

## ACOUSTIC COURTSHIP SIGNALS IN THE CARIBBEAN FRUIT FLY, *ANASTREPHA SUSPENS*A (LOEW)

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**Abstract.** Male Caribbean fruit flies, *Anastrepha suspensa* (Loew) produce two sounds in sexual contexts, calling songs and precopulatory songs. Calling song occurs during pheromone release from territories within leks and consists of repeated bursts of sound (pulse trains). Virgin female *A. suspensa* became more active in the presence of recorded calling songs. Activity during the broadcast of a heterospecific song did not differ from movement during periods of silence. A conspecific song typical of smaller males, i.e. conspicuous for its long periods between pulse trains, also failed to elicit more activity by virgin females than silence. Mated females were most active during silences. Unmated males had no obvious reaction to sound. Calling songs are apparently sexually important communications which females discriminate among and may use as cues for locating and/or choosing between mates. Precopulatory song is produced by mounted males just before and during the early stages of copulation. Males that did not produce such songs remained coupled for shorter periods, perhaps passing fewer sperm. Wingless (muted) males were more likely to complete aedeagal insertion if a recorded precopulatory song was broadcast. Calling song played at the same level (90 dB) had no significant effect on the acceptance of males, nor did precopulatory song at a lower SPL (52 dB). Precopulatory song may be used to display male vigour to choosing females.

A few acalypterate flies produce sounds to attract or court mates, e.g. the 'love songs' of *Drosophila* spp. (Bennet-Clark & Ewing 1970; Burk 1981). Certain Tephritidae have particularly rich, but poorly understood, acoustic repertoires. Males of *Anastrepha suspensa* (Loew), the Caribbean fruit fly or caribfly, make two sounds in sexual contexts. The calling song is produced by wing-fanning and is simultaneous with the emission of a pheromone by flies defending territories in male aggregations (leks); the precopulatory song is also produced by wing-fanning and is performed immediately prior to and during coupling (Webb et al. 1976; Burk 1983). Calling song consists of wing-generated pulse trains (ca 150 Hz) lasting a mean of 420 ms and repeated at mean intervals of 420 ms (Webb et al., in press), while precopulatory songs are continuous sounds (ca 170 Hz) lasting a few seconds to 25 min (Webb et al., in press).

Calling sounds have been regarded as artifacts of wing-mediated pheromone dispersal. However, sticky traps in field cages baited with recorded calling song capture more caged female Caribbean fruit flies than silent controls (Webb et al. 1983). We present further evidence that *A. suspensa* adults respond to calling song, and that their reactions have sexual significance and

do not result from an indiscriminating sensitivity to noise.

Precopulatory song has been considered the final acoustic element of courtship. Circumstantial evidence suggests that females judging male quality may use the intensity of sound to decide whether mounted males will be allowed to copulate (Burke & Webb 1983; Webb et al., in press). Similar songs produced by the African drosophilids *Zaprionus* spp. also are considered factors in intersexual selection (Bennet-Clark et al. 1980). We demonstrate that the precopulatory song does influence female choice of mating partners and that both song structure and sound pressure level (i.e. intensity) are important to a male's sexual success.

### Methods and Results

Flies were removed as 1-3-day-old virgins from colonies at the Insect Attractants, Behavior, and Basic Biology Research Laboratory, ARS/USDA, Gainesville, Florida. They were then kept in groups of 50 in 20 × 20 × 20-cm plastic screen-mesh cages until sexual maturity (10-14 days of age). Observations were made in an anechoic chamber. Temperatures averaged 26°C (± 1°SD).

### Calling Song versus Silence

Fifty caged virgin female flies were observed in a 20 × 20 × 20-cm screen-mesh cage. Activity

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in this and all calling sound experiments was quantified by scoring one count every time a fly crossed a line bisecting the cage. After a 5-min acclimation period, recorded calling song and alternating silences were presented in 5-min blocks for 30 min. Order of presentation was randomized. The 10-cm speaker was located overhead and the sound pressure level (SPL) of the song was 50 dB (0 dB re 20  $\mu$  Pa) measured in the centre of the cage (the mean SPL of a calling fly at 12 mm is circa 60 dB). Experiments began at 1300 EST (12 D scotophase beginning at 1730 hours). There were 19 replicates made.

**Results.** In all replicates, there was greater mean activity during the broadcast of calling song. Increases ranged from 3 to 315 movements (mean movements/fly/min of calling song = 0.41, mean movements/fly/min of silence = 0.37;  $P < 0.0005$ , Wilcoxon paired test; see Fig. 1).

#### Calling Songs of *A. suspensa* and *Dacus neohumeralis* Hardy versus Silence

In order to discover if reactions were the result of an indiscriminate sensitivity to noise, we com-

pared movement during conspecific and hetero-specific calling songs. That is, we wished to discover if any sound would cause a reaction in virgin females, or only those produced by conspecifics. In order to make the most conservative possible test we chose *D. neohumeralis* which, of the species available to us, had the song most similar to that of *A. suspensa* (mean pulse train duration = 118.5 ms, interval between pulse trains = 296.4 ms, fundamental frequency = 379 Hz). Fifty flies were exposed to 10 min of silence followed by either 10 min of *A. suspensa* or 10 min of *D. neohumeralis* calling song. Activity was measured by the number of crossings of a bisecting line. Five replicates of each song type were made.

**Results.** *A. suspensa* calling song resulted in an increase of activity (0.11 movements/fly/min of silence versus 0.14 movements/fly/min of calling song;  $P < 0.03$ , Wilcoxon paired test), while movement during the heterospecific calling song did not differ significantly from that during silence (0.14 movements/fly/min of silence versus 0.14 movements/fly/min of *D. neohumeralis* song;  $P > 0.25$ ).

#### Effects of Gender and Sexual Experience on Response to Different Conspecific Calling Songs

In order to determine if reproductive state influences the reaction to calling song, virgin females, mated females and unmated males were exposed in groups of 25 to 90-s blocks of two calling songs and alternating periods of silence for 30 min. The two songs differed conspicuously in the length of the interval between pulse trains (pulse train interval = PTI; short-PTI mean = 100 ms, long-PTI mean = 1115 ms; pulse train durations, 273 versus 277 ms). These songs were chosen because we believed they were representative of the songs made by large and small males respectively (Burk & Webb 1983). Large males are preferred as mates (Burk & Webb 1983). The SPL was 55 dB in the centre of the cage. Females were presumed mated after being kept with males during maturation (densities were kept equal by housing 25 males with 25 females). The activities of males, virgin females and mated females of the same age were observed. Seven replicates of each type were made. Because we felt the results from mated females were of particular importance, an additional four replicates were made. These results were nearly identical, so the data were pooled.

**Results.** There was a reversal in female activity following mating (Table I, Fig. 2). Virgin

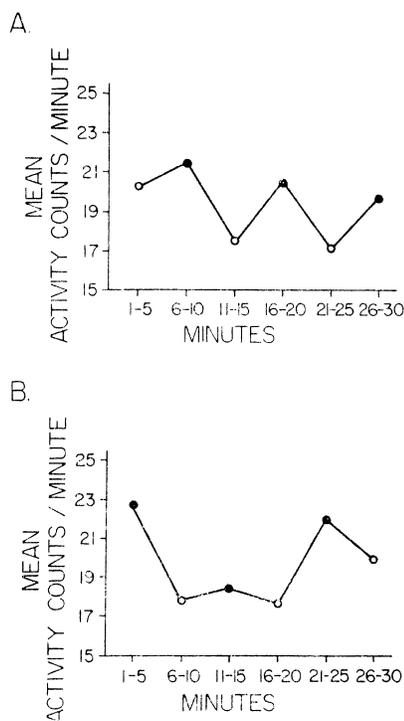


Fig. 1. Mean levels of female activity during alternating periods of broadcast conspecific calling song and silence. (A) represents replicates in which silence was offered first and (B) those in which song preceded silence.

Table I. Activity of Males and Virgin and 'Mated' Females Exposed to Silence or to Conspecific Calling Songs Differing Conspicuously in Pulse Train Interval (PTI)

	Mean movements/min/fly		
	Short PTI (mean $\pm$ SD)	Long PTI (mean $\pm$ SD)	Silence (mean $\pm$ SD)
Virgin females	0.64 $\pm$ 0.20	0.56 $\pm$ 0.20	0.55 $\pm$ 0.19
'Mated' females*	0.16 $\pm$ 0.20	0.18 $\pm$ 0.23	0.21 $\pm$ 0.26
Males	0.18 $\pm$ 0.05	0.17 $\pm$ 0.06	0.20 $\pm$ 0.07
			<i>P</i>
Virgin females			
Short PTI > silence			<0.05†
Long PTI versus silence			NS
Short PTI versus long PTI			NS
'Mated' females			
Short PTI < silence			<0.025
Long PTI < silence			0.01
Short PTI versus long PTI			NS
Males			
Short PTI versus silence			NS
Long PTI versus silence			NS
Short PTI versus long PTI			NS

\*'Mated' females were those kept with males for 8–12 days and presumed inseminated.

†Statistically tested by random block analysis of variance.

females moved about more during short-PTI calling song, while mated females were most active during silences. However, virgins did not react to a conspecific call conspicuous for its long pulse train interval. Males have no obvious reaction to calling song.

#### Effects of Male Presence and Insemination

It was possible that females kept with males in the previous experiment changed their behaviours because of the continuous presence of males rather than copulation itself. For instance, days of bombardment by male signals might result in habituation and dulling of response.

We performed two experiments to distinguish between the effects of male presence and actual insemination. First, males were allowed to signal toward females at close range but not to mate. To accomplish this, 10 males were kept in a 7.5  $\times$  7.5 cm screen cylinder with their own food and water. The cylinder was placed inside a standard rearing cage for 8–12 days with 50 virgin females. Female response to a short-PTI male calling song was measured at 10–14 days of age. Six replicates of 25 females each were made.

In the second experiment, females were allowed to mate but exposure to males was kept at a minimum. Females were released into a 30  $\times$  30  $\times$  30 cm screen cage along with approxi-

mately equal numbers of males. Mating pairs were immediately placed into vials. At the completion of copulation the females were retained and their mates discarded. Females had close contact with males for periods of only 30–120 min. Reactions to long- and short-PTI calling songs were measured 24 h later. Six replicates of 25 females each were made.

**Results.** Long-term exposure to sexually restrained males affected the reactions of virgin females to calling songs. The previously highly predictable reaction to short-PTI song failed to

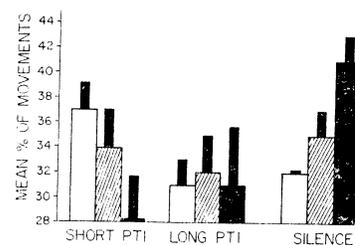


Fig. 2. The mean percentage of total activity that occurred during the acoustic broadcasts of short-PTI calling song, long-PTI calling song and silence. Virgin females (white bars), males (hatched bars) and mated females (wide black bars) are compared. Standard errors are represented by the narrower black bars.

occur (mean 0.12 movements/fly/min of broadcast, versus 0.17 movements/fly/min of silence).

Females that were known to have mated but had only minimum exposure to male sexual signals had patterns of response to song very similar to those females that had long periods of access to sexual partners and were presumed mated (0.51 movements/fly/min of broadcast, versus 0.69 movements/fly/min of silence;  $P=0.03$  Wilcoxon paired test).

#### The Effect of Precopulatory Song on Acceptance of Males

Circumstantial evidence suggests that the precopulatory song is used as a last cue in courtship, i.e. that females decide to allow penetration by mounted males on the basis of song quality (Burk & Webb 1983; Webb et al., in press). In order to demonstrate the influence of precopulatory song on female propensity to reject mounted males, we adapted experiments performed by Bennet-Clark & Ewing (1970) on *Drosophila* 'love singing'.

Male wings were clipped in the region of the alula, resulting in a much reduced sound output (<8% of original intensity). Five such wingless males and five winged females were placed in  $7.5 \times 7.5$ -cm wire-screen cylinders. Two cylinders at a time were observed during a 30-min period of silence or acoustic treatment. The order of treatment was alternated between replicates. Successful and unsuccessful mating attempts were recorded (the latter were defined as a male being dislodged from a female's back after at least one second of mounting). Our acoustic treatments consisted of the following sounds: (1) Precopulatory song at 90 dB (measured at the cage top); this broadcast was an attempt to recreate the signal that dealation had removed from courtship. Ninety dB was our estimate of the precopulatory song sound pressure level at the female's arista, which are known hearing organs located approximately 2 mm from the mounted males' wings (Sivinski & Webb, unpublished data); this figure may be an underestimate, since it fails to take into account the effect of near-field particle velocity on the female's ability to detect vibration. (2) Calling song at 90 dB; by broadcasting conspecific calling song (circa 450 ms sound followed by 390 ms of silence), we expected to discover if the form of the signal was important or if other sounds perceived at the appropriate moment were sufficient stimuli. Note that the SPL of short-duration intermittent sounds is difficult to measure, owing

to the inertia of the meter. We consider 90 dB to be a reasonable estimate rather than an accurate measurement of our broadcast. (3) Precopulatory song at 52 dB; relatively quiet precopulatory broadcasts were used to determine if SPL was important in female sexual decisions. Respective totals of 250, 200 and 190 males were observed, to give similarly sized samples of sexual interactions, i.e. 175, 179 and 184 mountings.

**Results.** Precopulatory song broadcast at 90 dB increased the proportion of males allowed to copulate (Fig. 3a). Calling song played at the same SPL, however, had no significant effect on male acceptance, showing that the structure of the signal is important (Fig. 3b). The precopu-

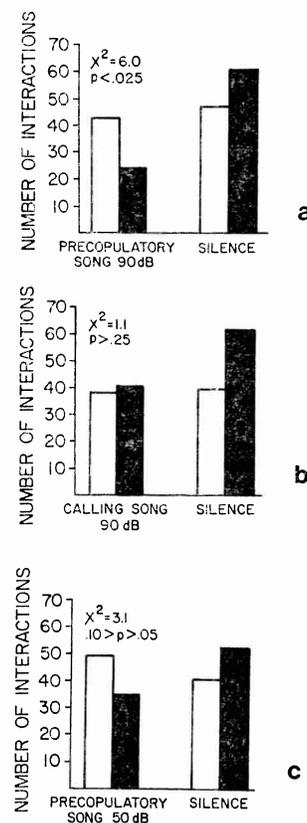


Fig. 3. The numbers of successful and unsuccessful mating attempts made during periods of silence and broadcast sounds. White bars represent matings, black bars rejections. (a) Precopulatory song at 90 dB, an attempt to recreate the signal of an intact mounted male; (b) calling song at 90 dB, a high-volume broadcast of an inappropriate signal; (c) precopulatory song at 50 dB, a low-intensity broadcast of an appropriate signal.

latory song played at a lower SPL did not significantly affect male acceptance (Fig. 3c).

### The Effect of Precopulatory Song on Copulation

Intact male Caribbean fruit flies occasionally do not produce precopulatory songs (for example, 3% of a sample of 125 observed by Webb et al., in press). Using males from a long-established laboratory colony, we followed the copulations of 68 males that did not sing and 134 that did. We also observed 20 wild males that did not sing and 18 that did, and looked for differences in sexual success between singers and muted individuals.

**Results.** Laboratory-reared males that produced precopulatory songs had longer matings, 33.2 min versus 27.2 min ( $t$ -test,  $P < 0.05$ ; see Fig. 4). There was no significant difference, however, in our smaller sample of wild males (37.4 min versus 31.6 min). Two possible advantages of longer mating durations are that more ejaculate may be transferred and perhaps that the female may be less likely to remate (see Farias et al. 1972 on the tephritid *Ceratitis capitata* (Wied.); also Thornhill 1977 on Mecoptera). In the previous experiment, wingless males and their mates that heard 90-dB precopulatory song had copulation durations that bordered on being significantly longer than coupling that occurred in silence (mean 33.4 versus 25.9 min,  $N = 14, 15$ ;  $t$ -test,  $P = 0.08$ ).

### Discussion

The consistently greater activity of virgin females when exposed to calling song might be either an effect of a sensitivity to noise, or an adaptive re-

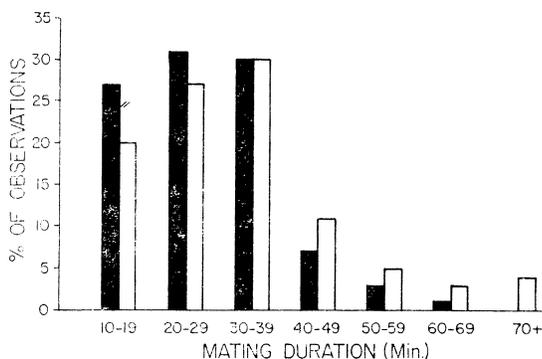


Fig. 4. The distribution of mating durations of laboratory-reared males that produced or failed to produce a precopulatory song. White bars represent singing males, black bars silent males.

sponse to a potential mate. The latter suggests an ability to discriminate between sounds. A comparison of reactions to conspecific and heterospecific calling sounds revealed that only *A. suspensa* song generates greater activity than silence. Further evidence of discrimination was the ability of one conspecific song (short-PTI) to elicit activity while another (long-PTI) did not (Table I). We chose these songs in the belief that PTI was inversely correlated with the sexually attractive character of large male size (Burk & Webb 1983; only weakly correlated, however, in Webb et al., in press). The lack of female response to a long-PTI song may be due to 'lack of interest' in the broadcast of a low-quality mate (see Burk & Webb 1983).

Further support for the contention that calling song serves as sexual advertisement is given by the different reactions of receivers with disparate reproductive interests (Fig. 2, Table I). Mated females are generally less active and move most during periods of silence, while males show no significant differences in activity. The decline in movement by mated females during calling song may be a means of avoiding the harassing attention of unwanted suitors.

There can be no further doubt that calling song has sexual significance. We are unsure however of its precise role. Attempts to demonstrate orientation or movement toward song in the laboratory have been unsuccessful (unpublished data). But data obtained under semi-natural conditions in field cages suggest that females are attracted to calling song (Webb et al. 1983).

The demonstration that 90-dB broadcasts of precopulatory song increased the acceptance of males is proof of its role in courtship. Circumstantial evidence had suggested that females choosing mates listened for signs of vigour in male singing. The songs of successful males are louder, and have a narrower frequency bandwidth than those of rejected males (Burk & Webb 1983; Webb et al., in press). Large males are preferred as mates, and the sound pressure level of singing is correlated to weight (Webb et al., in press). The statistical lack of response to 52-dB broadcasts is further evidence that less intense sounds are less effective in eliciting female sexual cooperation. Our finding that song form is important (i.e. the failure of loud calling song to increase male acceptance) may illuminate a previous and unexpected finding that the fundamental frequency of precopulatory song, unlike that of calling song, is uncorrelated to male size

and has a much lower variance than in calling song (Webb et al., in press). One interpretation is that females are sexually selected to prefer a particular song form that all males strive to produce.

Besides the initial effect of precopulatory song on acceptance, singing may affect the quality of the subsequent copulation. It seems possible that the singing males we observed to mate longer (and perhaps pass larger ejaculates) than non-singers, did so because they sang. The nearly significant increase in copulation durations for wingless (muted) males whose mates heard the precopulatory song broadcast is suggestive.

As with the calling song, there can be no further doubt that the precopulatory song serves a role in the courtship of Caribbean fruit flies.

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