

Acoustic Signals Produced During Courtship in *Diachasmimorpha* (= *Biosteres*) *longicaudata* (Hymenoptera: Braconidae) and Other Braconidae

JOHN SIVINSKI AND J. C. WEBB

Insect Attractants, Behavior, and Basic Biology Research Laboratory,
USDA-ARS, Gainesville, Florida 32604

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ABSTRACT Sounds produced by courting males of three species of Braconidae as they approach and then mount females differ in frequency, sound duration, and intervals between sounds. *Diachasmimorpha longicaudata* (Ashmead), a parasitoid of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), has a sexually dimorphic response to broadcasts of recorded "approach song" that suggests the sound has a sexual function. Sounds directed at other males have longer pulse trains (buzzes) and shorter intervals between pulse trains than those sung to potential mates. *D. longicaudata* males have more oval wings than females, as is the case in some acoustically signaling Diptera. *Microplitis croceipes* (Cresson) and *Cotesia marginiventris* (Cresson) produce multiple frequency sounds that result in a warbling effect. The song of the latter species is very complex and includes two distinct forms of pulse trains.

KEY WORDS Insecta, mating sounds, sexual selection, parasitism

AMONG THE PARASITIC HYMENOPTERA, male wing vibrations are an almost universal component of courtship (Matthews 1974, 1975; Gordh & DeBach 1978). The function of such movements has not always been clear. In the ichneumonid *Campoletis sonorensis* (Cameron), wing beating pulls air from in front of the male over his dorsal and posterior surfaces. Such an airflow could aid in orientation to female odors (Vinson 1972). Although the vibrations are similar to the more familiar acoustic courtship signals of Diptera (e.g., Sivinski et al. 1984), evidence is mixed that the wings might generate sexually important sounds. Dealated male *Aphytus* species achieve 100% insemination in spite of the mutilation (Rao & DeBach 1969), whereas similarly treated *Nasonta vitripennis* (Walker) suffer a reduction in, but not an elimination of, copulation (Miller & Tsao [1974], see also Kitano [1975] on *Apanteles glomeratus*). Dealation of the chalcid *Brachymeria intermedia* (Nees) diminishes the intensity of sounds produced by males, but apparently it does not influence male sexual success (Leonard & Ringo 1978). Removal of male wings influences female behavior in the braconid *Cardiochiles nigricaps* Viereck. Females are more likely to decamp when confronted with wingless males and so sounds may have a quieting effect on females (Vinson 1978, see also Miller & Tsao 1974).

The following describes the forms of male sound produced by three species of Braconidae: *Diachasmimorpha* (= *Biosteres*) *longicaudata* (Ashmead), *Microplitis croceipes* (Cresson), and *Cotesia marginiventris* (Cresson). For one of these, the widely established tephritid fruit fly biocontrol agent *D.*

longicaudata, the sexual significance of sound, its social plasticity, and means of production also are examined.

Materials and Methods

Diachasmimorpha longicaudata were obtained from colonies maintained at the University of Florida Institute of Food and Agricultural Sciences, Agricultural Research and Education Center, Homestead, Fla.; from the Department of Zoology, University of Florida, Gainesville; and from the Insect Attractants, Behavior, and Basic Biology Research Laboratory, USDA-ARS, Gainesville. *M. croceipes* and *C. marginiventris* were taken from colonies at the latter-named facility. Insects were held individually and recorded within 72 h of eclosion. When feeding was required, they were fed honey and given water on dampened wicks. Voucher specimens from the colonies are in the author's collection, which will be placed in the Florida State Arthropod Collection, Department of Plant Industry, Gainesville. Statistical analysis was performed either with a statistical computer package (SAS Institute 1982) or through tests described by (Zar 1974).

Description of Sounds. Recordings of songs were made in an anechoic chamber with a Brüel and Kjaer (B&K, Marlborough, Mass.) Model 4145 condenser microphone (frequency response of 5 hertz [Hz] to 20 kilohertz [kHz] \pm 3 decibels [dB]; open-circuit sensitivity of -25.5 dB referred to about 50 millivolts [mV] per pascal [Pa]). The microphone was coupled through a cathode follower to a B&K

Model 2610 preamplifier and then onto a Hewlett-Packard (Palo Alto, Calif.) 3964 tape recorder. Data were analyzed for frequency content with a Nicolet (Madison, Wisc.) 660A fast Fourier transform computing spectrum analyzer. Pulse train durations and intervals were measured with a Honeywell (Minneapolis, Minn.) Model 1858 oscillographic recorder. Recordings were made throughout the last half of the 12-h photoperiod. Temperature averaged ($26 \pm 1^\circ\text{C}$), and relative humidity ranged from 38 to 58%.

At the time of recording, *D. longicaudata* and *M. croceipes* were held in screen wire cylinders (6.5 by 4.5 cm) and the microphone was placed 1 cm from the cage. With both species, two males and a female were in the cage at any one time. After female was mounted or 15 min passed without acoustic activity, the insects were discarded and replaced. Songs sung by 10 male *D. longicaudata* approaching other males, and songs sung by 20 males approaching females, were recorded. These approach songs were generally performed when the singing male was within 1 cm of another individual. They were often continued as the insects moved about the container. Twelve songs sung by heterosexually mounted males as they climbed upon the female (precopulatory songs) also were obtained. Ten male-to-female *M. croceipes* approach songs were recorded, as were three precopulatory songs and three male-to-male interactions. The smallest of the three species, *C. marginiventris*, was recorded in a screen and plastic tube cage that fit over the end of the microphone. Eleven different male-to-female approach songs were recorded.

Function of Songs. To demonstrate that sound and not merely wing movement has behavioral significance, a tape-recorded approach song was broadcast to *D. longicaudata* and their responses noted. Six individuals (either all male or all female) were placed in a screen wire cage (7.5 by 7.5 cm), and their activity was quantified by counting the number of times they crossed a bisecting line. The sound pressure level of the broadcast was 55 dB in the center of the cage, the approximate volume of a song at 1 cm from the singing insect. After allowing the wasps 5 min to become settled, three 2-min sound and silent periods were presented alternately. To show that any response was not a general reaction to sound, we compared the activities of females and males. That is, any difference in the reactions of the two sexes was thought more likely to be due to differences in their sexual interests rather than in their propensity to be startled by the broadcast. Ten replications for each sex were made.

Sexual Dimorphism of Wings. In some acoustically communicating Diptera, male wings tend to be more oval than female wings (Sivinski & Webb 1985a). To determine if a similar sexual dimorphism occurred in *D. longicaudata*, wings were removed, placed on a projection microscope, and the length and width were marked out on

paper and then measured. Because males tend to be smaller than females, the possibility of a wing shape allometry needs to be considered. Size was estimated by measuring a hind tibia, and this was then correlated with the ratio of wing length/width. Fourteen individuals of each sex were examined.

Results

Description of Sounds: *Biosteres longicaudata*. In the field, males were found in aggregations that gave the appearance of leks. Males alone or approaching conspecifics on the surfaces of leaves performed bouts of wing fanning (see also Buckingham and Sharkey (in press)). Likewise, in the laboratory, males approaching other females or other males produced an intermittent buzzing sound, "an approach song" (Fig. 1 and 2). Other wing beating sounds were made by males immediately after mounting females. Because of the change of context these have been dubbed "precopulatory songs." Both songs consist of pulse trains (=PT, i.e., buzzes; pulse train duration = PTD) separated by pulse train intervals (PTI). The frequencies of the approach songs sung near males and females were the same (male: \bar{x} = 176 Hz, SD = 7.6 Hz; female: \bar{x} = 177 Hz, SD = 11.5 Hz, *t* test, $P < 0.04$; PTI. Female \bar{x} = 50 msec, SD = 8 msec; vs male \bar{x} = 70 msec, SD = 20 msec, *t* test, $P < 0.007$).

However, there were differences. Pulse trains were significantly longer and pulse train intervals significantly shorter in the presence of females (PT—female: \bar{x} = 90 msec, SD = 16 msec; male: \bar{x} = 80 msec, SD = 20, [*t* test, $P < 0.04$]. PTI—female: \bar{x} = 50 msec, SD = 8 msec; male: \bar{x} = 70 msec, SD = 20 msec [*t* test, $P < 0.007$]). The duration of female-directed songs, i.e., the number of pulse trains in a series, was greater (female: \bar{x} = 6.5 sec, SD = 3.9 sec; male: \bar{x} = 1.5 sec, SD = 0.8 sec; *t* test, $P < 0.001$).

Precopulatory songs differed from approach sounds in being shorter, generally less than a dozen pulse trains, and these pulse trains were shorter (approach: \bar{x} = 87.2 msec, SD = 20.8 msec, $n = 9$; precopulation: \bar{x} = 63.5 msec, SD = 10.9 msec, $n = 9$; Wilcoxon paired-sample test, $P = 0.005$) and separated by longer intervals (approach: \bar{x} = 51.5 msec, SD = 6.4 msec, $n = 9$; precopulation: \bar{x} = 112.7 msec, SD = 99.1 msec, $n = 9$; Wilcoxon paired *t* test, $P = 0.025$). Precopulatory songs also have lower frequencies (approach: \bar{x} = 178.4 Hz, SD = 9.1 Hz, $n = 9$; precopulation: \bar{x} = 166.6 Hz, SD = 11.8 Hz, $n = 9$; Wilcoxon paired *t* test, $P = 0.05$).

***Microplitis croceipes*.** The context of sound production was similar to that described for *D. longicaudata*; however the songs are distinctively lower in frequency (Fig. 3; male to female: \bar{x} = 122 Hz, SD = 12.6 Hz; male to male: \bar{x} = 129 Hz, SD = 8.8 Hz; precopulatory: \bar{x} = 131.7 Hz, SD = 1.4 Hz) and had a "warbling" quality in the longer

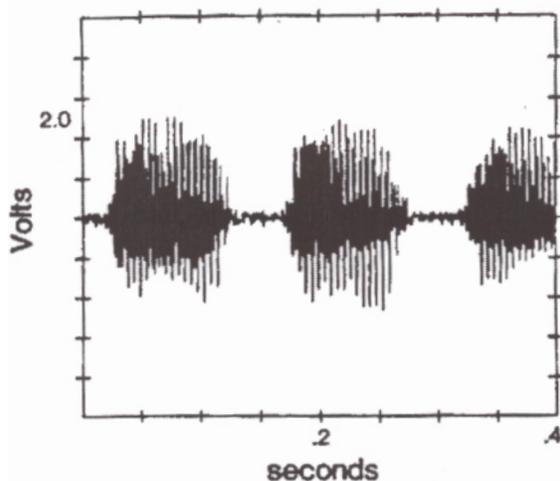


Fig. 1. The approach song of *Diachasmimorpha longicaudata*. The X axis represents 0.4 s. The Y axis is amplitude in volts.

pulse trains. This warbling is caused by an apparent merging of a series of shorter pulse trains, with resulting changes in pulse amplitude. Lower frequencies coincide with regions of low amplitude. Pulse train durations of songs directed toward females average 380 msec (SD = 280 msec) and the intervals 59 msec (SD = 58 msec).

Cotesia marginiventris. This courtship song is one of the more complex insect sounds that we have recorded. It typically consists of a series of short, rapidly repeated pulse trains (PT: \bar{x} = 30 msec, SD = 10 msec; PTI: \bar{x} = 20 msec, SD = 10 msec) that sometimes merge to form a relatively low frequency warble (\bar{x} = 243 Hz, SD = 93 Hz), i.e., a continuous sound with periodic changes in pulse amplitude. These short pulse train warbles often preceded and/or followed higher-amplitude, higher-frequency pulse trains that occurred in groups of one to eight (number of PT: \bar{x} = 3.3, SD

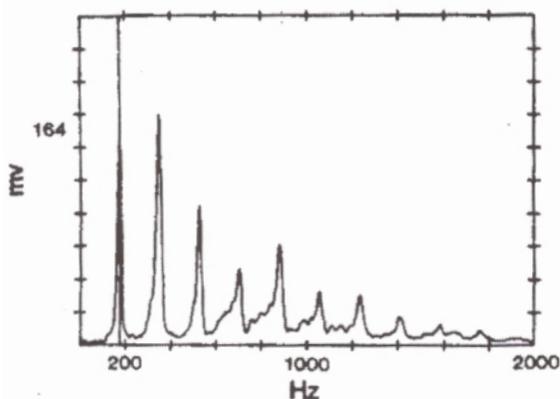


Fig. 2. A frequency analysis of the approach song of *Diachasmimorpha longicaudata*. The X axis represents frequency in hertz. The Y axis is amplitude in millivolts.

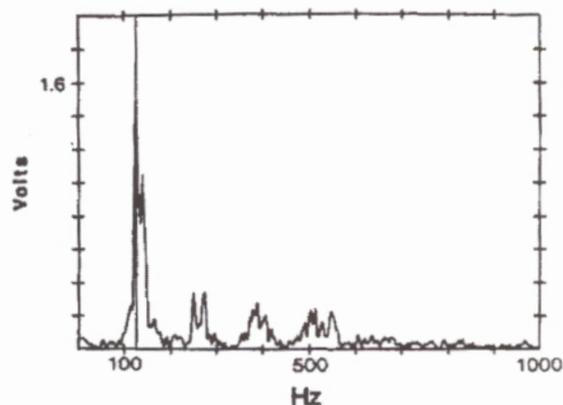


Fig. 3. A frequency analysis of the approach song of *Microplitis croceipes*. The X axis represents frequency in hertz. The Y axis is amplitude in volts.

= 1.5; frequency: \bar{x} = 314 Hz, SD = 48 Hz; PTD: \bar{x} = 31 msec, SD = 50 msec; PTI: \bar{x} = 30 msec, SD = 10 msec). These later pulse trains contain changes in amplitude and frequency that result in a twanging sound reminiscent of a mouth harp (see Fig. 4 and 5).

Function of *D. longicaudata* Songs. Females became more active during broadcasts of song than during intervening periods of silence (moves per min per insect during song, \bar{x} = 0.31; moves per min per insect during silence, \bar{x} = 0.21; Wilcoxon paired-sample test, $P < 0.05$). Males, on the other hand, became more quiescent during broadcast of song (moves per min per insect during song, \bar{x} = 0.53; moves per min per insect during silence, \bar{x} = 0.68; Wilcoxon paired t test, $P < 0.025$).

Sexual Dimorphism of the Wings. Male wings are broader (length/width—male: \bar{x} = 1.87; fe-

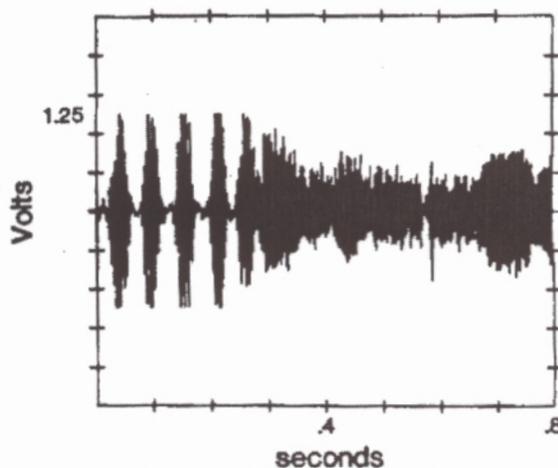


Fig. 4. The two pulse train forms in the approach song of *Cotesia marginiventris*. The higher frequency sound is to the left followed by a series of shorter, lower frequency pulse trains. The X axis represents time. The Y axis is amplitude in volts.

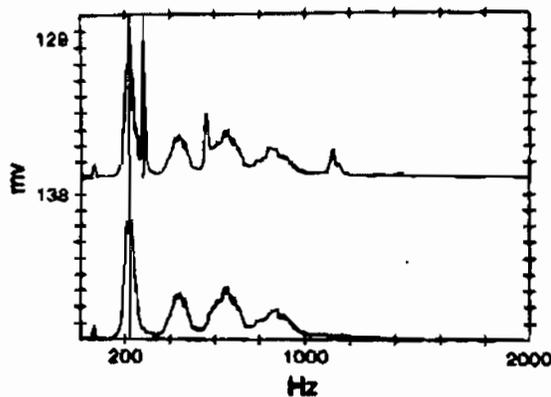


Fig. 5. A frequency analysis of the sounds produced by *Cotesia marginiventris*. The bottom level considers the short, relatively low frequency component alone. The upper level shows the addition of the relatively high frequency pulse train. The X axis represents frequency in hertz. The Y axis is amplitude in millivolts.

male: $\chi^2 = 1.95$, Kruskal-Wallis χ^2 approximation, $P = 0.03$). There was no significant relationship between body size and wing shape (total $r = -0.40$, $P = 0.10$; male $r = -0.21$, $P = 0.59$; female $r = -0.50$, $P = 0.17$).

Discussion

The acoustic signals of *D. longicaudata* and its confamilials have a number of parallels with the more familiar courtship songs of acalypterate Diptera. A close resemblance is with the papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker. As in the braconids, males produce songs of repeated pulse trains as they approach another individual and immediately after mounting a female (Sivinski & Webb 1985b). Short range singing, i.e., in the immediate presence of another insect, is characteristic not only of *T. curvicauda* but also of a number of *Drosophila* spp. (Bennett-Clark & Ewing 1970). There is also a pulse train series "approach song" of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (although this species also makes a higher pitch continuous "calling song" in the absence of other flies, Webb et al. 1983b). This pattern differs from that typically displayed by the chalcidoid parasites where most sounds are emitted by mounted males (Matthews 1975).

However, *D. longicaudata* has a even greater range of contexts in which it produces sound than *T. curvicauda* and other insects that sing only nearby approach songs. Wing fanning-sound production occurs even in insects when alone (which has also been interpreted as a means of pheromone dispersal; Buckingham & Sharkey 1988). Similar behavior is typical of the Caribbean fruit fly, *Anastrepha suspensa* (Loew). The "calling song" of this species, which is produced both when the insect is alone and in the presence of conspecifics, serves as

an attractant as well as a courtship display (Webb et al. 1983a, Sivinski et al. 1984).

As in the intermittent songs of Diptera, there is a considerable range of frequency composition within the sounds produced by the three braconids. The approach song of *D. longicaudata* generally has a single frequency. This is in contrast to the multiple frequencies in the song of *C. marginiventris*, with its two forms of pulse trains and the changing frequency within the high amplitude pulse train. A somewhat similar two-frequency pulse train occurs in the calling song of the Caribbean fruit fly. The two frequencies of the flies' pulse trains have been postulated to be an adaptation for avoiding vegetative filtering (Sivinski & Webb 1985a, see Michelsen et al. 1982). Mating of *C. marginiventris* in the field is undescribed and the advantages, if any, of escaping vegetative filtering are unknown.

Also like acoustically signaling tephritids, male *D. longicaudata* have more oval wings than females. Comparative evidence in Diptera suggests that this sexual dimorphism may be an adaptation for sound production (Sivinski et al. 1984). The adaptive significance of the different reactions of males and females to broadcast sound is unclear. The increased female activity is similar to the response of Caribbean fruit fly to calling song. Papaya fruit fly and *Drosophila melanogaster* Meigen females become more quiescent (von Schilcher 1976, Sivinski & Webb 1985a) in the presence of male song. It has been suggested that the increased movement of the Caribbean fruit fly female is a plausible reaction to a distance signal while the relative stillness of the others is due to a female "awaiting" further stimuli from an approaching suitor (Sivinski & Burk in press). Females of another braconid, *Cardiochiles nigriceps*, appear to be quieted by male wing vibration (Vinson 1978).

The differences in *D. longicaudata* songs sung near males and females are again reminiscent of Diptera behavior. In the Caribbean fruit fly, females prefer large males, large males sing songs with shorter pulse train intervals, females are more likely to react to short interval songs, and males in the presence of females will decrease their intervals (Burk & Webb 1983, Sivinski & Webb 1986). This change may be due to males with an audience expending more energy to appear as attractive, i.e., large and energetic, as possible. Male *D. longicaudata* also decrease their pulse train interval when approaching a female, and the variance in intervals is considerably less (the standard deviation of male-directed intervals is 2.5 times that of the female-directed). The increased pulse train series duration of female-directed songs could simply be due to differences in receivers. Males appear to be more likely to decamp when approached by singers, resulting in a shortening of the song.

Precopulatory song is known to be important in determining whether or not a male will complete an insemination in the Caribbean fruit fly where

it is thought to be the last and critical component of courtship (Sivinski et al. 1984). However, the diminution of mounted chalcidoid precopulatory song does not always influence male sexual success (Rao & DeBach 1969, see however Miller & Tsao 1974). In *D. longicaudata* and *M. croceipes*, we can say little beyond noting that its form is distinct from the approach song.

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