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Eavesdropping Parasitoids Do Not Cause the Evolution of Less Conspicuous Signaling Behavior in a Field Cricket

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Abstract

Males of many species produce conspicuous mating signals to attract females, but these signals can also attract eavesdropping predators and parasites. Males are thus expected to evolve signaling behaviors that balance the sexual selection benefits and the natural selection costs. In the variable field cricket, *Gryllus lineaticeps*, males sing to attract females, but these songs also attract the lethal parasitoid fly *Ormia ochracea*. The flies use male crickets as hosts for their larvae, primarily search for hosts during a 2 h period following sunset and prefer the same song types as female crickets. We tested whether males from high-risk populations reduce the risk of parasitism by singing less frequently or by shifting their singing activity to a time of the night when the risk of parasitism is low. We compared male singing activity and its temporal pattern between six high-risk and six low-risk populations that were reared in a common environment. There was no effect of parasitism risk on either total male singing activity or the temporal pattern of male singing activity. Males from high-risk populations thus sang as frequently as males from low-risk populations. These results suggest that sexual selection on male singing behavior may be substantially stronger in high-risk populations than in low-risk populations. It is possible that other traits may have evolved to reduce parasitism risk without compromising mate attraction.

Keywords: Field cricket, *Gryllus lineaticeps*, Natural selection, *Ormia ochracea*, Parasitoid fly, Sexual selection, Singing activity

Many male animals advertise the direct and indirect benefits they will provide to females using conspicuous mating signals (Bradbury & Vehrencamp 1998; Gerhardt & Huber 2002). However, eavesdropping predators can also use these mating signals to locate the signaller (Birch 1978; Dixon & Payne 1980; Harris & Todd 1980; Greany & Hagen 1981; Lloyd & Wing 1983). These conflicting sources of sexual and natural selection often force males to compromise between the benefit of attracting a mate and the cost of attracting a predator. For example, predation can favor the evolution of reduced signaling activity (Cade & Wyatt 1984; Cade 1991), and can cause a shift in the time of signaling to periods when the predators or parasites are less active (Endler 1987; Bertram et al. 2004; Vélez & Brockmann 2006), a reduction in the conspicuousness of male signals (Endler 1983; Bertram et al. 2004), or a switch to signaling modalities that are less conspicuous to the predator (Morris 1980; Morris & Beier 1982). Although this conflict between sexual and natural selection has important effects on the evolution of male signaling behavior, studies of the evolutionary consequences are relatively rare, partly because they require large comparative studies or long-term laboratory or field studies (e.g. Ferguson & Fox 1984; Reznick et al. 1990; Hasselquist 1998; Grant & Grant 2002).

The interaction between the tachinid parasitoid fly, *Ormia ochracea*, and its field cricket hosts (multiple species of the genera *Gryllus* and *Teleogryllus*) offers an excellent opportunity to investigate the effect of conflicting selection on the evolution of male signals and signaling behavior. Both female crickets (Alexander 1961) and female flies (Cade 1975) orient to male cricket mating songs. Females orient to male songs to locate mates, while flies orient to male songs to locate hosts for their larvae. Once a fly has located a male, it deposits larvae on and around the male (Cade 1975). Male crickets are infested either by larvae deposited on his body (Cade 1975), or by picking up larvae deposited nearby (Beckers et al. 2011; C. M. Martin & W. E. Wagner, Jr., unpublished data). When larvae contact a male cricket, they burrow into it, feed primarily on its muscles and fat tissue, and kill it within 7–10 days (Adamo et al. 1995).

Ormia ochracea ranges from Florida to California and Hawaii, U.S.A., and uses at least 10 field cricket species as hosts across this range (Cade 1975; Walker 1986; Walker & Winkler 1991; Zuk et al. 1993; Wagner 1996; Hedrick & Kortet 2006; Sakaguchi & Gray 2011). In California, *O. ochracea* uses the variable field cricket, *Gryllus lineaticeps*, as a host (Wagner 1996). The crickets are nocturnal and sing from dusk till dawn, whereas the flies primarily orient to male song during the first

2 h after sunset (Cade et al. 1996; O. M. Beckers & W. E. Wagner, Jr., personal observations). Parasitism rates of male *G. lineaticeps* can be as high as 60% (Martin & Wagner 2010). Only some populations, however, are attacked by *O. ochracea* (see below). There is thus geographical variation in the importance of this source of natural selection on male singing behavior.

Ormia ochracea not only orients to *G. lineaticeps* song, but also differentially orients to the song types preferred by females (i.e. higher chirp rates and longer chirp durations; Wagner 1996; Wagner & Basolo 2007a, b). Females appear to prefer these song types because, under some conditions, the males that produce them transfer seminal products that increase female fecundity and life span (Wagner & Harper 2003; Tolle & Wagner 2011). It is unknown whether or why the flies benefit from orienting to these song types. Because the flies prefer the same song types as females, the relatively straightforward prediction is that males in populations with a high risk of parasitism should produce lower chirp rates and shorter chirp durations. Surprisingly, however, males from parasitized populations produce songs that are highly attractive to the flies (O. M. Beckers & W. E. Wagner, Jr., unpublished data). In the present study we tested whether fly parasitism has affected the evolution of male singing activity. Parasitism may not have had the predicted effect on male song characters because males have evolved alternative mechanisms to reduce their risk of parasitism, such as a reduction in overall singing activity or a shift in the temporal pattern of singing to time periods when few flies are searching for hosts, as has been shown for other species of field crickets attacked by *O. ochracea* in different geographical regions (Cade & Wyatt 1984; Cade 1991; Zuk et al. 1993; Bertram et al. 2004; but see Kolluru 1999). To test the effect of parasitism on male singing activity, we conducted a comparative study that included six high-risk and six low-risk populations of *G. lineaticeps*. The crickets were reared in a common environment to examine evolved differences in singing activity between the two types of populations. We predicted that males from the parasitized populations would show lower overall singing activity and/or sing less frequently during the first 2 h after sunset, the time period in which fly activity is highest (Cade et al. 1996; O. M. Beckers & W. E. Wagner, Jr., personal observations).

Methods

Cricket Populations and Parasitism Status

Gryllus lineaticeps is the most abundant species of field cricket in California and ranges from southern Oregon to the southern tip of Baja California (Weissman et al. 1980). Reproductive activity occurs from the early summer to late autumn (Weissman et al. 1980), whereas *O. ochracea*, when present at a location, orients to male song for approximately 3–4 weeks in late summer or early autumn (Paur & Gray 2011; W. E. Wagner, Jr., personal observation). We collected crickets from 12 populations distributed along the coast and the Central Valley of California (for locations of the populations, see Supplementary Figure S1). Populations were separated by an average distance of 221 km, as well as by physical barriers such as the coastal and transverse mountain ranges.

We used two methods to determine parasitism status. First, we broadcast *G. lineaticeps* songs with temporal and spectral characters known to attract *O. ochracea* in California (Wagner 1996; Wagner & Basolo 2007b). The stimulus consisted of a natural pulse (duration of 11 ms, dominant frequency of 5.17 kHz) that was copied eight times (while keeping the pulse intervals constant at 4 ms) to synthesize chirps of 116 ms duration. The chirps were separated by gaps of silence of 217 ms, resulting in a chirp rate of 3.0 chirps/s. These parameter values are within

the natural range for *G. lineaticeps* chirps (Wagner 1996). We broadcast the songs at an amplitude of ~93 dB SPL (at 30 cm from the loudspeaker), which is substantially higher than the amplitude of natural cricket song (approximately 70 dB SPL at 30 cm; W. E. Wagner, Jr., unpublished data). We used high-amplitude broadcasts to increase the probability of attracting flies. Second, we collected males and females from each population (for numbers of collected animals see Supplementary Table S1) over 3 years of sampling (2008–2010) and held them in individual containers for 14 days. *Ormia ochracea* larvae typically emerge from their cricket host within 10 days of infestation (Adamo et al. 1995). We sampled most of the 12 populations multiple times over 3 years using both methods: in 2008 (August 8–September 21), in 2009 (June 6–August 30), and in 2010 (June 29–September 12). We sampled each population at least twice in the 3-year period and sampled some populations more frequently in the context of other studies (see Supplementary Table S1). The time windows of sampling each year corresponded approximately to the earliest and latest dates of fly activity observed over the past 10 years (W. E. Wagner, Jr., unpublished data). We categorized a population as high risk if one or both of the following criteria were met: (1) there was evidence of two or more flies at the site (attracted to broadcasts and/or known to have parasitized male *G. lineaticeps*) across all years of sampling, or (2) any female *G. lineaticeps* was parasitized. The second criterion is a strong indicator of parasitism risk because females show a much lower risk of being parasitized ($\leq 6\%$) than males (60%; Martin & Wagner 2010). One of our study populations (RSV) was previously known to be a high-risk population according to the criteria outlined above (Wagner & Basolo 2007b; Martin & Wagner 2010; see Supplementary Table S1). For the remaining populations, we categorized the populations as low risk if neither of these criteria was met. As a result, we included populations with a very low likelihood of fly parasitism in the low-risk category (e.g. one male cricket infested over at least 3 years of sampling) and we therefore use the terms 'low risk' and 'high risk' instead of 'not parasitized' and 'parasitized' to describe the populations in this study. Note that our criteria for categorization represent threshold values rather than quantitative measures of parasitism risk. Therefore, we stopped sampling a given population in a given year as soon as either criterion was met (for sampling between 2008 and 2010). High- and low-risk populations that have been vigorously sampled for more than 3 years (low-risk populations: ACD and SDG; high-risk population: RSV) or that had multiple male and female crickets infested (high-risk population: CYC) support our choice of categorization criteria: the singing activity measures from these populations are similar to the other high- and low-risk populations that were sampled over a shorter period.

Rearing of Crickets

We collected 18–80 females from each population between 2004 and 2009 to establish laboratory colonies at the University of Nebraska-Lincoln. Most of the collected females mated with one or more males prior to collection. Each female was placed in a 16 × 26 × 17 cm family container (Pla-House, Oscar Enterprises, Inc., Gardena, California, U.S.A.), which was provisioned with a paper towel substrate, a cotton-plugged water vial, ad libitum cat chow (Nestlé, Purina PetCare Company, St Louis, Missouri, U.S.A.) and moist vermiculite (Premium Grade, SunGro Horticulture Distribution, Inc., Bellevue, Washington, U.S.A.) for oviposition. The offspring of the field-collected females constituted the first laboratory generation. Siblings were reared together in the family container, and late-instar juveniles were transferred to individual containers, 9 × 16 × 11 cm (Pla-House). Individual containers were provi-

sioned with a paper towel substrate and cardboard shelters, and crickets were provided with water and food as described above. After reaching maturity, pairs of unrelated males and females were placed in family containers for mating and oviposition. The offspring of each pair constituted the second laboratory generation. Subsequent generations were propagated using this procedure. We maintained breeding records for each population and arranged matings to avoid inbreeding.

We sorted late-instar male juveniles from their family containers and placed them into individual containers. We checked the individual containers daily and noted the date on which the final moult occurred. We thus knew the adult ages of all individuals used in the study. All individuals were kept in environmental chambers with a reversed 14:10 h day:night cycle and ambient temperatures that varied between 21.1 °C and 27.7 °C. Males become sexually mature and start singing within 7 days of the final moult, and *Gryllus* males can live up to 3–4 weeks as adults under natural conditions (Murray & Cade 1995). All males used in our study were 7–21 days of adult age. We measured the singing activity of 56–68 males from each population (mean = 60) and we recorded up to four males from the same full-sibling family. The number of families ranged between 20 and 32 per population (mean = 26.2). Our research adhered to the ASAB/ABS guidelines for the use of animals in research, the legal requirements of the U.S.A. and all guidelines of the University of Nebraska.

Because environmental conditions during development might influence male singing behavior, we only tested males reared in the common environment of the laboratory. Furthermore, because environmentally based maternal and paternal effects can influence offspring traits, we only tested males whose parents were also reared in the common environment of the laboratory. Variation among populations in our study can thus be largely attributed to evolved genetic differences.

Experimental Set-up and Protocol

We measured male singing activity for 5 h following the start of the dark portion of the light cycle. In California, host searching by *O. ochracea* is highest in the 2 h following sunset and declines substantially afterwards, resulting in a very low risk of parasitism after the first 2 h (W. E. Wagner, Jr., personal observation; see also Cade et al. 1996). At the beginning of each trial, we placed 20 males in their individual containers in a circular arrangement on the counters of the experimental room. The cages were separated by a distance of 0.5–1 m, which is within the range of natural inter-male distances (W. E. Wagner, Jr., personal observation). Males were given 20 min of acclimation in the darkened room before we started data collection. We then sampled male singing activity every 10 min during the 5 h trial. During each sampling period, we monitored each male for 10 s. If a male sang during the 10 s period, he was recorded as 'singing;' otherwise, the male was recorded as 'not singing.' Males were sampled in a clockwise direction within each sampling period, and each subsequent round of sampling was started with the male immediately to the right of the first male sampled in the previous round. We used a dim red .ashlight to move from male to male. Male singing activity did not seem to be affected by the light or our movement. The ambient temperatures in the testing room ranged between 20.0 °C and 25.0 °C, which fall within the range of temperatures experienced by the crickets at night in the field (O. M. Beckers & W. E. Wagner, Jr., personal observations). We included 'ambient temperature' in our models to account for the variation in temperature among testing days (see below). Because the availability of males of the appropriate age from a given population could not be controlled, and because we did not want to collect data from more than four males per full-sibling fam-

ily, there were testing periods during which there were fewer than 20 males from which we could collect data. To maintain a constant number of males per trial, supplementary males were placed in the testing room so that 20 males were present in all of our trials. These supplementary males were the siblings of males used in previous testing periods. All males tested during a given 5 h trial were from the same population.

Statistical Analyses

First, we compared total male singing activity between high-risk and low-risk populations using a linear mixed model. The dependent variable was the number of sampling periods a male was observed singing during the 5 h of observations. This count variable was modelled using a Poisson error distribution. The fixed factors were parasitism risk (high or low risk), adult age and ambient temperature at the beginning of the observation period. Our model also included two random factors: population and family nested within population.

Second, we compared the temporal pattern of male singing activity between high- and low-risk populations. The dependent variable was the number of sampling periods a male was observed singing during a given 1 h period. This count variable was modelled using a Poisson error distribution. The fixed factors were parasitism risk (high or low risk), hour (which tested for a linear effect of hour), hour² (which tested for a nonlinear effect of hour), adult age, ambient temperature at the beginning of the observation period, the interaction between parasitism risk and hour, and the interaction between parasitism risk and hour². Our model also included three random factors: population, family nested within population and individual nested within family.

After testing the models including all fixed and random factors, we removed stepwise all nonsignificant interactions and fixed factors. Since we were primarily interested in the effects of parasitism risk, we present the effect of parasitism risk from the full model and then present the results of the reduced model. All statistical analyses were performed using the xt-mepoisson function of Stata v.10 (StatCorp, College Station, Texas, U.S.A.).

Results

First, we compared total male singing activity between high- and low-risk populations. There was not a significant effect of parasitism risk on total singing activity ($\chi^2 \leq 0.05$, $P = 0.831$; Figure 1). After the stepwise removal of nonsignificant fixed effects from the model, male age and ambient temperature were significant predictors of male singing activity; older males sang more frequently, and males sang less frequently at higher temperatures (Table 1). Population and family also had significant effects on total singing activity (Table 1).

Second, we compared the temporal pattern of male singing activity between high- and low-risk populations. In both high- and low-risk populations, male singing activity increased during the first hours of the night, but then plateaued at the third hour following sunset (Figure 2). Singing activity was not significantly affected by parasitism risk ($\chi^2 \leq 0.04$, $P = 0.851$), the interaction between parasitism risk and hour ($\chi^2 \leq 2.89$, $P = 0.089$), or the interaction between parasitism risk and hour² ($\chi^2 \leq 0.16$, $P = 0.690$). There was thus no evidence that singing activity of males from high- and low-risk populations differed over time within a night (Figure 2). There was also no significant effect of ambient temperature on hourly singing activity ($\chi^2 \leq 2.66$, $P = 0.103$). After the stepwise removal of nonsignificant fixed factors from the model, hour, hour² and male age were significant predictors of male singing activity (Table 2).

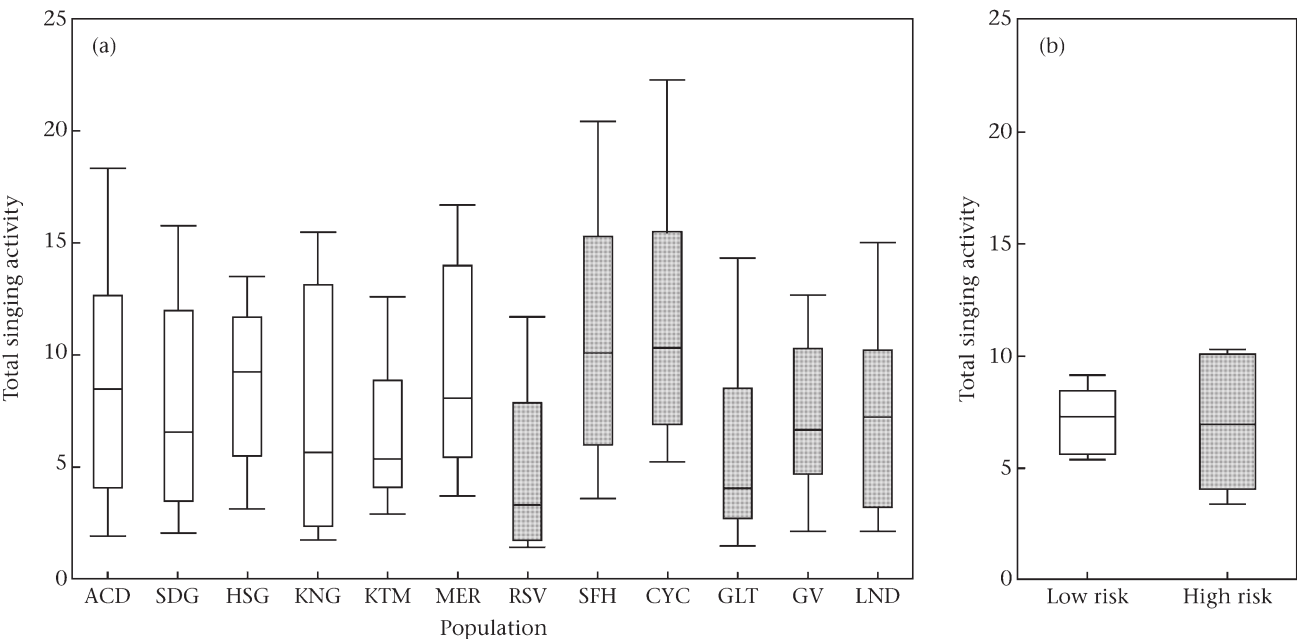


Figure 1. Total nightly singing activity of male *G. lineaticeps* from populations with low (white bars) and high (gray bars) parasitism risk. (a) Box plots show the median, top and bottom quartiles, and the 10th and 90th percentiles for each population. Abbreviations of population locations are indicated on the X axis (see Supplementary Figure S1; also see Wagner et al. 2012). (b) Box plots show the median, top and bottom quartiles, and the 10th and 90th percentiles of the population means ($N = 6$ for each parasitism environment).

As before, older males sang more frequently. Population, family and individual also had significant effects on male singing activity (Table 2).

Discussion

Natural and sexual selection often have conflicting effects on the evolution of male mating signals (e.g. Endler 1983; Wagner 1996; Zuk et al. 2006), and it is often thought that male signaling behavior should represent a compromise between these sources of selection. This might not be true, however, if males evolve alternative mechanisms to minimize their risk of predation (e.g. Belwood & Morris 1987; Hedrick 2000; Lewkiewicz & Zuk 2004), thereby allowing them to simultaneously attract females and avoid predators. Male *G. lineaticeps* from the same high-risk populations that were used in this study produce song types that most likely increase rather than decrease their risk of parasitism by *O. ochracea* (O. M. Beckers & W. E. Wagner, Jr., unpublished data). In the current study, we tested whether males from these high-risk populations compensate for the risk of parasitism by singing less frequently, either throughout a night or during the time of night when the risk of parasitism is highest. Contrary to our expectations, males from high-risk populations showed no overall reduction in singing activity, and did not sing less frequently during the

2 h following sunset, the period during which most of the parasitoid flies search for hosts (W. E. Wagner, Jr., personal observation; see also Cade et al. 1996).

In contrast to our results, in other North American species of field crickets that are parasitized by *O. ochracea*, parasitized individuals show reduced singing activity (Cade & Wyatt 1984; Cade 1991; Kolluru 1999), sing less frequently shortly after sunset (French & Cade 1987; Bertram et al. 2004; Vélez & Brockmann 2006) and/or sing less during times of the year when *O. ochracea* are active (Vélez & Brockmann 2006). In addition, male Polynesian field crickets introduced to Hawaii, where they are parasitized by introduced *O. ochracea*, shift their singing activity to periods when *O. ochracea* are less

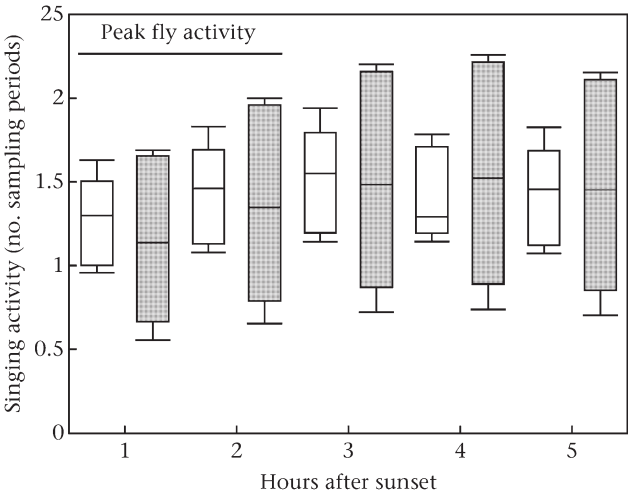


Figure 2. Number of sampling periods that male *G. lineaticeps* from populations with low (white bars) and high (gray bars) parasitism risk were observed singing following sunset (i.e. hourly singing activity). Box plots show the median, top and bottom quartiles, and the 10th and 90th percentiles of the population means ($N = 6$ for each parasitism environment) for each hour after sunset.

Table 1. Results of reduced linear mixed models examining fixed and random effects on total singing activity of male *G. lineaticeps* from high-risk and low-risk populations

Fixed effects	Coefficient	SE	χ^2_1	P
Age	0.032	0.005	42.82	0.000
Temperature	-0.181	0.042	18.74	0.000
Random effects	Estimate	SE	χ^2_1	P
Population	0.108	0.057	1159.75	0.000
Family	0.626	0.067	1636.740	0.000

Regression coefficients are shown for fixed effects; variance estimates are shown for random effects.

Table 2. Results of reduced linear mixed models examining fixed and random effects on the temporal pattern of singing activity of male *G. lineaticeps* from high-risk and low-risk populations

Fixed effect	Coefficient	SE	χ^2_1	P
Age	0.037	0.013	8.38	0.004
Hour	0.240	0.048	24.57	0.000
Hour ²	-0.032	0.008	17.32	0.000
Random effect	Estimate	SE	χ^2_1	P
Population	0.065	0.041	20.63	0.000
Family	0.213	0.081	8.37	0.004
Individual	1.216	0.117	1460.63	0.000

Regression coefficients are shown for fixed effects; variance estimates are shown for random effects.

active, compared to males from nonparasitized populations in French Polynesia and Australia (Zuk et al. 1993). Furthermore, a wing mutation that has spread in one of the Hawaiian populations has led to a near complete loss of singing (Zuk et al. 2006). Predation has had similar effects on male signaling behavior in some other animals (e.g. fish: Endler 1983; frogs: Tuttle & Ryan 1982; Tuttle et al. 1982; moths: Spangler et al. 1984; Spangler 1988; katydids: Faure & Hoy 2000). Our results are thus incongruent with both the expected effect of predation on male signaling behavior and the effect observed in some other animals.

There are several nonmutually exclusive explanations for our unexpected results. First, *O. ochracea* may only have recently begun to use *G. lineaticeps* as a host, and there may not have been sufficient time for males to evolve modified patterns of singing activity to reduce their risk of parasitism (sensu Adamo 1999; Kolluru et al. 2002). The historical pattern of association between *O. ochracea* and the various *Gryllus* species that it uses as host is not known. However, we know that *O. ochracea* was introduced to Hawaii within the last 100-1,000 years (Otte & Alexander 1983; Kevan 1990), and that male *T. oceanicus* in Hawaii rapidly evolved changes in their singing activity to reduce their risk of parasitism (Zuk et al. 1993, 2006; see above). Therefore, it is surprising that male *G. lineaticeps* have not reduced their singing activity in response to parasitism. Second, male *G. lineaticeps* may have evolved alternative mechanisms to reduce their parasitism risk, allowing them to maintain a high level of signaling activity despite the parasitism risk. For example, males from parasitized populations may be more likely to stop singing when parasitoid cues are detected, or they may take longer to resume singing (e.g. Hedrick 2000; Lewkiewicz & Zuk 2004), respond more aggressively to nearby flies, or show greater grooming behavior to remove fly larvae (Vincent & Bertram 2010). Third, by rearing males in a common environment, we precluded environmental effects on male singing behavior, but we also precluded genotype-environment interaction effects. It is possible that males from high-risk populations are more sensitive to environmental cues predictive of host searching by the flies and that they reduce their singing activity during the limited time that the flies are active. These cues were not present in the laboratory environment. And fourth, sexual selection might be stronger in high-risk populations, and thus, the benefits of singing (i.e. attracting mates) might compensate for the costs of singing (i.e. attracting lethal flies). This hypothesis is consistent with our results showing that males from high-risk populations produce songs that are more attractive to female crickets but also more attractive to the flies (O. M. Beckers & W. E. Wagner, Jr., unpublished data). However, the reason why sexual selection might be stronger in high-risk populations is not clear.

There are two other explanations for our results that we can provisionally reject. First, it is possible that our sample size was insufficient to detect differences in singing activity. We assayed the singing activity of 720 males from 12 populations (a mean of 60 males per population). We should thus have had sufficient statistical power to detect any biologically meaningful differences in singing activity, as we were able to detect differences in male song characters (O. M. Beckers & W. E. Wagner, Jr., unpublished data). Second, it is possible that gene flow among populations has prevented male singing activity from diverging between high- and low-risk populations. There are, however, detectable differences in multiple male song characters between the populations (Wagner et al. 2012; O. M. Beckers & W. E. Wagner, Jr., unpublished data), so it seems unlikely that gene flow is sufficiently high to prevent divergence in singing activity.

In conclusion, there is no evidence that the pattern of male singing activity in *G. lineaticeps* has diverged between parasitized and nonparasitized populations: males from high-risk populations did not sing less frequently, either throughout a night or during the period that flies are most active. It is possible, however, that fly parasitism has favored either alternative behavioral mechanisms or phenotypic plasticity in singing activity to reduce the risk of parasitism. Alternatively, sexual selection may be stronger in high-risk populations, favoring high levels of singing activity despite the parasitism cost. Predictions about the evolutionary consequences of a given source of selection are often difficult to make because adaptations can occur through a variety of mechanisms, and traits that evolve in a given population can depend on which mutations arise first (Hoekstra 2006). Furthermore, a given source of selection can vary spatially and temporally, multiple sources of selection may be correlated, and trait correlations may limit the extent to which a given trait can evolve in response to selection (Wagner et al. 2012). Thus, predictions about trait evolution may require a comprehensive understanding of many factors that might affect how a trait evolves. In fact, given the complexity of these factors, it is somewhat remarkable that simple predictions about trait evolution are often supported.

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Supplementary Material

Supplementary material for this article follows the References.

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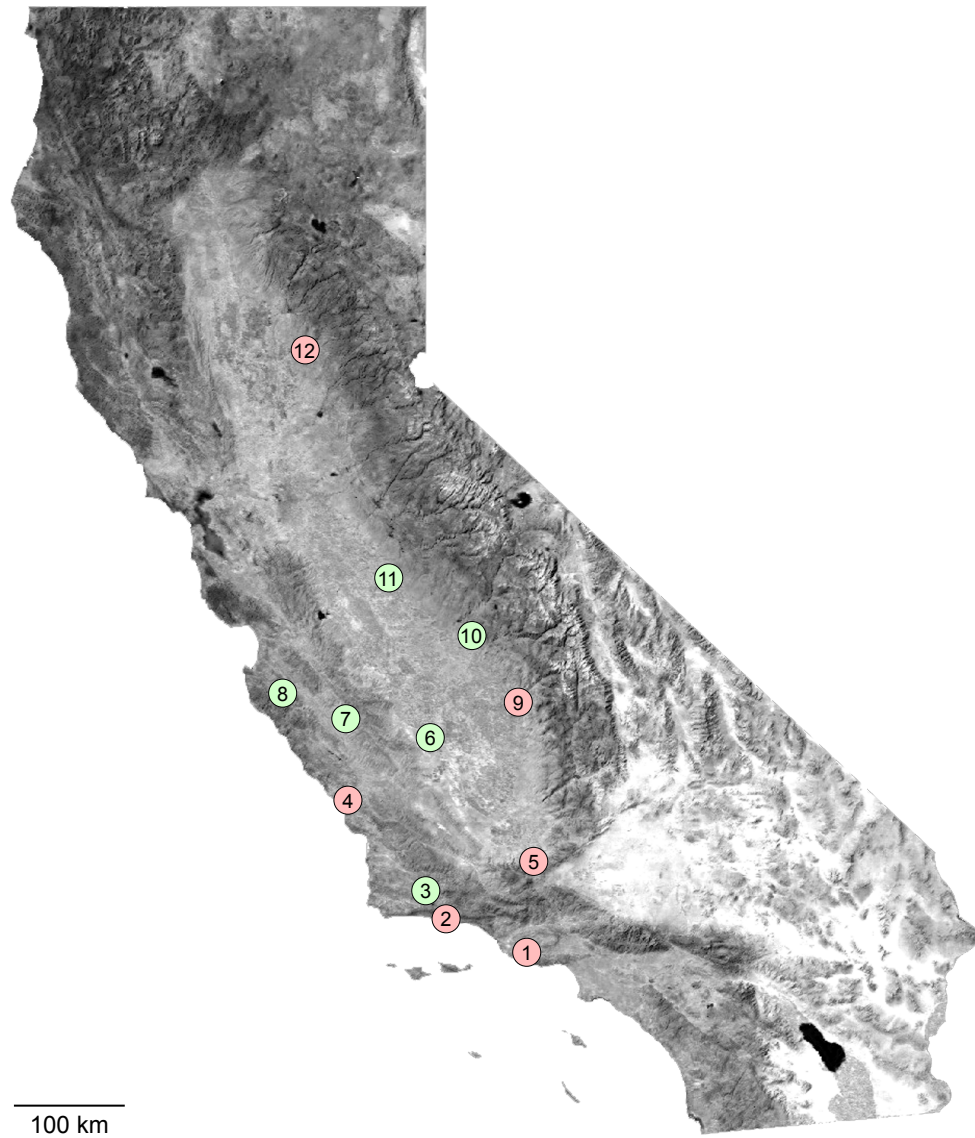


Figure S1. Locations of *G. lineaticeps* populations in California used in our study with high (red circles) and low (green circles) parasitism risk (latitude, longitude): (1) Rancho Sierra Vista, Santa Monica Mountain National Recreation Area, near Newbury Park, **RSV** (34°9'16.56"N, 118°58'26.76"W); (2) Santa BarbaraShores County Park, Goleta, **GLT** (34°25'22.08"N, 119°53'50.64"W); (3) Sedgwick Reserve, near Santa Ynez, **SDG** (34°41'9.2394"N, 120°2'12.84"W); (4) Whale Rock Reservoir, near Cayucos, **CYC** (35°28'23.16"N, 120°52'16.68"W); (5) Grapevine, **GV** (34°56'20.0394"N, 118°54'5.76"W); (6) Kettleman City, **KTM** (36°0'25.56"N, 119°59'33.3594"W); (7) King City, **KNG** (36°9'57.5994"N, 120°53'1.32"W); (8) Hastings Natural History Reservation, near Carmel Valley, **HSG** (36°23'18.9594"N, 121°33'5.4"W); (9) Lindcove near Visalia, **LND** (36°18'45.7194"N, 119°4'15.5994"W); (10) Academy, east of Clovis, **ACD** (36°50'14.2794"N, 119°30'34.56"W); (11) Merced, **MER** (37°21'39.24"N, 120°25'57"W); (12) Sierra Foothill Research and Extension Center, Browns Valley, **SFH** (39°15'7.5594"N, 121°18'47.5194"W). Map modified after Wagner et al. (2012).

Table S1

Populations of *Gryllus lineaticeps* sampled to determine risk of *Ormia ochracea* parasitism

Population	Year	No. of days sampled	No. of flies attracted to broadcasts	No. of crickets collected (males/females)	No. of crickets parasitized (males/females)
Low risk					
ACD	2004	3	0	5/15	0/0
ACD	2005	2	0	3/24	0/0
ACD	2006	1	0	10/14	0/0
ACD	2007	2	0	0/8	0/0
ACD	2008	2	0	1/7	0/0
ACD	2009	3	0	15/66	0/0
ACD	2011	1	0	4/21	0/0
HSG	2008	4	0	15/22	1/0
HSG	2009	9	0	46/43	0/0
HSG	2010	4	0	20/11	0/0
KNG	2008	2	0	11/26	0/0
KNG	2009	3	0	55/57	0/0
KNG	2010	1	0	4/7	0/0
KTM	2008	2	0	10/18	0/0
KTM	2009	4	0	9/44	0/0
KTM	2010	2	0	3/6	0/0
MER	2008	3	0	17/24	1/0
MER	2009	5	0	42/67	0/0
MER	2010	3	0	26/24	0/0
SDG	2004	1	1	0/1	0/0
SDG	2005	1	0	25/0	0/0
SDG*	2006	7	0	0/8	0/0
SDG*	2007	14	0	98/126	0/0
SDG*	2008	8	0	55/137	0/0

Table S1 *continued*

Population	Year	No. of days sampled	No. of flies attracted to broadcasts	No. of crickets collected (males/females)	No. of crickets parasitized (males/females)
High risk					
CYC	2008	4	0	4/14	0/0
CYC	2009	4	0	33/57	1/3
GLT	2007	3	0	0/14	0/0
GLT	2008	3	0	11/34	0/1
GLT	2009	1	0	0/2	0/0
GV	2007	3	3	0/8	0/0
GV	2008	2	0	1/7	0/0
GV	2009	1	0	3/6	0/0
GV	2010	4	0	13/24	0/0
LND	2008	2	0	4/3	0/0
LND	2009	4	0	46/53	0/1
LND	2010	3	0	19/43	0/0
RSV	2006	11	92	No collection	NA/NA
RSV	2007†	8+	59	22/104	13/1
RSV	2008†	~15	NA	0/49	0/3
RSV	2009	5	48	No collection	NA/NA
RSV	2010	6	57	No collection	NA/NA
RSV	2011	10	27	No collection	NA/NA
SFH	2008	4	0	8/18	0/0
SFH	2009	4	0	19/55	0/1
SFH	2010	3	0	19/70	0/0

Sampling before 2008 was done in the context of other studies. Abbreviations for locations as in Fig. S1.

* C. Mitra (personal observation).

† Data from Martin & Wagner (2010).