

Death comes suddenly to the unprepared: singing crickets, call fragmentation, and parasitoid flies

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Male field crickets are subject to a delicate dilemma because their songs simultaneously attract mates and acoustic predators. It has been suggested that in response, crickets have modified various temporal song parameters to become less attractive to acoustic predators. We investigated whether crickets with chirping (versus trilling) song structures are less likely to attract acoustically orienting parasitoid flies. Experimentally, we evaluated the phonotactic quest of the parasitoid fly *Ormia ochracea* in response to broadcast cricket calls, presented both simultaneously (choice paradigm) and sequentially (no-choice paradigm). Flight trajectories were recorded in darkness using three-dimensional active infrared video tracking. The flies showed remarkable phonotactic accuracy by landing directly on the loudspeaker. The introduction of acoustic fragmentation that resembles calls of many chirping crickets altered the flies' phonotactic accuracy only slightly. Our results document differential attraction between trilling and chirping cricket songs and quantitatively demonstrate that chirping songs, if presented alone, do not impair the efficiency (temporal investment and landing accuracy) of the flies' phonotactic quest. This study shows that song fragmentation is no safeguard against acoustic parasitism. We conclude that, in general, a cricket may reduce predation only if its neighbors are acoustically more conspicuous, chiefly by amplitude. *Key words*: communication, *Gryllus*, *Ormia*, parasitism, phonotactic behavior, trajectory analysis. [*Behav Ecol* 13:598–606 (2002)]

Both female field crickets and the larviparous parasitoid flies *Ormia ochracea* (Diptera, Tachinidae, Ormiini) rely on acoustic cues to detect and find singing male crickets (Cade, 1975; Robert et al., 1992; Walker and Wineriter, 1991). Once a fly homes in on her host, she lays her larvae on and around the cricket. The larvae enter the host, feed, and grow, and after approximately 7–10 days they emerge and pupate, killing the cricket (Adamo et al., 1995). Male crickets are therefore faced with the dilemma of attracting mates on the one hand and avoiding parasitoids on the other.

Several possibilities have been suggested as to how male crickets could signal under conditions that reduce the risk of exploitation by acoustic predators. One such strategy found in some males of *Gryllus integer* (Orthoptera, Gryllidae) is to remain silent while staying near callers (Cade, 1975, 1979). These silent males, called “satellites,” intercept females as they move toward callers and thus avoid both parasitization and the cost of signaling. Another possible strategy is to shift the seasonal (Burk, 1982) or the diel (Cade et al., 1996; Zuk et al., 1993) calling pattern in relation to the phenology and abundance of parasitoid flies. Greater attention has been directed to the idea that crickets might have modified various temporal song parameters to become less attractive to acoustic predators (e.g., Bailey and Haythornthwaite, 1998; Lehmann and Heller, 1998; Zuk et al., 1993, 1998). The idea that parasitization can be reduced through song modification has found some support in the finding that female flies show a

“preference” for certain song types (Lehmann and Heller, 1998; Wagner, 1996).

Field studies by Walker (1986, 1993) have revealed that gravid females of *O. ochracea* were attracted in greatest numbers to the trilling songs of *Gryllus rubens*. Songs of other candidate hosts were much less attractive relative to the simultaneous broadcast of a *G. rubens* song. It is therefore conceivable that *O. ochracea* discriminates between cricket species on the basis of their calling song. By examining the attractiveness of varied songs in the chirping field cricket species *Gryllus lineaticeps*, Wagner (1996) found that female flies favor songs with a higher chirp rate, longer chirp duration, and higher chirp amplitude. This study suggests that *O. ochracea* may not only distinguish between potential host species, as found by Walker (1993), but also displays differential attraction to certain song variations within a single host species. Wagner (1996) then suggested that higher chirp rate and longer chirp duration may be easier for flies to localize and that ultimately female flies may minimize search costs by orienting to such songs. Proximally, these search costs should be directly reflected in the flies' flight behavior in terms of spatial accuracy and temporal investment.

Thus far, field data on fly phonotaxis have been methodologically confined to choice experiments where alternative stimuli were presented simultaneously. The comparison between stimuli presented at the same time provides a sensitive and adequate measure of differential attraction. Because alternative stimuli may interfere at the perceptual level and possibly mask one another, differential attraction alone does not test whether flies minimize search costs by orienting to one chosen stimulus. Thus, to address the question of search efficiency in response to variable song structures, complementary experiments are desirable. Such experiments may consist in the sequential, no-choice presentation of test stimuli while the flight behavior is documented in standard and reproducible conditions. Differences in flight behavior can then be

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interpreted as a function of the acoustic parameters modified in the test songs. Such experiments are naturally complementary because, in the field, crickets call simultaneously as well as in sequence. By combining choice experiments with no-choice experiments, more can be learned about the relationship between acoustic signaling and the constraints imposed by eavesdropping parasitoids.

In the present study, we first examined, in the context of choice experiments, whether the trilling song of the primary host (*G. rubens*) is more attractive than chirping calls. Second, using no-choice experiments, we investigated the flies' phonotactic behavior in terms of their search efficiency (temporal investment and landing accuracy) and structure of flight trajectories. We compared the results from the choice and no-choice paradigms, and we conclude that song fragmentation alone is no safeguard against acoustic parasitism.

METHODS

Animals and rearing conditions

The parasitoid flies, *O. ochracea*, used were reared in the Laboratory of Bioacoustics at the University of Zürich. The founder flies of the colony originated from Gainesville, Florida, courtesy of T. J. Walker. We kept animals at a light regime of 16:8 hr day:night cycle, 26°C, and 60% relative humidity and provided them with nectar (Vita-Rich instant nectar for hummingbirds) and water ad libitum. We performed experiments only on gravid females, as no positive phonotaxis is observed for other sex or age classes.

Experimental arena

We conducted all behavioral experiments in an indoor room (length 6.8 m × width 4.9 × height 4.0 m) with walls covered with high-frequency absorbing foam (Maag Technik AG, type 1135). Inside this room, a flight cage (length 4.5 m × width 2.6 m × height 3.0 m) formed the actual experimental arena (Figure 1A). To enhance the fly's contrast against the background for three-dimensional video tracking, we covered the background uniformly with black cotton sheets. The only objects placed in the experimental arena were a starting platform, one or two loudspeakers (each mounted in a cabinet; length 30 cm × width 30 cm × height 8 cm) on the ground and two video cameras protruding through the roof (Figure 1A).

Data acquisition

A single fly was placed on the platform, and placidly stayed there, motionless, until the test stimulus was presented. As soon as sound was broadcast through one or both loudspeakers (Radio Shack Super Tweeter, No. 40-1310B, 4 cm diameter), the fly took off and landed on the active loudspeaker a few seconds later.

Because *O. ochracea* is crepuscular and nocturnal (Walker, 1993), and to exclude visual cues, we conducted all experiments under LED infrared illumination (875 nm peak wavelength), providing light only to the cameras of the tracking system. We recorded flight trajectories using two infrared-sensitive video cameras with pan-tilt optics (Sony LSX PT 1; Figure 1A). Camera tracking and trajectory data acquisition was achieved using Trackit Stereo software (Fry et al., 2000). We sampled the spatial position of the fly every 20 ms. By an additional custom-written LabVIEW 5.0 (National Instruments) interface, we were able to use the positional information to control the sound stimulus as a function of the fly's position in space (Müller and Robert, 2001). Hence, our set-

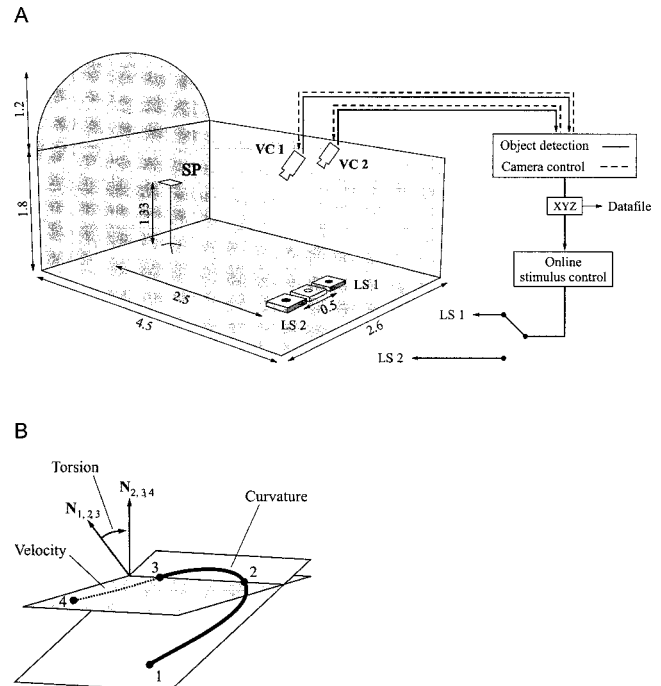


Figure 1

Experimental setup for recording flight trajectories and online stimulus control and variables describing the three-dimensional flight trajectories. (A) Flight cage with starting platform (SP) and two loudspeakers (LS 1 and 2) housed in cabinets (length 30 cm × width 30 cm × height 8 cm). We recorded flight trajectories with two pan-tilt infrared video cameras (VC 1 and 2). Positional data were saved on disk and streamed to a second computer to control stimulus presentation. Measurements are given in meters. (B) Estimation of velocity, curvature, and torsion by the technique of finite helix fit (Crenshaw et al., 2000). Torsion was defined as the angular change between the subsequent normal vectors $N_{1,2,3}$ and $N_{2,3,4}$ of the planes given by points 1–3 and 2–4, respectively.

up allowed us not only to record flight trajectories as such, but also to perform interactive experiments.

Preliminary experiment

We estimated how short a chirp could be so that a fly could still detect and locate it. A single chirp is a short series of sound pulses followed by silence. We then built our chirping cricket calls on the basis of the shortest possible chirp. To assess the minimal number of sound pulses necessary for the fly to detect and locate a sound source, we used an interactive paradigm: the flight space of the fly was separated in two by the introduction of a virtual plane (Figure 2). The plane was imaginary but had a dedicated logical function analogous to a light barrier. The fly was first attracted to the loudspeaker LS 1 by playing a continuous control trill (Figure 3A). When the fly crossed the plane, she elicited the switch over from one loudspeaker (LS 1) to the other (LS 2). As a reaction to the new sound source, the fly changed her course, turned back, and eventually landed on LS 2 (Figure 2A). We then systematically reduced the total number of sound pulses in the trill played from LS 2. Providing the fly with a train of only five sound pulses still caused a distinct orientation reaction (Figure 2B). Fewer sound pulses failed to induce any oriented turn (Figure 2C). Based on this evidence ($n = 7$), we then constructed our model songs as described in the following sections.

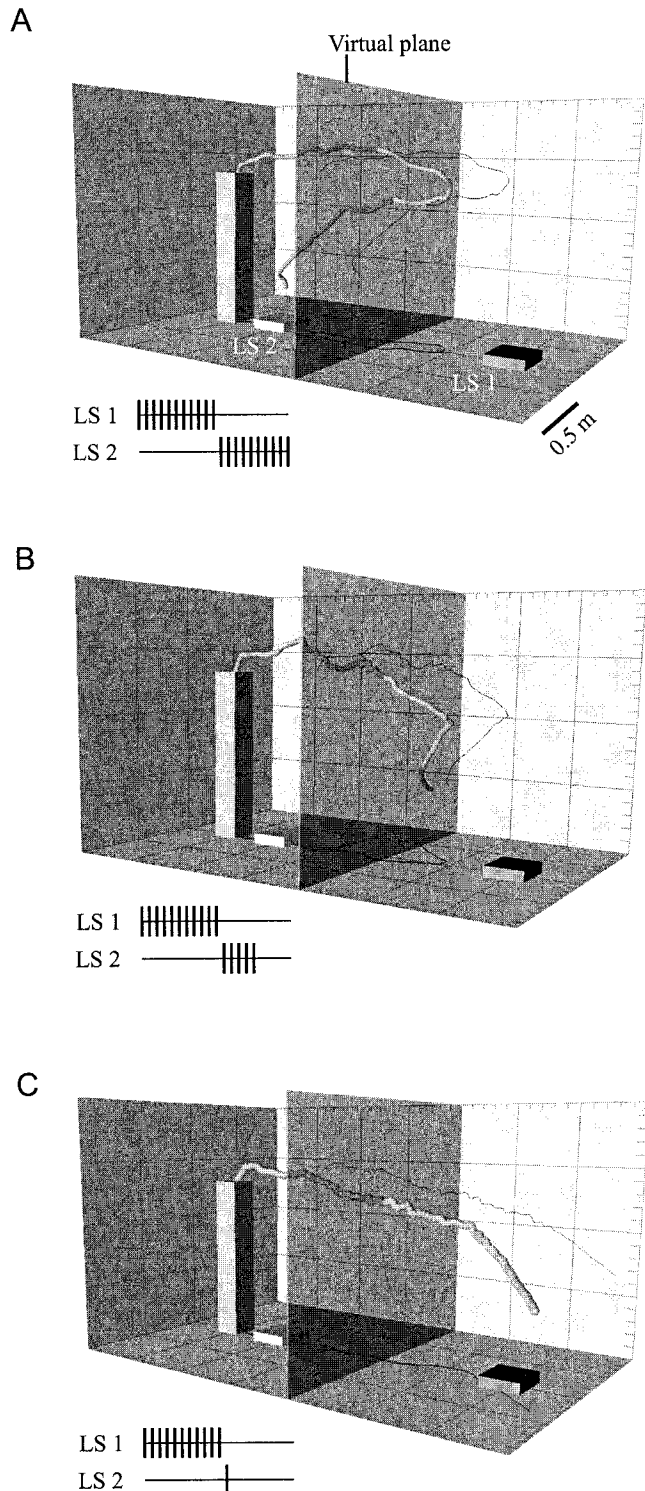


Figure 2

Minimal number of sound pulses necessary for the fly to localize the artificial cricket sound. As the fly intersected the virtual plane, the presentation of the cricket model song was switched from LS 1 to LS 2. This arrangement allowed us to test the fly's reaction to a defined number of sound pulses presented in free flight. (A) The fly was first attracted to loudspeaker LS 1 and upon switching, LS 2 played the same song. As a result, the fly changed its course, turned back and landed on LS 2. (B) If, upon switching, LS 2 played a short burst of only five sound pulses, the fly still showed a distinct turn. (C) Providing the fly with less than five sound pulses, the stimulus elicited no clear response.

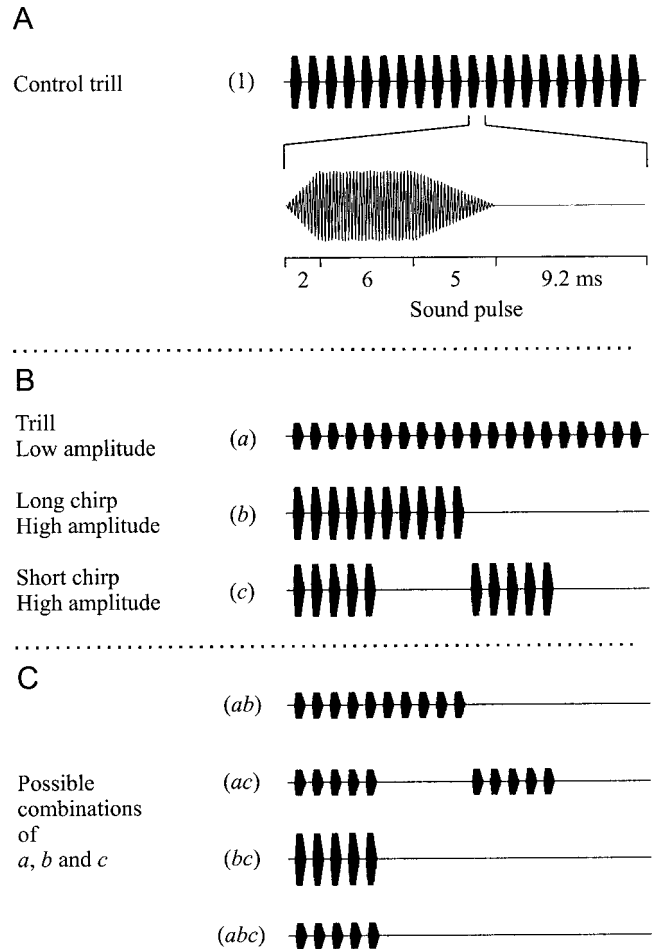


Figure 3

Waveforms of the stimuli used in the experiments. (A) Calling song modeling a male field cricket, *G. rubens*. The control trill (1) consisted of 45 pulses/s of 4.8 kHz carrier frequency at 82 dB SPL. (B) Songs mimicking other types of field crickets. Songs were created by reducing the control trill in one of the following factors, amplitude (*a*; 76 dB SPL), repetition rate (*b*; 2.25 pulse trains/s) or pulse train length (*c*; 5 pulses). (C) Possible combinations of factors *a*, *b*, and *c*. These combinations allowed testing interactions between factors *a*, *b*, and *c*.

Choice experiments

In the choice experiments we exposed 24 female flies to six treatments. Each treatment was a choice experiment between cricket calls simultaneously broadcast from two loudspeakers (LS 1 and 2; Figure 1A). We randomized the treatments to exclude time and sequence effects.

We presented all pairs of stimuli once to each fly in a randomized order and exchanged the broadcast stimuli between LS 1 and LS 2 to obtain an equal number of presentations per loudspeaker for each treatment. On the basis of the landings on one of the two loudspeaker cabinets, we could determine which of the two stimuli was more attractive. We compared the number of flies successfully attracted to each of the stimuli with a two-tailed binomial test.

Choice experiments encompassed the simultaneous presentation of the control trill with stimulus *a*, *b*, or *c* and, complementarily, of stimuli *a*, *b*, and *c* against each other (Figure 3 and Table 1).

Control trill

The natural calling song of *G. rubens* is a trill in which sound pulses are repeated at a constant rate and are packaged in

Table 1
Stimuli used in the experiments

Treatment	Stimulus	Amplitude	Repetition rate	Pulse train length
1	Control trill	82	4.5	10
<i>a</i>	Trill	76	4.5	10
<i>b</i>	Low amplitude			
	Long chirp			
<i>c</i>	High amplitude			
	Low repetition rate	82	2.25	10
<i>ab</i>	Short chirp	82	4.5	5
	High amplitude			
<i>ac</i>	High repetition rate			
	Long chirp	76	2.25	10
<i>bc</i>	Low amplitude			
	High repetition rate			
<i>abc</i>	Short chirp	76	4.5	5
	Low amplitude			
<i>abc</i>	High amplitude			
	Low repetition rate			
<i>abc</i>	Short chirp	82	2.25	5
	High amplitude			
<i>abc</i>	Low repetition rate			
	Short chirp	76	2.25	5
<i>abc</i>	Low amplitude			
	Low repetition rate			

Amplitude in decibels (dB) SPL, re 20 μ Pa, measured at a distance of 15 cm from the loudspeaker. Repetition rate given per s; pulse train length given as number of pulses.

long pulse trains of about 2–30 s (Doherty and Callos, 1991; Walker, 1993). Accordingly, we generated a model calling song using the program SoundEdit 16 (Macromedia; 16-bit resolution, 44.1 kHz sampling rate). As in the natural song, the duration of a single pulse was 13 ms, followed by a gap of silence of 9.2 ms. The carrier frequency was 4.8 kHz. The sound pulse had a linear onset ramp of 2 ms and a linear offset ramp of 5 ms. We looped the single pulses so that we obtained a continuous pulse train of 45 pulses/s (control trill; Figure 3A).

Control trill versus *a*, *b*, or *c*

We simultaneously presented the control trill with stimulus *a*, *b*, or *c* (Figure 3B and Table 1). Each of the three stimuli (Figure 3B) differ in one acoustical property from the control trill. Stimulus *a* is also a continuous trill but only half the intensity of the control (i.e., 6 dB SPL less). Stimulus *b* is a chirping call with long chirps but equal intensity as the control. The chirps had a length of 10 sound pulses followed by an equal length of silence. Stimulus *c* was again a chirping call, but the chirps were shorter (i.e., half the length as in *b*). As for the control trill and the other chirping call (*b*), we adjusted the sound pressure level to 82 dB SPL.

We measured the intensities of the acoustic stimuli with a TES 1352 sound level meter. The instrument was regularly calibrated with a Brüel & Kjær sound level calibrator (type 4231). We adjusted sound pressure levels (SPL) in decibels (re 20 μ Pa) at 15 cm above the loudspeaker.

Test stimuli *a*, *b*, and *c*

We constructed *a*, *b*, and *c* by halving either amplitude, repetition rate, or pulse train length in the control trill. Thus, the overall acoustic energy of stimuli *a*, *b*, and *c* became equal. Simultaneous presentation of stimuli *a*, *b*, and *c* against each other forced the flies to choose between sound stimuli that varied only in their information content. Therefore, these stimuli allowed us to test the effects of amplitude (*a* vs. *b* and

c), repetition rate (*b* vs. *a* and *c*), and length of pulse train (*c* vs. *a* and *b*).

Sequential experiments

In the sequential experiments (no-choice), we examined the phonotactic flight performance of 14 flies in the presence of a single sound stimulus. The stimuli were broadcast from a single loudspeaker (center cabinet in Figure 1A). In total, we tested eight different treatments for each fly (Figure 3 and Table 1): the control trill, the three test stimuli (*a*, *b*, and *c*), and all possible combinations (*ab*, *ac*, *bc*, and *abc*). We randomized the treatments to exclude time and sequence effects. We constructed a 2³-factorial design (Bailey, 1995) using one block per fly, each of which contained exactly eight plots, one for each treatment. The advantage of using this type of design is that we obtained a broad picture of the effect of each of three song parameters (amplitude, repetition rate, and pulse train length) in the different conditions furnished by variations in the other parameters. If the song parameters were not independent of one another, we collected at once all the information about the nature of the interaction. Because we were interested both in the effects on search efficiency and flight behavior, we chose a set of five descriptors (see below) from each recorded trajectory. The estimates of each descriptor were subjected to a three-way repeated-measures ANOVA (von Ende, 1993) investigating the effects of high versus low amplitude (*a*), high versus low repetition rate (*b*), and long versus short pulse trains (*c*), both separately and in different combinations. In the repeated-measures ANOVA, the effect \times fly interaction was used as the error term for the respective effect.

We examined the following two descriptors to estimate the effects on search efficiency: (1) landing accuracy—the distance (m) between the landing position and the center of the active loudspeaker; and (2) flight duration—the time (s) elapsed between takeoff and landing. Also, we investigated the flight trajectory alone. One concise approach to doing so is the analytical technique of finite helix fit (Crenshaw et al., 2000; Figure 1B), whereby a three-dimensional trajectory is completely described by its velocity, curvature, and torsion. We thus measured (3) speed—the mean magnitude of flight velocity expressed in m/s; (4) curvature—the average curvature (rad/m) along the entire flight trajectory; and (5) torsion—the average torsion (rad/m) along the entire flight trajectory.

We performed all data processing and the according statistical analyses using the software package R 1.2.2 (General Public License; <http://www.R-project.org>) running on a LINUX platform. The level of significance, α , was set at 0.05. The significance level for each of the five descriptors (see above) were adjusted according to the Bonferroni correction (i.e., $\alpha' = \alpha/5 = 0.01$).

RESULTS

Choice experiments

In the first series of choice experiments, the goal was to see whether the control trill is more attractive than any one of the modified stimuli *a*, *b*, or *c*. For all pairs of stimuli, we found significant attraction to the control trill (two-tailed binomial tests: control vs. *a*, 20 out of 24 flies; $p = .0015$; control vs. *b*, 22 out of 24 flies, $p = .0002$; control vs. *c*, 20 out of 24 flies, $p = .0015$; Figure 4A). This result demonstrates that the model song of the primary host (*G. rubens*) is more attractive than any other alternative song (*a*, *b*, or *c*) when broadcast simultaneously.

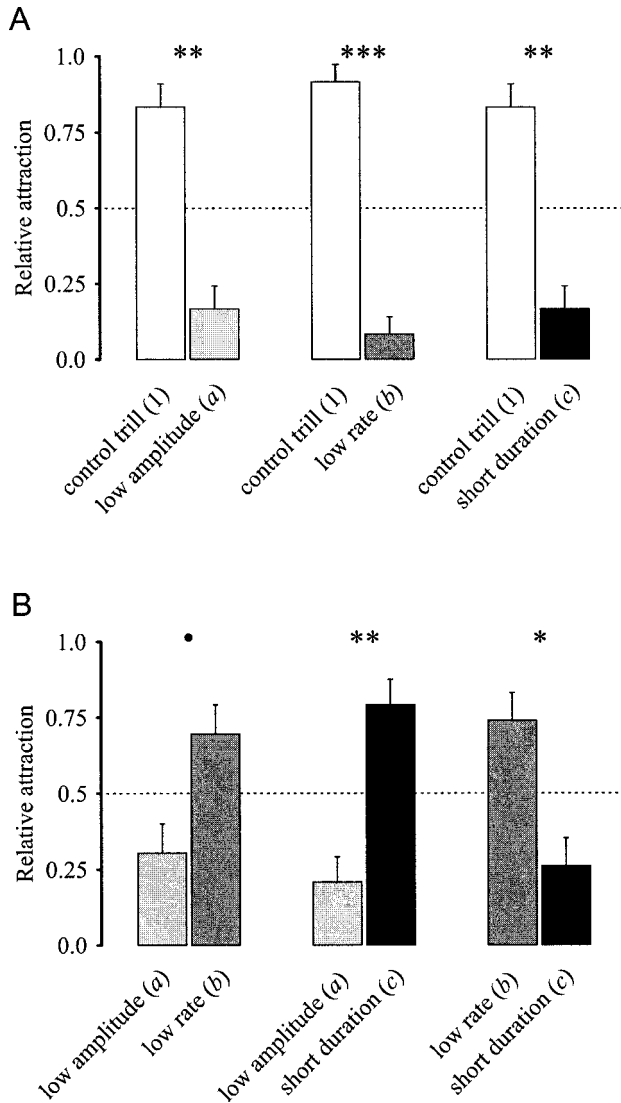


Figure 4
Proportion of parasitoid flies attracted to different stimuli in paired choice experiments. (A) The control trill (1), resembling the natural cricket call most, always attracted more flies when presented together with one of the test stimuli *a*, *b*, or *c*. (B) Among the control stimuli, *b* was preferred over *a*, *c* over *a*, and *b* over *c*. *** $p < .001$, ** $p < .01$, * $p < .05$, * $p < 0.1$; see text for statistical tests. Error bars represent SDs about the mean of relative attraction.

In a second series, we presented test stimuli of equal sound energy per unit of time, but that differed in their informational value (Figure 4B). The data reveal a tendency of higher attraction for amplitude when the flies had the choice between a trill of lower amplitude and a long chirp of high amplitude (*a* vs. *b*: two-tailed binomial test; 16 out of 23 flies, $p = .0639$). Flies were also more attracted to high amplitude when they were given a choice between a trill of low amplitude and a short chirp of high amplitude (*a* vs. *c*: two-tailed binomial test, 19 out of 24 flies, $p = .0067$). Comparing two chirp types, flies clearly showed more attraction to longer chirps (*b* vs. *c*: two-tailed binomial test; 17 out of 23 flies, $p = .0227$).

Altogether, these choice experiments suggest that flies are attracted to songs of higher amplitude. Among chirps of equal amplitude, flies are preferentially attracted to longer chirps.

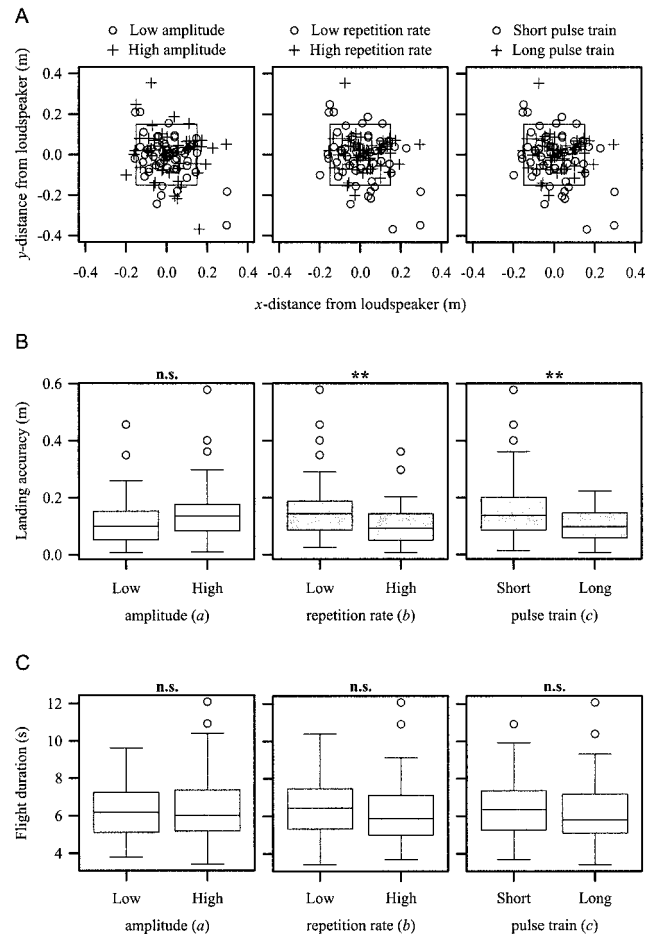


Figure 5
Search efficiency as a function of amplitude (*a*), repetition rate (*b*), and pulse train length (*c*) (112 trajectories from 14 individuals). (A) Map of landing positions. Square box: loudspeaker cabinet. One single outlier (landing accuracy = 0.58 m, cf. boxplots below) was omitted from the map to enhance the visibility of all other landing positions. (B) Boxplots yielding the independent influence of amplitude (*a*), repetition rate (*b*), and pulse train length (*c*) on landing accuracy measured as the distance between landing position and center of loudspeaker. The centerline through each box represents the median. Boxes represent the interquartile distance (IQD). The whiskers extend to the extreme values of the data (i.e., $\pm 1.5 \times$ interquartile distance from the median). Open circles indicate possible outliers. (C) Data for flight duration measured as the time elapsed between takeoff and landing. ** $p < .002$ (Bonferroni-corrected α levels); see text and Table 2 for statistical tests.

Sequential experiments

Based on a sample of 14 individuals (112 trajectories), we found that landing accuracy decreased for low repetition rate, as well as for short pulse trains (Figure 5A,B and Table 2). Together, these data imply that chirping reduces landing accuracy in general. All interaction terms in the repeated-measures ANOVA were nonsignificant (Table 2). Hence, the differences in landing accuracy simply add up if the test song was a combination of songs *b* and *c*. Although the differences in landing accuracy caused by a change in one of the factors were significant, they were rather small (Figure 5A,B). The differences in landing accuracy between low and high levels were only 5.7 cm (SE = 5.2) for factor *b* and 5.8 cm (SE = 4.9) for factor *c*. This is still remarkably precise because the mean path length between the starting platform to the loud-

Table 2
Three-way repeated-measures ANOVA of the effects of amplitude (a), repetition rate (b), and pulse train length (c) on landing accuracy and flight duration

Source	df	Landing accuracy (m)		Flight duration (s)	
		F	p	F	p
Amplitude (a)	1, 13	2.583	.1320	0.2053	.6579
Repetition rate (b)	1, 13	16.389	.0014	6.9847	.0203
Pulse train length (c)	1, 13	19.796	.0006	0.6259	.443
Interaction (ab)	1, 13	0.066	.8013	0.0537	.8203
Interaction (ac)	1, 13	0.044	.8365	0.0034	.9545
Interaction (bc)	1, 13	2.172	.1644	1.9199	.1892
Interaction (abc)	1, 13	0.195	.6659	0.1115	.7438

Data are the landing accuracy (distance from center of loudspeaker in m) and flight duration (time elapsed from takeoff to landing in seconds). Level of significance $\alpha' = 0.01$ (Bonferroni correction).

speaker extended to 3.85 m (SE = 0.26). Apart from a few outliers (indicated by the circles in the box plots), all flies landed close to the loudspeaker irrespective of stimulus condition (Figure 5A,B). Remarkably, flight duration was not significantly affected by any change of the song parameters *a*, *b*, or *c* (Figure 5C and Table 2).

Comparing flight trajectories in response to a continuous trill or chirps, we found that flies tend to fly straighter if attracted to chirping cricket calls (Figure 6). Typically, within a flight to a continuous trill, we recognize three phases: a take-off phase, a cruising phase, and a landing phase. Inspection of the trajectory's three-dimensional representation and its projections reveals that the fly was first gaining altitude and then approached the sound source with a meandering path. Closing in on the sound source, the fly initiated a spiral descent to the loudspeaker. Compared to the control situation, the trajectory to stimulus *b* did not show such transitions in the flight pattern except for the short takeoff phase. Instead, the fly flew straight to the loudspeaker with less alterations of her flight course. The repeated-measures ANOVA supports

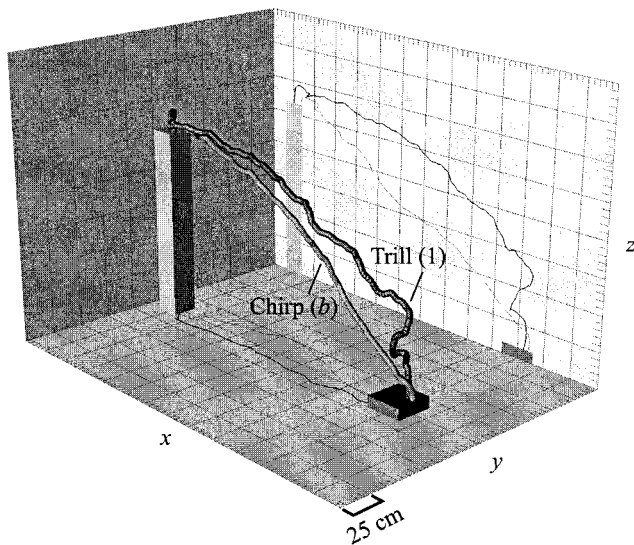


Figure 6
 Examples of flight trajectories from the same individual first tested with a control trill (1) and then with a chirp (test stimulus *b*, Figure 3 and Table 1). In response to a trill, the flight trajectory revealed more changes in flight direction than that to a chirp.

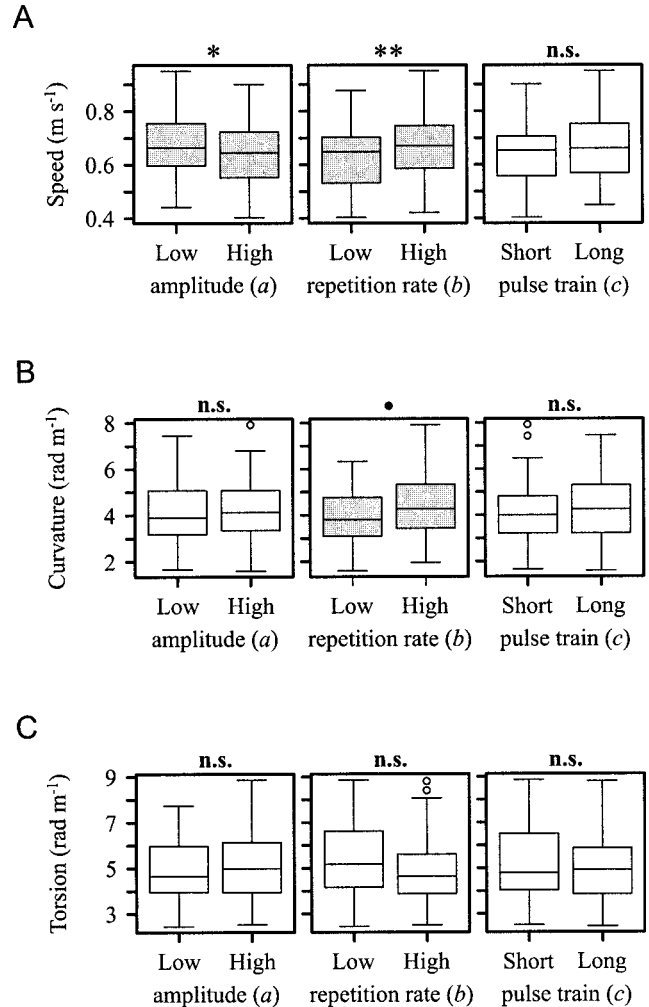


Figure 7
 Three-dimensional flight pattern as a function of amplitude (*a*), repetition rate (*b*), and length of pulse train (*c*) (112 trajectories from 14 individuals). (A) shows the effects on mean velocity, (B) on mean curvature, and (C) on mean torsion. The centerline through each box represents the median. Boxes represent the interquartile distance (IQD). The whiskers extend to the extreme values of the data (i.e., $\pm 1.5 \times \text{IQD}$ from the median). Open circles indicate possible outliers. ****** $p < .002$, ***** $p < .01$, **•** $p < .02$ (Bonferroni-corrected α levels); see text and Table 3 for statistical tests.

the impression obtained from Figure 6. The analysis reveals that both speed and curvature were affected by reducing the number of chirps (Figure 7A,B and Table 3). The flies flew slower, but tended to fly straighter. Hence, we may regard a straight flight path as the flies' behavioral response to the fragmentation of a continuous trill. Similarly to repetition rate, speed was reduced for high amplitudes, whereas curvature and torsion were not affected (Figure 7B and Table 3). The repeated-measures ANOVA yielded no effect on torsion for any change in the factors *a*, *b*, or *c* or for any of the possible interaction (Figure 7C and Table 3).

DISCUSSION

Four main points summarize the results of this study. First, the call of the primary host *G. rubens* was more attractive than any other alternative stimuli. Second, high intensity was a more attractive song characteristic than trilling. Everything else being equal, flies chose long over short chirps. Third, in

Table 3
Three-way repeated-measures ANOVA of the effects of amplitude (*a*), repetition rate (*b*), and pulse train length (*c*) on speed, curvature, and torsion

Source	df	Speed (m/s)		Curvature (rad/m)		Torsion (rad/m)	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Amplitude (<i>a</i>)	1, 13	9.3352	.0092	0.0051	.9443	3.0094	.1064
Repetition rate (<i>b</i>)	1, 13	19.506	.0007	8.7171	.0112	4.054	.0652
Pulse train length (<i>c</i>)	1, 13	3.0482	.1047	1.645	.2220	1.8156	.2009
Interaction (<i>ab</i>)	1, 13	0.3772	.5497	0.0894	.7696	0.0722	.7924
Interaction (<i>ac</i>)	1, 13	0.1338	.7204	0.8665	.3689	1.3705	.2627
Interaction (<i>bc</i>)	1, 13	0.3288	.5761	0.3243	.5788	6.1304	.0278
Interaction (<i>abc</i>)	1, 13	0.603	.4513	0.0367	.851	0.085	.7752

Data are the averaged values from the finite helix fit parameters; speed, curvature, and torsion along each trajectory. Level of significance $\alpha' = 0.01$ (Bonferroni correction).

no-choice experiments, phonotaxis and search efficiency were altered little in response to substantial fragmentation of the primary host song. Finally, flight trajectories imply that flies coped with the loss of acoustic information due to song fragmentation by keeping their flight direction.

Methodological considerations about choice versus no-choice experiments

The study of preference by choice experiments presupposes that all stimuli are perceivable when presented singly or together. Hence, it is useful to employ both simultaneous (choice) and sequential (no-choice) stimulus presentation to first establish perceptual capacity for each stimulus (no-choice) and then highlight differential phonotactic responsiveness (choice). Moreover, sequential stimulus presentation provides information about the relationship between call fragmentation and search costs. Field conditions have promoted choice experiments because they are simple to perform and require less time to gather a sufficiently large sample. Admittedly, comparison between stimuli presented at the same time provides sensitive and adequate information on differential attraction. Yet choice experiments may not rule out differential perception of the sound stimulus by the animal. Also, choice experiments present a disadvantage in that they do not unravel whether a no-preference stimulus is associated with increased search costs. On the other hand, the no-choice paradigm does not permit one to single out the part of differential attraction that is due to the specific test song parameter. Because the attractiveness of a stimulus, as determined from behavior, may well rely on the animal's evaluation of several properties of the stimulus, little can be said about the relative attractiveness of any particular stimulus property (Doherty, 1985). Hence, both methods are clearly complementary; they have their biological relevance because flies may encounter crickets calling alone or in aggregations.

Song structure and phonotaxis

In accordance with previous studies (Cade, 1981; Gray and Cade, 1999b; Ramsauer and Robert, 2000; Wagner, 1996; Walker, 1993), we found that female flies are differentially attracted to different song types. Yet differential attraction alone does not explain the reasons—ultimate and proximate—for the flies' capacity to distinguish song structures. We see at least three potential explanations as to why female flies distinguish different cricket songs. First, songs may convey some information about host quality that in turn reflects suitability for the flies' offspring. Second, the flies may find some songs psychoacoustically easier to locate and thus benefit from

a more effective acoustic search. Third, the flies' acoustic perception may be constrained to specific song types and therefore may be less sensitive to songs departing from a typical template.

In crickets, it has been suggested that by favoring males that produce more syllables per chirp, female crickets select males with higher pathogen resistance ability (Ryder and Siva-Jothy, 2000). Likewise, calling songs could provide parasitoids with information about host quality. Other parasitoids are known to discriminate between hosts based on host quality (for review, see Godfray, 1994). The number of pulses per trill in male *G. integer*, the major host for *O. ochracea* in Texas, is not related to male size or age (Gray and Cade, 1999a). Consequently, Gray and Cade (1999b) concluded that male quality is unrelated to the number of pulses per trill. Alternatively, they suggest that both female crickets and female flies adaptively minimize search costs by preferring the most common song. This conclusion is intriguing because the flies are gregarious parasitoids and may deposit a clutch of larvae on a host even when a host has already been parasitized (Adamo et al., 1995). In addition, cricket size seems to influence neither pupal weight nor the flies' survival to adulthood (Hage, 1998). Hence, the fly's reproductive success is expected to depend rather on her search efficiency which, in turn, is directly related to her auditory capacity and to the cricket's acoustic conspicuousness. For crickets, there is evidence that females search to minimize their search cost by also reducing predation risk (Hedrick and Dill, 1993). The composition of search costs for parasitoid flies has not been evaluated. On the basis of choice experiments alone, it is quite tempting to believe that song preference reflects search costs. Surprisingly, our data suggests that search costs vary little between different song types. Hence, other explanations, ultimate and proximate, ought to be considered as well.

Overall, flies are more attracted to songs with higher amplitudes and longer chirps. In response to a single test stimulus, flies are slightly less efficient in terms of spatial accuracy at low repetition rates and short pulse trains (Figure 5A,B). Because mean flight speed also changes as a function of stimulus properties (i.e., high amplitude and low repetition rate; Figure 7A), it may seem at first glance that overall search efficiency is indeed affected. Yet flight trajectories were highly variable, and the total amount of time elapsed between take-off and landing was not significantly different between treatments. Hence, the introduction of acoustic fragmentation in the cricket's trill alters the flies' phonotactic success little. Providing the flies with only 12.5% of the initial acoustic information of the control trill (control trill vs. stimulus *abc*; see Figure 3A,C) the flies remain capable of locating the sound source rather precisely. Upon landing, flies proceeded with

their phonotactic search by walking straight to the center of the loudspeaker.

The fact that flies tend to keep their flight course in response to song fragmentation may seem counterintuitive at first but bears significance for the questions raised here. Earlier studies (Müller and Robert, 2001) have shown that the flies' orientation toward the sound source also persists after complete interruption of the sound stimulus, demonstrating their remarkable capacity of pursuing a silenced sound source. This most intriguing persistence in orientation suggests that the flies are endowed with a behavioral strategy to accommodate the fragmentation (or even the absence) of acoustic information. Thus, as already pointed out by Walker (1993), chirping is no safeguard against acoustic parasitism.

Our data also do not support the ideas that flies show preference for calls they find easier to locate (Zuk et al., 1998) and that they spend less time doing so (Lehmann and Heller, 1998). Why, then, do *Ormia* flies show preferential phonotactic behavior when exposed to alternative songs? Differential attraction to higher intensities (here for *a* over both *b* and *c*) corroborates previous field studies (Cade, 1981; Wagner, 1996). In this context, Forrest and Raspet (1994) developed a model explaining that differential attraction may solely be based on relative loudness. Taking into consideration relative loudness and source spacing (Forrest and Raspet, 1994; Figure 5A), their model exactly predicts the relative attraction of 0.83 observed here.

Apart from song amplitude, *O. ochracea* also shows a comparable differential attraction for longer chirp duration (i.e., *b* over *c*; Figure 4B), as found here and in the field (Wagner, 1996). Surprisingly, chirping calls (*b* and *c*) were more attractive when presented simultaneously with a continuous trill of lower amplitude. We suggest that chirps of high amplitude impose a masking effect on the low-amplitude trill. Although masking effects have not been studied in detail in acoustic parasitoids (Ramsauer and Robert, 2000), studies in crickets have revealed that auditory masking is an important and complex issue in insect communication. For example, Römer et al. (1989) found that the song of *Hemisaga denticulata* was suppressed in the presence of the singing bushcricket *Mygalopsis marki*. Additionally, song conspicuousness in *M. marki* is affected by the competing acoustic activity of conspecifics (Dadour, 1989). Clearly, such masking may have negative effects on reproductive success if one's song becomes less conspicuous to conspecifics. In contrast, the impact of acoustic predators may favor reduced conspicuousness, which in turn ought to result in increased survival and reproductive success.

These results show that individual male crickets will benefit from song fragmentation only in some situations. Song fragmentation will reduce conspicuousness only in the presence of acoustic neighbors, conspecific and heterospecific, that are themselves more conspicuous chiefly by way of song amplitude. The flies' behavior highlights how hazardous it is for a keen, but unprepared, cricket to fill up a competitor's acoustic space.

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