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Males and females evolve riskier traits in populations with eavesdropping parasitoids

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Abstract

Predation and/or parasitism often limits the evolution of conspicuous male traits and female preferences because conspicuous traits can attract predators or parasites and it is costly for females to associate with males that attract predators or parasites. As a result, males and females in high-risk populations are expected to evolve safer mating behaviors compared to individuals from low-risk populations. We tested this antagonistic selection hypothesis in the field cricket *Gryllus lineaticeps*. Males produce chirped songs, and both female crickets and the eavesdropping parasitoid fly *Ormia ochracea* prefer faster chirp rates. The flies attack the field crickets late in the breeding season and parasitized crickets die. We used a common garden rearing design to test for evolved differences in songs and preferences between high- and low-risk populations. In contrast to predictions of the antagonistic selection hypothesis, males from high-risk populations produced faster (riskier) chirp rates and females preferred faster chirps. We suggest that late-season parasitism selects for increased investment in reproductive traits to maximize reproduction before the advent of parasitoid activity (“late-season parasitism hypothesis”), which would at least explain riskier female preferences and potentially riskier male songs in the high-risk populations. Predation and parasitism may thus have diverse and unexpected effects on the evolution of reproductive behavior, depending upon the temporal pattern of predator- or parasite-induced mortality.

Significance statement

Mating signals are typically conspicuous and not only attract partners but also predators and parasites. Even the silent mating partner may experience predation or parasitism by associating with the signaler. Under these circumstances, it is commonly assumed that natural and sexual selection act in opposite directions, effectively *limiting* the evolution of conspicuous signals and preferences. We demonstrate that an eavesdropping parasitic fly caused the evolution of preferences, and potentially songs, in a field cricket in the opposite, *more conspicuous*, direction than predicted by antagonistic selection. We argue that the temporal pattern of parasitism in relation to the reproductive season likely causes this unexpected evolutionary pattern. We propose the late-season parasitism hypothesis as an alternative to the antagonistic selection hypothesis, which might better explain more conspicuous mating trait values in other species that experience seasonal predation or parasitism.

Keywords — Sexual selection, Predators, Parasitoids, Male signals, Female preferences

Introduction

Most animals have predators and/or parasites, and selection commonly favors traits that reduce an individual's risk of being detected, captured, consumed, and/or parasitized. Predation, for example, is known to affect the evolution of animal morphology, movement and dispersal, foraging behavior, parental care behavior, and social behavior (Langerhans 2006). Importantly, predation and parasitism are also thought to be major factors that limit the evolution of sexually selected male signals (Andersson 1994; Zuk and Kolluru 1998). Females often prefer to mate with males with conspicuous, extreme, signals (Ryan and Keddy-Hector 1992) because these conspicuous males frequently provide material or genetic contributions that increase female or offspring fitness (Andersson 1994; Wagner 2011). These conspicuous male signals, however, can increase a male's risk of being detected and thus attacked by an eavesdropping predator or parasite (Zuk and Kolluru 1998). Because males with signals that are attractive to females are more likely to attract predators or parasites, females may also have a higher risk of predation or parasitism when near these males (Pocklington and Dill 1995; Martin and Wagner 2010). As a result, natural selection due to predation or parasitism is often expected to limit the evolution of conspicuous, attractive male signals and strong female preferences for these signals (Andersson 1994; Lande 1981; Kirkpatrick 1982). This has been shown, for example, in multiple studies of the guppy, *Poecilia reticulata* (male signals: Endler 1980, 1983; female preferences: Stoner and Breden 1988; Houde and Endler 1990; Endler and Houde 1995). We will refer to this as the “antagonistic selection hypothesis.”

We tested the evolutionary consequences of parasitism in the variable field cricket, *Gryllus lineaticeps*. Males in this species produce a chirped calling song to attract females, and previous data from some populations indicated that females prefer male songs with higher chirp rates and longer chirp durations (Wagner 1996; Wagner and Reiser 2000; Wagner et al. 2001; Wagner and Basolo 2007a; Beckers and Wagner 2011a). Females benefit from these preferences: in low-nutrition environments, males with these song characters transfer seminal fluid products that increase female fecundity (chirp rate) or longevity (chirp duration; Wagner and Harper 2003; Tolle and Wagner 2011). However, a phonotactic parasitoid fly, *Ormia ochracea*, is present in some populations. Adult female flies orient to male song, and lay larvae on and around the male (Cade 1975). When the larvae contact a cricket, they enter the cricket's body, feed on the host's organs, and emerge 7–10 days later, inevitably killing the host (Cade 1975; Adamo et al. 1995). Most cricket reproductive activity, such as female mating and oviposition activity (Adamo et al. 1995), and male singing activity (Beckers and Wagner 2011b), is substantially reduced within 1 to 3 days of infestation. Since both males and females mate multiple times throughout their lives (Wagner 2001), parasitism results in a substantial reduction of their lifetime reproductive success. While females are parasitized at a lower rate than males, females can be parasitized when they approach a male (Martin and Wagner 2010), likely by picking up larvae deposited by a fly around the male prior to the female's arrival at the male (Beckers et al. 2011). Up to 65% of males, and 6% of females, can be parasitized at any given time (Martin and Wagner 2010). Importantly, the flies, like female crickets, prefer higher chirp rate songs (Wagner 1996; Wagner and Basolo 2007b). Thus, males producing high chirp rates, and the females that approach these males, have a higher risk of parasitism (Martin and Wagner 2010), resulting in opposing natural and sexual selection on both male song and female preferences, providing an opportunity to test the evolutionary predictions of the antagonistic selection hypothesis.

We tested the effect of fly parasitism on the evolution of male song characters and female preferences in *G. lineaticeps* by comparing these male and female traits between six populations that experience a high parasitism risk and five populations that experience a low parasitism risk. We used a common garden rearing design to test for evolved differences in male and female behavior. A previous study indicated that fly parasitism had no effect on the evolution of male singing activity; in the laboratory, males sing as frequently in high-risk populations as in low-risk populations (Beckers and Wagner 2012). Predictions about the evolution of male song characters in *G. lineaticeps* are complicated by the presence of a genetically based tradeoff between chirp rate and chirp duration. Because of this tradeoff, an evolutionary increase in one trait requires an evolutionary decrease in the other

(Wagner et al. 2012). Previous work, however, indicates that chirp rate has a greater effect than chirp duration on both mate attraction (Wagner and Basolo 2007a) and parasitoid attraction (Wagner 1996). A fast chirp rate with short-duration chirps is thus a more attractive but riskier trait combination than a slow chirp rate with long-duration chirps. According to the antagonistic selection hypothesis, we predicted that males from high-risk populations would produce safer but less attractive songs (i.e., slower chirp rates and longer chirp durations) and that females from high-risk populations would express preferences for slower chirp rates that are safer but yield lower fecundity benefits.

Methods

Populations and sampling

We collected *G. lineaticeps* from 11 populations in California (Figure S1; supplementary materials). The populations were separated by an average linear distance of 221 km (range 32.0–604.6 km) and, in some instances, by physical barriers such as mountain ranges. We sampled each population at least twice in 2 years between 2008 and 2011, and some of the populations had been sampled for 5–16 years in the context of this and other studies (ACD, CYC, GLT, GV, and RSV). For the latter populations, parasitoids have been either consistently present or consistently absent, except for one population that has been periodically parasitized (GV), which we categorized as “high-risk” because it fulfilled the below-mentioned criteria. The parasitism status of each population used in this study was determined in a previous study (Beckers and Wagner 2012). In brief, we used two methods to determine parasitism status. First, we broadcasted *G. lineaticeps* songs that readily attract *O. ochracea* in California at each field site multiple times in each year of sampling during the late summer and fall when fly activity typically occurs in California (Paur and Gray 2011). The amplitude of the broadcast was ~ 93 dB SPL (at 30 cm from the loudspeaker), which is substantially higher than the natural call amplitude (~ 70 dB SPL at 30 cm, WEW, unpublished data) to increase the chances of attracting nearby flies. Second, we collected males and females from each population and checked for the emergence of fly larvae from the crickets. We categorized a population as high-risk if we found evidence of two or more flies attracted to males (i.e., two or more flies attracted to song broadcasts and/ or two or more parasitized males) across all the years of sampling. We also categorized a population as high-risk if we found evidence of at least one parasitized female. The latter criterion is a strong indicator of parasitism because females are substantially less likely to be parasitized than males ($\leq 6\%$ vs. 65% ; Martin

and Wagner 2010). If neither of these criteria was met, we categorized the populations as low-risk. We included two populations (MER, HSG) with a very low likelihood of fly parasitism in the low-risk category (i.e., one male cricket infested over at least 3 years of sampling). Because of these criteria, we use the terms “low-risk” and “high-risk” instead of “not parasitized” and “parasitized” to describe the populations (Beckers and Wagner 2012). These criteria for categorization represent threshold values rather than quantitative measures of parasitism risk and we stopped sampling for a given population when any of these criteria was met (for summarized data on parasitism rates, see Table S1).

Rearing methods

We collected between 18 and 80 females from each population between 2004 and 2009 to establish laboratory colonies at the University of Nebraska-Lincoln (for details on rearing procedures, see Beckers and Wagner 2012) and subsequently supplemented the colonies with between 6 and 70 females in 2010. Most of the females collected had mated at least once prior to collection and thus laid eggs in the laboratory. We raised crickets in family groups and separated late-instar nymphs into individual containers prior to their final molt. Males and females were kept in separate rooms and females were therefore acoustically naïve at the time of testing. We recorded the date of the final molt, and thus knew the adult ages of all individuals used in our experiments. We maintained genealogical records for all individuals and we propagated the colonies by mating unrelated males and females within each population. We reared all experimental individuals in the common environment of the laboratory, which allowed us to minimize environmental effects on male and female traits, and all test crickets had parents that were reared in the common environment of the laboratory, which allowed us to minimize environmentally based maternal and paternal effects on male and female traits. We did not notice reproductive seasonality in the lab (i.e., matings that were set up in the lab typically resulted in offspring independent of the time of the year). Variation among populations can thus be attributed primarily to evolved, genetically based, differences. All males were between 7 and 15 days of adult age when their songs were recorded and all females were between 7 and 12 days of adult age when their preferences were tested. Animals from all populations were recorded or tested between 2009 and 2011, which is the time period over which the fly sampling occurred (see above).

Male song traits

To obtain measures of male song characters, we housed each male in a plastic container that was covered with an acoustically transparent wire mesh lid

and placed the container inside one of 10 Styrofoam rectangular coolers (50 × 33 × 40 cm; for details on recording procedures, see Wagner et al. 2012). The coolers were lined with acoustic foam to prevent males from hearing singing males in adjacent containers. A microphone (Sennheiser ME64 K6P, Sennheiser GmbH and Co. KG, Hannover, Germany or Schriber SA-568, NRG Research Inc., Merlin, OR, USA) was suspended above each cricket through a hole in the lid of the Styrofoam box. Each male was recorded during the dark portion of the light cycle when males typically sing. The microphones were connected through a 10-channel recording board (Micro 1401 and expansion ADL 12, both Cambridge Electronic Design Ltd., Cambridge, UK) to a personal computer (Macintosh G3). Songs were digitized with a sample rate of 12,500 Hz. The songs were analyzed using a customized script for Spike 2.0 software (Cambridge Electronic Design Ltd., 1995). We recorded the songs of 839 males (mean = 76.4 males/population, range = 71–83) and measured chirp rate and chirp duration for each male. We used a mean of 32.6 full sibling families per population and recorded between 1 and 4 males per full sibling family (mean = 2.4 males/family).

Female song preferences

We tested the population-level song preferences of females in a semi-anechoic chamber (2.2 × 2.2 × 2.7 m; w × l × h). The chamber was equipped with a dim red light and a video camera (Lorex SG4915R), both of which were mounted on the ceiling. The camera was connected to a TV/VCR system (Sylvania SRC20134AC) outside the chamber where we observed female behavior. In one of the corners of the arena, we drew a “speaker circle” with a radius of 26 cm. We placed the loudspeaker broadcasting the acoustic stimulus in the center of the circle. Between the speaker circle and the arena wall was a space of 3 cm to allow crickets to walk along the arena wall without entering the circle.

At the beginning of each trial, we placed the female under a cup (radius = 4 cm) that was attached to a string that ran through the ceiling to the outside of the testing chamber. This allowed us to raise the cup and release the female without entering the chamber. The cup was located inside a release circle ($r = 5$ cm) in the center of the arena, at a distance of 1.14 m from the loudspeaker. First, we tested if the female was responsive to acoustic stimulation using a standard song (chirp rate = 3.0 chirps/s, chirp duration = 120 ms; Wagner and Basolo 2007a). We acclimated the female under the cup for 10 min. After the acclimation period, we released the female by remotely lifting the cup. During the following 10 min, we recorded whether the female entered the speaker circle and how long she stayed in the circle. A female was considered unresponsive if she did not leave the release circle within 10 min, never entered the speaker circle, or spent less than 15 s inside the

speaker circle. For females from low-risk populations, 766 of 994 females responded to the standard song as outlined above. For females from high-risk populations, 859 of 1131 responded to the standard song. There was no significant difference between low- and high-risk populations in the probability that a female responded (mixed effects logistic regression with parasitism environment as the fixed effect and population and family nested within population as random effects: $X_1^2 = 0.02$, $P = 0.882$).

Of these females, 1100 were immediately tested (100 females/ population) in a second trial to estimate population-level preferences for chirp rate and chirp duration (note that the additional females mentioned above were part of a separate study not reported here). Female preferences can be described by female preference functions (Wagner 1998). These preference functions measure how female attraction to a set of male traits varies with trait values, and preference functions can be open (i.e., females prefer higher and/or lower trait values) or closed (i.e., females prefer intermediate trait values; Wagner 1998; Lande and Arnold 1983; Blows and Brooks 2003; Brooks et al. 2005; Rodríguez et al. 2006). In addition, preferences for different traits in the set may be correlated, i.e., female attraction to one trait may depend on the value of a second trait (Blows and Brooks 2003; Brooks et al. 2005). We measured the attraction, i.e., the time associated with each stimulus (e.g., Wagner and Basolo 2007a) of the females to a set of 25 song stimuli that contained all possible combinations of five chirp rates and five chirp durations, covering 95% of the natural variation in these song characters (for a description of the stimuli see Wagner and Basolo 2007a). We used a full factorial design so that we could test for effects on the interaction between chirp rate and chirp duration on female responses. Females were acclimated to the test stimulus for 10 min as described above and then released. For each female, we measured the time spent inside the speaker circle during the 10-min trial. Each female was tested with only one stimulus and each stimulus was tested with four different females from each population. Females that did not leave the release circle within 10 min of lifting the cup or did not enter the speaker circle were not included in subsequent analyses. Our analyses thus consisted of 718 females (mean = 65.3 females/ population, range = 58–76). We used a mean of 36.3 full sibling families per population and tested the responses of between 1 and 5 females per full sibling family (mean = 1.8 females/family). To minimize observer bias, blinded methods were used when all behavioral data were collected and analyzed.

Ethical note and permits

Our research adhered to the ASAB/ABS guidelines for the use of animals in research, the legal requirements of the USA, and all guidelines of the University of Nebraska-Lincoln. Animals were carefully collected, handled, and

maintained in the laboratory under proper conditions. After testing, animals were used for breeding to maintain lab colonies.

Statistical analyses

We compared male song traits and female preferences between low- and high-risk populations using linear mixed models (STATA 13.1). Song traits and preferences were modeled using Gaussian (male chirp rate and chirp duration) or negative binomial (preference tests) error structures.

Male song

Male songs were analyzed immediately following each recording using a customized script for the software Spike 2.0 (Cambridge Electronic Design Ltd., 1995). In a small number of recordings, some of the individual pulses within a chirp showed amplitude fluctuations that may have caused our initial analysis script to count a single pulse as two pulses. We adjusted the script used to analyze the songs during data collection to exclude any potential ambiguity of the pulse measurements. The first script was used to analyze the songs of 221 males, and the second script was used to analyze the songs of 618 males. Because the song files were not stored after analysis, we could not re-analyze the first set of songs using the second script. However, to control for potential effects this change in script may have had on our results, we included “script” as a fixed factor in the statistical analyses (see below). To confirm that the patterns we report were not biased by this procedure, we repeated our analyses using only males recorded with the second script. The statistical analyses with and without “script” as a fixed factor, as well as those using songs that were only analyzed with the second script, yielded the same significant results, including the “parasitism environment” effect.

There was significant variation among populations in male mass (linear mixed model with mass as the dependent variable, parasitism environment as a fixed effect, population as a random effect, and family nested within population as a random effect; population effect: $X_1^2 = 58.9$, $P < 0.001$). Because the size of an individual might affect its behavior, we were interested in separating the within- and among- population effects of size on behavior. The within-population effect tests whether size is correlated with behavior within a population, while the among-population effect tests whether evolved differences in size among populations are associated with evolved differences in behavior. We thus derived two separate predictor variables for each individual from the mass measures (van de Pol and Wright 2009, Rabe-Hesketh and Skrondal 2008): mean population mass (the mean of the individual’s population) and within-population deviation in mass (the individual’s deviation from the population mean).

We tested the effect of parasitism environment on male song characters using mixed effect models (*xtmixed*). Our initial models included the following fixed factors: parasitism environment, script, recording temperature, adult age at recording, mass (mean), and mass (deviation). The model also included the following random factors: population, and family nested within population. We then compared the initial models to reduced models using AIC and selected the model with the lowest AIC value. Parasitism environment, however, was retained in all models as it was the factor of primary interest, and the random factors were retained in all models given the nature of the study.

Female preference

We tested the effect of parasitism environment on the time that females spent in the circle around the speaker broadcasting the 25 test stimuli that varied in chirp rate and/or chirp duration using multilevel mixed effects negative binomial regression (*menbreg*). Our initial model included the following fixed factors: parasitism environment, testing temperature, age, mass (mean), mass (deviation), stimulus chirp rate (cr), the square of stimulus chirp rate (cr²), stimulus chirp duration (cd), the square of stimulus chirp duration (cd²), cr × cd, parasitism × cr, parasitism × cr², parasitism × cd, parasitism × cd², and parasitism × cr × cd. We included linear and quadratic effects in the models to test for differences in preference function shapes (e.g., for open versus closed preferences). Because variation in female responsiveness can confound measures of preference when using single stimulus response tests (Wagner and Basolo 2007a), we also included the time that females spent in the circle during the broadcast of the standard stimulus (first trial) as a fixed factor. There was significant variation among populations in female mass ($X_1^2 = 25.1, P < 0.001$). Because the size of an individual might affect its behavior, we were interested in separating the within- and among-population effects of size on behavior (see above). The model also included the following random factors: population, and family nested within population. We then compared the initial model to reduced models using AIC and selected the model with the lowest AIC value. However, we retained the following factors, regardless of their effect on the model AIC, because we were specifically interested in their effects on female responses: parasitism environment, stimulus chirp rate (cr), the square of stimulus chirp rate (cr²), stimulus chirp duration (cd), the square of stimulus chirp duration (cd²), cr × cd, parasitism × cr, parasitism × cr², parasitism × cd, parasitism × cd², and parasitism × cr × cd. In addition, the random factors were retained in all models given the nature of the study.

Illustrating the results

In order to illustrate how a given factor affected a given trait, we used the observed fixed and random effects from the appropriate statistical model to derive a predicted value for that trait for each individual (*predict*). We then calculated the mean \pm SEM for the predicted values of the trait for each group of interest. Our figures show the model-implied differences between treatment groups for each trait and more closely reflect the results of the statistical analyses.

Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Results

Male song characters

In the high-risk populations, chirp rates ranged between 1.01 and 3.26 chirps/s and chirp durations between 0.076 and 0.167 s, while in the low-risk populations, chirp rates ranged between 0.52 and 2.95 chirps/s and chirp durations ranged between 0.078 and 0.167 s. Males from high-risk populations produced songs with significantly faster chirp rates and significantly shorter chirp durations than males from low-risk populations (Fig. 1a and Table 1a, b). There were no significant effects of mass on chirp rate. Within populations, larger males produced longer chirp durations (Table 1b). However, those populations with larger males produced shorter chirp durations (Table 1b).

Female song preferences

Female attraction was unaffected by chirp duration, by the interaction of parasitism environment and chirp duration, by the interaction of parasitism environment and the square of chirp duration, and by the interaction of parasitism environment, chirp duration, and chirp rate (Table 2). There was, however, a significant effect of the interaction between parasitism environment and chirp rate on female attraction, and a significant effect of the interaction between the parasitism environment and the square of chirp rate

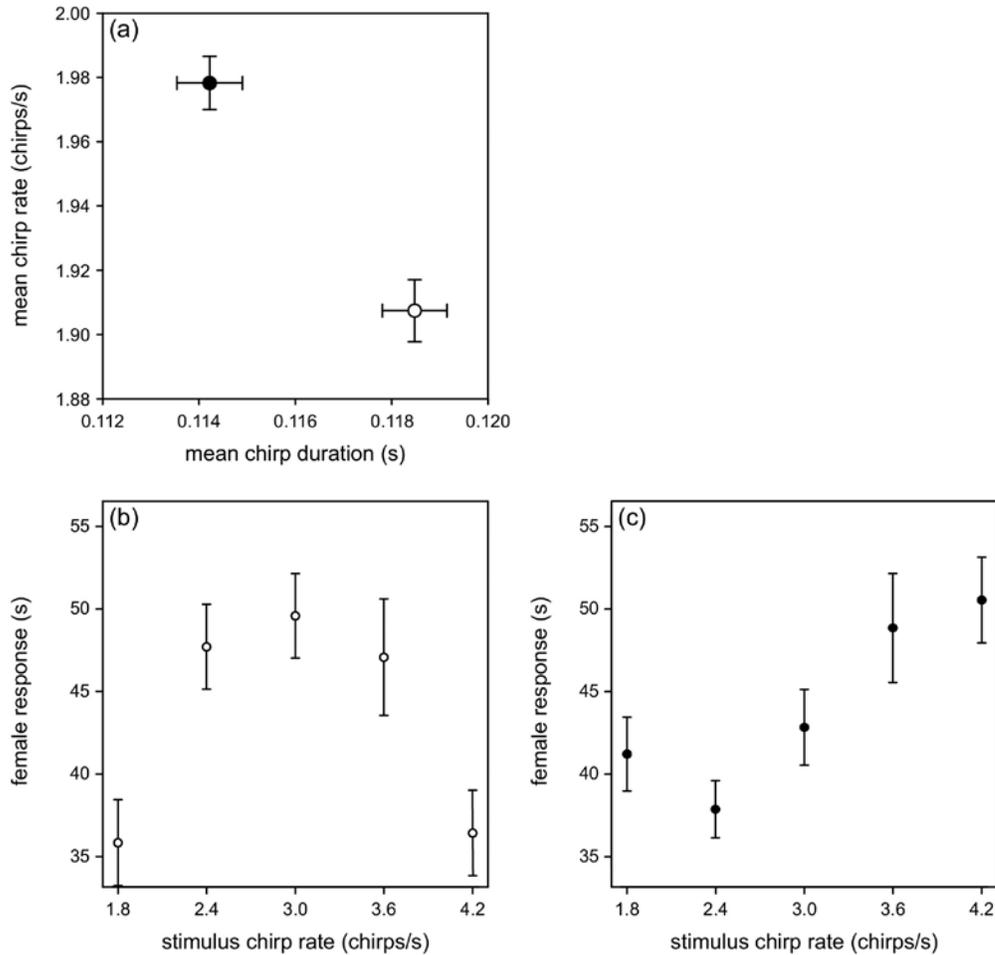


Fig. 1. Effects of parasitism by *Ormia ochracea* on the evolution of male and female traits in *Gryllus lineaticeps*. Shown are the mean (\pm SE) predicted male song characters and female responses based on empirical Bayes prediction. **(a)** Chirp durations and chirp rates of males from low-risk (\circ) and high-risk (\bullet) populations. We recorded 459 males from six high-risk populations and 380 males from five low-risk populations. **(b)** Effect of chirp rate on female responses in low-risk populations. **(c)** Effect of chirp rate on female responses in high-risk populations. For each chirp rate, we tested 74–84 females from high-risk populations and 58–73 females from low-risk populations

on female attraction. Females from low-risk populations preferred intermediate chirp rates; they had a strong closed component to their chirp rate preferences (Fig. 1b, figure S2). In contrast, females from high-risk populations preferred higher chirp rates; they had a strong open component to their chirp rate preferences (Fig. 1c, figure S2). Females from high-risk populations thus preferred faster male chirp rates, which is inconsistent with

Table 1. Effects of parasitism environment on (a) male chirp rate and (b) male chirp duration in *G. lineaticeps*. Data were analyzed using linear mixed models with a normal error distribution. Temperature is the recording temperature. Script is the analysis script used to analyze the recording. Mass (mean) is the mean population mass. Mass (deviation) is deviation from the mean population mass. Fixed effects were tested using Wald tests. Random effects were tested using a likelihood ratio test that compared models with and without the random effect. Regression coefficients are presented for the fixed effects while variance component estimates are presented for the random effects

(a) Chirp rate				
<i>Fixed effects</i>	<i>Coefficient</i>	<i>SE</i>	X_1^2	<i>P</i>
Parasitism	0.1189	0.0430	7.63	0.006
Script	- 0.1200 0	.0337	12.63	< 0.001
Mass (mean)	0.4915	0.2327	2.65	0.104
<i>Random effects</i>	<i>Estimate</i>	<i>SE</i>	X_1^2	<i>P</i>
Population	0.0013	0.0017	1.10	0.294
Family	0.0184	0.0065	9.65	0.002
(b) Chirp duration				
<i>Fixed effects</i>	<i>Coefficient</i>	<i>SE</i>	X_1^2	<i>P</i>
Parasitism	- 0.0073	0.0028	6.83	0.009
Script	0.0039	0.0014	8.13	0.004
Temperature	- 0.0047	0.0016	8.56	0.003
Mass (mean)	- 0.0383	0.0195	3.86	0.050
Mass (deviation)	0.0089	0.0034	6.72	0.010
<i>Random effects</i>	<i>Estimate</i>	<i>SE</i>	X_1^2	<i>P</i>
Population	0.000012	0.000007	14.26	< 0.001
Family	0.000058	0.000010	47.85	< 0.001

the antagonistic selection hypothesis. There was also a significant effect of the interaction between chirp rate and chirp duration on female attraction (Table 2), indicating that female attraction to one trait is affected by the value of the other trait. However, because there was not a significant effect of the three-way interaction between parasitism environment, chirp rate, and chirp, there is no evidence that the pattern of correlated preference differed between parasitism environments. Our ability to detect a three-way interaction, however, was limited by the small number of females tested with each stimulus. Finally, within populations, larger females spent less time around the speaker broadcasting song, and thus showed weaker responses to male songs.

Table 2. Effects of parasitism environment on female attraction to varied male song characters in *G. lineaticeps*. Data were analyzed using a linear mixed model with a negative binomial error distribution. Mass (deviation) is deviation from the mean population mass. The fixed effects were tested using Wald tests. The random effects were tested using a likelihood ratio test that compared models with and without the random effect. Regression coefficients are presented for the fixed effects while variance component estimates are presented for the random effects

<i>Fixed effects</i>	<i>Coefficient</i>	<i>SE</i>	X_1^2	<i>P</i>
Time in standard test	0.0028	0.0005	35.54	< 0.001
Mass (deviation)	- 0.9336	0.2829	10.89	0.001
Parasitism	3.8822	3.6941	1.10	0.293
Chirp rate	4.7043	1.6084	8.55	0.003
Chirp rate ²	- 0.4907	0.2166	5.13	0.024
Chirp duration	0.3450	0.5223	0.24	0.623
Chirp duration ²	- 4.02e ⁻⁶	0.0003	0.00	0.991
Chirp rate * chirp duration	- 0.0164	0.0076	4.68	0.031
Parasitism * chirp rate	- 2.4608	0.9809	6.29	0.012
Parasitism * chirp rate ²	0.2851	0.1325	4.63	0.031
Parasitism * chirp duration	- 0.0152	0.0535	0.08	0.777
Parasitism * chirp duration ²	- 1.1e ⁻⁵	0.0002	0.00	0.961
Parasitism * chirp rate * chirp duration	0.0076	0.0046	2.78	0.095
<i>Random effects</i>	<i>Estimate</i>	<i>SE</i>	X_1^2	<i>P</i>
Population	0.0038	0.0107	0.16	0.694
Family	0.2494	0.0611	26.39	< 0.001

Discussion

In this study, we tested whether antagonistic selection has favored the evolution of slower but safer male chirp rates and the evolution of female preferences for slower but safer chirp rates. Unexpectedly, we found the opposite pattern in male songs and female preferences: male and female *G. lineaticeps* have evolved riskier signals and riskier signal preferences in response to parasitism by *O. ochracea*. These results cannot be entirely explained by the antagonistic selection hypothesis. First, we propose a different hypothesis, the “late-season parasitism hypothesis,” that could better explain some of our findings. Second, we discuss how our results relate to this and the antagonistic selection hypothesis.

The late-season parasitism hypothesis

In contrast to the predictions of the antagonistic selection hypothesis, we argue that parasitism (or predation) might sometimes facilitate the evolution of risky male signals and risky female signal preferences. In particular, the temporal pattern of parasitism might affect how natural selection acts on male and female reproductive traits. Individuals commonly do not invest maximally in current reproduction because of a fundamental tradeoff with future reproduction (Williams 1957). When parasites are present throughout the breeding season, selection should favor the evolution of less risky male signals and female preferences, as predicted by the antagonistic selection hypothesis, because individuals that express these traits can incur a substantial cost in terms of both current and future reproduction. However, when parasites are primarily present during the late portion of the breeding season, selection may favor the evolution of risky signals and signal responses because, if an individual's future reproductive prospects are low, it may pay to invest heavily in current reproduction (e.g., in highly attractive male signals and female preferences for these signals) in order to reproduce at a high rate prior to the onset of parasite-related mortality. We refer to this pattern as the late-season parasitism hypothesis.

In *G. lineaticeps*, parasitism is temporally limited: the crickets breed from the early summer to early fall (Paur and Gray 2011; Weissman et al. 1980), whereas the flies primarily attack the crickets in the late summer (Paur and Gray 2011). The breeding season in parasitized populations essentially ends 2–3 weeks after the flies begin attacking the crickets because parasitism removes most singing males from the population, whereas substantial numbers of polyandrous females (Wagner et al. 2001) remain, some of which have never mated, some of which are searching for additional mates (WEW and OMB, personal observations). Thus, parasitism may favor enhanced reproduction earlier in the breeding season before the parasites begin searching for hosts. Our hypothesis predicts that males from high-risk populations should produce more attractive but riskier songs (i.e., faster chirp rates and shorter chirp durations) and that females should express chirp rate preferences that are riskier but yield higher fecundity benefits (i.e., faster chirp rates). Note that field crickets typically live for a few weeks in the field (Murray and Cade 1995). As a result, selection due to fly parasitism primarily occurs on individuals who are reproductively active later in the breeding season.

Riskier male signals

There are at least two non-mutually exclusive reasons why selection may have favored high-risk male signals in populations of *G. lineaticeps* with a higher risk of predation. First, natural selection due to late-season predation in high-risk populations may have favored male investment in the most attractive signal combination, i.e., a high chirp rate but short chirp duration (Wagner and Basolo 2007a; Wagner et al. 2012), because producing this attractive signal type allows males to maximize mate attraction and reproduction before parasitism causes high mortality at the end of the breeding season. This explanation would be consistent with the proposed late-season parasitism hypothesis. Second, the strongly directional female preferences in high-risk populations may have favored higher chirp rates in these populations. However, this increase in chirp rate in high-risk population may be counteracted by the increased risk of parasitism (Wagner 1996), resulting in only a small increase in chirp rate in these population, as reported here. This explanation is consistent with the antagonistic selection hypothesis. Given that the late-season parasitism hypothesis and the antagonistic selection hypothesis make identical predictions for male song evolution when females from high-risk populations express more strongly directional preferences, further work would be necessary to distinguish between them.

In a previous study, we tested the prediction of antagonistic selection hypothesis that male *G. lineaticeps* from high-risk populations will sing less frequently, particularly early in the night, to avoid parasitism (Beckers and Wagner 2012). Unexpectedly, males from high-risk populations sang as frequently as males from low-risk populations, and males from both types of populations showed the same temporal pattern of singing activity across a night. This high, rather than reduced, level of singing activity in high-risk populations would contribute to higher rates of reproduction as proposed here by the late-season hypothesis. As for our chirp rate results, this similar level of singing activity for males from low- and high-risk populations could also be consistent with the antagonistic selection hypothesis if females sample male singing activity across a night, and if females preferentially mate with males that sing more frequently.

The differences between low- and high-risk populations in male song characters were relatively small (difference in chirp rate = 0.07 chirps/s; difference in chirp duration = 0.004 s) and are likely of little biological importance. It is noteworthy, however, that males from high-risk populations do not produce safer song types given that fly parasitism, while only occurring late in the season, has a major effect on male reproductive success. Selection for early reproduction and/or selection to produce attractive song types must be strong enough to counteract the effects of fly parasitism.

In contrast to *G. lineaticeps*, males of the field cricket *Teleogryllus oceanicus*, that is attacked by *O. ochracea* in Hawaii, have evolved a safer, non-singing phenotype in response to fly parasitism rather than riskier song types (Zuk et al. 2006; Tinghitella 2008). The temporal pattern of fly parasitism, however, may be substantially different for the two prey species. In North America, *O. ochracea* are highly seasonal, attacking field crickets in the later stages of the cricket's breeding season (Paur and Gray 2011; Cade et al. 1996). In contrast, *O. ochracea* females in Hawaii appear to orient to male song whenever males are singing (M. Zuk, personal communication). This difference in the seasonality of male parasitism risk may at least partially explain the strikingly different evolutionary outcomes of fly parasitism in the two species of field crickets.

The chirp rates measured in our experimental lab animals were about 1 to 1.5 chirps/s slower than those of field animals from some of the same populations (Wagner et al. 2012), where mean chirp rates are typically around 3 chirps/s (Wagner 1996; Wagner and Reiser 2000; Wagner et al. 2012). We noticed a similar effect of reduced chirp rates in laboratory reared *G. lineaticeps* males in a previous study (Wagner and Harper 2003). As a result of this reduction, males in the field in low-risk populations produce chirp rates near the peak preference of females, whereas males in the field in high-risk populations produce chirp rates well below the peak preference of females reported here. The reason for this reduction in chirp rate in the lab is not clear, but could be related to nutrition, the absence of temperature and humidity variation, and/or the social isolation of males during song recordings. However, since all experimental animals were reared and recorded under the same conditions, a general rearing or social isolation effect likely had similar effects on males from low- and high-risk populations.

In contrast to male chirp rates, the preferences of our experimental lab females seemed to be matched with the average chirp rates of males observed in the field (Wagner 1996; Wagner and Reiser 2000; Wagner et al. 2012), at least for females from low-risk populations. It is possible, however, that female preferences, like male chirp rates, shifted in lab-reared individuals. For example, females in the field might have higher peak chirp rate preferences than seen in our lab-reared females. Note, that even if peak female preferences were shifted in all of the laboratory populations, females from high-risk populations were still significantly more attracted to faster singing males compared to low-risk females, and the general shapes of the preference functions (open vs. closed) differed substantially between the two types of populations. However, additional work will be necessary to determine if female preferences differ for lab and field females, and if so, how this translates into differences in sexual selection in low- and high-risk populations.

Riskier female preferences

Females from high-risk populations displayed open chirp rate preference functions with highest attraction to male songs with the highest tested chirp rates (3.6 and 4.2 chirps/s; Fig. 1c), which also correspond to the highest chirp rates of wild males (Wagner 1996; Wagner and Reiser 2000; Wagner et al. 2012). In contrast, females from low-risk populations were centered at an intermediate chirp rate (3.0 chirps/s), which correspond to the average chirp rates of wild males (Fig. 1b). Since *O. ochracea* displays preferences for faster chirp rates (Wagner 1996), female field crickets from high-risk populations incur association costs related to these preferences (Martin and Wagner 2010) late in the breeding season when the flies are searching for hosts. The late-season association costs with riskier males, however, may be outweighed by benefits of mating with males exhibiting these extreme phenotypes. In *G. lineaticeps*, males that produce fast chirp rates also transfer fecundity-enhancing seminal fluid products to females during mating, allowing females to produce more eggs (sensu Wagner and Harper 2003). Preferences for more exaggerated male sexual traits in high-risk populations in *G. lineaticeps* are consistent with the late-season parasitism hypothesis but not the antagonistic selection hypothesis that would predict a shift toward safer rather than more risky chirp rates.

There are two non-mutually exclusive reasons why late-season parasitism would favor female preferences for fecundity-enhancing (fast-chirping) males in *G. lineaticeps*. First, parasitism on females late in the season might directly select for increased reproduction earlier in the season, explaining the preferences for faster chirp rates. Second, the disappearance of males due to parasitism late in the season might favor early reproduction and therefore preferences for fast-chirping males. Since parasitism rate of female *G. lineaticeps* is substantially lower than that of males (6 vs 65%; Martin and Wagner 2010), we suggest that the disappearance of males rather than the direct risk of being parasitized has likely had a stronger influence on the evolution of the preferences in high-risk populations of *G. lineaticeps*.

Gryllus lineaticeps is a flight polymorphic field cricket (Weissman et al. 1980), and some individuals can fly long distances. Even though our populations were separated from each other by 200+ km (and physical barriers in some instances; see figure S1; supplementary materials), there is potentially gene flow among populations, which would tend to limit the extent to which populations can diverge. Given the likelihood of gene flow among populations, the highly divergent preferences of females from low- and high-risk populations are remarkable: selection to mate with high chirp rate males in high-risk populations would have to be strong to overcome the potentially homogenizing effects of gene flow.

Alternative explanations

The differences in male songs and female preferences between high-risk and low-risk populations could potentially be explained by sources of selection correlated with, but unrelated to, parasitism risk. For example, if parasitized populations have higher cricket densities, male-male competition may be more intense, favoring more attractive signals, and female preferences might be more directional because females have more choices at higher densities. We did not, however, notice a consistent relationship between parasitism status and density in the field (OMB and WEW personal observations). Furthermore, the higher parasitism risk of males (Martin and Wagner 2010) toward the end of the breeding season causes the operational sex ratio to become strongly female-biased, which would reduce the intensity of male-male competition in high-risk populations. However, we cannot rule out the presence of other sources of selection explaining the pattern of signals and preferences in high-risk populations.

It is also possible that selection has favored the evolution of behavioral plasticity rather than the evolution of mean phenotypes. For example, males might adjust their chirp rates and singing activity, and females might adjust their preferences, based on the perceived risk of parasitism in the field (e.g., Gong and Gibson 1996; Johnson and Basolo 2003). In the field cricket *G. texensis*, females (but not males) can sense the presence of the parasitoid (Vincent and Bertram 2010). If males and females express less risky behavior when the parasitoids are present, this could reduce the cost of parasitism, a hypothesis that we did not test. Males and females could also potentially use indirect cues to assess parasitism risk, such as day length and temperature. We reared crickets on a 14:10 h light/dark cycle, which is typical of late July, and in the absence of any direct parasitoid cues. The parasitoid flies can attack field crickets in late July in some years, but they usually do not begin attacking the field crickets until August (Wagner unpublished data). So, at a day length that typifies a low-risk of parasitism, and in the absence of direct parasitoid cues, males from high-risk populations produced higher chirp rates than males from low-risk populations, and females from high-risk populations expressed preferences for higher chirp rates than females from low-risk populations. Thus, even if selection due to fly parasitism has favored the evolution of behavioral plasticity, our results indicate that selection has also favored the evolution of higher male chirp rates and directional female chirp rate preferences in high-risk populations.

In summary, our results suggest that parasitism, and by extension predation, can potentially have diverse and sometimes unexpected effects on the evolution of male and female reproductive behavior, namely the evolution of exaggerated female preferences and male traits. Our data suggest that male and female *G. lineaticeps* have evolved trait values that ultimately

increase their chances of being killed by eavesdropping parasitoids late in the season, because these same traits likely increase reproduction earlier in the season when the parasitoids are not present. Considering the temporal pattern of parasitism, we argue that the late-season parasitism hypothesis provides a potentially useful framework for understanding the evolution of seemingly costly signals and preferences. While many animals incur a relatively constant risk of predation or parasitism across the breeding season, in many others, predation or parasitism is transient or seasonal (e.g., Kingsolver and Srygley 2000; McCutchen 2002; Remmel et al. 2009; Titsaar et al. 2013). Thus, the temporal pattern of predation or parasitism may have a major effect on how sexually selected traits evolve.

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