

## **Parasitized Male Field Crickets Exhibit Reduced Trilling Bout Rates and Durations**

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### **Abstract**

The reproductive compensation hypothesis suggests that recently parasitized animals could offset future reproductive losses by increasing their current reproductive effort. We test this hypothesis by determining how male Texas field crickets (*Gryllus texensis*) alter their mate attraction displays following parasitism by acoustically orienting female parasitoid flies *Ormia ochracea* (Diptera, Tachinidae, Ormiini). Larval tachinid parasitoids cause little damage in phase I of host infestation. However, substantial host damage occurs in phase II, which results in parasitoid emergence and host death. We predicted that recently parasitized crickets would increase their mate attraction behaviour over pre-parasitism levels to enhance their abilities to attract a mate in phase I. Contrary to our prediction, during phase I neither total signalling time, trilling bout duration, trilling bout rate or amplitude changed from pre-parasitism levels. During phase II male crickets had significantly reduced total signalling times, and produced calls of significantly shorter duration at significantly slower trilling bout rates. Our results suggest that male Texas field crickets do not compensate for their shortened lifespan by increasing their reproductive effort following parasitism.

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### **Introduction**

Organisms that use acoustic displays to attract mates are susceptible to parasites that host-locate using acoustic cues. Once parasitized, significant life history changes can occur (Minchella & Loverde 1981; Forbes 1993; Agnew et al. 2000). Post-parasitism reproductive changes have been traditionally viewed as by-products of parasitic damage or adaptations by parasites to reduce predation (Fritz 1982; Minchella 1985; Horton & Moore 1993). Alternatively, reproductive changes could represent host adaptations (Forbes 1993; Poulin 1995). The reproductive compensation hypothesis suggests that recently parasitized animals offset future reproductive losses by increasing their current reproductive effort

(Minchella & Loverde 1981). Here we test the reproductive compensation hypothesis against the alternative hypothesis that parasitism depresses host reproductive behaviour by determining how male Texas field crickets (*Gryllus texensis*) alter their mate attraction displays post-parasitism.

Male Texas field crickets use two alternative strategies to attract mates: callers produce conspicuous acoustic signals, whereas satellites sit silently alongside signalling males and attempt to intercept attracted females (Cade 1975). Callers are regularly attacked by acoustically orienting gravid female parasitoid flies *Ormia ochracea* (Diptera, Tachinidae, Ormiini) (Cade 1975). Parasitism rates vary from 9–26% (Cade 1975, 1979, 1984; Adamo et al. 1995). Gravid flies have directional tympanal hearing organs that allow host location (Robert et al. 1992, 1996a,b, 1998; Robert & Hoy 1994; Miles et al. 1995). Once the host is located, the fly deposits planidia (first instar larvae) on and around the cricket (Cade 1975). In phase I of parasitism, the planidia burrow into the cricket, usually lodging themselves inside thoracic flight muscles, where they appear to cause little damage (Adamo et al. 1995). Phase I lasts approx. 3 d, and then the larvae leave the thorax and migrate to the cricket's abdomen. Phase II lasts approx. 4 d, during which time the larvae feed on the cricket's fat body and muscle, causing extensive damage (Adamo et al. 1995). The parasitoid then makes a hole in the cricket's cuticle and departs (Adamo et al. 1995). Cricket death ensues, usually within 3 h of parasitoid departure (Wineriter & Walker 1990; Robert et al. 1992; Adamo et al. 1995).

Given that parasitoids cause little muscle damage in phase I of host infestation, crickets may be able to increase their current reproductive effort to offset future losses resulting from their shortened life span (Minchella & Loverde 1981). Contrary to the reproductive compensation hypothesis, however, all behaviours examined to date indicate that reproductive effort either remains constant or is reduced. Adamo et al. (1995) showed that in phase I of parasitism both the time for males to initiate courtship singing and the time for females to mount males was unaltered. A related species (*Teleogryllus oceanicus*) also parasitized by *O. ochracea* does not alter the number of successful mountings, the number of hatchlings produced, or their metabolic rate in phase I. They do, however, produce fewer spermatophores and devote significantly less total mass to reproductive tissue (Kolluru et al. 2002). These studies provide little support for the reproductive compensation hypothesis.

Little research has focused on whether parasitized crickets alter their signalling behaviour. Cade (1984) found that while parasitoid infestation depressed total signalling time in Texas field crickets in phase II, total signalling time was unaltered in phase I. Kolluru et al. (2002) found signalling activity (percentage time spent signalling over an approx. 12-h monitoring period) in *T. oceanicus* dropped significantly the first day of parasitism and remained reduced throughout parasite infection. To date, the only research that has examined other signalling parameters suggests the potential for field crickets to enhance their calls following parasitism. In a study designed to examine whether *O. ochracea* females are preferentially attracted to calls exhibiting certain

signalling parameters, Zuk et al. (1998) recorded and collected male *T. oceanicus* from natural populations across several of the Hawaiian Islands. Males that were parasitized naturally prior to collection had significantly longer songs, with longer long chirps, short chirps, and pulses than males that had avoided parasitoid infestation (Zuk et al. 1998). Zuk et al. (1998) proposed that female flies prefer male crickets with these song characteristics. However, as Zuk et al. (1998) pointed out, the differences between parasitized and unparasitized crickets could also result from male crickets increasing their investment in mate attraction following parasitism.

Here we compare the signalling behaviour of male Texas field crickets, parasitized following an initial 2-d period of acoustic monitoring, with the signalling behaviour of control males to determine whether parasitized male crickets increase their trilling amplitude, trilling bout duration, or bout rate. We selected trilling amplitude, bout rate, and bout duration as signalling parameters because all should be energetically costly to enhance (Hoback & Wagner 1997), and because female crickets appear to be preferentially attracted to males that signal loudly, for long periods, with signals produced at fast rates. Field-based sound traps are more successful at attracting female crickets when broadcast at a high amplitude (Cade 1979, 1981; Walker 1986), suggesting that female crickets are preferentially attracted to loud calls. A field-based arena study revealed that mating success correlates positively with male cricket total signalling time in low-density populations (Cade & Cade 1992). Given that total signalling time is the product of trilling bout rate and bout duration, female Texas field crickets may be preferentially attracted to calls produced at a high rate and long duration. This prediction has been supported by research on a congener, *G. lineaticeps*. Female crickets prefer male chirps produced at higher rates, longer durations, and higher amplitudes (Wagner 1996). Male Texas field crickets may therefore be able to enhance their current reproductive success by increasing their investment in any or all of these signalling components.

### Methods

Male Texas field crickets and were collected at the bright lights of a driving range in Austin, TX, in Sep. 2003. Parasitoid flies were collected using a fly trap (Walker 1989) with speakers playing *G. texensis* song at the Brackenridge Field Laboratory of University of Texas at Austin. Collected flies and crickets were brought to our laboratory at Arizona State University.

Male crickets were placed in individual containers with food, water and a shelter. Their acoustic mate signalling behaviour was monitored over at least nine nights. Acoustic signalling was monitored each night from 18:00 h until 10:00 h the following day using an electronic acoustic recorder (EAR) (Bertram et al. 1996; Bertram & Johnson, 1998). Each cricket was housed in a 500-ml wax-coated 'ice-cream cup' paper container with food and water. A microphone was hung 5 cm above each cricket, within the container. The EAR

selected a microphone, read the container's noise level (amplitude), amplified the signal, converted it from AC to DC, filtered it, and then recorded it to disk. Only one microphone was turned on at a time, and each microphone was sampled eight times a second. Samples were averaged over each second, resulting in a second-by-second signalling history for each individual throughout the course of every evening.

Following two nights of monitoring, males were removed from the EAR, parasitized, and then returned to the EAR for at least another week of acoustic monitoring. To parasitize crickets, we removed planidia from the gravid female fly by dissecting her abdomen and freeing the planidia (Adamo et al. 1995). After one to three planidia had adhered to fine-point forceps, we placed them on the soft underside area of the cricket between the front two legs (Adamo et al. 1995). We attempted to hand parasitize 44 crickets. Males successfully infested by the larval parasitoids were classified as our parasitized treatment ( $n = 23$ ). All parasitized crickets died following parasitoid departure, usually at day 7 post-parasitism. Males in which the planidia failed to penetrate the integument were classified as our control treatment ( $n = 21$ ). All control crickets were killed 10 d 'post-parasitism'. They were carefully dissected to ensure that they had not been parasitized following Adamo et al. (1995).

The second-by-second signalling history collected for each individual on each night monitored was used to determine total signalling time (time spent signalling during each of the 16-h monitoring periods), average duration of trilling bouts (number of minutes trilling without taking at least a 1-min break), trilling bout rate (number of trilling bouts/h), and average trilling amplitude (dB). Data were analysed statistically with JMP 5.1 software (JMP 5.1, SAS Institute Inc., Cary, North Carolina, USA). We tested results for normality using the Shapiro–Wilk statistic, and then log-transformed total signalling time, bout duration, and bout rate data to meet the assumptions of normality.

To determine if males altered their signalling behaviour as parasitism progressed we conducted a series ( $N = 4$ ) of two-way repeated-measures ANOVAs, with parasitism phase as the repeated factor, and treatment (parasitized or control) as the other main effect on all signalling parameters. There were three parasitism phases: pre-parasitism – two nights of monitoring prior to parasitism; phase I – the first three nights of acoustic monitoring following parasitism, during which time the parasitoid typically resides in the thorax (Adamo et al. 1995); and phase II – nights 4–7 following parasitism when the parasitoid typically resides in the abdomen and substantial damage occurs (Adamo et al. 1995). Because of the use of multiple tests, we employed a Bonferroni-adjusted significance level of  $p < 0.0125$ .

For both treatments we also ran a series of ANOVAs with phase (pre, phase I, and phase II) as the factor to allow us to identify significant differences among phases using all-pairs Tukeys-Kramer tests. To show whether the signalling behaviour differed across treatments within each of the phases, we ran ANOVAs for each parasitism phase with treatment (parasitized or control) as the factor. Statistical significance levels were Bonferroni corrected to  $p < 0.0025$  to account for the multiple tests ( $N = 20$ ).

## Results

Overall, our repeated-measures ANOVA revealed a statistically significant interaction between parasitism phase (pre-parasitism, phase I and phase II) and treatment (parasitized vs. control) for total signalling time, trilling bout duration, and Trilling amplitude, but not bout rate (Table 1).

Contrary to the reproductive compensation hypothesis, parasitized male Texas field crickets did not increase their acoustic mate signalling behaviour following parasitism. In phase I of parasitism, when the parasitoid resided in the host's thorax, parasitized males' signalling behaviour did not change from pre-parasitism levels (Table 2). However, during the second phase of parasitism, when the parasitoid resided in the host's abdomen, crickets dramatically reduced most aspects of their acoustic signalling behaviour. Parasitized males signalled for significantly shorter amounts of time and produced trilling bouts of shorter durations (Table 2). There was a non-significant trend for parasitized males to signal at reduced trilling rates. Parasitism appeared to have no significant effect on trilling amplitude.

Control males' acoustic mate signalling behaviour did not change significantly across the three phases of parasitism (Table 2). There was a non-significant trend during the pre-parasitism phase of the experiment for the control males to signal with less vigour than the parasitized males: control males had lower total signalling time, shorter bout durations, and produced bouts at a lower rate (Table 2).

*Table 1:* Repeated measures analysis of variance results with phase (pre-parasite, phase I and phase II) as the repeated factor, and treatment (parasitized or control) as the other main effect for all signalling parameters. Significance levels are Bonferroni corrected to values of  $p < 0.0125$  to account for multiple tests ( $N = 4$ )

	df	<i>F</i>	p
Total signalling time (min)			
Treatment	11	0.50	0.4936
Time	10	14.96	0.0010
Treatment × time	10	9.47	0.0049
Bout duration (min)			
Treatment	11	0.62	0.4459
Time	10	18.12	0.0005
Treatment × time	10	13.19	0.0016
Bout rate (no. bouts/h)			
Treatment	11	0.04	0.8495
Time	10	1.93	0.1960
Treatment × time	10	1.58	0.2537
Trilling amplitude (dB)			
Treatment	11	0.00	0.8838
Time	10	3.60	0.0665
Treatment × time	10	17.44	0.0005

*Table 2:* Average values with standard deviation in parentheses for each treatment (control and parasitism) and parasitism phase (pre-parasitism, phase I, and phase II). Statistical differences across treatments but within parasitism phase reside below averages, and statistical differences across parasitism phases but within treatments lie to the right hand side of the mean values. Significant differences (Bonferroni corrected to  $p < 0.0025$ ) between parasitism phases are represented by superscripted letters, phases with different superscripts are significantly different (all pairs Tukeys–Kramer test significant differences require  $p < 0.05$ )

Parameter	Treatment	Pre-parasitism	Phase I	Phase II	<i>F</i>	<i>p</i>
Signalling time	Control	40.54 (52)	72.83 (99)	93.17 (127)	0.20	0.82
	Parasite	111.81 (112) <sup>a</sup>	97.18 (120) <sup>a</sup>	29.03 (86) <sup>b</sup>	12.18	<0.0001
	<i>F</i>	3.42	0.45	4.46		
	<i>p</i>	0.07	0.51	0.04		
Bout duration	Control	7.91 (9)	12.39 (18)	8.87 (12)	0.36	0.70
	Parasite	15.36 (16) <sup>a</sup>	14.17 (15) <sup>a</sup>	5.88 (14) <sup>b</sup>	11.08	<0.0001
	<i>F</i>	2.67	0.53	3.94		
	<i>p</i>	0.11	0.47	0.06		
Bout rate	Control	0.33 (0.25)	0.34 (0.21)	0.43 (0.36)	0.01	0.99
	Parasite	0.5 (0.33)	0.4 (0.31)	0.2 (0.14)	4.83	0.01
	<i>F</i>	2.20	0.07	3.05		
	<i>p</i>	0.15	0.79	0.09		
Bout amplitude	Control	63.78 (3)	65.28 (4)	65.97 (4)	1.98	0.15
	Parasite	65.49 (3)	66.20 (3)	63.66 (3)	2.58	0.09
	<i>F</i>	2.97	0.86	3.19		
	<i>p</i>	0.09	0.36	0.08		

## Discussion

The reproductive compensation hypothesis predicts that once a host becomes parasitized it should enhance its reproductive efforts to offset future losses (Minchella & Loverde 1981). We predicted that recently parasitized crickets would increase their mate-attraction behaviour over pre-parasitism levels to enhance mate attraction in phase I of parasitism. Contrary to our prediction, neither total signalling time, bout duration, bout rate, nor trilling amplitude changed in comparison with pre-parasitism levels or in comparison with uninfested controls. During phase II of parasitism, when substantial host abdominal tissue damage occurred (Adamo et al. 1995), mate-attraction behaviour declined. Phase II males had significantly lower total signalling times, and produced bouts of significantly shorter duration at decreased rates. Overall these results suggest that the ability of parasitized crickets to reproduce declines a few days after parasitism and that crickets are unable to compensate for the fitness losses they will experience by increasing their current reproductive effort.

Why do crickets not show an adaptive response to tachinid parasitism by increasing their investment in mate-attraction behaviours after attack by gravid female flies? There are at least three possible explanations. First, cricket response to tachinids may not have had time to evolve because the parasitoid–cricket

interactions may be relatively recent (Adamo 1999). Secondly, larval parasitoids may actively or passively depress host signalling behaviour, masking the host's response (Adamo et al. 1995). Forbes (1996) and Perrin et al. (1996) recommend examining the host's metabolic responses to parasitism to determine whether changes in host behaviour result from host or parasitoid-induced responses. Although the metabolic rates of uninfested and infested *G. texensis* have yet to be measured, *T. oceanicus* infested with larval *O. ochracea* appear to have lower resting metabolic rates than uninfested crickets (Kolluru et al. 2002). Therefore, infested crickets may be unable to increase their reproductive effort because of a reduced metabolic capacity for breaking down storage tissue needed for reallocation to reproduction (Kolluru et al. 2002). Thirdly, the cricket's immune response may not be activated in the first phase of parasitism, so that the cricket may not receive the cues necessary to enhance their mate attraction calls (Adamo 1999; Kolluru et al. 2002). During phase I, larval parasitoids enter the cricket and embed in thoracic muscle. Because they stay out of the hemocoel they are thought to evade the host's immune response and thereby avoid encapsulation (Vinson 1990).

While Texas field cricket males do not alter their mate attraction behaviour following parasitism, our results demonstrate that phase II of parasitism should constrain the male's ability to successfully attract a mate. Parasitism by tachinid flies represents a significant fitness cost to crickets several days before parasitoid-induced death. Because the crickets are not compensating for these fitness costs by enhancing their signalling behaviour immediately following parasitism, crickets should experience strong selection to avoid parasitoid infestation.

What is the evidence that crickets attempt to avoid parasitoid infestation? Several of the males that we hand-parasitized did not succumb to parasitism. Instead, they showed no indication that they had ever been exposed to the parasitic planidia. Lack of infestation might have resulted from damage to the planidia during transfer, improper planidia placement on the crickets integument, or the use of immature planidia. However, it is also possible that control and parasitized treatments reflect non-random sampling. Lack of infestation in control males may reflect intrinsic differences in quality, where males avoid parasitoid infestation by careful grooming or having an integument too thick for most planidia to penetrate. If failure to be parasitized reflects some intrinsic difference in quality, then whatever confers this heightened resistance could also affect calling behaviour. Parasitoid resistant males may be less prone to acoustical fatigue. This hypothesis remains to be tested.

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