had been exposed to 0, 2.5, 5, 7.5, or 10 kR, respectively. After irradiation, the pupae were placed in a cage and flies that emerged during the following 2 h were discarded. Males that emerged during the subsequent 24 h were held for experimentation. Males irradiated at 5 kR 24 and 48 h before eclosion were also compared because the earlier the irradiation occurs in the pupal state, the greater the possibility that normal development and sound production will be disrupted.

Flies were kept in 20 x 20 x 20-cm screen-mesh cages containing water and yeast hydrolysate. The temperature was 20-22°C and photoperiod 12L:12D. At 10 days of age, the calling sound was recorded on magnetic tape. Males were then individually placed in 7.5-cm diam, 8-cm high screen wire cages. The next day an unirradiated virgin female was added to the cage. Sexual behaviors were noted and a recording of the precopulatory sound was made (similar to Burk and Webb 1983). The sound pressure level (SPL) in dB (0 dB re 20  $\mu$  Pa) of the precopulation song was measured at a distance of 12 mm from the fly. The SPL of the calling song was not determined because the pulse train duration was generally shorter than the response time of the SPL meter, a problem that did not occur with the relatively continuous precopulatory song. After mating, flies were killed and weighed.

After 30 min of observation, the males were placed into four categories of sexual success: copulated on first attempt, copulated after initial failure(s) to mount, attempted mounting(s) but failed, and never attempted to mate.

Signals were detected with a 25-mm Brüel & Kjaer® (B&K) Model 4145 condenser microphone that had a frequency response of 5 Hz to 20 kHz  $\pm$  3 dB and an open circuit sensitivity of -25.5 (0 dB re 1V per pa). The microphone was coupled through a cathode follower to a B&K model 2608 microphone amplifier. The output of the amplifier was interfaced to a Hewlett-Packard 3964A® tape recorder where the analog data were stored. Data were analyzed with a Nicolet® 660A dual channel Fast Fourier Transform (FFT) computing spectrum analyzer equipped with a Nicolet 160C 5 1/4-in storage disc drive. The following factors were calculated from the analysis of the recorded data: fundamental frequency, total voltage under the spectrum analysis curve, percent waveform distortion, bandwidth or frequency spread of the first harmonic (fundamental frequency), pulse train duration, and pulse train interval. For the definition of these factors, see glossary of terms in Webb et al. (1984). Statistical analysis was by regression, analysis of variance, *t*-test and correlation analysis.

## RESULTS

Gamma radiation had no discernible effect on many behaviors known or suspected to be related to sexual success (see Sivinski et al. 1984, Webb et al. 1984). There was no correlation between radiation dosage and time to mate after introduction of female ( $r = -.08$ ,  $n = 100$ ,  $p = .43$ ), nor duration of mating ( $r = .05$ ,  $n = 95$ ,  $p = .62$ ), and the more irradiated males were just as likely to attempt mounting a female (no. of mounts/ $\times$  against no. of males  $n_{0.0} = 57$ ,  $N_{2.5} = 45$ ,  $N_s = 56$ ,  $N_{7.5} = 29$ ,  $N_{10} = 34$ ;  $r = -.44$   $p > .10$ ).

Acoustic signal production and signal structures were influenced by radiation. Calling sound production was decreased (Fig. 1a). The interval between pulse trains became longer with increased radiation rising from a mean length of .41 sec at 0 kR ( $n = 51$ ) to .59 sec at 10 kR ( $n = 34$ ) (Fig. 1b;  $p = .02$ ). The structure of the sound itself was also affected. A pulse train typically consisted of two fundamental frequencies, a high frequency component followed by a generally shorter segment of lower frequency. While the higher frequency was unchanged by radiation, the lower frequency became progressively lower as dosages increased ( $F = 11.0$ ,  $p < .001$ ). As a result the difference between the high and low frequencies was significantly less in controls ( $\bar{\chi}$  control 17,  $S = 6.8$ ,  $n = 37$  vs.  $\bar{\chi}$  irradiated 21 Hz,  $S = 91.$ ,  $t = -3.0$ ,  $p = .004$ ,  $n = 128$ ). There was no evidence that flies with less difference between their high and low frequencies had greater sexual success ( $F = 1.37$ ,  $p > 0.25$ ).

Previous examination of irradiated flies indicated that at 5 kR the low frequency was often absent altogether (Sharp and Webb 1977). We found no evidence that flies irradiated with any dosage up to 10 kR within 24 h of eclosion differed in the ratio of energies produced at high and low frequencies (such ratios indicate the relative proportions of the two frequency segments;  $F = 0.5$ ,  $p = 0.49$ ). However, when flies were exposed to 5 kR 24 to 48 h before emergence, as they were in Sharp and Webb (1977), the lower frequencies were significantly shorter or of less amplitude or both ( $\bar{\chi}$  .32,  $S = 0.31$ ,  $n = 25$  vs.  $\bar{\chi}$  .54;  $S = 0.43$ ,  $n = 25$ ,  $t = -2.3$ ,  $p = .02$ ).

The sound pressure level of the precopulatory sound was not significantly changed by radiation ( $\bar{\chi}$  control 57 dB  $S = 14.7$ ,  $n = 11$  vs.  $\bar{\chi}$  irradiated 48.7 dB,  $S = 18.9$ ,  $n = 47$ ,  $t = 1.4$ ,  $p = 0.17$ ); neither was the distortion of the song (i.e., the proportion of the total energy that lies under the fundamental frequency curve;  $F = 0.00$ ,  $p = 0.98$ ). However, the fundamental frequency at 7.5 and 10 kR is lower than that of controls as well as those of lower radiation dosages ( $F = 5.1$ ,  $p < 0.03$ , Fig. 2).

Precopulatory sounds were significantly longer in flies that had been irradiated at 10 kR ( $F = 3.2$ ,  $p < 0.02$ , Fig. 3).

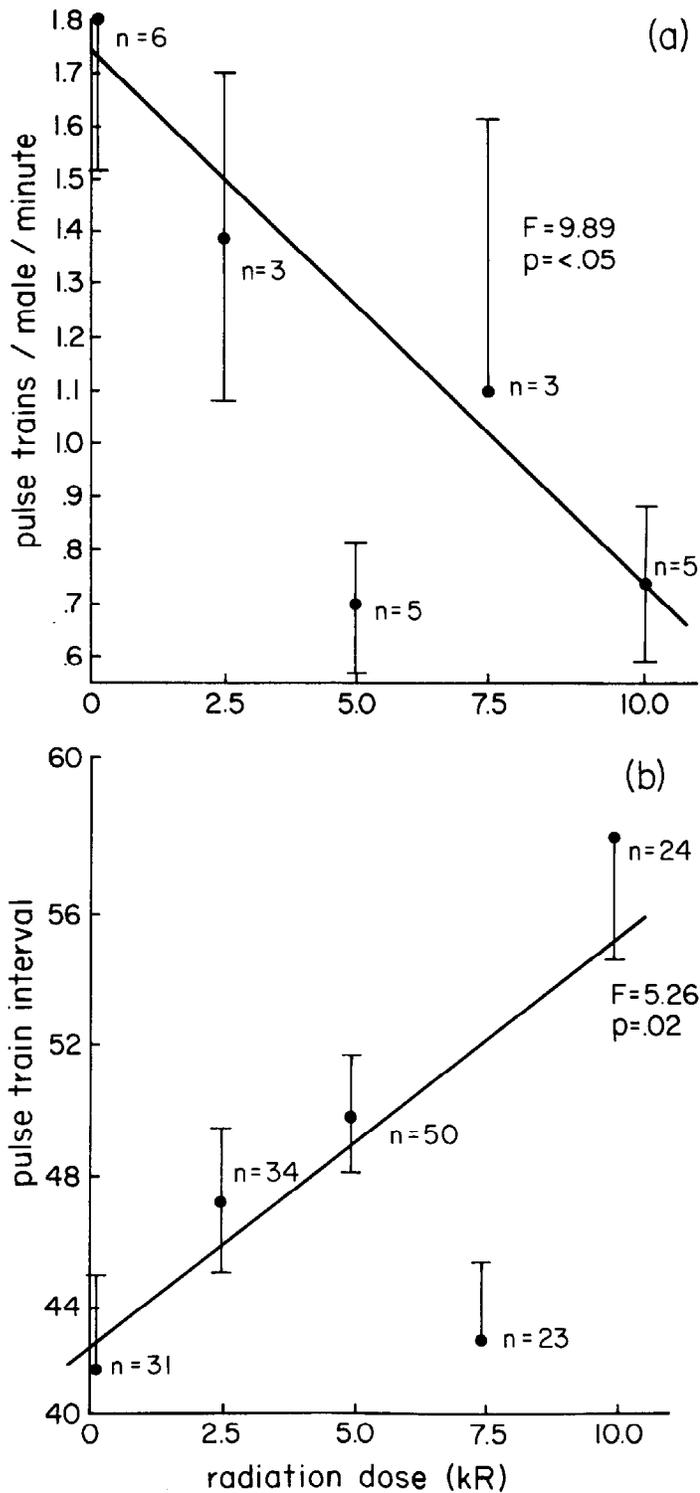


Fig. 1. a). The mean production of calling song pulse trains by 30 males over 5 min with various exposures to radiation,  $n$  = number of replications. b). The mean interval (in hundredths of a sec) between pulse trains in the calling songs of males exposed to various dosages of radiation. Cross bars represent  $\pm$  SE.

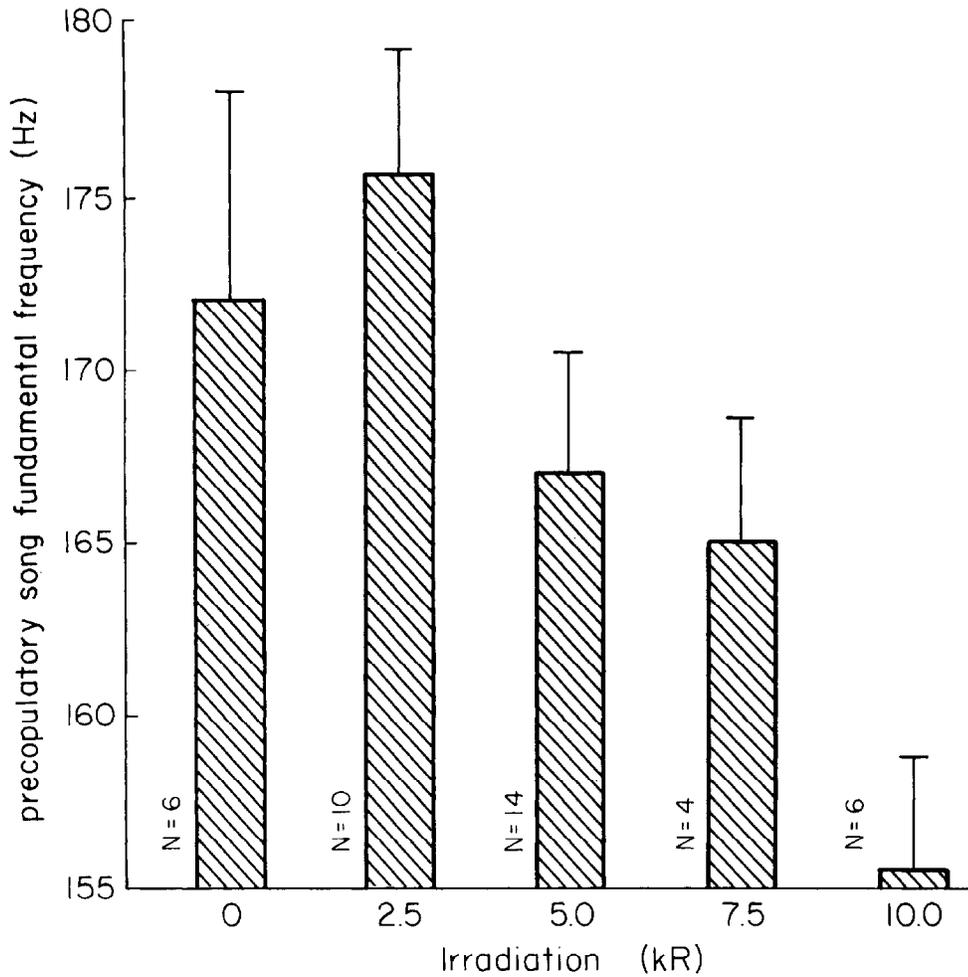


Fig. 2. The mean fundamental frequency of the precopulatory song of males exposed to various dosages of radiation. Thinner bar represents SE.

#### DISCUSSION

Certain changes in the sounds of irradiated flies may result in a less effective sexual broadcast signal. The increase in pulse-train interval could be particularly disadvantageous. Virgin females react less to sounds conspicuous for their long pulse-train interval (Sivinski et al. 1984), and males invest in presumably more energy-expensive, short-interval songs when placed next to females (Sivinski and Webb 1986). It appears that the longer pulse trains are indicative of a less vigorous fly. The decreased song production in irradiated flies would presumably result in such males being less detectable by searching females.

Broad bandwidth of the first harmonic is a correlate of poor sexual performance in the Caribbean fruit fly (Webb et al. 1984). Improperly sifted Mediterranean fruit fly pupae sometimes develop as adults, a condition known as "droopy wing" that is reflected in a broader bandwidth (Webb et al., unpublished data, see Ozaki and Kobayashi 1981). While we found no significant expansion of the bandwidth, the increasing distance between high and low frequencies with irradiation might be due to damage analogous to, but less dramatic than, that causing droopy wing.

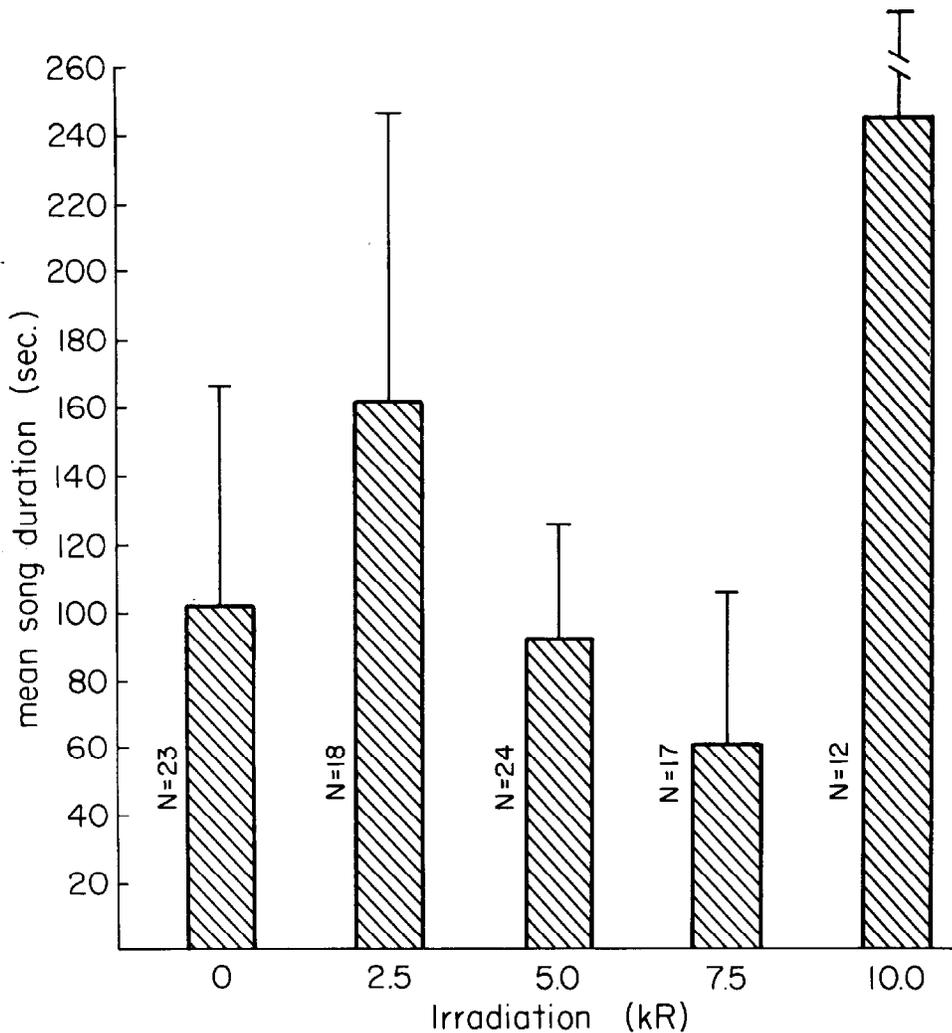


Fig. 3. The mean duration of precopulatory song in males exposed to different levels of radiation. Thinner bar represents SE (SE at 10 kr = 66).

The loss of the low frequency component of the calling sound in flies irradiated 24-48 h before eclosion but not in those irradiated within 24 h emphasizes that damage to signals and behavior can be minimized by irradiating as late as possible during the developmental process.

The intensity, frequency and duration of the precopulatory song are known to influence male acceptance by females (Sivinski et al. 1984). While intensity of precopulatory sound production is unchanged, radiation may otherwise effect the endurance of the producer. The increased duration of the sound signal might be indicative of its poor performance as an advertisement (Webb et al. 1984). Precopulatory sounds often continue until the male genitals completely penetrate the female, and long song may be indicative of a male having difficulty inserting his aedeagus into an uncooperative female. Another possible flaw is the lower frequency of irradiated fly sound. There is speculation that frequency is important to the effectiveness of the signal in eliciting female acceptance. Precopulatory sound frequency has a low variance compared to the calling song

and is not correlated to size, an outstanding feature of calling sound frequency (Webb et al. 1984). This may reflect selection for a precise signal.

Given that radiation has modified signal characteristics in ways that ought to make them less attractive, it is surprising that sexual success was affected so little. The artificiality of the cage arena could account for some of the discrepancy. For instance, loss of range in a calling sound makes little difference when flies are constrained within several cm of each other. Then, too, the years of colonization may have bred less discriminating females.

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