

## Phonotaxis to Amphibian Vocalizations in *Culex territans* (Diptera: Culicidae)

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Ann. Entomol. Soc. Am. 101(1): 95–103 (2008)

**ABSTRACT** *Culex territans* Walker (Diptera: Culicidae) acquires bloodmeals from amphibian hosts. Females overwinter as inseminated adults and exit diapause in New Jersey when spring peepers (*Pseudacris crucifer*) are calling. We tested the hypothesis that *Cx. territans* uses amphibian vocalizations as a long-distance attractant. Two thirds of females oriented toward sound across all experiments. Females allowed to orient toward or away from a frog call, bird song, live frog, or control (a plugged in compact disc player) exhibited positive phonotaxis only to the frog call. Females exhibited positive phonotaxis to calls of *P. crucifer*, *Hyla versicolor* (northern gray tree frog), *Bufo americanus* (American toad), and *Rana clamitans* (green frog), but they were not attracted to calls of *R. catesbeiana* (bullfrog), *R. sylvatica* (wood frog), or control. Multiple regression analysis showed that call frequency is the best predictor for phonotaxis, with pulse duration and call amplitude increasing the attractiveness of the source. When exposed to *P. crucifer* calls at increasing sound intensity levels, females oriented to calls in the range of 50–75 dB, with particle velocities of 0.02–0.3 mm/s, indicating that phonotaxis occurs at distances >5 m from the source.

**KEY WORDS** *Culex territans*, attraction, amphibian, mosquito hearing, mosquito

*Culex territans* Walker (Diptera: Culicidae), a widely distributed species of mosquito in the United States, takes bloodmeals from amphibians (Crans 1970). Crans (1970) frequently observed female *Cx. territans* blood feeding on the spring peeper, *Pseudacris crucifer* (Wied-Neuwied), in nature. *Cx. territans* overwinters as an adult inseminated female in New Jersey, and exit from diapause in the early spring correlates with the mating calls of *P. crucifer* (unpublished data). Therefore, early season host-seeking behavior occurs at a time when preferred hosts are vocalizing.

Use of auditory cues in host-seeking behavior has been documented in the Diptera for Corethrellidae (McKeever and French 1991), Tachinidae (Muller and Robert 2001), and Sarcophagidae (Kohler and Lakes-Harlan 2001). McKeever and French (1991) found blood-feeding flies in the genus *Corethrella* were attracted to the calls of several species of tree frogs from which they acquire bloodmeals. Kohler and Lakes-Harlan (2001) reported that the parasitic fly *Emblemasoma auditrix* Shewell locates cicada hosts acoustically. Muller and Robert (2001) found parasitic flies, *Ormia ochracea* (Bigot), were attracted to the calls of the male cricket hosts. These authors reported *O. ochracea* were accurate in locating hosts in complete darkness and could gauge the direction and distance in three dimensions.

Sound attraction has been examined in several mosquito species. Gibson and Russell (2006) found female *Toxorhynchites brevivalpis* Theobald altered wing beat frequency in response to the male flight tones. Gopfert et al. (1999) found female *Aedes aegypti* (L.) were sensitive to specific frequencies that did not correspond to the wing beat of males. Thongrungrat (1990) examined sound trapping of *Culex tritaeniorhynchus* Giles at 530 Hz, collecting 70% more females with sound. Leemingsawat et al. (1988) showed similar results, with an increase in females at higher frequencies between 800 and 1,000 Hz. Borkent and Belton (2006) recently reported collecting *Uranotaenia lowii* Theobald females in traps baited with frog calls. Toma et al. (2005) had similar results, with 863 female mosquitoes collected in traps baited with frog calls, as opposed to five females without.

Mosquitoes use olfactory, visual, humidity, temperature, and tactile cues in host-finding and recognition (McIver 1982). The use of these cues depends on the distance from a host (Lehane 1991). Dekker et al. (2005) categorized stimuli as a long-range attractant if sensed at >5 m and as short range at <1 m. At long distances, host odor and CO<sub>2</sub> guide mosquitoes to the bloodmeal source (Gillies and Wilkes 1969, Lehane 1991). As mosquitoes approach their host, short-distance cues, including vision, temperature, and odor, become increasingly important (Lehane 1991). Mosquitoes can detect sounds in near-field ranges of <30 cm (Clements 1999). Observations by Smith and

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Gadawski (1994) indicated that male *Aedes provocans* (Walker) could detect females at greater distances. Recent reports of female mosquitoes collected in traps baited with frog calls (Toma et al. 2005, Borkent and Belton 2006) suggest that mosquitoes can detect sounds further than 1 m, corresponding to the far-field range.

Auditory behavior in insects requires an accurate ability to use mechanoreception to detect and recognize specific sound cues (Robert and Gopfert 2002). Mosquitoes sense vibrations in the air by using their antennae (Gopfert and Robert 2001), which in turn are transmitted to the Johnston's organ. The Johnston's organ is a chordotonal organ located at the base of the antennae (Yack 2004), which detects near-field sounds within one or two wavelengths of the source. It includes of four types of scolopidia that differ in number and structure in male and female mosquitoes (Clements 1999). There is also variation in scolopidia type, structure, and number between different species of mosquitoes. Both near-field and far-field (sound pressure) receptors are chordotonal organs. Far-field receptors have independently evolved in several species of Diptera where there is a selective pressure to locate a vocalizing host (Lakes-Harlan et al. 1999, Kohler and Lakes-Harlan 2001, Yack 2004), suggesting a far-field receptor could exist in nematoceran Diptera.

If the antennal resonance frequency of a given species of mosquito is determined, then sound trapping could potentially target that species for surveillance. Sound waves travel in a predictable pattern from a point source, and they are less limited by wind direction than odors (Fishbane et al. 1996). Sound decreases by 6 dB as source distance doubles (Marten and Marler 1977, Bailey 1991). Gerhardt (1975) examined the distance attenuation of calls for 20 species of frogs and found the expected 6 dB was consistent for most species in field conditions. Depending on the initial volume, type of sound, and background noise, there is variation in how far a sound can be detected. However, Brenowitz et al. (1984) found that a *P. crucifer* chorus could be detected within 3.3 ha (8 acres) around the source. Therefore, sound could potentially attract mosquitoes from greater distances than most conventional trapping methods.

We tested the hypothesis that *Cx. territans* females use amphibian vocalizations as a long-distance attractant that can be detected at over 5 m. We propose that positive phonotaxis toward amphibian vocalizations may allow *Cx. territans* to locate an amphibian blood-meal.

## Materials and Methods

**Laboratory Colony Maintenance.** *Cx. territans* were colonized from larvae and adults field-collected throughout New Jersey, based on techniques described by Benache (1970). All stages of development were maintained at 24°C at a photoperiod of 16:8 (L:D) h. Larvae were reared in shallow pans and daily fed ground rat chow (Purina Mills, St. Louis, MO).

Water was changed daily. Pupae were transferred to glass bowls, and placed in 0.23-m<sup>3</sup> emergence cages. Adults were provided a continuous supply of 10% sucrose, which was removed 24 h before experimentation to increase host-seeking behavior.

**Experimental Arena.** The test arena consisted of two 0.029-m<sup>3</sup> cages separated by a 30- by 12-cm tube. A 2.5-cm hole in the tube center provided a port to release mosquitoes. In the distance attenuation study, a longer tube was used (76 by 8 cm), with a wider release port (8 cm). The frog calls and bird songs were played from CDs. Mating calls from New Jersey frogs and toads (Golden and Bunnell 2002) were used. Common bird songs (sparrows, finches, grosbeaks, and buntings) from eastern and central North America (Peterson 1990) were played on an Audiovox CDA1361 (60 Hz, 14 W, 120 V) portable compact disc player (Audiovox Corp., Hauppauge, NY.), at an amplitude of 80 ± 2 dB. A mature male bullfrog (*Rana catesbeiana* Shaw) was used for the live frog. To determine the effect of background noise and attraction of CD player emissions, a control consisted of a plugged in CD player that was not playing a compact disc. For consistency, the compact disc player, live frog, and control were placed 30.5 cm away from the experimental cage, and under the same lighting and temperature conditions (24.7 ± 0.7°C). Experiments were conducted in low light conditions, with a single overhead light 2.5 m away from the test arena. Background noise levels were recorded using a digital sound level meter (Fisher Scientific, Pittsburgh, PA). During each trial, only one cage contained either the treatment or the control. The opposite cage was empty. Seven-day-old *Cx. territans* were introduced into the tube and subjected to either the treatment or control for 15 min before the number of mosquitoes in each cage was counted. Those mosquitoes moving into the cage containing the treatment or control were counted as toward, and those moving into the empty cage were counted as away. Females not moving into either cage were recorded as no response.

**Comparison of Different Treatments.** If amphibian vocalizations are used as a long-distance attractant, then significantly more females would orient in the direction of the vocalizations compared with the control. To remove direction as a confounding variable, treatments (frog calls, bird songs, live frog, and control) were replicated five times each in the left and right directions. Twenty females were used for each of the 10 replications (200 females for each treatment or control), resulting in 800 total females examined in this experiment.

**Comparison of Different Frog Calls.** We compared the responses of mosquitoes orienting to calls of six frog species. If vocalizations of particular frog species are used as an attractant, the attractiveness of calling individuals to host-seeking mosquitoes is predicted to differ among the frog species. We predicted that if *Cx. territans* orients toward sound, then there are components of sound that increase the attractiveness. The frog calls tested included *R. sylvatica* LeConte (wood frog), *R. catesbeiana* (bullfrog), *R. clamitans* Latreille

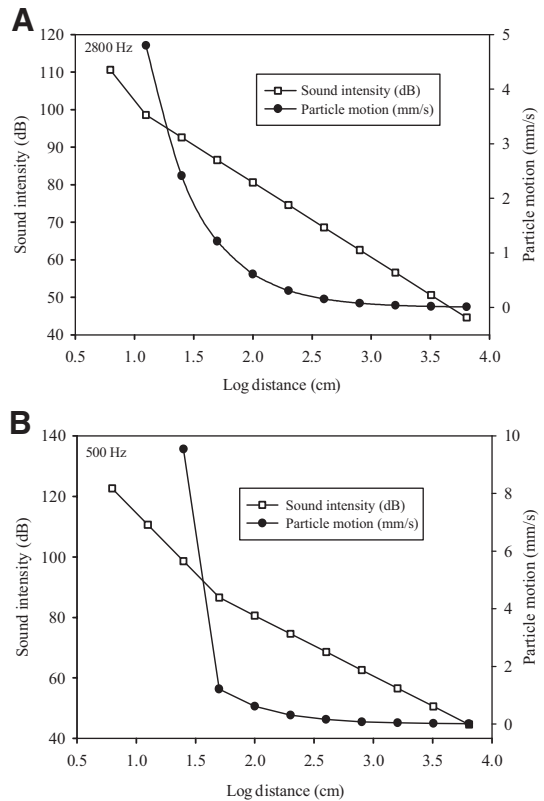
(green frog), *Bufo americanus* Holbrook (American toad), *Hyla versicolor* LeConte (northern gray tree frog), *P. crucifer* (spring peeper), and a control. Twenty-five females were used for each of the five replications (125 females), resulting in 875 females examined in this experiment.

We performed a comparison study to examine the responses of *C. pipiens pipiens* L. orienting toward *P. crucifer* calls and a control. Twenty-five females were used for each of the five replications (125 females), resulting in 250 females examined.

**Distance Attenuation and Attraction of Sound-Specific Cues.** We examined the response of *Cx. territans* to decreasing intensity of *P. crucifer* calls. If vocalizations are used as a long distance attractant, then an optimum distance away from the source at which vocalizations are most attractive could be predicted. We examined the response of females at increasing decibels (46.8, 50, 54.8, 60.7, 63, 69, 73.5, and 76.7 dB). Sound intensity was recorded with a digital sound level meter (Fisher Scientific), by using the reference pressure and frequency for human hearing (20  $\mu$ PA, 1 KHz). Each sound intensity level was replicated three times with 50 females per dB level (150 females), resulting in 1,200 females examined in this experiment.

Using the inverse square law, distance attenuation was extrapolated from the decibel data. Field recorded decibel levels were made for *P. crucifer* at 50 and 100 cm away. We examined the literature for measurements on *P. crucifer* decibel levels (Gerhardt 1975, Brenowitz et al. 1984, Wilczynski et al. 1984). We chose the lower mean of 86.6 dB at 50 cm, even though values above 95 dB at 50 cm are not that uncommon (Gerhardt 1975). Based on the literature, there are two dominant frequencies in *P. crucifer* recordings; 2,800 Hz is the dominant frequency of the vocalization, and 500 Hz is the dominant frequency of wind and white noise detected in the recordings. We defined near-field as the distance in which the sound pressure of a particular frequency ( $f$ ) travels through one wavelength ( $\lambda$ ), where  $\lambda = v_{\text{sound}}/f$  (Fishbane et al. 1996). The speed of sound through air was calculated as  $v_{\text{sound}} = 331.5 + 0.6 T$  ( $^{\circ}$ C), where  $T$  is 24.3 $^{\circ}$ C during our experiments. From our calculations  $v_{\text{sound}} = 346.1$ , resulting in a near-field distance of 12 cm for 2,800 Hz, and 69 cm for 500 Hz. Using these values we were able to determine sound pressure levels (SPLs) and particle velocity levels (PVLs) for each frequency at increasing distances (Fig. 1). The SPL decreased 12 dB within near-field and 6 dB in far-field, and PVL decreased 18 dB within near-field and 6 dB in far-field for every doubling of the distance (Clements 1999). Particle velocity ( $u$ ) was converted to meters per second using the formula  $u = I/p$ , where  $I$  is the sound intensity (Watts per square meter), and  $p$  is the sound pressure (newtons per square meter).

**Field Trials.** If vocalizations are used as a long-range attractant, then more females would be predicted to be collected in mosquito traps baited with sound than in comparable traps without sound. Trap types examined included Center for Disease Control and Prevention (CDC) miniature light traps, pickle jar traps,



**Fig. 1.** Sound attenuation for the two dominant frequencies of *P. crucifer* recordings. Measurements of SPLs and PVLs are shown for 2,800 Hz (A) and 500 Hz (B) at increasing distances. Note that the distance on the x-axis has been logarithmically transformed. Far-field decibel values are from Brenowitz et al. (1984), Wilczynski et al. (1984), and Gerhardt (1975). The transition from near-field to far-field (one wavelength) at 2,800 Hz and 500 Hz occurs at 12 cm ( $10^{1.1}$ ) and 69 cm ( $10^{1.84}$ ), respectively.

and resting boxes. Three traps of each type contained an Audiovox portable CD player. The players were set to continuously play *P. crucifer* calls at 92 dB. The pickle jar traps were tested in Warren County New Jersey. The CDC light traps and resting boxes were examined in the Pine Barrens region of New Jersey. Five field trials were conducted. Because we were unsuccessful using CDC light traps and pickle jar traps to collect *Cx. territans*, only one trial was conducted for each of these two trap types.

**Statistics.** Data were analyzed using SPSS software (SPSS Inc. 2005). To determine whether a particular sound was attractive to mosquitoes, we used a  $t$ -test to compare the number of females orienting toward and away from the sound source for all experiments. In the first experiment, we were interested in determining which treatments were most attractive, and we compared treatments to each other and to the control using a two-way analysis of variance (ANOVA). Only the females entering the cage containing the treatment or control were used in the statistical analysis. In addition, we compared the number of females orient-

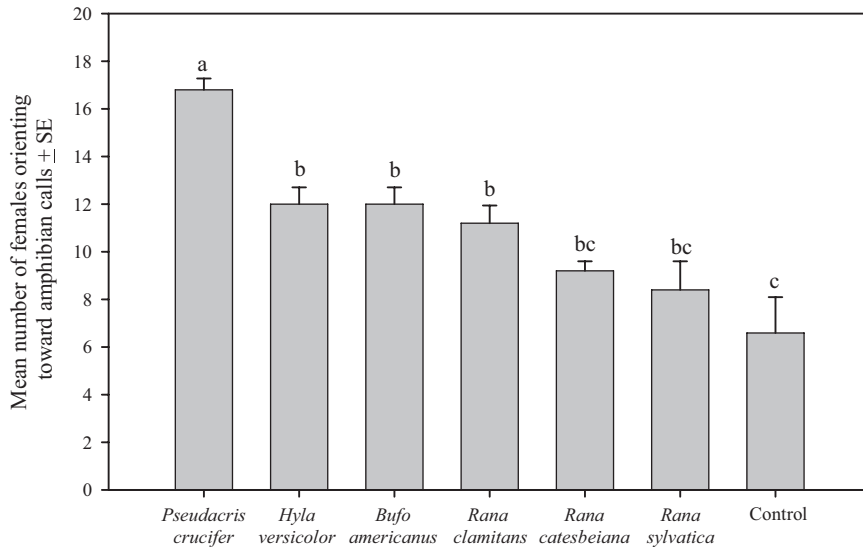


Fig. 2. *Cx. territans* orientation to different frog calls. Responses were analyzed using analysis of variance. Numbers followed by same letter are not significantly different.

ing toward treatments in the left direction versus the right direction. For all analysis, a Tukey's test was used to compare means.

In the second experiment, we were interested in determining which frog calls were most attractive. We compared the number of females orienting toward each treatment to each other and the control using a one-way ANOVA. A Tukey's test was used to compare means. A stepwise multiple regression analysis was performed to determine which characteristics of sound (frequency, pulse duration, call duration, call amplitude, and call rate) could predict phonotaxis in *Cx. territans* females.

For the distance attenuation study, curvilinear regression analysis was performed for both near-field and far-field data to determine a trend. The best-fit line was determined using the  $P$  values and  $R^2$  values obtained from the curve estimation regression analysis.

Those traps baited with sound were compared with those without sound using a  $t$ -test. A chi-square test was performed to determine whether the week of sound trapping influenced the number of mosquitoes collected, by comparing the number of *Cx. territans* females collected in resting boxes at three other sites not baited with sound. All resting box sites were located within the New Jersey Pine Barrens.

## Results

We examined a total of 2,875 *Cx. territans* females for phonotaxis in the laboratory. When provided with a sound source, 70.3% moved in the direction of the sound, as opposed to 29.6% that moved away from the sound ( $P < 0.001$ ). Orientation occurred regardless of the source of the sound. Although birds are not a preferred host for *Cx. territans*, a large proportion

(60%) of females oriented toward the bird songs. Although *Cx. territans* readily feed on *R. catesbeiana* in the laboratory, only 58% of the females oriented toward the live frog in the 15-min period. The number of females orienting toward the control averaged 51% across all experiments.

Significantly more *Cx. territans* females oriented to frog calls ( $F = 5.07$ ;  $df = 3, 36$ ;  $P = 0.005$ ), compared with bird songs, live frog, or the control. There was a 30% increase in the number of females orienting to the frog calls compared with the other treatments. The original design compared responses in both left and right directions. Neither direction ( $F = 1.54$ ;  $df = 7, 32$ ;  $P = 0.223$ ) nor the interaction ( $F = 1.33$ ;  $df = 7, 32$ ;  $P = 0.28$ ) were significant, so the data were collapsed to include 10 replicates each. In both the two-way ( $F = 5.29$ ;  $df = 7, 32$ ;  $P = 0.004$ ) and one-way designs ( $F = 5.07$ ;  $df = 3, 36$ ;  $P = 0.005$ ), the effects of call type were significant.

Females ( $F = 15.04$ ;  $df = 6, 28$ ;  $P < 0.001$ ) were attracted to the calls of *P. crucifer*, *H. versicolor*, *B. americanus*, and *R. clamitans* compared with the control (Fig. 2). Females were not attracted to the calls of *R. sylvatica* or *R. catesbeiana*. *P. crucifer* attracted more mosquitoes (16.8 females) than the other treatments. *H. versicolor* (12.0 females) and *B. americanus* (12.0 females) attracted the second highest number of mosquitoes. These frogs have high-frequency vocalizations compared with the other species examined (Table 1). A comparison study was conducted with *Cx. pipiens*, and there was no significant difference between those orienting toward *P. crucifer* (mean  $\pm$  SE,  $5.88 \pm 0.88$ ) compared with the control ( $6.3 \pm 1.4$ ).

The multiple regression analysis showed that orientation was positively correlated with frequency ( $P < 0.001$ ) (Table 2). There was a slight correlation with call rate, calling period, and call amplitude. Call

**Table 1. Characteristics of select amphibian vocalizations**

Species	Dominant call frequency (HZ)	Call amplitude (db)	Call duration (s/call)	Pulse duration (pulses/s)	Call rate (calls/h)	Calling period in New Jersey	References
<i>P. crucifer</i>	2,600–3,500	80.2–97	0.10–0.30	Continuous	600–990	Mar.–May	Brenowitz et al. 1984, Wilczynski et al. 1984, Gerhardt 1975, Marshall et al. 2003, Gessner and Stiles 2001
<i>H. versicolor</i>	1,800–2,200	86–100.5	0.475–0.875	16–25	600–1400	May–July	Gerhardt 1975, Fellers 1979, McLister 2001, Gerhardt et al. 2000, Taigen and Wells 1985, Gessner and Stiles 2001
<i>B. americanus</i>	1,300–2,000	90–115	6.0–10.8	30–40	60–100	Mar.–June	Howard and Young 1998, Gerhardt 1975, Moffat and Capranica 1976, Blair 1958, Gessner and Stiles 2001
<i>R. clamitans</i>	350–450	84	0.16	Continuous	300–1800	April–Aug.	Bee et al. 2000, Bee and Perrill 1996, Gessner and Stiles 2001
<i>R. catesbeiana</i>	200–400	64–76	0.37–0.97	Continuous	600–2000	April–July	Capranica and Moffat 1977, Capranica 1965, Simmons 2004, Gessner and Stiles 2001
<i>R. sylvatica</i>	Not available	Not available	Not available	Not available	Not available	Mar.–May	Gessner and Stiles 2001

duration and pulse duration did not correlate with the number of females orienting. We developed a model based on these data (Table 3). Although frequency was sufficient to predict orientation ( $R^2 = 0.7, P < 0.001$ ), the best-fit model included frequency, pulse duration, amplitude, and error ( $R^2 = 0.85, P < 0.001$ ). This model best explains the data, suggesting that frequency is the best predictor for orientation, with pulse duration and amplitude increasing the attractiveness of the source.

The call of *P. crucifer* was analyzed as a potential long-distance attractant. The highest (76-dB PVL, 1.8 m,  $u = 0.4$  mm/s) and lowest dB levels (46-dB PVL, 40 m,  $u = 0.01$  mm/s) attracted fewer mosquitoes ( $F = 3.91; df = 7, 16; P = 0.05$ ) than those in the middle range (50–75-dB PVL, 2–28 m,  $u = 0.02$ –0.3 mm/s). The number of *Cx. territans* females orienting ( $y$ ) to *P. crucifer* call sound intensity ( $x$ ) was described by a quadratic trend [ $y = -0.033 (\pm 0.01)x^2 + 4.3 (\pm 1.27)x - 105.49 (\pm 38.4); R^2 = 0.456, P = 0.002$ ] (Fig. 3).

**Table 2. Linear associations between number of females orienting and call characteristics of amphibians by using stepwise multiple regression**

Characteristic	Pearson correlation	P value
Frequency	+0.836	<0.001
Call duration	-0.082	0.349
Pulse duration	-0.069	0.372
Call rate	-0.414	0.020
Calling period	-0.527	0.003
Call amplitude	-0.393	0.026

The number of mosquito females orienting to spring peeper calls decreased with distance away from the source (Fig. 4). A cubic trend described the number of *Cx. territans* females orienting ( $y$ ) to frog calls as a function of distance from the source [ $y = 0.001 (\pm 0.001)x^3 - 0.053 (\pm 0.045)x^2 + 0.772 (\pm 0.738)x + 29.6 (\pm 2.75); R^2 = 0.422, P = 0.011$ ]. The optimum distance at which *Cx. territans* responded to vocalizations was between 2 and 32 m from the source. As particle velocities reach below 0.05 mm/s, the number of females orienting toward frog calls rapidly decreases (Fig. 5).

Neither the CDC light trap nor the pickle jar trap collected *Cx. territans* in any of the trials. Placing a compact disc player of *P. crucifer* calls next to resting boxes resulted in an increase in the number of *Cx. territans* females with sound (69%) compared with those without sound (31%). Note that even with small collection sizes (25 females) our data were significant at  $P = 0.07$ . A  $\chi^2$  test showed sound trapping increased the observed number of *Cx. territans* collected ( $\chi^2 =$

**Table 3. Comparison of models predicting the number of females orienting toward frog calls**

Model	Model predictors	R-squared	F-value	P value
1	Frequency	0.698	53.175	<0.001
2	Frequency	0.777	38.428	<0.001
3	Pulse duration	0.845	38.183	<0.001
	Frequency			
	Call amplitude			

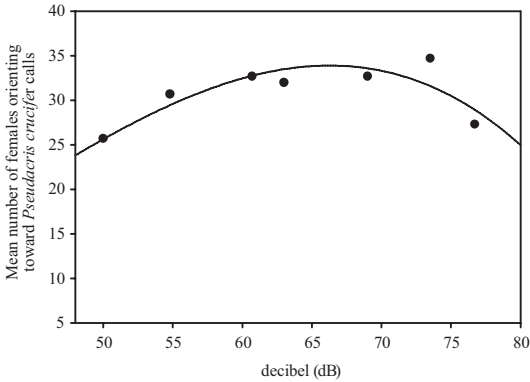


Fig. 3. Influence of frog call intensity of orientation of *Cx. territans*.

59.84,  $P < 0.001$ ). Our highest collection of *Cx. territans* (15 females) occurred in resting boxes baited with sound, compared with the mean of 2.8 females in resting boxes without sound.

Discussion

Our laboratory study shows that *Cx. territans* females are attracted to sound. The proportion of female mosquitoes orienting to sound, regardless of the source, was greater than that moving away from the sound source. Both male and female mosquitoes use a Johnston's organ to sense airborne vibrations (Clements 1999). Gopfert and Robert (2000) found that female mosquitoes are almost as sensitive to sounds as males.

Fewer females oriented toward the live frog in the 15-min period compared with the frog calls and bird songs. *Cx. territans* readily blood-feed on *R. catesbeiana* in the lab; however, females do not attempt blood-feeding immediately and will continually land on *R. catesbeiana* throughout a 3-h period. Because *Cx. territans* have fewer chemoreceptors (McIver 1970) than other species of *Culex*, females might not orient toward most odors. Experiments were conducted in an

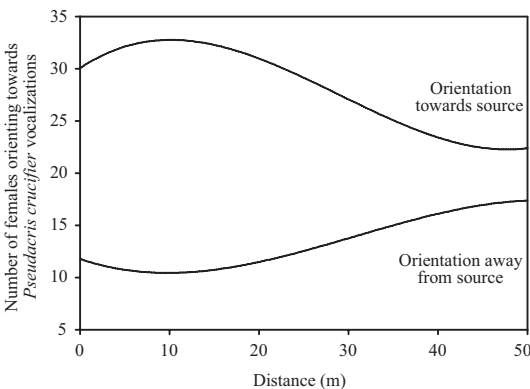


Fig. 4. *Cx. territans* orientation to *P. crucifer* calls at increasing distances from source.

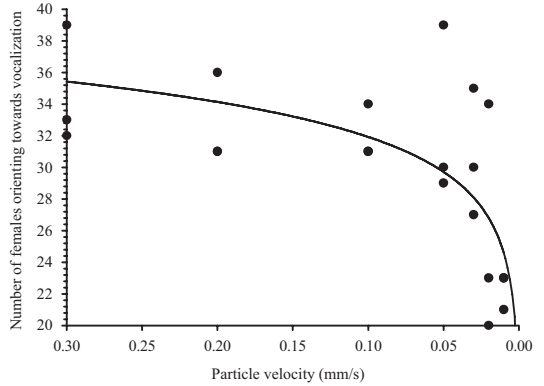


Fig. 5. Effect of particle velocity on the orientation of female *Cx. territans*.

open environment in the laboratory, as opposed to a wind tunnel. Therefore, mosquitoes did not fly upwind to the amphibian odor.

Females were attracted to the calls of *P. crucifer*, *H. versicolor*, and *B. americanus*, three species commonly heard calling in New Jersey. *P. crucifer* are among the earliest amphibians to call in the spring, beginning to call in New Jersey from March to early April (Gessner and Stiles 2001), corresponding to when *Cx. territans* exit hibernation and seek their first bloodmeal. Resting boxes reveal the first collections of adult female *Cx. territans* coincide with the calling of *P. crucifer*.

Phonotaxis was correlated with higher frequency vocalizations. A curve estimation test indicated that the data showed a linear trend. As the frequency increases, then the number of females orienting increases. *P. crucifer* calls had the highest frequency examined (2.6–3.5 kHz), and they were also the most attractive to *Cx. territans*. Fewer females oriented toward the lower frequency calls of *R. catesbeiana* (200–400 Hz) and *R. clamitans* (350–450 Hz). Most frogs and toads produce calls in frequencies ranging from 100 Hz to 5–6 kHz (Capranica 1965). Bird songs are produced in frequencies ranging from 80 to 10.7 kHz (Narins et al. 2004). This overlap in frequencies may explain why *Cx. territans* females were orienting toward bird songs in the laboratory. Because birds sing during the day and amphibians call during the evening, the nocturnal feeding behavior of *Cx. territans* would reinforce orienting only toward amphibians.

Prior studies show female mosquitoes respond to specific sound frequencies. Gopfert et al. (1999) found that the antennae of female *Ae. aegypti* respond to frequencies  $\approx 230$  Hz, with a second peak  $\approx 1,500$  Hz; the lower frequency being the resonant frequency of the antennae, and the higher being the resonant frequency of the antennal hairs (Gopfert et al. 1999). Gibson and Russell (2006) found *T. brevipalpis* females respond to frequencies  $\approx 400$  Hz. Resonance frequency can be influenced by age, temperature, size of antennae, and stiffness of the cuticle (Gopfert and Robert 2001). The antennal hairs do not indepen-

dently receive sounds (Belton 1994), and it is the resonant frequency of the antennae that stimulates the Johnston's organ, with the antennal hairs increasing surface area for sound reception (McVean 1991). If these resonance frequencies can be determined for a target species of mosquito, then sound can be added to traps to improve surveillance techniques. Although we were unsuccessful in sound trapping for *Cx. territans*, Borkent and Belton (2006) collected large numbers of *U. lowii* by adding frog calls to CDC miniature light traps.

Although *Cx. territans* females were attracted most to the vocalizations of *P. crucifer*, it is unclear what frequency in the call was the most attractive to host-seeking individuals. The *P. crucifer* vocalization is made up of a range of frequencies from 0 to 4 kHz (Wilczynski et al. 1984), with a dominant peak  $\approx 2.5$ –3.5 kHz. We used the dominant frequency in the statistical analysis, although the full range was present in our treatment. For *P. crucifer*, Wilczynski et al. (1984) and Schwartz and Gerhardt (1998) showed a second dominant peak at 500 Hz, which both authors attributed to wind and white noise. Because we were using a CD of field-recorded amphibians, it is likely we had the same noise conditions in our recordings. This white noise might correspond to the resonant frequency of the antennae. Therefore, if the white noise stimulated the Johnston's organ, the higher frequency vocalizations might have increased the attractiveness of the recorded calls by stimulating the antennal hairs. The CD player might emit low frequency components that stimulate the Johnston's organ; however, female *Cx. territans* did not orient toward all vocalizations. If female mosquitoes responded to sounds produced by the CD player, then higher frequency vocalizations increased the attractiveness of the source.

Near-field receptors detect air-borne vibrations; typically lower frequencies within one wavelength of the source (Yack 2004). The two peak frequencies in the *P. crucifer* vocalization were 500 Hz ( $\lambda = 69.2$  cm), and 2800 Hz ( $\lambda = 12.3$  cm). If females were using only near-field receptors, they would not be able to detect calls at  $>69$  cm for the low-frequency, and  $>12$  cm for the high-frequency component of the *P. crucifer* call. In our experiments, the CD player was placed 30.5 cm from the cage. Females were never within one wavelength of the high-frequency band. Given the cage and tube sizes in our arena, the females were released 99 cm away from the CD player. This would be within two wavelengths of the low-frequency band, but not the high-frequency band. If females were only responding to the low frequency band and white noise, then there would be no significant difference between treatments, suggesting females can detect particle velocities at distances greater than two wavelengths. This hypothesis seems plausible considering the high sound intensity levels of amphibian vocalizations compared with female wing beats. It would be worthwhile to repeat experiments with the CD player  $>70$  cm away from the release port.

Call and pulse durations did not influence the number of female mosquitoes orienting toward calls. *P.*

*crucifer* has a short continuous peep, lasting 0.3 s (Gerhardt 1975). *R. clamitans* calls, which were not attractive to *Cx. territans* females, also have a short continuous peep lasting 0.16 s (Bee and Perrill 1996). *B. americanus* calls, which were attractive to *Cx. territans* females, has a long series of trills lasting 6–10 s (Gerhardt et al. 2000). However, multiple regression analysis showed pulse duration may explain the data if coupled with other characteristics. When combining frequency, pulse duration, and call amplitude into a model, 84.5% of the variation in the mosquito responses was explained. This finding indicates that *Cx. territans* may orient toward multiple parameters of a host's call and supports other studies of phonotaxis in Diptera. DeVries and Lakes-Harlan (2005) found that flies were attracted to several features of the host's calling song, the most important of which was frequency. However, pulse duration and pulse repetition rate may also allow further discrimination of hosts (DeVries and Lakes-Harlan 2005). *Cx. territans* will obtain bloodmeals from a variety of amphibian species, indicating females do not need accurate discrimination of host species.

Call amplitude did not show a strong correlation to the number of females orienting, but it increased the attractiveness of the call based on our model. Our data suggest that amplitude can influence the distance from which the sound can be detected. Vocalizations by particular frog species may be a mid to long-range attractant, with higher numbers of host-seeking mosquito females orienting toward vocalizations between 50–75-dB PVL. This amplitude corresponds to a mosquito female at 2–28 m away from the point source. The degree of attraction may also increase with higher call amplitudes ranging from 90 to 100 dB (2–5 cm), the call amplitude reflecting the distance attenuation of the particular frequency (Gerhardt 1975). The distance at which a sound can be detected depends on the frequency, intensity, and attenuation of sound (Webster et al. 1992). A sound output of 90 dB SPL at a distance of 1 m, which has a typical 6 dB decrease for every doubling of the distance, has the potential to be detected at 1,000 m from the source (Webster et al. 1992) by a sound pressure detector. During calling periods, *P. crucifer* space themselves apart from other calling males based on their call amplitude (Brenowitz et al. 1984). This behavior maximizes their active calling space and maximizes the advertisement call of the entire chorus. Call amplitudes of a *P. crucifer* chorus can potentially reach 100 dB on a humid spring night, with a potential to be detected with a sound pressure receptor from a kilometer away.

The Johnston's organ is a near-field receptor that detects particle velocities and not sound pressures (Tischner and Schief 1955). Even though the tympanic membrane and vertebrate ears can detect far-field sound pressures, the mosquito antenna detects near-field particle velocities (Clements 1999). Our data show that as particle velocity approaches 0.05 mm/s, the attractiveness of the vocalization rapidly decreases. In addition, there was little difference between those female mosquitoes responding to particle

velocities above 0.05 mm/s, corresponding to 60 dB. These values seem reasonable considering male mosquitoes respond to female wing beats at 45 dB (Charlwood and Jones 1979). Typical background noise, which can affect sound detection, may be  $\approx$ 40–60 dB (Mankin 1994) or 30–50 dB (Brenowitz et al. 1984), depending on location. Amplitudes of the female wing beats might be similar to typical background noise.

The near-field sounds are most-likely detected by *Cx. territans*; however, far-field receptors also are present in dipterans. The chordotonal organs for near-field and far-field hearing are structurally similar, and they differ by connectivity, attachment, cap structure, and type of scolopidia (Yack 2004). The mosquito Johnston's organ is made up of four different types of scolopidia, the most of which are type A and B scolopidia (amphinematic), which are used for detecting particle velocities. Mosquitoes have few type C scolopidia (mononematic), which are similar in structure to far-field receptors. Far-field receptors have independently evolved in several species of Diptera where there has been selective pressure to locate a vocalizing host (Lakes-Harlan et al. 1999, Kohler and Lakes-Harlan 2001, Yack 2004). Because several nematoceran Diptera, including *Corethrella*, *Cx. territans*, and *Ur. lowii* have vocalizing hosts, and they have been collected in traps baited with frog calls (McKeever and French 1991, Borkent and Belton 2006), it would be worthwhile to further study the ultrastructure and function of the chordotonal organs in these species.

### Acknowledgments

We thank L. McCuiston for help in mosquito rearing, W. Staudenger, S. Healy, and S. May for help in the collection of *Cx. territans*, and the New Jersey Mosquito Control Association and Northeastern Mosquito Control Association for financial support.

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Received 16 February 2007; accepted 4 September 2007.