

Effect of continuous rearing on courtship acoustics of five braconid parasitoids, candidates for augmentative biological control of *Anastrepha* species

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Abstract The courtship acoustics of five species of parasitoid wasps (Hymenoptera: Braconidae), potential candidates for augmentative biological control of *Anastrepha* (Schiner) species (Diptera: Tephritidae), were compared between recently colonized individuals and those continuously reared 70–148 generations. During courtship, males of these parasitoid species fan their wings and produce a series of low amplitude pulses. The first series of 15 or more continuous courtship pulses was used to measure the pulse duration, frequency, and interpulse interval (IPI) from the beginning, middle, and end of the pulse series. Each parameter was compared between young and old colonies, and among species. Several differences in

courtship acoustics were detected in colonies that had been continuously reared. The pulse duration at the end of the pulse series was longer in old colonies for *Doryctobracon crawfordi* (Viereck) (Hymenoptera: Braconidae), but shorter for old colonies of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). The IPI of the middle pulse was shorter in old colonies of *Opius hirtus* (Fischer) (Hymenoptera: Braconidae), and was also shorter at the last pulse for old colonies of both *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae) and *D. longicaudata*. The duration of the middle pulse distinguished the three native species, and separated the two introduced species from each other. We discuss our findings in light of their biological and applied implications, particularly those dealing with quality control of mass-reared parasitoids.

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Introduction

Fruit flies (Diptera: Tephritidae), including several species of the genus *Anastrepha* (Schiner), are among

the most important insect pests in the neotropics and subtropics, and can hinder agricultural trade between countries (Aluja and Mangan 2008). *Anastrepha ludens* (Loew), the Mexican fruit fly, can inflict substantial losses in fruit crops including citrus and mango (Aluja 1994; Aluja et al. 1996; Birke et al. 2006). Management programs for *A. ludens* have been implemented in Mexico to support “fly-free export zones” and minimize the risk of their introduction into the US (Riherd 1993). These programs include insecticide-based suppression, sterile insect technique (SIT), and augmentative and classical biological control (Krafsur 1999; Thomas et al. 1999). Augmentative biological control programs that mass rear and release parasitoids can enhance naturally occurring biological control of *A. ludens* and other *Anastrepha* pest species (Sivinski 1996; Montoya et al. 2000; Ovruski et al. 2000). The exotic parasitoids *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) and *Diachasmimorpha tryoni* (Cameron) are currently mass produced in Mexico (Reyes et al. 2000; Montoya and Cancino 2004), and several native parasitoids including *Doryctobracon crawfordi* (Viereck), *Opius hirtus* (Fischer), and *Utetes anastrephae* (Viereck) (all Hymenoptera: Braconidae: Opiinae) are being evaluated for mass production and augmentative biological control of *A. ludens* (García-Medel et al. 2007; Aluja et al. 2008, 2009).

The performance of mass reared insects produced for augmentative biological control can decrease over the generations due to adaptation to laboratory conditions and behavioral changes (Gandolfi et al. 2003; Kölliker-Ott et al. 2003; Bloem et al. 2004). Changes in host finding and acceptance behaviors as well as courtship behavior of mass produced insects have been investigated (Richerson and Cameron 1974; Sivinski et al. 1989; Geden et al. 1992; Kölliker-Ott et al. 2003; Rull et al. 2005; Bloem et al. 2006). Such changes could indicate the need to replace or renew laboratory strains with wild individuals.

Male parasitoid wasps including a number of species in the family Braconidae exhibit wing fanning behavior toward females during courtship. This behavior has been observed for males of the five parasitoids in the present study, *D. crawfordi*, *O. hirtus*, *U. anastrephae*, *D. longicaudata*, and *D. tryoni*. All five species are solitary, koinobiont, endoparasitoids of *Anastrepha* larvae (Ovruski et al.

2000). Female parasitoid wasps respond to airborne and substrate-borne male courtship signals, and presence of these signals improves mating success (van den Assem and Putters 1980; Sivinski and Webb 1989; Field and Keller 1993; Joyce et al. 2008). The artificial rearing environment could affect the transmission and detection of courtship vibrations and thus impact rearing efficacy, or influence selection for life history traits, which could impact subsequent success of the biological control agent when released in the field (Sgrò and Partridge 2000; Joyce et al. 2008).

The objective of this study was to determine whether long term mass rearing of the five parasitoid wasps in this study will significantly change their courtship acoustic behaviors. Male produced courtship sounds from young cultures of each parasitoid species were recorded and compared to those produced by males of the same parasitoid species from older colonies, which were laboratory reared for 70–140 or more generations. Courtship parameters were also compared among species. Knowledge of parasitoid courtship behavior and how it may change over time in rearing systems could contribute to developing better rearing systems, and could be used to monitor behavioral changes of parasitoids maintained in experimental colonies as well as those produced for augmentative biological control.

Materials and methods

Insect collections and rearing

Three parasitoid species native to Mexico, *D. crawfordi*, *O. hirtus*, and *U. anastrephae* were reared from field collected fruit infested with *Anastrepha* spp. The younger colony of *D. crawfordi* was collected from yellow chapote [*Casimiroa greggii* (S. Watson) F. Chiang: Rutaceae], in Santa Rosa Canyon, Ejido Cruz y Cruz, in Nuevo Leon, Mexico, and from la Oveja Canyon, Tamaulipas, Mexico. The older colony of *D. crawfordi* was reared from *Anastrepha fraterculus* (Wiedemann) and *Anastrepha striata* Schiner on guava (*Psidium guajava* L.; Myrtaceae) and *A. ludens* on orange (*Citrus sinensis* L.; Rutaceae) collected from Llano Grande and Tejería, in the Municipality of Teocelo, Veracruz, Mexico. *Doryctobracon crawfordi* has a distribution from Mexico to northern South America (Ovruski et al. 2000). The younger colonies

of *O. hirtus* originated from collections on yellow chapote (*C. greggii*) in Juan Rincón, Tamaulipas, Mexico, while the older collection was obtained from *Anastrepha cordata* Aldrich larvae from *Taberna-montana alba* Mill. (Apocynaceae) at Playa Escondida and Sontecomapan, Los Tuxtlas, Veracruz, Mexico. *Opius hirtus* occurs in Mexico, Costa Rica and Dominican Republic (Hernandez-Ortiz et al. 1994). *Utetes anastrephae* was collected from *Anastrepha obliqua* (Macquart) on jobo (*Spondias mombin* L.; Anacardiaceae) in the Municipality of Llano Grande and Teocelo, Veracruz, Mexico, for both young and old colonies. The distribution of *U. anastrephae* ranges from Florida, USA to Argentina (Ovruski et al. 2000). All three species occur in central Veracruz, Mexico (Hernandez-Ortiz et al. 1994; Lopez et al. 1999). The year of each parasitoid collection is listed in Table 1.

The first introduced species, *D. longicaudata*, native to the Indo-Pacific, was imported into Mexico in 1956, and is now distributed throughout the country (Aluja et al. 2008). The young colony of *D. longicaudata* was collected from *Anastrepha* spp. on guava (*Psidium guajava* L.; Myrtaceae), in San Julián, Veracruz, Mexico, while the old colony was obtained from the CONAFRUT laboratories in Xalapa, Veracruz, Mexico. The second exotic parasitoid, *D. tryoni*, also native to the Indo-Pacific, was introduced to Mexico in 1988 and 1994 (Ovruski et al. 2000). *Diachasmimorpha tryoni* was donated by the Mexican Campaña Nacional Contra Moscas de la Fruta, in Metapa de Dominguez, Chiapas, Mexico. The colony in Mexico originated from the USDA-ARS mass-rearing program in Honolulu, Hawaii, USA. Only an old colony of *D. tryoni* was available for recordings.

Field collected fruit fly infested fruit were maintained in plexiglas cages (30 × 30 × 30 cm) in a rearing room at 24–26°C, 70 ± 5% RH, and a 12:12 photoperiod until parasitoids emerged. Parasitoid colonies thereafter were reared and maintained in cages constructed of a plexiglas frame (30 × 30 × 30 cm) and thin, well aerated mesh walls (amber mesh C62A033; SI Performance Fabrics, now Propex, Chattanooga, Tennessee, USA) with the front side covered by thin plastic wrap (Kleen Pack®; Kimberly Clark de México S.A. de C.V.). Each mating and oviposition cage of adult parasitoids consisted of 40–60 parasitoids, with a 2♀:1♂ adult ratio in each cage. Cages were provided with 8-day-old *Anastrepha ludens* larvae and diet in a sandwich-type device, which was replaced every 36 h. Adult parasitoids were provided with a honey and water mixture (70% honey: 30% water). Approximately ten cages were maintained for each age group (young, old) of each parasitoid species, which maintained a cohort size of ~600 adult parasitoids for each generation, with the exception of *D. longicaudata* which was maintained using ~1000 individuals per generation. *Anastrepha ludens* larvae were used as hosts for rearing all parasitoid species. Adults flies were maintained in a laboratory at 27 ± 1°C and 70 ± 5% RH, with a 12:12 h photoperiod, while larvae and pupae were kept at 30 ± 1°C and 75 ± 5% RH in a separate, completely dark room. Full details of rearing *A. ludens* are described in Aluja et al. (2009).

Each parasitoid species had colonies of two age groups. One was initiated with specimens collected recently from the field, and will hereafter be called the ‘young’ colony for each species (Table 1). The second age group was reared at least several years in

Table 1 Parasitoid species used for courtship recordings and analyses, and the number of generations each species was reared in captivity

Parasitoid species	Introduced or native to Mexico	Generations reared	Year of collection	Colony age
<i>D. crawfordi</i>	Native	9	2006	Young
		135	1995	Old
<i>O. hirtus</i>	Native	8	2006	Young
		148	1995	Old
<i>U. anastrephae</i>	Native	41	2003	Young
		96	2001	Old
<i>D. longicaudata</i>	Introduced	8	2006	Young
		70	2001	Old
<i>D. tryoni</i>	Introduced	131	Unknown	Old

the laboratory and will hereafter be referred to as the ‘old’ colony (Table 1). In the case of *D. tryoni*, only an old colony was available for recording (Table 1).

Courtship recordings

Parasitoid adults were obtained for recordings by isolating individual parasitized puparia in small plastic petri dishes (40 mm diam × 20 mm height) with vermiculite. Petri dishes were checked daily for emerging adults, to ensure their virgin status prior to recording trials. Virgin adults were maintained in all male or all female cages for each species prior to recordings. Parasitoids used for courtship recordings were 3–6 days old, and were considered sexually mature if a male exhibited wing fanning when he was placed near a female. Each recording consisted of a pair from the same age class, i.e. young colony males with young females, or old colony males with old females. Recordings were randomized among species, and were alternated between young and old age classes.

A courtship recording of each male–female pair was conducted in a plastic arena (40 mm diam × 40 mm height), with transparent plastic sides and an open top and bottom, which were covered with white chiffon fabric (Hancock Fabrics, Chiffon Georgette, 100% polyester). The arena was 0.5 cm above a condenser microphone (AKG, Model C-1000, Nashville, Tennessee, USA), with a frequency response of 20–20,000 Hz ± 2 db. Courtship sounds were recorded onto cassette tape with a portable cassette recorder (Sony TC-D5M), and then digitized on a PC at a sampling rate of 44 kHz. During courtship, males fan their wings when in close proximity to a female, and wing fanning produces low amplitude pulsing sounds. The courtship recording began once a male and female were placed together in the arena. Recordings continued for 15 min, or were terminated if mating occurred sooner. All wasps were used for only one recording trial. The number of males recorded from each young or old colony ranged from 11 to 15. The recording room was maintained at 27 ± 2°C and 75 ± 5% RH, and all recordings occurred between 08:00 and 11:00 a.m.

Measurement of courtship acoustic parameters

The first series of 15 or more consecutive pulses produced by male wing fanning during courtship was

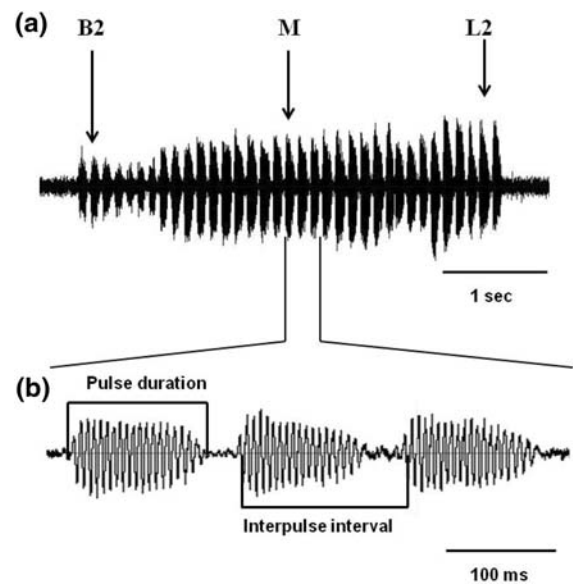


Fig. 1 **a** The first series of pulses produced by male wing fanning during courtship of *Diachasmimorpha longicaudata*, one of the five parasitoid species recorded. The courtship sound components B2 (second from the beginning), M (middle) and L2 (second from the last) were used for measurements of pulse duration, pulse frequency and IPI for all of the five parasitoid species; **b** the pulse duration and the IPI near the M pulse

used for courtship acoustic measurements. Three components of the first series of pulses were used, which were the second pulse from the beginning of the series (beginning 2, hereafter ‘B2’), the middle pulse of the series (hereafter ‘M’), and the second to the last pulse at the end of the series (last 2, hereafter ‘L2’) (Fig. 1). When there were an even number of pulses in the series, the middle pulse (M) was calculated using the formula $[(\text{total pulses}/2) + 1]$. The sound editing software Adobe Audition 2.0 (Syntrillium Software, San Jose, California, USA) was used to measure pulse duration (ms), pulse frequency (Hz), and interpulse interval (IPI) (ms) (the time from the beginning of a one pulse to the start of the next pulse) for each of these three components (B2, M, L2).

Data analysis

Data were analyzed with SPSS (version 16.0) using a two-way ANOVA with parasitoid species (four species) and age (time reared; young or old) as main effects (SPSS 2001). A fifth species, *D. tryoni*, was not included in the two-way ANOVA, as only an old colony was available for recording (Table 1). When

Table 2 Two-way ANOVAs for the influence of parasitoid species and rearing age (young or old) on three courtship parameters, pulse duration, pulse frequency, and IPI

Courtship parameter	Location of measurement	Factor	<i>F</i>	df	<i>P</i>
Pulse duration	B2	Species	10.09	3, 98	<0.001*
		Age	0.02	1, 98	0.90
	M	Species	53.86	3, 98	<0.001*
		Age	2.61	1, 98	0.11
	L2	Species	21.62	3, 97	<0.001*
		Age	4.62	1, 96	0.03*
Pulse frequency	B2	Species	5.77	3, 99	0.001*
		Age	1.07	1, 99	0.30
	M	Species	11.24	3, 98	<0.001*
		Age	0.84	1, 98	0.36
	L2	Species	18.21	3, 97	<0.001*
		Age	0.08	1, 97	0.79
Interpulse interval	B2	Species	3.32	3, 99	0.02*
		Age	1.97	1, 99	0.16
	M	Species	32.17	3, 98	<0.001*
		Age	6.52	1, 98	0.01*
	L2	Species	7.72	3, 97	<0.001*
		Age	6.83	1, 97	0.01*

Each parameter was measured at three locations in the first series of pulses. An * indicates significance at $P < 0.05$

age significantly influenced a courtship parameter (see “Results” section, Tables 2, 3; pulse duration at L2, and IPI at M, L2), *t*-tests were used to compare the means between the old and young colony within each species (Table 3).

Each courtship component (B2, M, L2) varied significantly among species for all three parameters measured (see “Results” section, Table 2). However, only the middle pulse (M) was used to subsequently examine differences among species. The middle pulse was consistently loud and subject to less variation in volume than the beginning or end of the series of pulses. The M duration, frequency and IPI were compared among species using Tukey’s HSD tests. Data from young and old colonies were pooled for both the comparisons of pulse duration and frequency among the five species. As colony age significantly influenced the M interpulse interval, comparisons among species were run separately for young and old colonies. All comparisons were made at a 0.05 significance level.

Results

Influence of colony age on courtship acoustics

Colony age significantly influenced the L2 pulse duration, and the M and L2 IPI duration (Table 2). The L2 pulse duration was significantly longer in the old colony than the young colony of *D. crawfordi* ($t = 2.09$, $df = 28$, $P = 0.04$), and shorter in the old colony than the young colony of *D. longicaudata* ($t = 2.16$, $df = 24$, $P = 0.04$) (Table 3). The M IPI was significantly shorter in the old colony than the young colony of *O. hirtus* ($t = 2.16$, $df = 25$, $P = 0.04$), while the IPI at L2 was significantly shorter for the old cultures than the young ones of both *D. longicaudata* and *U. anastrephae* ($t = 2.51$, $df = 24$, $P = 0.02$; $t = 2.69$, $df = 20$, $P = 0.01$, respectively) (Table 3). A number of courtship parameters did not vary between young and old colonies, and these included the B2 and M pulse duration, the B2, M and L2 pulse frequency, and the IPI at B2 (Tables 2, 3).

Influence of parasitoid species on courtship acoustic signals

Pulse duration, pulse frequency and IPI varied significantly among species (Tables 2, 3). There were significant differences among species in the M pulse duration ($F = 43.82$, $df = 4, 113$, $P < 0.001$), which was shortest for *O. hirtus*, intermediate for *U. anastrephae* and *D. longicaudata*, and longest for *D. crawfordi* and *D. tryoni* (Fig. 2a). The M pulse duration separated all three native species (*D. crawfordi*, *O. hirtus* and *U. anastrephae*), and also the two introduced species (*D. longicaudata* and *D. tryoni*). However, the M pulse duration did not separate pairs of native and introduced species, such as *D. crawfordi* and *D. tryoni*, or *D. longicaudata* and *U. anastrephae* (Fig. 2a). The M pulse frequency also varied significantly among species ($F = 14.73$, $df = 4, 113$, $P < 0.001$) (Fig. 2b). *Doryctobracon crawfordi* and *D. longicaudata* had the highest recorded frequencies, while *O. hirtus*, *U. anastrephae* and *D. tryoni* had lower frequencies (Fig. 2b). The range of pulse frequencies for M was from ~128 to 175 Hz. The M interpulse intervals varied significantly among species for both young ($F = 11.29$, $df = 3, 47$, $P < 0.001$) and old colonies ($F = 46.99$,

Table 3 The mean (\pm SE) for the pulse duration, pulse frequency and IPI for three components of courtship, B2, M, and L2

Species		Courtship parameter	Young colony	Old colony	<i>P</i> value
<i>D. crawfordi</i>	B2	Pulse duration (ms)	61.94 \pm 7.48	62.93 \pm 6.96	0.92
		Pulse frequency (Hz)	150.16 \pm 4.04	161.13 \pm 11.07	0.34
		Interpulse interval (ms)	118.94 \pm 10.09	113.40 \pm 7.87	0.67
	M	Pulse duration (ms)	118.50 \pm 4.00	111.47 \pm 5.25	0.48
		Pulse frequency (Hz)	165.34 \pm 2.68	175.26 \pm 9.86	0.33
		Interpulse interval (ms)	180.81 \pm 4.41	176.87 \pm 5.00	0.56
	L2	Pulse duration (ms)	96.53 \pm 4.41	111.20 \pm 5.47	0.04*
		Pulse frequency (Hz)	160.37 \pm 3.95	165.38 \pm 5.08	0.50
		Interpulse interval (ms)	189.07 \pm 8.54	186.20 \pm 6.76	0.79
<i>O. hirtus</i>	B2	Pulse duration (ms)	44.08 \pm 3.02	44.57 \pm 2.87	0.91
		Pulse frequency (Hz)	139.95 \pm 3.18	134.59 \pm 4.14	0.32
		Interpulse interval (ms)	137.69 \pm 5.19	134.14 \pm 6.28	0.67
	M	Pulse duration (ms)	59.85 \pm 4.22	50.86 \pm 2.77	0.08
		Pulse frequency (Hz)	143.75 \pm 2.69	141.96 \pm 1.85	0.58
		Interpulse interval (ms)	140.08 \pm 8.22	121.00 \pm 3.79	0.04*
	L2	Pulse duration (ms)	63.54 \pm 4.87	53.00 \pm 4.75	0.13
		Pulse frequency (Hz)	140.95 \pm 1.91	136.64 \pm 1.99	0.13
		Interpulse interval (ms)	163.92 \pm 14.54	146.79 \pm 13.05	0.39
<i>U. anastrephae</i>	B2	Pulse duration (ms)	77.10 \pm 4.58	65.50 \pm 4.20	0.07
		Pulse frequency (Hz)	149.53 \pm 2.02	146.78 \pm 2.53	0.42
		Interpulse interval (ms)	134.80 \pm 6.47	125.30 \pm 7.00	0.34
	M	Pulse duration (ms)	91.30 \pm 4.95	79.92 \pm 6.46	0.19
		Pulse frequency (Hz)	151.65 \pm 2.78	148.36 \pm 2.59	0.40
		Interpulse interval (ms)	140.50 \pm 5.91	133.83 \pm 6.28	0.45
	L2	Pulse duration (ms)	93.20 \pm 7.68	79.42 \pm 6.15	0.17
		Pulse frequency (Hz)	147.70 \pm 2.96	147.64 \pm 2.28	0.98
		Interpulse interval (ms)	160.60 \pm 8.08	136.25 \pm 4.83	0.01*
<i>D. longicaudata</i>	B2	Pulse duration (ms)	67.00 \pm 6.66	79.14 \pm 6.23	0.20
		Pulse frequency (Hz)	155.73 \pm 4.92	172.79 \pm 10.63	0.18
		Interpulse interval (ms)	146.00 \pm 8.21	130.00 \pm 7.73	0.26
	M	Pulse duration (ms)	94.75 \pm 4.39	99.14 \pm 6.74	0.60
		Pulse frequency (Hz)	164.74 \pm 3.05	175.57 \pm 10.40	0.36
		Interpulse interval (ms)	157.33 \pm 4.92	146.50 \pm 5.49	0.16
	L2	Pulse duration (ms)	94.42 \pm 6.55	72.43 \pm 7.57	0.04*
		Pulse frequency (Hz)	158.06 \pm 3.20	154.64 \pm 4.34	0.54
		Interpulse interval (ms)	165.33 \pm 5.62	138.86 \pm 8.46	0.02*

t-tests were conducted between young and old colonies (within a row). Significance is indicated by an * ($P < 0.05$)

df = 4, 62, $P < 0.001$) (Fig. 2c). For young colonies, the IPI of *D. crawfordi* was longer than the other three species (Fig. 2c), while *D. tryoni* followed by *D. crawfordi* had the longest IPIs of older colonies. Older colonies tended to have shorter IPIs than younger colonies, although this was only significant in several cases (Table 3; Fig. 2c).

Discussion

Several significant changes in courtship acoustics were observed for older colonies compared to younger ones. Changes in courtship acoustics might be expected for those species reared longer in the laboratory, such as *D. crawfordi* and *O. hirtus* (135

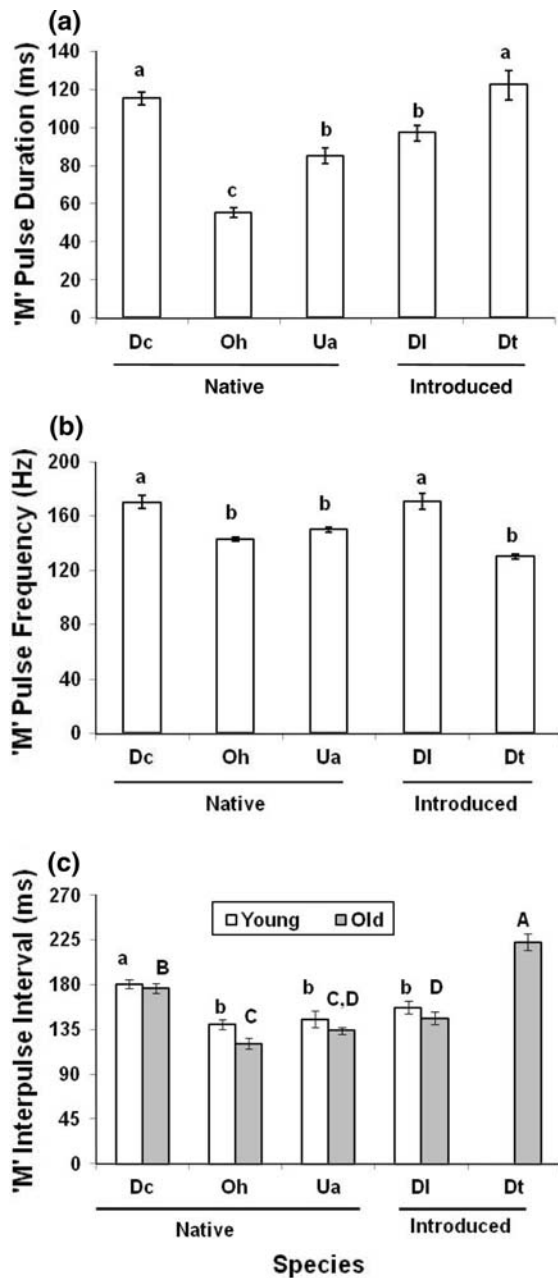


Fig. 2 Courtship acoustic parameters measured at the middle pulse (M) in the first series of pulses produced by male wing fanning during courtship. Comparisons of each parameter are among species. The mean (\pm SE) for **a** pulse duration **b** pulse frequency and **c** IPI. Lower case letters (a, b) indicate significant differences among species (one-way ANOVA, Tukey's HSD tests, $P < 0.05$). Lower case letters (c) indicate significant differences among young colonies, while upper case letters indicate significant differences among old colonies (one-way ANOVA, Tukey's tests, $P < 0.05$). Dc, *D. crawfordi*; Oh, *O. hirtus*; Ua, *U. anastrephae*; Dl, *D. longicaudata*; Dt, *D. tryoni*

and 148 generations, respectively) compared to *U. anastrephae* and *D. longicaudata* (96 and 70 generations, respectively). However, each species had at least one significant change detected in the courtship acoustic pattern after continuous rearing of at least 70 generations. Most observed changes were the shortening of pulse durations or IPIs, except in one instance when the L2 pulse duration of *D. crawfordi* was longer for males from old colonies. No significant changes were detected in courtship frequencies over the course of long term rearing for the four species.

Mass rearing has influenced courtship acoustics in other insects (Sivinski et al. 1989; Briceño et al. 2002). The fundamental frequency of courtship acoustics was significantly lower in mass reared *Ceratitis capitata* flies than in wild flies, although it was suggested the change might be attributable to the larger size of mass produced flies (Sivinski et al. 1989). Changes in intermittent buzz durations during courtship of *C. capitata* were detected between a wild Costa Rican population and one laboratory reared seven years, and also between a wild Hawaiian population and one laboratory reared 40 years (Briceño et al. 2002). However, the direction of change in duration was not consistent for the two populations. Mass reared flies from Costa Rican had longer buzz durations than wild flies (152 vs. 113 ms, respectively), while mass reared Hawaiian flies had shorter buzz durations than wild types (95 vs. 113 ms). One explanation for the longer courtship buzz duration for *C. capitata* was to help overcome background noise (Cayol 2000).

Behavioral changes in laboratory reared insects could occur by adaptation to the laboratory environment either through environmental or genetic influences (Huettel 1976; Geden et al. 1992; Sgrò and Partridge 2000; Evenden et al. 2002; Gandolfi et al. 2003). Olfactory responses to host odors by female parasitoids were influenced by both genetic and environmental influences (Gandolfi et al. 2003; Wang et al. 2003). However, host acceptance by the parasitoid *Hyssopus pallidus* (Hymenoptera: Eulophidae) declined when reared on an artificial diet, but was restored by rearing on the natural host, suggesting that any genetic changes were insignificant (Gandolfi et al. 2003). Few have studied the genetic basis of parasitoid behavior (Beukeboom and van den Assem 2001; Olson and Andow 2002; Wang et al.

2003; Shuker et al. 2007). Populations of a parasitoid species with significantly different courtship acoustic signal patterns could be used to investigate the genetic basis of the inheritance of song characteristics. In this study, founder population sizes of the five parasitoid species may have been sufficiently large to minimize any negative genetic influences such as drift or inbreeding. In addition, each generation of parasitoids had 600 or more individuals, while *D. longicaudata* had colony sizes of 1,000 or more adults.

There are a number of selective contexts that could account for changes in acoustic courtship signals. For example, artificial and natural rearing substrates influence vibrational communication and mating in other arthropods including parasitoid wasps (Miklas et al. 2001; Elias et al. 2004; Seeley et al. 2005; Joyce et al. 2008). The courtship acoustics produced by male wing fanning in parasitoids have both an airborne and substrate-borne component (Leonard and Ringo 1978; van den Assem and Putters 1980; Sivinski and Webb 1989; Field and Keller 1993; Joyce et al. 2008), and both types of signals are known to be detected by Hymenoptera (Greenfield 2002). Female *D. longicaudata* responded to airborne sound replay of male courtship sounds (Sivinski and Webb 1989), while female *Cotesia rubecula* (Marshall) need to detect substrate-borne vibrations from males in order for mating to occur (Field and Keller 1993). Similarly, mating frequency for the parasitoid *Cotesia marginiventris* (Cresson) was highest on substrates which best transmitted males courtship vibrations (Joyce et al. 2008).

Substrates used for rearing could influence courtship vibration transmission and mating for the parasitoid wasps in this study. Substrate density is known to influence the transmission of insect produced vibrations (Fischer et al. 2003). Rearing cages in this study consisted of a Plexiglas (frame) and had thin, well aerated mesh walls which were less dense and more elastic than host plant leaves, such as citrus, mango and guava, the presumed substrate for courtship of opiines attacking fruit flies (Sivinski and Petersson 1997). Transmission of courtship vibrations through the cage substrate could be louder in amplitude or longer in duration than courtship vibrations transmitted in natural host plant leaves, as was the case when male *C. marginiventris* courted on chiffon material and host plant leaves (Joyce et al.

2008). Differences between the vibration transmission properties of laboratory rearing substrates and host plant leaves could cause alterations in the courtship signals of insects laboratory reared for many generations on artificial substrates. The vibrational signals of parasitoids are influenced both by substrate and temperature (Kroder et al. 2006). The diminished ability of laboratory reared parasitoids to produce the proper courtship vibration pattern, or adaptation of vibrational signals to laboratory temperatures, could affect their mating ability when field released for augmentative biological control.

The five parasitoid species recorded here all had a long series of pulses that is repeated numerous times during courtship, similar to the series shown for *D. longicaudata*. The middle pulse duration separated the three species native to Mexico (*D. crawfordi*, *O. hirtus*, and *U. anastrephae*), and also the two introduced species (*D. longicaudata*, and *D. tryoni*). Courtship acoustic patterns during male wing fanning are species specific for many parasitoid species (Leonard and Ringo 1978; van den Assem and Putters 1980; Sivinski and Webb 1989). Significant differences in courtship acoustic parameters might be expected among the native, sympatric species, which may use courtship vibrations as a species isolation mechanism (Greenfield 2002). Variation in courtship signals observed in young and old colonies of native species could be due to geographic variation in collection localities. However, both the young and old populations of *U. anastrephae* were collected at the identical location, yet the L2 interpulse interval was shorter in older colonies. The middle pulse duration observed here for young colonies of *D. longicaudata* (95 ms) was similar in duration to that reported previously for the approach song of *D. longicaudata* (pulse train duration 90 ms, Sivinski and Webb 1989). The pulse duration in the middle of a series of courtship pulses could be used to compare populations or strains of a species where cryptic species are suspected. Fundamental frequencies for the larger parasitoids in this study (*D. crawfordi* and *D. longicaudata*) were higher than those produced by the smaller parasitoids (*O. hirtus* and *U. anastrephae*). Generally, an inverse relationship is expected between insect size and the dominant frequency of airborne or substrate-borne courtship vibrations produced by insects (Cocroft and DeLuca 2006). Parasitoids that were larger in size in this study did not

have lower frequency courtship acoustic signals. However, vibrational signals used by parasitoid wasps are thought to be under myogenic control, and thus may not relate directly to the size of the parasitoid (Kroder et al. 2006).

Long term rearing resulted in several changes in the temporal components of the courtship acoustic behavior of the parasitoids in this study. Insect rearing facilities could consider behavioral attributes of insects as a method for monitoring quality. Consideration of the founding population sizes of colonies and the rearing substrates could help maintain critical behaviors such as courtship.

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